Improved Tomato Breeding Lines Adapted to Organic Farming Systems Have Enhanced Flavor, Yield, and Disease Resistance

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Why they did not use organic seed was the lack of desirable traits available in an organic variety. It has been established that conventional breeding objectives can differ from organic breeding objectives and breeding for the specific needs of organic systems is essential to developing high-performing varieties for organic agriculture (Ceccarelli 1994; Lammerts van Bueren and Myers 2012). Environmental conditions on conventional farms are relatively similar to each other because of higher and more uniform use of synthetic fertilizers and pesticides, whereas more variable management practices in organic systems lead to field conditions that differ more significantly among organic farms. Because of the difference in the production conditions and the market objectives, the desirable traits for a variety differ for organic and conventional farmers.

Flavor is the top priority for tomato growers in the Midwest, followed by disease resistance, crack resistance, and nutritional value (Hoagland et al. 2015). In terms of disease concerns, 67% of the organic farmers responding to the survey found early blight (Alternaria solani) (EB) difficult to control, while 72% found Septoria leaf spot (S. lycopersici) (SLS), 58% found leaf mold (Passalora fulva) (LM), and 33% found late blight (Phytophthora infestans) (LB) hard to control. Powdery mildew (Oidium neolycopersici) (PM) was not identified as an important pest in this survey, but it has appeared to spread more recently in greenhouse and high tunnel tomato production, making it a potential disease of concern for current and future farmers. Genetic resistance to PM has been found to be related to six monogenic genes (Bai et al. 2005) and three polygenic Quantitative Trait Loci (QTLs) (Bai et al. 2003), and none of these are known to be present in the parental lines of the tomato breeding lines evaluated in this research. EB is a disease that is particularly important to organic farmers because there are few products that can help with prevention, and it is even more complicated to stop the spread once the fungus cycle has started. LM is a fungal disease that has become predominant in high tunnels due to the high relative humidity and low ventilation that can potentially occur depending on the management (Sudermann et al. 2022). Combining disease resistance, high yields, and good flavor in tomato has proved challenging. SLS resistant tomato varieties have been developed, but they do not always have acceptable flavor. Although organic direct-market farmers report that flavor is the most critical trait that they consider when choosing varieties, they also require varieties that have sufficient production potential and disease resistance. According to a survey of Wisconsin organic vegetable farmers carried out in 2012 by Lyon et al. (2015), disease tolerance was the highest-ranked trait regarding priorities for plant breeding, followed by insect tolerance and yield.

Decentralizing the breeding process and involving farmers in trials can result in improved organic breeding outcomes (Casals et al. 2019; Dawson et al. 2011). Participatory plant breeding (PPB) enables farmers and breeders to develop varieties that are adapted
to local conditions, and selection and trials can happen both in research stations and on-farm. PPB was initially used to support economically disadvantaged farmers in the Global South who were not benefitting from nonparticipatory, conventional breeding programs (Bellon 2006). A participatory approach can address several challenges related to conventional crop improvement. Decentralization of the environments where selection is carried out is key to developing varieties adapted to diverse agricultural systems. PPB itself promotes the diversification of environments and integration of multiple actors in the breeding process, working toward a more geographically and stakeholder decentralized variety development. Including farmers early in the breeding program can greatly accelerate relevant improvements, especially if they are experienced in the nuances of their production systems and market preferences. PPB approaches have been successful both in the Global North and South. Colley et al. (2021) identified 47 projects across the United States, Canada, and Europe, including trials with 22 crops. In the United States alone only, PPB has aided in the development of new varieties, genetic diversification, and conservation of staple crops such as apples, tomatoes, maize, oats, peppers, potato, and wheat, among others. A successful example of PPB is the development of ‘Who Gets Kissed’ sweet corn, in which organic farmers defined the priority traits for the breeding program, then researchers and a public sector plant breeder in Wisconsin carried out the initial population development. The project culminated with the release of a new variety that farmers in other states such as Oregon, Washington, California, and New Mexico have continued to adapt to their local conditions (Shelton and Tracy 2015).

Our project emerged as an initial step toward wider collaborative organic breeding efforts that can meet the overall demand for reliable organic tomato varieties while developing high-performing varieties that are specifically adapted for organic farming in the Upper Midwest. Previous work identified promising tomato varieties (Healy 2016; Hodge et al. 2019) that were chosen as parental varieties for our participatory breeding project. Organic farmers hosted production trials of our breeding lines; chefs evaluated their culinary qualities; and research trials assessed yield, production traits, and response to plant diseases. This article presents the results of the project and analyzes the potential of the breeding lines to be released as varieties or used as genetic resources for future tomato breeding efforts.

### Materials and Methods

#### Plant material

We evaluated 10 advanced breeding lines developed under organic management (Healy et al. 2017; Hodge et al. 2019). These lines were selected under an organic high-tunnel management system during the summer seasons, and advanced without selection in a greenhouse each winter. The parental lines were selected for their high quality, particular flavor, disease tolerance, and yield. Defiant is an F1 hybrid that has high resistance to LB (resistance genes Ph-2 and Ph-3) and intermediate resistance to EB (Johnny’s Selected Seed 2021). OSA404 is a cross between “Wisconsin 55” (W155) and a disease-resistant North Carolina State inbred, selected by the Organic Seed Alliance for disease tolerance and flavor over several years. It was received in 2014 as an advanced line and maintained by selfing. A6 is a reselection of an Amish heirloom selected for Midwest–ern adaptation by Craig Grau, a retired plant pathologist at University of Wisconsin–Madison. ‘Japanese Black Trifele’ is an heirloom with a smoky flavor maintained by K Greene at the Hudson Valley Seed Company. ‘Crimson Sprinter’ is an heirloom from Ontario, CA, USA, with partial SLS resistance, earliness, and good flavor. ‘Defiant’ and ‘Japanese Black Trifele’ were also used as check varieties. A summary of the trialed breeding lines can be found in Table 1. Figure 1 shows a simplified version of the crossing diagram used to develop the 10 breeding lines evaluated in this project.

