PARTICIPATORY TOMATO IMPROVEMENT FOR ORGANIC SYSTEMS FOCUSED ON PRODUCTION AND CULINARY TRAITS

Ambar Carvallo Lopez

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List of Contents

GENERAL OUTLINE

The present thesis focuses on the evaluation of production, disease, and fruit quality traits of tomato breeding lines developed through a participatory plant breeding approach in the University of Wisconsin-Madison. We identified the need to develop tomato varieties that are specifically adapted for organic farming systems in the Upper Midwest in the US, focused specifically on fruit quality (flavor), disease resistance, and yield. We developed a tomato breeding program that focused on improving those traits and in this thesis I present the field trial results from the 2020 season, where we evaluated advanced breeding lines that had been selected previously by our lab members.

Chapter 1 presents a literature review including information about tomato breeding history, organic agriculture, and the benefits of using a participatory approach to breed tomatoes for organic agriculture. In Chapter 2, I outline the project where this analysis is encompassed, which started with a participatory tomato variety trial in the Upper Midwest and continued with a participatory breeding program to develop improved tomato varieties adapted to organic systems. Chapter 3 contains the main analysis and discussion of the data obtained of the 2020 field trial season, and Chapter 4 finalizes the document with an overall conclusion of the obtained results.

CHAPTER 1: BREEDING FOR ORGANIC TOMATO PRODUCTION: LITERATURE REVIEW

Economic importance of the tomato

Tomatoes are one of the most produced and consumed vegetables worldwide. In 2019 alone, 5.03 million hectares yielded 180 million tons of tomatoes worldwide (FAOSTAT, 2019). In the United States, tomatoes accounted for 36% (10.85 million tons) of total vegetable production in 2019. This data includes both tomatoes produced for processing and fresh markets. Tomatoes are a locally important crop in the Upper Midwest of the United States where this research is based. In 2014 the state of Wisconsin produced nearly 1.5 million pounds of tomatoes, and by 2015, 248.000 pounds were certified organic by the National Organic Program (USDA, 2015).

Tomato origin and domestication

The tomato is a vegetable commodity that has been studied by many researchers around the world, thus there is vast information about its domestication and breeding history. Despite this, its exact origin remains unclear, likely within the Andes region that includes modern-day Chile, Peru, Ecuador, and Colombia (Bai & Lindhout, 2007; Peralta & Spooner, 2006). Wild tomatoes have a wide geographic range encompassing variable habitats. Two species of wild tomato endemic to the Galapagos Islands *Solanum galapense*, and *S. cheesmaniae* are adapted to a warm and humid climate (Darwin et al., 2003), while other related species, like *S. chilense*, grow in the Atacama desert in the south of Peru and North of Chile in high temperatures and dry conditions (R. T. Chetelat et al., 2009).

One of the first reports of tomatoes grown for consumption was in Mesoamerica when Europeans captured the city of Tenochtitlan. Bernardino the Sahagun described tomatoes that were sold in the markets there as "… large ones and also very small ones, and all the kinds that exist, of many different varieties, as discussed in the text, such as yellow tomatoes, red ones, and those that are very ripe…" (Sahagun, 1577). This implies that the tomato had already gone through significant domestication before being taken to Europe in the 15th century, after which further and more intense domestication occurred in Europe during the 18th and 19th centuries (Bai & Lindhout, 2007). Today's diversity of sizes, shapes, colors, and flavors in tomatoes is the result of hundreds of years of domestication and breeding across different continents, climates, and cultures.

Solanum lycopersicum morphology and taxonomy

Tomatoes belong to the Solanaceae family, which includes more than 3000 species. It is the only cultivated species in the *Solanum* genus, and 12 wild relatives are part of the same genus and the section *Lycopersicon* (former genus of cultivated tomato). It is a diploid species with a relatively small genome size (around 950Mb). The tomato and its wild relatives have 12 chromosomes (2n=2x=24), and tomato chromosomes were first identified by Barton (1950). It is a perennial herbaceous plant that grows in warm climates, requiring around 45 days from germination to anthesis and 90-100 days to begin fruit ripening (Garcia et al., 2015). The growth habit of the plant ranges from indeterminate to determinate, and it can get as tall as 3 m. It produces perfect flowers, where the style can be shorter or larger than the tip of the anther cone, depending on the variety. The stigma is receptive from one to two days before to four to eight days after its own flower releases pollen, thus cross-pollination is possible (Garcia et al., 2015).

Nutritional value of tomatoes

Evidence shows that frequent consumption of tomatoes can prevent the development of chronic degenerative diseases such as cancer (Sahin & Kucuk, 2013). The fruits are mostly water (>90%), have a low-fat content (<0.5 g), and are rich in antioxidant molecules including carotenoids, ascorbic acid, vitamin E, and flavonoids (Table 1). In the carotenoid group, lycopene is the molecule that gives the characteristic red color to the fruit, and it has been widely studied because of its antioxidant properties. Lycopene also helps the tomato plant defend itself against diseases. Interest in the health benefits of tomatoes has led to breeding projects focused on improving the nutritional quality of the fruit. Frusciante et al. (2007), evaluated 12 advanced breeding lines and six open-pollinated cultivars, finding that that 10 of the 18 genotypes showed a high level of total carotenoids in an antioxidant analysis. With their data, they created the Index of Nutritional Quality (I_{QUAN}), proposed as a tool to inform breeding programs in selecting tomato genotypes for their nutritional qualities. Parallel research has also sought to identify the genes and quantitative trait loci (QTL) that control the accumulation of phytonutrients like lycopene (Sun et al., 2012), and fruit quality traits like degrees Brix and ascorbic acid (Sacco et al., 2013).

Table 1. Nutrients present in 100 g of Roma tomatoes (USDA, 2021)

Nutrient	**Average amount**	**Unit**
Water	94.7	g
Energy (Atwater General Factors)	22	kcal
Nitrogen	0.11	g
Protein	0.7	g
Total lipid (fat)	0.42	g
Ash	0.31	g
Carbohydrates		
Carbohydrate, by difference	3.84	g
Fiber	1	g
Minerals		
Calcium, Ca	10	mg
Iron, Fe	0.1	mg
Magnesium, Mg	8.1	mg
Phosphorus, P	19	mg
Potassium, K	193	mg
Sodium, Na	<2.5	mg
Zinc, Zn	0.08	mg
Copper, Cu	0.032	mg
Manganese, Mn	0.087	mg
Selenium, Se	<2.5	µg
Vitamins and other Components		
Vitamin C, total ascorbic acid	17.8	mg
Thiamin	0.056	mg
Riboflavin	<0.1	mg
Niacin	0.533	mg
Vitamin B-6	0.079	mg
Folate, total	10	µg
Vitamin A, RAE	24	µg

Carotene, beta	276	µg
Carotene, alpha	1	µg
Carotene, gamma	2	µg
Lycopene	2860	µg

Recently, purple tomatoes have gained interest not only because of their uncommon color but also because of the specific pigment type that generates the dark purple coloration. These soluble pigments are called anthocyanins, plant secondary metabolites that belong to the polyphenols class. They can be found on purple fruits or dark vegetables (e.g. berries, cherries, plums, grapes, purple sweet potato, black carrots, red cabbage, etc.) (Khoo, 2017). Purple tomatoes were initially obtained by promoting the expression of two transgenes from snapdragon, *Delila* (*Del*) and *Rosea1* (*Ros11*), where the fruit obtained contained high anthocyanin concentration, producing an intense purple coloration in both peel and flesh (Butelli et al., 2008). In parallel, a traditional breeding approach has also been used to achieve purple-colored tomato fruits, by crossing *S. lycopersicum* with different wild species that transferred the ability to produce small quantities of anthocyanins in the peel of cultivated tomatoes (Jones et al., 2003). High levels of anthocyanin offer benefits both to the plant and the consumer. For the plant, they act as antioxidant compounds that protect the plant against various types of environmental stress. Because of this, the anthocyanin content can be affected by environmental factors, such as light and temperature (Liu et al., 2018).