#### Line selection and advancement

In Summer 2017, 18 tomato varieties, including commercial lines and unreleased advanced germplasm, were planted in both the open field and high tunnel, and 22 crosses were made between the parental lines. Of the 22 crosses, 16 were successful. In Winter 2017–18, the first F1s were grown, and with the parental lines for the crosses that were not successful in the first round. Three plants per F1 cross were planted in winter, and seed was saved from at least one plant. The six remaining crosses were successfully made during that winter as well. In Summer 2018, the F2s obtained from the winter crosses and the F3s obtained from the seed advancement were planted in a high tunnel at the West Madison Agricultural Research Station (WMARS), totaling 22 families and 40 lines in total. The experimental unit consisted of three plants per breeding line, and each was replicated twice. One or two plants were selected per cross for advancement, taking into consideration productivity, flavor, and disease resistance. At this point, 55% of the lines were dropped from the program, keeping eight families and 18 lines total. The seed saved in Summer 2018 was advanced during Winter 2018–19, obtaining F3 and F4 seed. The F3 and F4 lines were planted in Summer 2019, totaling 18 lines. From Summer 2017, two families were dropped from the program, keeping six families and 10 lines in total. The 10 lines were advanced during Winter 2019–20, and the selected families and lines were planted in Summer 2020 and 2021 following the experimental design explained in the next section. Figure 2

**Table 1. Parent lines, generation, market type, and fruit color and size of 10 tomato breeding lines evaluated in 2020 and 2021 in organic high tunnel systems in the West Madison Agricultural Research Station, Verona, WI, USA.**

<table>
<thead>
<tr>
<th>Family name</th>
<th>Seed Parent</th>
<th>Pollen parent</th>
<th>Generation</th>
<th>Market class</th>
<th>Fruit color and size</th>
</tr>
</thead>
<tbody>
<tr>
<td>A6JB-F5-34</td>
<td>A6</td>
<td>‘Japanese Black Trifele’</td>
<td>F5</td>
<td>Slicer</td>
<td>Pink, large size</td>
</tr>
<tr>
<td>JBD-F5-28</td>
<td>‘Japanese Black Trifele’</td>
<td>Defiant</td>
<td>F5</td>
<td>Slicer</td>
<td>Brick red, medium size</td>
</tr>
<tr>
<td>JBD-F5-31</td>
<td>‘Japanese Black Trifele’</td>
<td>Defiant</td>
<td>F5</td>
<td>Slicer</td>
<td>Brick red, medium size</td>
</tr>
<tr>
<td>O4JB-F5-MV1-116</td>
<td>OSA 404</td>
<td>‘Japanese Black Trifele’</td>
<td>F5</td>
<td>Contemporary Heirloom</td>
<td>Dark pink, large size</td>
</tr>
<tr>
<td>O4JB-F6-5</td>
<td>OSA 404</td>
<td>‘Japanese Black Trifele’</td>
<td>F6</td>
<td>Slicer</td>
<td>Red, medium to large size</td>
</tr>
<tr>
<td>CSDE-F6-46</td>
<td>‘Crimson Sprinter’</td>
<td>‘Defiant’</td>
<td>F6</td>
<td>Slicer</td>
<td>Red, medium size</td>
</tr>
<tr>
<td>CSDE-F6-47</td>
<td>‘Crimson Sprinter’</td>
<td>‘Defiant’</td>
<td>F6</td>
<td>Slicer</td>
<td>Red, medium size</td>
</tr>
<tr>
<td>O4DE-F5-43</td>
<td>OSA 404</td>
<td>‘Defiant’</td>
<td>F5</td>
<td>Slicer</td>
<td>Red, medium size</td>
</tr>
<tr>
<td>O4DE-F5-44</td>
<td>OSA 404</td>
<td>‘Defiant’</td>
<td>F5</td>
<td>Slicer</td>
<td>Red, medium size</td>
</tr>
<tr>
<td>O4A6-F4-MV1-109</td>
<td>OSA 404</td>
<td>A6</td>
<td>F4</td>
<td>Slicer</td>
<td>Dark red, medium to large size</td>
</tr>
</tbody>
</table>

---

**Fig. 1. Summarized crossing diagram used to develop 10 tomato breeding lines in Madison, WI, USA.**
summarizes the selection and advancement process followed in this tomato breeding program. **Experimental design.** In 2020 and 2021, the advanced breeding lines were grown at the West Madison Agricultural Research Station, University of Wisconsin–Madison, Madison, WI, USA (43.06054765°N, 89.52376954°W, elevation 323 m) on land certified organic by the Midwest Organic Services Association since 2008. The high tunnel was on a level area, oriented north-south; the rows ran from east to west, with the long ends facing east and west. The high tunnel dimensions were 30 ft × 88 ft (9.1 m × 26.8 m) and covered ~2640 ft² (245.3 m²). The experiment was designed as a randomized complete block design. The experimental unit (plot) comprised three individual plants in 2020 and four individual plants in 2021. The experimental units were replicated twice in both years. The within-row plant spacing was 2 ft (0.61 m), and beds were 4 ft apart (1.2 m), center to center, with 2 ft (0.61 m) aisles. **Management.** In 2020 and 2021, the soil was amended according to soil nutrient analysis using Renaissance 11–0–0, an Organic Materials Review Institute (OMRI) approved feather meal fertilizer (PIC & Company, Ecological Land Care Inc., Rowley, MA, USA) to achieve a total rate of 125 lb N per acre (140 kg/ha). Before each planting, a cover crop of winter rye was grown and incorporated. In 2020, transplants were started by West Star Organics in Cottage Grove, WI, USA, a USDA-certified organic grower. In 2021, the transplants were started by Circadian Organics in Viroqua, WI, USA, also a USDA-certified organic grower. Transplants were planted on 12 May in both years. Beds were laid with drip irrigation and covered with black landscape fabric. The aisles were covered with straw mulch to control weeds. Plants were watered consistently 3 d a week during the first month, decreasing to twice a week from then on. Plants received between 0.75 and 1.0 inches of water per week.

Pruning was done weekly until the plants were ~5 ft tall. After this, pruning was done as required to maintain a two-lead trellis system. When the plants reached 4 to 5 ft tall, the basal leaves were pruned to increase the airflow of the canopy and to increase the distance between the leaves and the soil, with the purpose of diminishing the potential of disease development and spread. The plants were trellised using tomato clips to guide the plants up the twine attached to the cross-braces. The high tunnel temperature was closely monitored to decide when to open and close the sides. Side vents and doors were opened manually to maintain good ventilation and keep day-temperatures below 95 °F (35 °C) and above 65 °F (18 °C) during night. Higher than 95 °F daily temperatures can cause detrimental effects on pollen quality, pollination, flower abortion, and fruit set. **Harvest.** Harvest was done once a week, picking tomatoes that were at least in “pink stage” following the USDA definition (USDA 2005), which means that at least 30% of the fruit surface, in aggregate, shows a change in color from green to tannish yellow, pink, or red. Yield was recorded on a per-plot basis. At harvest, the tomatoes were subdivided into marketable and unmarketable fruit. Marketable fruit was counted and weighed, whereas unmarketable tomatoes were weighed and the reason(s) of unmarketability recorded. Weights are presented in kilogram/plant and tons/plant, where ton refers to metric ton throughout this article. Any fruit with signs of damage such as splitting, cat face, windowing, scarring, insect, rodent signs, and rots were considered unmarketable. A sample of fully ripe fruit was set aside for the quality evaluation. The percentage of unmarketable fruit by weight was also calculated for each plot.

**Flavor.** Tomato fruit flavor was evaluated weekly. In 2020, samples were packed individually for research staff to take home. This was implemented to follow the sanitary guidelines in effect due to the coronavirus pandemic. In 2021, research staff conducted in-person tasting in the field three times during the peak harvest season. The tasting group participated in a calibration exercise at the beginning of the season. This exercise included the recognition of the basic flavor components—sweet, acid, salty, bitter, and umami—at three different concentrations in water. Breeding lines were divided into different tasting groups based on shared parents and market similarities. When a breeding line did not have enough fruit for testing in its designated group and date, it was tasted later in the season. Only completely ripe fruit was used for flavor analysis. Fruit from each plot of each line was bulked in a composite sample. Tasters rated each sample on a 1 to 5 scale for sweetness, acidity, saltiness, bitterness, and umami, where 1 was very low perception and 5 was high perception of that flavor. Flavor intensity was also rated on a 1 to 5 scale, with 1 being low, and 5 being high intensity of a “tomato” flavor. Finally, after completing the tasting set, tasters were asked to return to each sample and rate it on an overall scale for flavor with 1 being very bad and 5 being excellent.

Because the taste testing includes the participation of people, an institutional review board (IRB) application was submitted to the University of Wisconsin–Madison for approval. The IRB committee qualified the study to be exempt from federal regulations under category 45CFR 46.101(b)(6): Taste and food quality evaluation (Pre-2018 Requirements), and the protocol was approved on 26 Aug 2014 and renewed every 3 years.

**Flavor analysis.** Fruit was used for flavor analysis. A sample of the fruit set apart for testing was saved and frozen to later measure citric acid equivalents (CA) and °Brix (as a measure of total dissolved solids) in the laboratory. This was to analyze and find any correlations between the taster’s flavor perception and some of the main elements, sugar, and acid, which contribute to tomato flavor. For both CA and °Brix, tomato juice samples were filtered through cheese cloth to remove excess solids. °Brix levels were tested using a digital refractometer (model no. 30051; Sper Scientific, Scottsdale, AZ, USA). Acidity content by volume was measured using an automatic titrator (model HI901C; Hanna Instruments, Smithfield, RI, USA) and is reported as g citric acid/100 g sample because CA is the main organic acid in tomatoes that contributes to titratable acidity and pH (Wilkinson et al. 2013). Twenty milliliters of each tomato sample were titrated, NaOH was used at a 0.1 N

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**Fig. 2. Selection and seed advancement diagram used to develop 10 advanced tomato breeding lines in Madison, WI, USA from 2017 to 2021.**
concentration, and the samples were titrated to a fixed endpoint of 8.1 pH.

Disease incidence and severity. Plots were scored every 2 weeks starting 8 weeks after transplanting. Diseased foliage was recorded using a 0% to 100% scale in 5% increments of leaf area affected by disease. A 0% score referred to a healthy plot that had no symptoms of foliar diseases. A 100% score referred to a plot where the plants were completely dead. The evaluation included EB, LM, PM, and SLS. The area under the disease progress curve (AUDPC) was calculated for each breeding line. The AUDPC was a quantitative summary of the disease intensity over time and is useful to compare values across plant varieties, years, locations, or management. The trapezoidal method was followed to calculate the AUDPC values for each disease for each breeding line by calculating the average disease intensity between each pair of adjacent data points (weekly) (Jeger 2004).