A summary of tomato breeding history

Because tomatoes are soft fruits which do not leave behind a clear archeological record, in contrast to grain crops, it is unknown how they evolved from wild species to plants with large and many-shaped fruits. According to Tanksley (2004), it is likely that people at the beginning of the domestication process selected large fruits, probably caused by mutations, which led to our present-day cultivars. The journey of tomatoes from Mesoamerica to Europe and other continents generated a severe genetic bottleneck, causing genetic limitations in today’s cultivated tomato. It is estimated that modern tomato contains less than 5% of the genetic variation of its relatives (Caicedo & Peralta, 2016). Regardless, the tomato went through the "domestication syndrome", where quantitative traits loci (QTL) related to fruit traits and growth habit have been identified. The fruit size changed dramatically from wild to cultivated tomato. The wild tomato has small berries, while the modern tomato has large, succulent fruits (Abewoy Fentik, 2017). Fruit size is controlled by a small number of loci, which is typical of most domestication-associated traits. (Koenig et al., 2013).

Wild tomato species are diploid and can be crossed with cultivated tomato to incorporate traits like disease and drought resistance in breeding programs (Caicedo & Peralta, 2016). They are entirely distributed in the Americas, mainly in South America, from Ecuador to northern Bolivia and Chile (Grandillo et al., 2012). Many of them have been sequenced and mapped, finding useful quantitative trait loci (QTLs) associated with disease resistance, including, but not limited to bacterial canker (*Clavibacter michiganensis ssp michiganensis*), early blight (*Alternaria solani*), grey mold (*Botrytis cinerea*), and many others (Grandillo et al., 2012). Introgressing QTLs of interest can also carry a cost, as genetic linkages can exist with other QTLs that may be beneficial or detrimental. Linkage can be broken through successive backcrossing, but it is often difficult, causing linkage drag to persist, especially if recombination is suppressed (Labate & Robertson, 2012). This has resulted in disease resistance still being a primary challenge for tomato breeding today and one of the critical traits desired by farmers that is difficult to achieve in combination with other desirable traits.

Formal tomato breeding started in the mid-20th, seed companies initially worked with open pollinated varieties, and the first hybrid cultivar was released in 1946, called 'Single Cross'. After this, most of the breeding work shifted to hybrid production, on the reasons being its efficiency to integrate traits of interest like disease resistance, uniformity, and flavor, obtaining overall better performing varieties (Bai & Lindhout, 2007). The objectives of tomato breeding have been diverse, targeting traits including yield, disease resistance and tolerance, abiotic stress resistance, and fruit quality. Increasing yields has been one of the central objectives of tomato breeding programs.

Breeders have taken advantage of heterosis on hybrid F1 lines, obtaining improved yields (Kumar et al., 2012). Following yield, disease and pest resistance have been of great interest in tomato breeding programs. Conventional and molecular marker-assisted selection have helped develop tomato lines resistant to late blight, tomato and yellow leaf curl disease, bacterial wilt, and others (Hanson et al., 2016). The variety Defiant, for example, was bred to inherit the genes *Ph-2* and *Ph-3* (Johnny's Selected Seeds, 2021a). Both genes have been identified to confer resistance to late blight in tomatoes (Wang et al., 2016; C. Zhang et al., 2014; Zhi et al., 2013). Zhi et al. (2013) mapped the *Ph-2* gene and identified flanking markers, which have been used for marker-assisted selection for late blight resistance. In the case of the *Ph-3* gene, Wang et al. (2016) evaluated the marker associated with the gene and concluded that it would be also useful for future marker-assisted selection.

Early blight (EB) is caused by the fungus *Alternaria solani*, and is a widespread foliar disease, especially in wet, humid conditions (Adhikari et al., 2017). EB is of particular concern for organic tomato producers in the Midwest. Hoagland et al. (2015) found in a survey that 82% of organic farmers identified EB as a problem, and 67% found it difficult to control. Because organic farmers have a limited range of pesticides, they rely on the use of resistant varieties to prevent the disease. Extensive screening of wild tomato varieties has identified sources of resistance to EB, including *S. pimpinellifolium*. Several accessions have shown moderate to high levels of resistance to EB, as well as other symptoms caused by the fungus (Foolad et al., 2008). Particularly, the accession LA 2093 of *S. pimpinellifolium* has shown high resistance to EB, and the F7 recombinant inbred line (RIL) of this accession crossed with a breeding line with moderate EB resistance resulted in significantly lower leaf defoliation (%) and AUDPC than the breeding line parent (Ashrafi & Foolad, 2015). In the same study, they identified 5 major QTLs for Early blight resistance, and for three of them the wild parent contributed the resistant allele. In the same study, Foolad & Ashrafi (2015) found a significant positive correlation (r = 0.49) between disease severity and fruit yield, where high-yielding plants appeared to have more disease, and a significant negative correlation between disease severity and earliness-immaturity, where late-maturing plants exhibited less disease. Both relationships are important and should be considered when breeding tomatoes for Early blight resistance.

Breeding to improve fruit quality has also been an objective of interest. This includes physical traits like size, shape, and color, as well as chemical factors like soluble solids, acidity, taste, and sensory factors (Abewoy Fentik, 2017). For example, Hagimori et al. (2005) developed a variety of tomatoes with high L-ascorbic acid (AsA) content by clonal selection. There is a wide range of fruit colors in tomatoes, and breeding programs have focused on developing newer colors that may increase beneficial components such as lycopene, beta-carotene, and anthocyanins. Manoharan et al. (2017) crossed two inbred lines, an orange variety with a brown variety, obtaining an F2 segregating in red, orange, brown, and orange-brown colored fruits, the orange-brown fruit having high beta-carotene and chlorophyll contents. In 2009 a new variety called "Indigo Rose" was released by Jim Myers, a tomato breeder for Oregon State University. It was bred using conventional breeding approaches, and it is described as the first "really" purple tomato, and it is the first tomato variety that has anthocyanins in its fruit. Anthocyanin is only produced in the areas of the fruit that are exposed to sunlight, and this variety has purple skin with orange flesh, which has a high content of carotenoids (Boaches & Myers, 2009).