Statistical analysis. The statistical analysis was carried out using RStudio Software (RStudio Team, Vienna, Austria). The information collected was analyzed using a mixed model analysis of variance (ANOVA) and least squares (LS) means were calculated for year, genotype, and the interaction between year and genotype. The model was defined as:

\[ y_{ijk} = \mu + G_i + Y_j + GY_{ij} + B_k(Y_j) + e_{ijk}, \]

where \( \mu \) represents the grand mean, \( G_i \) represents the main effect variety of the \( i^{th} \) genotype, \( Y_j \) represents the year effect of the \( j^{th} \) year, \( GY_{ij} \) represents the effect of genotype-by-year interactions, \( B_k \) represents the effect of the \( k^{th} \) block nested within year, with unexplained error \( e_{ijk} \). Dependent variables were production traits (marketable fruit count, average fruit weight, marketable yield, percent unmarketable yield), disease traits (AUDPC for EB, LM, PM, and SLS), and fruit quality traits (CA, Brix, and taste evaluation scores). The mixed model was built using the lmer function from the R package lme4 (Bates et al. 2015). The LS means were calculated using a mixed model, where the genotype was a fixed effect, the year was a random effect, the interaction between genotype and year was a random effect, and block and error were random effects. The LS means were calculated using the emmeans function from the emmeans package (Lenth 2023). Means comparison was done using Tukey’s multiple comparison procedure implemented in the emmeans package.

Correlations. The production, fruit quality, and disease traits were correlated to each other by calculating the Pearson correlation coefficient. The correlation matrix was calculated using the cor base function in R Programming Software. A year-to-year Pearson correlation coefficient was also calculated for each trait using the cor function in R. Correlations reported are Pearson coefficients (\( \rho \)).

Broad-sense heritability. Broad-sense heritability estimates the proportion of phenotypic variance (\( V_P \)) that is due to genetic causes (\( V_G \)) (Bernardo 2020) and was calculated as:

\[ V_P = \frac{V_G}{V_P}, \]

where \( V_G \) is the genotypic variance, and \( V_P \) is the phenotypic variance, which is calculated as:

\[ V_P = \frac{V_{Gy}}{\text{nyear}} + \frac{V_{Gc}}{\text{nrep} \times \text{nyear}}, \]

where \( V_{Gy} \) is the genotypic variance, \( V_{Gc} \) is the genotype-by-year variance, \( \text{nyear} \) is the number of years, \( \text{nrep} \) is the number of replications, and \( V_e \) is the error variance.

A linear model was built using the lm function in R, from which an ANOVA analysis was carried out. The ANOVA results provided each component in the model. With the means squares, the genetic variances were calculated using the variance formulas provided by Bernardo (2020):

\[ V_G = \frac{MS_{Genotype} - V_{Gy}}{r^2} = \frac{MS_{Genotype} - V_e}{r}, \]

where \( V_G \) is the genotypic variance, \( V_{Gy} \) is the genotype-by-year variance, \( V_e \) is the error variance, \( r \) is the number of replicates, \( e \) is the number of years, \( MS_{Error} \) is the error mean square, \( MS_{GE} \) is the genotype-by-year mean square, and \( MS_{Genotype} \) is the genotype mean square.

On-farm trials. The breeding lines evaluated in this project were trialed on-farm by six organic farmers in the Upper Midwest in 2020 and 2021. Farmers were sent enough seed to plant six plants per breeding line on their farm, following their normal agricultural management. The six plants were planted in a single plot, and each trial was planted within their normal production tomato area following the planting map that was provided (Supplemental Fig. 1), surrounded by other tomato varieties that farmers grew commercially. This allowed farmers to compare the breeding lines to the main tomato cultivars they would grow for market. The farmers were requested to submit their evaluations considering their own market needs (Supplemental Table 1).

The farmers grew the breeding lines under protected structures such as high tunnels or caterpillar tunnels. Throughout the season, the farmers took notes on overall yield, fruit quality, disease resistance, and earliness of each breeding line. Each farmer returned an evaluation form with notes on each line, and this feedback was used to understand the uniformity of the lines among different growing environments and to know how well these lines satisfied the needs of the farmers and the potential for variety release.

Results

Production

Genotype was a highly significant source of variation for all the production traits, which includes marketable count, marketable weight, proportion unmarketable by weight, and average fruit weight (Table 2). The broad-sense heritability was high for the production traits (Table 2). Year and genotype-by-year interaction were not significant sources of variation for these traits. Overall, the average marketable weight of all the breeding lines was 6.31 kg/plant and 6.11 kg/plant for 2020 and 2021, respectively, with no significant differences between years. No genotype-by-year interaction was observed for any of the production traits (Table 2).

In 2020, the check varieties ‘Defiant’ (8.64 kg/plant) and ‘Japanese Black Trifelé’ (8.12 kg/plant) had the highest marketable

Table 2. Broad-sense heritability (\( H^2 \)), significance of F tests of production, disease, and fruit quality traits and year-to-year correlation evaluated from 10 tomato breeding lines grown in an organic high tunnel system in 2020 and 2021, at the West Madison Agricultural Research Station, Verona, WI, USA.