Tomato season extension with high tunnels

High tunnels are a valuable season extension tool for vegetable growers (Hodge et al., 2019), and have been a success, especially in organic vegetable production. The structure provides multiple benefits, including growing season extension and protection against unfavorable weather (Carey et al., 2009). Importantly for organic tomato production, the high tunnel provides a higher accumulation of growing degree days (GDD), which can result in earlier maturity (O'Connell et al., 2012; Rogers & Wszelaki, 2012). Astroza (2021) compared high tunnel and open field organic production. The plants in the high tunnel were planted 34 days before those in the open field, and results showed that the high tunnel accumulated GDD in about the same amount of time or less than the field when the temperatures were lower. The high tunnel protects plants against rain and wind, which decreases disease propagation, especially those that spread through wet leaves and soil splashing upward during rainfall (Blomgren & Frisch, 2007; Rogers & Wszelaki, 2012). Astroza (2021) found significant differences in the incidence of Septoria leaf spot between open field and high tunnel tomato production. The mean area under the disease progress curve foliar disease coverage of this fungus was close to zero percent in the high tunnel by the end of the season, while in the open field it was 66% on average. Similarly, Hodge et al. (2019) found that the foliar disease coverage was about 78% of leaf area at mid-season in the open field, compared to 17% in the high tunnel. Also, because the high tunnel excludes rain, it is possible to maintain more uniform soil moisture, which decreases physiological fruit problems such as blossom end rot, radial split, and stem side cracking (Astroza, 2021).

Tomato flavor, lost and found

The qualities that affect the perception of tomato flavor are well known, including taste, smell, texture, appearance, and mouthfeel (Hoagland et al., 2015). Tomatoes have a characteristic sweet-sour taste, and flavor intensity is affected by multiple components, including reducing sugars, free acids, and volatiles. This last one is comprised of multiple less-known compounds that are harder to isolate and measure, making flavor analysis a complex process. Over the last decade, consumers have grown dissatisfied with the bland flavor of modern commercial tomatoes and seek alternatives, such as heirloom varieties. This decline in flavor can be explained in terms of genetic and environmental factors. Commercial tomato breeding has been focused on increasing yield, shelf life, firmness, and disease resistance rather than fruit quality. Flavor-associated components have been left aside, which has inadvertently led to a decline in flavor quality. Tieman et al. (2017) quantified flavor-related components in 398 modern, heirloom, and wild accessions, and were able to identify genetic loci that affect most of the target flavor chemicals. A total of 13 flavor-related volatiles were significantly reduced in the modern varieties relative to heirloom varieties. By genome-wide association study (GWAS), they found a significant negative correlation between fruit weight and sugar content. This correlation can be linked with the loss of high-sugar alleles during domestication as larger fruits were selected.

Incorporating flavor selection in a breeding program is challenging. As mentioned above, many components affect the perception of flavor, and there is a long way to go before we elucidate the interactions of all the components affecting the final product. Environmental factors also affect fruit quality, from irrigation, fertilization, and other agronomic factors to the ripening stage of the fruit at harvest and the methods of post-harvest handling. Tomatoes are usually stored at cold temperatures to extend the shelf life, and this chilling process has been found to negatively affect flavor quality by reducing the most important ripening-associated transcription factors (Zhang et al., 2016). Commonly, breeding programs focus on production or disease resistance traits in the early generations, leaving flavor selection for the advanced generations. Because flavor is difficult to fix late in the breeding process, breeders interested in improving fruit quality have to be careful about choosing suitable starting germplasm, and evaluate flavor in multiple stages rather than just at the final generations (Dawson & Healy, 2017).

Experiences in participatory plant breeding

Participatory plant breeding (PPB) is defined by Ceccarelli & Grando (2019) as “the participation of clients (most often, but not only, farmers) in all the most important decisions during all the stages of a plant breeding program…”. The main stages of a breeding cycle are shown in Figure 1. Shelton & Tracy (2016) expand upon this framework, explaining that PPB is a process of collaboration between farmers and formally trained breeders to tailor the focus traits to the farmers' needs. There are many forms of interaction between farmers and scientists throughout the breeding process, and this is designed to shift the focus of plant improvement to a local level. 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 is a methodology that was initially created in developing countries, where economically disadvantaged farmers were not benefitting from non-participatory, conventional breeding programs (Bellon, 2006). Farming in developing countries is constrained by limited input use, and varieties bred for conventional and high-input systems do not perform well when grown under severe stress (Dawson et al., 2008).

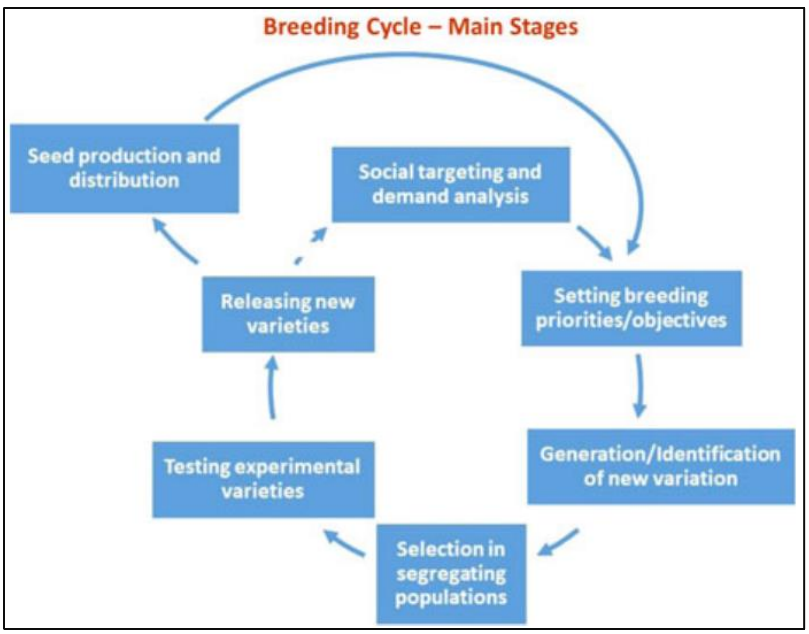

Figure 1. Main stages of a breeding program (Ceccarelli & Grando, 2019).

Organic systems in developed countries share many of the challenges of agriculture in developing countries. A large portion of the varieties used in organic agriculture were originally bred for conventional agriculture, which can be buffered with inputs, decreasing the environmental variability. This way, modern varieties are apparently bred for broad adaptation, but this does not apply to organic and low-input systems, where the environmental variability is much higher. Organic farming can benefit from increasing the genetic variability of the cultivated crops, and participatory breeding has been key to descentralize and incorporate valuable genetic material that can help develop heterogenous populations that can evolve specific adaptation to the local conditions (Dawson et al., 2011).

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 marginal agricultural systems. PPB itself promotes the diversification of environments and integration of multiple actors in the breeding process, working towards 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.

There are several examples of PPB in developing countries, many with positive outcomes. Abay & Bjørnstad (2009) carried out a participatory improvement of barley to adapt it for production in low-input systems in Ethiopia. They were able to identify the preferences, constraints, and potential of different varieties, with the input of farmers throughout the research. In Rwanda, the early involvement of farmers in a bean breeding program resulted in significantly higher-yielding varieties that were selected on-farm than the ones selected on-station and were also found to be retained longer by farmers than those selected by breeders (Sperling et al., 1993). The farmers' experience is a valuable resource, and their involvement can significantly improve the results of a plant breeding program, especially when developing varieties that adapt to local farming systems.

Even though PPB is often geared toward small-scale agriculture in developing countries, it is equally relevant in developed countries. Mendes-Moreira et al. (2017) compared farmer's and breeder's selection in a maize breeding program in Portugal, evaluating traits like ear shape, cob, ear weight, and overall yields, among others. They found that there wasn't a significant difference in genetic reduction between the different selections, but yield increase was only detected during farmer selection. Also working with maize, Shelton & Tracy (2015) carried out a recurrent selection for high yielding open-pollinated (OP) maize adapted for organic systems in the US. They focused on traits that were identified as key for maize improvement by an organic farmer, who collaborated in the recurrent selection and evaluation of the best breeding lines. They found promising traits in one of the populations, and further selections were needed to continue improving critical traits for organic farmers, including rust resistance, husk protection, and cold soil germination.

PPB has gained popularity in organic farming research. Organic crop production systems are often low-input, necessitating the use of high-yielding and disease resistance varieties for reliable production. However, most crop varieties are developed for conventional high-input systems, and don't perform equally well in more variable, comparatively resource-limited organic systems (Lammerts van Bueren & Myers, 2011). Organic farmers often work on small farms with diverse surrounding ecosystems and multiple microclimates in which mainstream conventional seed tends to underperform, and thus PPB has become a tool to develop programs that focus on improving traits that are of critical importance tothe specific needs of organic farmers (Dawson et al., 2008). In Germany, breeders and farmers collaborated to develop region-specific genotypes of spring faba bean for organic conditions. Farmers and breeders evaluated phenotypic traits and also gave a personal appreciation of the material. They found 18 superior genotypes out of 49, and there were two that were of high interest to the farmers, according to their evaluation. French farmers and researchers have experimented with PPB in different crops and regions, starting as a movement of farmers that aimed to re-establish their breeding-practices autonomy (Chable & Berthellot, 2003). In Brittany (western France), a collaboration was formed between organic farmers, professionals, and researchers to develop *Brassica* crops varieties adapted for organic farming. They evaluated different genetic resources for seed production, and farmers were able to take charge of population breeding by mass selection of broccoli and cauliflower. Seed production for variety trials was done in the agrobiological station of the institution associated with the program. Among other results, the program was able to diversity production by introducing new genetic material and creating new forms of population varieties (Chable et al., 2008). The use of a PPB approach in organic farming can help develop varieties that are adapted for the needs of organic systems and specific regional and local conditions.

Organic tomato breeding has also benefited by incorporating a participatory approach. In Collaserola, Spain, traditional tomato varieties have been displaced from commercial agriculture, and a collaboration between researchers and local farmers emerged to promote the local landraces by developing and trialing 5 experimental inbreds, the best line of which was further cultivated in the area of study (Casals et al., 2019). They were able to protect the landrace, storing seeds in a seed bank. In Austria, there have also been efforts to conserve and improve local landraces, mainly heirlooms historically maintained by small local farmers. A collaboration between farmers, advisors, and researchers was formed to develop a breeding program focused on improving disease resistance, particularly resistance to *Cladosporium fulvum* for indoor production and *Phytophthora infestans* for outdoor production. After making crosses between their local heirloom tomato varieties and disease resistant varieties, they carried out on-farm and on-station evaluations where farmers and researchers selected the best breeding lines. They were able to improve cultivars and identified the challenges of a PPB approach, setting a knowledge and experience baseline for future projects. Tomato is also an important horticultural crop in Italy, and the increasing demand for organic tomatoes led a PPB program to develop varieties adapted for specific organic microclimates (Campanelli et al., 2015). Farmers visually evaluated F2 and F3 generations, and selected single plants for seed advancement. The selected plants differed significantly between farmers and researchers, but even so, they were able to develop an F4 that significantly outyielded the commercial F1 hybrid used as a comparison. They developed a wider range of varieties *per* breeding cycle, considered to be wider than conventional plant breeding, contributing positively to the genetic diversity.

Organic tomato production in the US has increased in the last decades, and the seed industry has not been able to supply varieties that are well adapted to the conditions of the organic systems. Because the farming conditions and the farmers' needs vary from one region to another, it is important to define the key traits relevant to the breeding program. Hoagland et al. (2015) surveyed farmers to identify the key plant traits of interest for organic and conventional tomato growers. Organic and conventional growers ranked flavor as their top breeding priority, followed closely by disease resistance. In terms of fruit quality, the conventional growers were more concerned with appearance characteristics like crack resistance, color, and shape. Organic farmers, on the other hand, ranked nutritional quality higher than appearance traits. In regards to disease resistance, EB, Septoria leaf spot, and late blight (*Phytophthora infestans*) were top-ranked by organic and conventional farmers. Conventional farmers have the option of using synthetic pesticides when any of these diseases cause damage, while organic farmers rely on crop rotation and disease-varieties as a prevention strategy and use copper fungicides for control. The use of disease-resistant varieties is one of the most valuable tools for controlling foliar pathogens like these. Even though there are available disease resistant varietites for these specific diseases, it has been noted that organic growers may not adopt them because they are not adapted to their growing conditions, or lack the required fruit qualities, like good flavor, fruit shape, and color. This provides evidence that breeding solely for one trait and without farmers input does not guarantee that a new variety will have the success and broad use as intended. As resistant as a variety might be, it won’t sell well if the flavor is not good, and thus it won’t be used by small farmers that focus on providing high quality produce to their consumers.

The Seed to Kitchen Collaborative (SKC) is a PPB project that started at the University of Wisconsin-Madison, intending to evaluate how a participatory approach can benefit a breeding program when different stakeholders like chefs, farmers, and the public, participate at the distinct stages of a breeding project. The SKC organized hub trials at the West Madison and Spooner Agricultural Research Stations, and satellite trials at participating farms following a participatory variety selection model, with participatory selection by a subset of interested farmers in a few crops, particularly tomato. Hill (2020) found that farmers had an interest in participating in the project and that their participation percentage could increase when visiting their farms and engaging directly with them. Chefs' participation in variety tastings proved to be a valuable tool to assess their market potential that breeders might not perceive. Chefs also showed interest in evaluating breeding lines of the crop improvement programs that were currently being developed and in future projects and have tasted early generation breeding lines of tomato, carrot, beet, corn and potato. For other crops they have evaluated advanced generation breeding lines and experimental hybrids to provide feedback directly to breeders.

Conclusion

The tomato plant has experienced a genetic bottle-neck since it was domesticated in Mesoamerica and distributed throughout Europe and the rest of the continents. Breeding has been mainly focused on improving production traits like fruit weight and number of fruits per plant, as well as incorporating disease resistance genes. This has decreased overall fruit quality and flavor, causing consumers and home gardeners interests to shift towards local varieties often identified as heirlooms. Heirlooms are revered for their diverse shapes, colors, and intense flavor, but often have negative production traits such as fruit cracking, short shelf life and disease susceptibility that prevent their production-scale usage.