<table>
<thead>
<tr>
<th>Trait</th>
<th>( H^2 )</th>
<th>Genotype</th>
<th>Year</th>
<th>Genotype \times Year</th>
<th>Year correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marketable count</td>
<td>0.97</td>
<td>***</td>
<td>*</td>
<td>NS</td>
<td>0.97***</td>
</tr>
<tr>
<td>Marketable weight (kg)</td>
<td>0.91</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>0.83***</td>
</tr>
<tr>
<td>Average fruit weight (g)</td>
<td>0.99</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.98***</td>
</tr>
<tr>
<td>EB</td>
<td>0.67</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>0.66*</td>
</tr>
<tr>
<td>LM</td>
<td>0.71</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>0.51</td>
</tr>
<tr>
<td>PM</td>
<td>0.12</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>0.15</td>
</tr>
<tr>
<td>SLS</td>
<td>0.68</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.37</td>
</tr>
<tr>
<td>Brix</td>
<td>0.74</td>
<td>*</td>
<td>NS</td>
<td>**</td>
<td>0.57</td>
</tr>
<tr>
<td>CA (%)</td>
<td>0.94</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>0.87***</td>
</tr>
<tr>
<td>Appearance</td>
<td>0.55</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>0.91***</td>
</tr>
<tr>
<td>Texture</td>
<td>0.33</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>0.76*</td>
</tr>
<tr>
<td>Sweetness</td>
<td>0.27</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.57</td>
</tr>
<tr>
<td>Acidity</td>
<td>0.22</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>0.55</td>
</tr>
<tr>
<td>Bitterness</td>
<td>0.07</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>0.08</td>
</tr>
<tr>
<td>Umami</td>
<td>0.14</td>
<td>*</td>
<td>NS</td>
<td>***</td>
<td>0.06</td>
</tr>
<tr>
<td>Intensity</td>
<td>0.26</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>0.60</td>
</tr>
<tr>
<td>Overall</td>
<td>0.2</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>0.78**</td>
</tr>
</tbody>
</table>

CA = citric acid equivalent; EB = early blight; LB = late blight (Phytophthora infestans); LM = leaf mold (Passalora fulva); PM = powdery mildew (Oidium neolycopersici); SLS = Septoria leaf spot (S. lycopersici).

NS, *, **, *** nonsignificant or significant \( P \leq 0.05, 0.01, \) or 0.001, respectively.
weight values, followed by the breeding lines CSDE-F6.46 (7.39 kg/plant), O4DE-F5.44 (7.06 kg/plant), and O4DE-F5.43 (7.04 kg/plant), with no significant differences between any of these lines (Supplemental Table 2). In 2021, the lines with the highest marketable weight were O4DE-F5.44 (8.29 kg/plant), ‘Japanese Black Trifele’ (7.59 kg/plant), O4DE-F5.43 (7.54 kg/plant), ‘Defiant’ (7.21 kg/plant), and CSDE-F6.46 (6.61 kg/plant), with no significant pairwise differences. In terms of fruit size, O4DE-F5.43 had the highest fruit weight (398 g/fruit), followed by O4DE-F5.44 (387 g/fruit), with no significant difference between these lines. These varieties can be considered large-sized slicers. The CSDE-F6.46 (135 g/fruit) and CSDE-F6.47 (135 g/fruit) lines were not significantly different from the check varieties ‘Defiant’ (112 g/fruit) and ‘Japanese Black Trifele’ (152 g/fruit) (Supplemental Table 2). In terms of proportion unmarketable, CSDE-F6.46, JBDE-F5.31, and ‘Defiant’ had the lowest values (0.10, 0.11, and 0.12 respectively). The lines O4JB-F5-MV1.116 (0.48), A6JB-F5.34 (0.42), and O4A6-F4-MVI.109 (0.41) had the highest proportion unmarketable.

**Disease**

Genotype and year were both significant sources of variation for EB AUDPC. No significant genotype-by-year interaction was observed. In 2020 and 2021, JBDE-F5.28, ‘Defiant’, and ‘Japanese Black Trifele’ had the lowest EB AUDPC scores, but differed significantly only from O4JB-F5-MV1.116 with the highest score (637). Year was a significant source of variation, and overall, the AUDPC scores for EB were higher in 2020 than in 2021. The year 2020 had a higher number of intense rain and elevated temperature events than 2021. EB thrives on wet surfaces (leaves) and moderate temperatures (~27°C) (Foolad et al. 2008); thus, these results align with expectations. The breeding line JBDE-F5.28 had the lowest EB AUDPC score (125), and O4JB-F5-MV1.116 had the highest score (637) (Fig. 3). The broad-sense heritability of EB AUDPC was medium (0.67), with a high genetic variance. This can be explained by the high variability between the parental lines.

For SLS AUDPC, only year was a significant source of variation. In 2020, the overall SLS AUDPC score was 55, whereas in 2021, it was 398. Overall, CSDE-F6.47 had the highest score (459), and ‘Japanese Black Tri- fele’ had the lowest (53). Although there was not a statistically significant effect of genotype, the correlation among breeding line scores between years was moderate at 0.37, and broad-sense heritability was also moderately high (0.67), suggesting that with more consistent disease pressure, we might see more consistent differences among varieties.

For LM AUDPC, genotype was the only significant source of variation (Table 2), although there were not any significant pairwise differences. The AUDPC score ranged from 311 to 878 in 2020 and from 359 to 1379 in 2021. Overall, LM spread later in the season and did not seem to significantly affect yield or fruit quality. LM had a medium to high broad-sense heritability (0.71). This can be attributed in part to the variability between the parental lines.

For PM AUDPC, genotype, year, and the interaction between genotype and year were significant sources of variation. There was a significant difference between years, in the opposite direction of the SLS disease pressure. For PM AUDPC, the mean for 2020 was 838, and dropped to 140 in 2021. Overall, O4DE-F5.44 had the lowest PM AUDPC score (322), and CSDE-F6.47 had the highest (812). The broad-sense heritability for PM AUDPC was exceptionally low (0.12), which could be explained due to having a large proportion of the phenotypic variation explained by the genotype-by-year interaction (Table 2).