Organic vegetable farms are comparatively low-input systems with variable environmental, ecological, and human community factors that determine which variety traits are relevant depending on their location and the market that they sell to. Organic vegetable farmers in the Upper Midwest still rely partly on conventional seed for some of their crops and varieties because there are not enough organic options that have all the necessary traits. This is also true for organic tomato production, where traits like flavor and fruit quality are more valuable for organic farmers than conventional farmers. To fulfill the current needs, through a participatory approach, we are carrying out a breeding program with the objective of developing tomato varieties that excel in organic farming systems, have a high disease resistance, and have excellent flavor.

In chapter two I present the development of this program and its transition from participatory variety selection to participatory plant breeding and the results and feedback from farmers on their participation. I also describe future directions for this program. In chapter three, I present the analysis of the advanced tomato breeding lines developed in this program. Farmers were involved in setting priorities, parental variety testing through the SKC trials and feedback on lines, with some farmers conducting selection on their farm.

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CHAPTER 2: INSIGHTS ON ORGANIC PARTICIPATORY TOMATO BREEDING IN THE UPPER MIDWEST

INTRODUCTION

This project followed a participatory breeding approach to develop flavorful tomato varieties that excel in organic farming systems in the Upper Midwest. In this chapter I will detail the background and goals of the project, our research process, challenges we faced, and potential future directions the project for that could inform a shift of the prevailing plant breeding paradigm from a centralized model controlled by universities and private companies to a more collaborative and decentralized model involving small farmers, chefs, and diverse communities.

Organic tomato production in the Upper Midwest has continuously increased during the last decade, reaching a total production of 15.206 tons and total sales of $2.8 million, according to the 2017 Census of Agriculture (USDA, 2020). This increase in organic tomato production acreage has been fueled by rising consumer demand for organic produce, which has also catalyzed the development of tomato production systems under hoop houses, high tunnels, caterpillar tunnels, and other structures that boost crop marketability by extending the growing season and improving production efficiency and fruit quality.

The market for organic produce has grown by double digits annually since the mid-1990s, but the organic seed sector has not kept pace with these trends. In the 2016 State of Organic Seed Report, OSA found that 82% of organic vegetable farmers still depend on conventional seed for some portion of their production. Myriad reasons explain this persistent lag in organic seed usage; the most commonly cited reason (in a survey of a representative sample of 10% of organic farmers nationwide) was that specific varieties with desirable traits were unavailable in an organic form (Hubbard & Zystro, 2016). The survey results demonstrate a clear need to develop varieties for organic production systems and to improve regional seed systems. They also point to a need for better trialing, as independent breeders and regional seed companies may have breeding lines with these traits, but they do not have the testing infrastructure to make farmers and seed companies with a larger distribution area aware of their existence. These independent breeders and small seed companies need access to more cost-effective trialing options and show desire for training on scaling-up seed production or licensing varieties to mid-sized retail seed companies with the capacity for larger scale organic seed production and sales.

Independent breeders and small regional seed companies are an underappreciated sector of the organic seed industry but are critical to producing varieties that are regionally adapted and suited to organic systems. Often participatory breeding takes the form of breeders seeking out farmers to conduct on-farm trials. This model works well for crops for which a formal breeding program exists in a region. But, because of the lack of investment in plant breeding in general, and for organic systems in particular, there are many crops for which there are no public or private sector breeding programs in the Upper Midwest. The Upper Midwest does have a strong presence of vegetable breeders, often focusing on conventional processing varieties, including the largest public sector vegetable breeding group in the country at UW Madison. The lack of breeding programs for organic fresh market vegetables is not due to a lack of interest at the university, but rather results from a shortage of resources that is unlikely to improve in the near future. Many other regions of the country suffer from the same under-investment so new models developed will be helpful in many regions and crops.

This points to a need for new models to develop varieties for organic farmers, as it is highly unlikely that either public sector institutions or larger seed companies will be able to establish formal breeding programs for a critical majority of important crops within a region. Expecting full time organic vegetable farmers to all become farmer-breeders and develop their own varieties for crops where they have inadequate variety choices is also unrealistic. Vegetable farmers are incredibly busy during the growing season, and while they may be interested in conducting selection on their farms, most are not able to add another full-time job as breeders to their more-than-full-time work as farmers. Independent breeders (who may have started as full-time farmers) and small seed companies provide the means to bridge this gap and will be most effective when they are able to collaborate with networks of farmers interested in evaluating early generation crosses on their farms, with public sector researchers and breeders that can give them access to more advanced techniques and resources. They may also find it advantageous to work with larger scale organic seed companies that have access to larger seed markets and tools for managing the logistics of larger volume seed production and sales.

Access to diverse breeding material is another critical component of developing high-performing varieties for organic agriculture. Independent plant breeders, regional seed companies and seed savers' networks often have large collections of germplasm, and multiple varieties in the pipeline. However, they may not have resources to trial their breeding lines across diverse environments, collaborate with farmers, expose varieties to potential seed company partners, or navigate the commercialization process. In the Upper

Midwest, the Seed to Kitchen Collaborative and Seed Savers Exchange Networks each include 60 to 70 farmers. These networks have been used successfully for variety trialing and seed saving, but members of both groups have expressed growing interest in improving their functionality for collaborative plant breeding. This demonstrates farmers' increasing interest in deeper engagement with the seed system and a growing recognition that current organic variety offerings are insufficient for the diversity of organic farms (Hubbard & Zystro, 2016; Lammerts Van Bueren et al., 2011).

In response to the needs identified previously, in collaboration with farmers, breeders, and chefs, this project aims to develop tomato varieties that are adapted to organic systems in the Upper Midwest.

PROJECT DESCRIPTION AND METHODS

This project had two main phases, A and B. Phase A was the Participatory Variety Selection (PVS) process, and phase B was the Participatory Plant Breeding process (Figure 1). The PVS phase consisted of a variety trialing process where farmers evaluated the growth performance of different tomato varieties on-farm, and chefs provided qualitative feedback about the flavor, culinary, and market potential of the varieties evaluated at the research station. From this evaluation, the most promising varieties were used as parental lines for the PPB phase. In the PPB phase, crosses were made following a diallelic scheme, and these where advanced on-station and trialed both on-station and on-farms. Following are more details on the specifics of each phase.

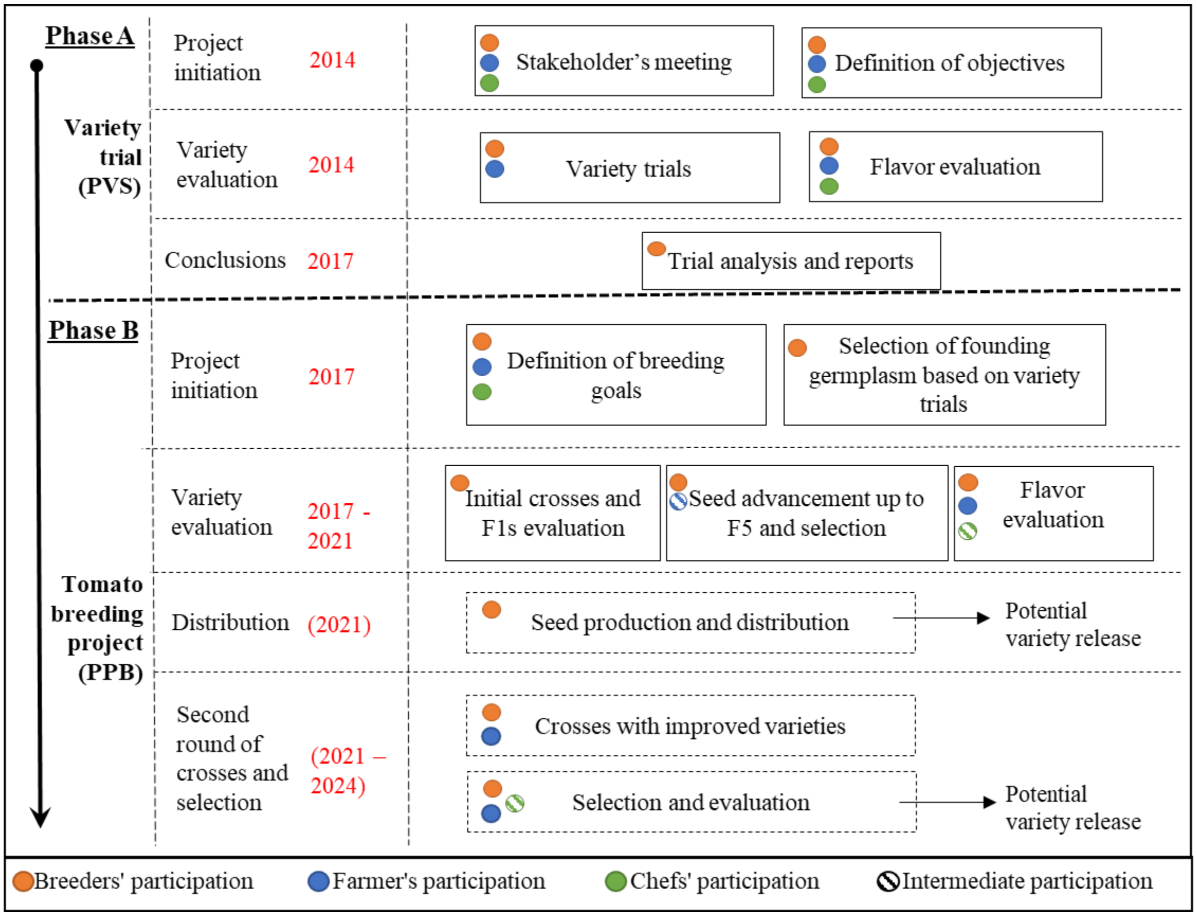

Figure 2. Process diagram of the Participatory Varietal Selection phase (PVS) and the Participatory Plant Breeding phase (PPB) used in this tomato breeding project.

Phase A: Participatory Variety Selection

This project aims to improve tomato varieties that are adapted for organic systems in the Upper Midwest. The founding germplasm was chosen through a participatory varietal selection process as part of the Seed to Kitchen Collaborative (SKC). The SKC is an ongoing participatory research project involving farmers, breeders, and chefs in the upper Midwest. It was founded in 2013 by five chefs, five farmers, and seven UW Madison plant breeders. Since then, it has expanded to include 56 farmers and 63 gardeners, a core of 10 to 15 chefs, and 21 breeders (Hill, 2020). A major component of this project is a process of collaborative selection for improved flavor, a trait highly valued by consumers (Tieman et al., 2017). Commercial breeding companies often focus on production, appearance, and storage traits, leaving flavor evaluation to the final stages of selection process, when it is often difficult to improve as a trait. With that in mind, one of the specific goals of SKC is to develop better methods to evaluate and select for flavor and culinary quality as central foci of early-stage breeding processes.

Farmer's participation in PVS

Participating farmers receive seed of different varieties of their previously requested crops, instructions on how to integrate the trials into their growing systems, and data collection forms to be returned post-harvest. They are allowed to keep and sell the produce from the trials and are asked to return their completed variety evaluations at the end of the season. Evaluations include qualitative assessments of key traits such as germination performance, vigor of growth, disease resistance, productivity, flavor, and overall impressions about the variety, including their likelihood of growing it again. In the PVS process informing Phase A of our project, results of the returned evaluation forms were integrated with the variety data from the West Madison and Spooner research station hub trials.

Chefs' participation

To evaluate the culinary and marketable potential of the varieties, SKC works with chefs to identify potentially useful traits that breeders might overlook. Monthly variety tastings were hosted in various restaurants around Madison, with the Dawson Lab handling organization and set up. In each tasting, five to six varieties were evaluated using a Qualtrics survey developed specifically for this activity. The lab crew did a taste calibration activity, and after tasting all the varieties, a smaller set including the best rated ones were given to the chefs for evaluation. This rapid sensory evaluation including professional experts (chefs) and a semi-trained panel (lab crew), made it possible to sample a large number of varieties, at a significantly lower cost compared to laboratory analysis. Hill (2020) found that after participating in this process, chefs were interested in learning more about plant breeding and ways to access new or un-released varieties.

Phase B: Participatory Plant Breeding

Organic farmers have variety needs that differ from one to another depending on the target market, their local agricultural and environmental conditions, and personal preferences. Even though the number of organic tomato farmers has increased during the past decade, they are still considered a niche market in terms of seed and variety development, as seed companies often prefer to produce seed that can perform well under a wide range of conditions, rather than selecting for adaptation to local microclimates and unique community preferences. Historically, universities have played an important role in developing and releasing crop varieties that are improved for a specific trait or adapted to certain regions. The problem arises when they try to license a variety through big seed companies, that are interested in selling seed that is “adapted” for a wide range of environmental conditions, and not for the regional or local requirements. Adaptation is in quotation marks because the seed that is produced for mass cultivation is bred under and for conventional, high-input farming system which are not representative of the variable growing conditions and nuanced customer demands present on small-scale, organic market farms. The alternative that we propose is for the university to collaborate with independent small-scale plant breeders that know the local conditions and traits of interest, and that might also be interested in generating partnerships with universities and farmers to develop locally adapted varieties that could be released under an open-source or joint Intellectual Property Rights (IPR) model.

Parental Varieties

The key traits important to variety development for organic tomato growers are fruit quality (including flavor), disease resistance, and yields, according to the survey carried out by Hoagland et al. (2015). This information along with the results of the PVS process informed our choices of the most promising founding germplasm for our breeding program to select tomato varieties adapted for the diverse requirements of organic tomato growers in the Upper Midwest. We chose 8 varieties to use as parental material based on their overall performance and the traits that the farmers were more interested in, including disease resistance, heirloom-type fruit, medium to large slicers, novelty colored fruit such as purple and black, and great flavor. Defiant is an early and very productive variety with intermediate resistance to early blight (*Alternaria solani*), it has a decent flavor and many farmers still like it because of the other beneficial traits. OSA404 is a small slicer developed by the Organic Seed Alliance with good flavor, it did well in the variety evaluations, and it works for farmers that sell for that market. A6 is a pink Amish heirloom-type variety selected by a Craig Grau and adapted for the cooler Midwestern temperatures. Crimson Sprinter is an heirloom type with quantitative resistance to Septoria leaf spot (*Septoria lycopersici*). Japanese Black Trifele is a very productive heirloom-type variety with excellent flavor. P321 is a cross between Indigo Rose and Ananas Noir developed by Jim Myers at Oregon State University, it is a small slicer with yellow flesh and anthocyanin pigmentation in the skin. Finally, Summer Sunrise is a dwarf-type with yellow medium-sized slicer with incredible flavor. We crossed them and obtained 7 families that were grown and advanced in the research station. A summary table with the parental varieties' information can be found in Appendix A.