**Fruit quality**

Genotype was a significant source of variation for ‘Brix and CA. Year and genotype-by-year interaction were not significant sources of variation for either trait (Table 2). Genotype was a significant source of variation for all the taste tasting traits evaluated. For appearance, O4JB-F5.5 had the highest score (4.2); for texture, CSDE-F6.47 and CSDE-F6.46 had the highest scores (3.8); for sweetness, CSDE-F6.47 had the highest score (3.4) for acidity, CSDE-F6.47 had the highest score (3.4); for bitterness A6JB-F5.34, CSDE-F6.46, and O4JB-F6.35 had the lowest score (1.7); for umami, CSDE-F6.47 had the highest score (3.2); for flavor intensity, CSDE-F6.47 had the highest score (3.8); and for overall flavor, CSDE-F6.47 had the highest score (3.7) (Supplemental Table 2). The broad-sense heritability of the tasting attributes was low in general (Table 1). This illustrates the challenge of selecting for flavor because it is hard to obtain enough data from taste evaluations to have sufficiently high heritability for selection. However, tasting evaluations provide information that is not available in other ways. In this case, combining information from tasting evaluations with indirect selection based on correlated laboratory measurements might be a more efficient approach. The correlation among overall flavor, flavor intensity, and ‘Brix is high (Fig. 4), and the broad-sense heritability of ‘Brix is moderately high, suggesting that indirect selection may help increase genetic gain for flavor when selecting on multiple traits.

**Correlations**

Marketable weight was negatively correlated with ‘Brix (−0.45), CA (−0.44), acidity (−0.31), and flavor intensity (−0.27). ‘Brix is positively correlated with sweetness (0.43) and flavor intensity (0.73). CA is positively correlated with acidity (0.79) and flavor intensity (0.86) (Fig. 4). The overall flavor rating was highly correlated with ‘Brix (0.57), CA (0.69), sweetness (0.85), umami (0.65), and flavor intensity (0.92) (Fig. 4). Average fruit weight also had a negative correlation with
Brix (−0.68) and CA (−0.85). This means that larger fruit had lower 'Brix and CA. Something similar can be observed in the correlations between average fruit weight and flavor intensity (−0.72), or overall flavor ratings (−0.51). These negative correlations may suggest the trade-offs between size and fruit quality; improving fruit quality could mean reducing the fruit size if fruit size is not controlled during selection. In terms of year-to-year correlations, all the production traits had high significant values (>0.8, Table 2). EB AUDPC had a medium year-to-year correlation (0.66). Of the fruit quality traits, CA (%), appearance, and overall flavor had high significant year-to-year correlation coefficients.

### Results by family

'Crimson Sprinter' by 'Defiant' (CSDE) Family. CSDE-F6.46 and CSDE-F6.47 both had high marketable weight (7.00 and 6.32 kg/plant, respectively), with no significant differences with each other or when compared with 'Defiant' (7.87 kg/plant). Both lines had some of the lowest proportion unmarketable of all the breeding and check lines (Supplemental Table 2). The lines were not significantly different for EB AUDPC scores compared with 'Defiant'. High LM AUDPC scores were observed in 2021 (Supplemental Table 3) but were not significantly different from any of the other check varieties or breeding lines. The spread of LM was late in the season, with overall incidence higher than 40% starting week 35 in 2021 (23 Aug). Line CSDE-F6.47 had the highest flavor intensity (3.8) and overall flavor (3.7) scores. Line CSDE-F6.47 was not significantly different in yield from its sister line CSDE-F6.46 and had significantly higher scores for flavor intensity and overall flavor. In this case, improving flavor did not translate into a lower marketable weight, as was expected with the obtained negative correlation between production and fruit quality traits. The farmers shared similar observations. They considered this family to have a good acid/sweet balance, and for some, it was the best tasting family of all. Fruit size on-farm was medium, as it was at on-station trials, with an average fruit weight of 135 g (Supplemental Table 2). The line CSDE-F6.46 averaged 69.5 ton/ha, making it a strong candidate for potential release. A comparable commercial variety is 'Defiant', one of the parental lines for these breeding lines. In 2016, 'Defiant' had a yield of 69.42 ton/ha in 2016 (Seed to Kitchen 2016), 72.00 ton/ha in 2020, and 59.17 ton/ha in 2021.

OSA404 by 'Japanese Black Trifele' (O4JB) Family. The two breeding lines of this family were significantly different from each other for most traits. O4JB-F6-5 performed significantly better for most traits, so the following discussion focuses exclusively on this line. O4JB-F6-5 had high marketable weight (6.13 kg/plant) and was not significantly different from 'Defiant' or the CSDE family lines. O4JB-F6-5 had a low proportion unmarketable. This line was highly rated in the tastings. In appearance it got the highest score. The flavor intensity score was 3.1, while the overall flavor score was 2.9, both falling into the median range. There were no significant differences in EB AUDPC scores compared with 'Defiant' and the CSDE family lines. The same was observed for LM AUDPC. Farmers liked the size and the flavor of this breeding line, which was similar to the heirloom parents. For some, this was the best breeding line for production traits, with sustained good flavor (Table 3). 'Genuine' is a comparable commercial variety. It produces a large beefsteak-type tomato with an average fruit weight of 265 g/fruit, and has broad disease resistance, although based on ratings from our trials it does not have good flavor intensity (Seed to Kitchen 2016). 'Paul Robeson' and 'Pruden’s Purple' are two heirloom-type varieties that farmers like in the Upper Midwest, due to their consistently highly preferred flavor, although some find 'Pruden’s Purple' to be too large. Previous trials at the same research station show 'Paul Robeson' with a marketable weight of 33.25 ton/ha (Seed to Kitchen 2016), and 'Pruden’s Purple' at 43.9 ton/ha (Seed to Kitchen 2016). O4JB-F6-5 had significantly higher marketable yield (60.9 ton/ha) with similar fruit quality characteristics, making it a breeding line with high potential for commercial release, especially for local gardeners and diversified organic farmers. 'Japanese Black Trifele' by 'Defiant' (JBDE) Family. The breeding lines JBDE-F5.28 and JBDE-F5.31 were not significantly different from each other for any of the production traits. The average marketable weight was 5.19 and 5.18 kg/plant, respectively, lower than the CSDE lines and ‘Defiant’. These lines had a very low proportion of unmarketable fruit (<0.14) (Supplemental Table 3). These lines performed similarly to the CSDE family lines in terms of EB AUDPC and LM AUDPC scores. Flavor intensity scores were above 3.0, and overall flavor was 3.0 and 2.9, respectively. Farmers liked the flavor but noted that the fruit of these lines had a tougher skin than other breeding lines. Productivity varied from farm to farm (Table 3).

A6 by 'Japanese Black Trifele' (A6JB) Family. The breeding line A6JB-F5-34 was characterized for producing large, pink-colored tomatoes. Coming from a cross between two heirloom varieties, it can be classified as a contemporary heirloom. Its yield was not the highest (4.55 kg/plant), but the general-public tasters liked the fruit because of its color, size, and intense flavor.

### Discussion

The high broad-sense heritability values observed for the productivity traits and some of the diseases in this study have important implications for future breeding programs. The production traits marketable weight, marketable count, and average fruit weight all had high broad-sense heritability. This indicates that in our organic high tunnel system, we are seeing consistent variety rankings and low levels of genotype-by-environment interactions. However, it is important to consider the limitations of these heritability values. The study evaluated the lines in only one
Disease resistance is a high priority, both for outdoor and high tunnel production. Although critical in the field, EB and SLS do not appear to be of concern in high tunnels due to the physical protection the tunnel provides, limiting free water on leaves and the potential spread of spores (Foolad et al. 2008). The advanced tomato breeding lines evaluated here showed low to medium EB and SLS infection, without a significant decrease in yield. The advanced lines evaluated in this project showed a medium level of tolerance to LM and PM, and the onset and spread of the disease was late enough in the season that it can be concluded it did not affect overall marketable weight. Good agricultural management, such as opening the sides of the tunnel in the mornings to promote air flow, can slow down the spread of the pathogen. Interestingly, the heritability of PM AUDPC (0.12) was lower than the other diseases. This could be because there was little genetic variance in the F1s; none of the parental lines, and therefore the breeding lines, had a known resistance gene or QTL that could confer significant differences in the disease scoring between breeding lines. Additionally, the correlation of breeding line scores between years for PM AUDPC was negative, which may be a result of the significant variability in PM pressure between years and genotype-by-year interactions observed for the lines evaluated. The large variance due to genotype-by-environment interactions negatively affects the calculated heritability.

For quality traits, although heritability values were low, we found strong correlations that may help simplify evaluation, while still allowing selection on quality in early generations. The correlation between overall flavor and flavor intensity was 0.91 ($P < 0.001$). Baldwin et al. (1998) found equivalent results, obtaining an 0.71 correlation between the same traits. This suggests that consumers prefer more intensely flavored fruit. Merscher (2020) also found a significant correlation between overall flavor and flavor intensity (0.74) and overall flavor and sweetness (0.7) when evaluating pink and red slicer tomato varieties. In parallel, CA was highly correlated with acidity (0.82), flavor intensity (0.87), and overall flavor (0.70), and $^\circ$Brix was highly correlates with flavor intensity (0.72) and overall flavor (0.56). This suggests that $^\circ$Brix and CA could be good measures to predict flavor. An analysis of specific sugars, such as glucose and sucrose, could potentially give better estimates of flavor. It is important to note that the perception of flavor was also highly correlated to the $^\circ$Brix/CA ratio because the acid content plays an important role in the taste of sugar during the consumption of tomato fruits (Wang et al. 2023). The correlation found between $^\circ$Brix and CA with overall flavor acceptance is high enough in this analysis to warrant its future use for flavor predictability calculations alongside sensory evaluations. In addition, the high correlation between flavor intensity and overall liking may allow for a simplified taste test during early generations, allowing researchers to evaluate more breeding lines while still conducting a more extensive taste evaluation on advanced lines such as we present in this article. Similar to what Merscher (2020) found, the use of tasting analysis offered flexibility to the researchers, allowing us to answer simple questions about the flavor components of the tomato lines, rather than carrying out time-consuming volatile compound analysis. Interestingly, the overall flavor perception and texture had a high year-to-year correlation, whereas the individual flavor components did not (Table 2).

In general, flavor and marketable weight were negatively correlated. The correlation of marketable weight with CA was −0.45 and with $^\circ$Brix was −0.44. Prior research using whole-genome sequencing and genome-wide association analysis has allowed researchers to associate the loss of high-sugar alleles with domestication and improvement, as larger fruits were selected (Tieman et al. 2017). This means that the selection for larger fruits produced the loss of specific alleles that contribute to higher $^\circ$Brix content, making current selection for both higher fruit weights and higher soluble solids content difficult. De Souza et al. (2012) evaluated the yield, $^\circ$Brix, and CA of the F$_{1}$S obtained from a diallelic cross between five parental lines. They obtained a correlation of −0.18 between plant yield and $^\circ$Brix and −0.13 between plant yield and CA.