Participating farmers

A small group of farmers participated in the advanced breeding evaluation. All of them have diversified vegetable organic systems, and shared similar needs in terms of variety traits, like improved flavor, disease resistance, and yields.

- **Voss Organics** is a certified organic sub-acre urban farm located in Madison, WI. They focus on produce and seedling production, and sell their products in farmers markets, restaurants, and Willy Street Co-op. Mark Voss, the owner, was one of the farmers that selected and saved seed from 2 families of the breeding lines that were sent to him.
- **Luna Circle** is a certified organic farm with 3 acres in vegetable production 25 miles north of Madison, WI. Tricia Bross, the owner, manages hoop houses and open fields that grow a variety of produce throughout the year. She sells in farmers markets and through a pick-your-own approach.
- **Riverbend Farm**, run by Greg Reynolds, is 30-acre certified organic farm located 30 miles west of Minneapolis, MN, and is managed using a 4-year crop rotation of vegetables, grains, and cover crops. They run a CSA and sell to restaurants, food co-ops, and schools. Greg Reynolds, the owner, works in seed selection, seed-saving, and

preserves local varieties. For our project, he selected single plants of the families that he liked the most and saved seed for his own farm and for on-station evaluation.

- **Cattail Organics**: Owner Kat Becker runs this 50-acre certified organic farm in the Northwest corner of Marathon County, involving a 3-season CSA program offering vegetables, flowers, and seedlings.
- **Nature and Nurture Seeds,** located in Ann Arbor, Michigan, is a seed company focused on organic certified seed, heirlooms, open pollinated varieties, and Midwest adapted varieties. They are a Seed Company Partner of the Open-Source Seed Initiative (OSSI). They grow their seed in a 122-acre certified organic farm, which features education and outreach about organic gardening, food biodiversity, and locally adapted varieties. We worked with Erica Kempter, one of the owners of the farm.
- **Amy's Acre** in Caledonia, WI, is a certified organic 2-acre farm that produces mixed vegetables. Produce is sold directly to restaurants and at farmers markets. They also have 10 acres of pasture and 4 acres transitioning to hop production. Amy Wallner, the owner and operator of the business, prefers heirloom tomato varieties.

Participating farmers received 6 breeding lines and the varieties were selected according to their fruit quality preferences, such as the color, shape, and flavor of the fruit. This way, each farmer received a distinct group of breeding lines. After the harvest season ended, each farmer returned an evaluation form or communicated through e-mail or phone call to report on how the varieties performed in their system, including any insights on production approaches that worked for them during the season. Their evaluations of flavor, disease resistance, yield, and earliness were the most relevant for this project. The instructions sheet we sent the farmers can be found in Appendix B. The suggested plot maps can be found in Appendix C, and the management and breeding lines evaluation forms in Appendix D. All the returned evaluation forms can be found in Appendix E.

Timeline of the breeding process

First crosses and F1s

The parental lines were planted in certified organic open field and high tunnel management systems at the West Madison Agricultural Research station, near Madison, WI. The varieties Defiant, OSA 404, A6, Crimson Sprinter, and Japanese Black Trifele were crossed following a diallel scheme, and P3-2-2-1 was crossed with Summer Sunrise. All the F1s were grown and evaluated under certified organic management on-station. See crossing scheme in Appendix F.

Seed advancement and selection

Seed advancement was done on-station, and after each field trial selection was done focusing on flavor, disease resistance, and yields. Flavor was evaluated by crew members on-station.

F3 and F4 generations

After the breeding families were advanced to generations 3 and 4 on-station, seeds of selected families were sent to farmers for on-farm trial evaluation and selection. Farmers filled out a form where they evaluated the varieties by observation, not with quantitative data. They were also offered the option to do their own family or single plant per family selection, based on their preferences. We received seeds from single plant selection from 2 farmers, which we continued to advance and evaluate on-station, separately from the selections made in parallel in the station.

F5 and F6 generations

F4 selected families were advanced and trialed under certified organic management on-farm and on-station. These generations were evaluated in the field season of 2020 and the data and analysis is further analyzed and discussed in Chapter 3.

DISCUSSION

Past and current challenges

Reach a greater diversity of farmers

Most of the farmers that participated both in the PVS and the PPB phases of this project became involved through organic agriculture conferences and informal gatherings of vegetable growers. Historically, most attendees at organic grower conferences have been white, rural farmers, and only in recent years have growers from more diverse racial and experiential backgrounds begun attending these activities. Even though all are invited to participate in our project, it has been difficult to connect with potential participants from outside the established network of predominantly white, rural growers who often have existing generational access to land and agricultural resources. Considering that a core ethic of participatory plant breeding is to help catalyze resilient farming systems with sufficient versatility to suit the many human elements of a given region, it is essential to very intentionally reach outside of organic farming's current sphere of influence and crowd-source variety selection from within the many communities that have been historically marginalized and denied agricultural resources and learning opportunities. Developing outreach events to actively invite BIPOC, Latinx, and LGBTQ+ communities into the conversation and to collaborate should be part of our future project objectives. These events could be informal listening sessions, organic farming conferences geared to these communities, and extension events to explain our current project and other similar programs that are developing at UW-Madison. These would help us as researchers to further understand the specific challenges and needs that these communities have and would help develop a more holistic approach to improving tomato varieties.

Diversifying the language in which knowledge resources are shared can also be a good strategy to connect with the Latinx community. Re-connecting with farmers that are part of the Dane County Centro Hispano could be a way to widen the network and learn more about their farming experience, their agricultural practices, and what tomato traits are of interest to them. As the project continues, it will be in the agenda to create new resources like video tutorials and guides in Spanish to connect with the Hispanic community.

Members of our lab are also involved with projects like the Afrodiasporic garden at Eagle Heights and the West Madison Display Garden, and with the Intertribal Agriculture Council, with which we have collaborated on chef evaluations of several crops. Working with those connections, it would be interesting to learn if and how these communities integrate tomato cultivation into their intercropping systems. Different cuisines highlight different traits in each crop, so it would be beneficial to foster and deepen the networks to exchange agricultural and culinary knowledge.

More than 50 farmers participate in the SKC trials each year, including the ones that have specifically trialed the tomato breeding lines evaluated in this project. Reaching out to those that grow tomatoes as part of their agricultural system and that are in the Upper Midwest could be a way of integrating more farms into this specific tomato breeding program.