Table 3. Summary of on-farm evaluations of tomato breeding lines grown by organic farmers in the Upper Midwest United States in 2020 and 2021.

<table>
<thead>
<tr>
<th>Family</th>
<th>General</th>
<th>Flavor</th>
<th>Productivity</th>
<th>Fruit characteristics</th>
<th>Other comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Crimson' × 'Defiant'</td>
<td>Competitive with weeds, healthy</td>
<td>Good acid/sweet balance, tough skin; best of all</td>
<td>High–moderate</td>
<td>Medium size</td>
<td>Late</td>
</tr>
<tr>
<td>(CSDE)</td>
<td></td>
<td>Good, sweet flavor Heirloom appearance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSA404 × 'Japanese Black Trifele' (O4JB)</td>
<td>Good size; great disease resistance to EB</td>
<td>Some medium, some larger sized</td>
<td>High–good</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBT × 'Defiant' (JBDE)</td>
<td>Healthy plants</td>
<td>Very tasty, sweet flavor, tough skin</td>
<td>Moderate; very productive for one farm Low</td>
<td>Medium size</td>
<td></td>
</tr>
<tr>
<td>A6 × JBT (A6JB)</td>
<td>Typical heirloom; not good disease resistance; prone to rot</td>
<td>Sweet, good flavor</td>
<td>Big, heirloom type</td>
<td>High in fruits, some purples; soft fruit</td>
<td></td>
</tr>
<tr>
<td>OSA404 × A6 (O4A6)</td>
<td>Decent leaf disease resistance</td>
<td>Very sweet</td>
<td>Variable</td>
<td>Medium sized</td>
<td>Took a long time to ripen; soft fruit; radial cracking and green shoulders</td>
</tr>
</tbody>
</table>
Tieman et al. (2012) found that aroma volatiles make important contributions to the perceived sweetness of tomato fruits. By reducing unpleasant volatile compounds, which may have simpler genetic inheritance than perceived sweetness, breeders could more easily improve consumer perception of flavor (Zhao et al. 2019). However, this would require the development of molecular markers for the relevant volatile compounds in appropriate genetic backgrounds.

Selecting only for higher marketable weight without evaluating flavor could negatively affect the flavor perception by consumers, confirming Klee and Tieman’s (2013) findings. This can make the future selection process difficult because the main objectives of this breeding program are to generate varieties with high yields and excellent flavor, adapted to organic high tunnel systems. For this reason, it is important to evaluate flavor early in selection, even if heritability is lower, to avoid eliminating breeding lines with good flavor. Working with indirect measures such as ‘Brix and CA in addition to simplified flavor evaluation may also help maintain excellent flavor in early generations of selection while also selecting for production and disease resistance.

From verbal communication with the tasters, the breeding lines had good flavor overall, and overall flavor scores were higher than for the check varieties in the trial. We received similar feedback from the farmers who tried the breeding lines in their production systems: overall flavor was good, and flavor was outstanding in some of the breeding lines. Before the COVID pandemic, the breeding program did regular evaluation of breeding lines with local chefs. This was interrupted in 2020 and 2021 due to pandemic restrictions. We resumed in a limited way in 2022 and 2023 and received feedback from chefs on overall flavor of the advanced lines, similar to farmer evaluations of the lines. This was done after the completion of the study reported here but served to confirm the selections we had made based on chef involvement before the pandemic and our research team flavor evaluations. This demonstrates that this type of selection program is a promising starting point for flavor improvement while maintaining adequate production and disease tolerance.

The participatory aspect to this research was key to achieve well-adapted tomato varieties. Both the researchers and the participatory organic farmers defined the objectives of this breeding program. Even though a participatory breeding approach is not exclusive to an organic breeding program, it is rare to find a conventional breeding program that involves farmer selection. The selection and evaluation of the breeding lines in organic environments on-station and on-farm make this tomato-breeding program different from a typical conventional breeding program. Evaluation and selection were conducted in and for organic farming systems. In this project, we prioritized fruit quality and adaptation to the specific conditions of organic high tunnels in the Upper Midwest. Trials conducted at research stations may not always be the most representative of organic production environments, even when certified organic. For this reason, we also prioritized having more on-farm evaluations over a higher number of on-station locations, providing us with farmers’ input that was key to carry out the selection process.

With the data collected on-station, on-farm, and from the taste tastings, there are four breeding lines that show high potential for commercial release: CSDE-F6.47, O4JB-F5.5, JBDE-F5.28, and O5DE-F5.43. These lines are being further evaluated on-station and on-farm, and depending on the additional data obtained, some or all of them will continue the process to be released as varieties to have available for farmers.

Conclusions

Overcoming the negative correlation between yield and fruit quality traits such as ‘Brix, CA, sweetness, flavor intensity, and overall flavor persists as a breeding challenge in the improvement of tomato varieties. The use of good tasting heirloom varieties as parental lines showed positive results in obtaining breeding lines with a favorable combination of yield and flavor, such as CSDE-F6.46 and CSDE-F6.47. Tomato breeding lines adapted to organic farming systems are key to organic farmers achieving the production goals and fruit quality standards that they need for their markets. The best breeding lines evaluated in this experiment will be further evaluated on-station and on-farm, and different options will be explored for their future commercialization. These lines are uniform enough that they can also be used as parent lines in future efforts, considering their improved fruit quality and sustained moderate to high marketable weight.

References Cited