COVID-19 Pandemic restrictions

Engaging with farmers throughout the growing season and beyond key to learning about their current economic, material, and social needs, the dynamics of their customer markets, and their personal and community well-being. Frequent in-person communications that were part of the PVS and early PPB phases became impossible after the COVID-19 pandemic started in 2020. During the pandemic, small farmers saw an overall increase in the demand for fresh produce, especially through alternative sales venues such as online vending and direct order pickup and delivery. These changes, added to the challenges of new sanitation and social distancing protocols, made it hard for some growers to continue with trials or maintain involvement as originally planned. Due to pandemic precautions, staff from our project did not visit participating farms during 2020 and the first half of 2021. The pandemic also prevented us from featuring different tomato varieties, including breeding lines, in dishes prepared by local chefs at our Farm to Flavor event. This limited our audience for variety tastings and the development of new connections and collaborations between the different stakeholders of the seed improvement process. It was possible to have carry out a modified version of the crew tasting for the breeding lines. Normally, the tastings would be carried out in the lab offices or in the field, in a group setting where all the samples would be labeled, and everyone would take taste each variety following the survey instructions (Figure

2). In 2020, we modified the protocol to adjust it to the COVID-19 safety precautions, and instead of doing an in-person tasting, we prepared samples for each person separately (Figure 3). To evaluate the appearance, we included a photo of each variety in the Qualtrics survey. Everyone received an instructions sheet and a QR code to access the survey (Figure 4).

Figure 3. Regular tasting set up, year 2019.

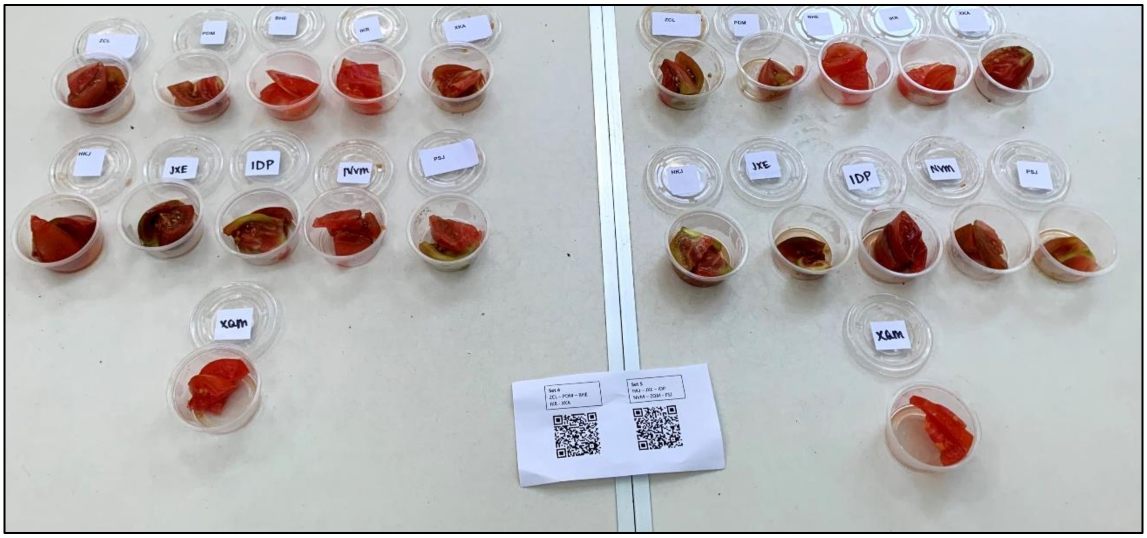

Figure 4. Individual tasting of two sets of tomato breeding lines.

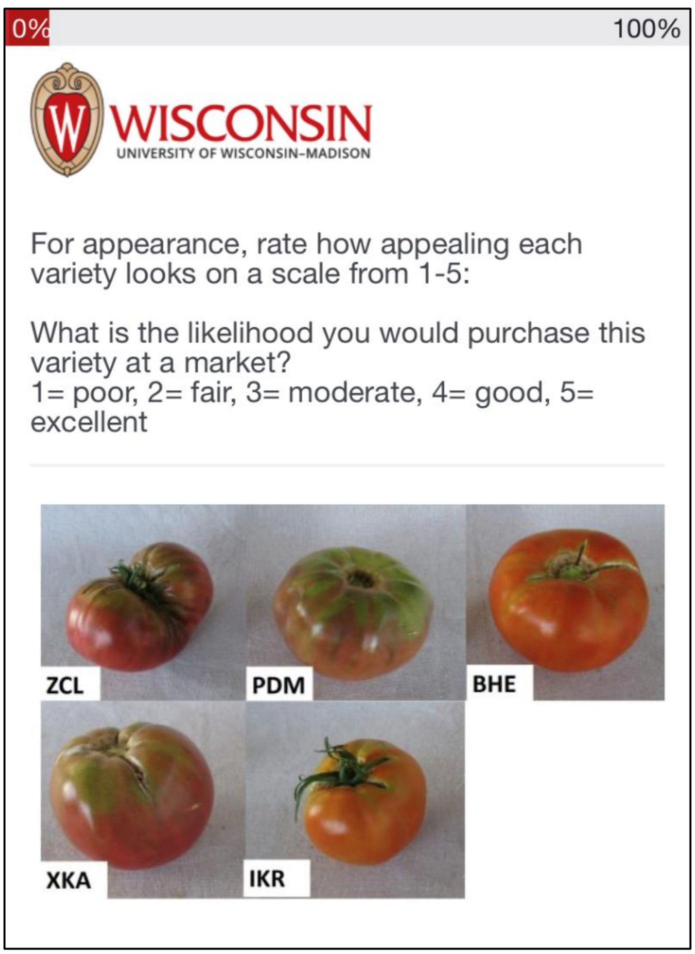

Figure 5. Qualtrics survey used in 2020 including photos of each tomato variety.

Recommendations for the future of this project

Improving the networks with farmers and chefs will be crucial to strengthen the participatory and collaborative aspect of this project. This will entail increasing direct communication by visiting the farms, and more outreach events like informal gatherings and conferences. Up until now, most of the seed advancement and variety selection has been carried out on-station, with limited on-farm trialing and fruit quality evaluation, which was heavily affected by the COVID-19 pandemic, as discussed earlier in this chapter. New crosses will be carried out among the best breeding lines, and we expect to collaborate more closely with farmers, where they can actively select and evaluate the new generations. This will help us evince the agricultural differences between farms, and how that affects the selection process. Similarly, it will help recognize the similarities and differences between the selections made on-farm and on-station. After each selection, in addition of carrying out crew tastings, we hope to integrate chefs further in this process, to connect the farmer's perspective with the end user, chefs in this case, and analyze the culinary potential of the varieties selected at the same time as the marketability.

Farmer's feedback on the project

Improve researcher-farmer communication

Similar to the challenges that we identified, a more stable and continous communication with farmers is something they could benefit from. Offering more frequent updates about the breeding process and involving them in on-station activities could be a great way of keep contact, as well as visiting their farms. Visits are a valuable activity to learn not only about the farmer's growing system, but to acquire a deeper insight into their needs in terms of the farming system overall, and also the needs they visualize in the different crops they grow. Hill, (2020) also identified communication strengthening as key to improve the collaboration with the involved farmers. Further, she noted that farmers usually have crop specific questions regarding various aspects of the growing operation, and thus it is expected that the visiting researcher comes with some sort of preparation. To improve the outcomes of such visits, it is suggested to the specialist to communicate with the farmer previously to the visit, this way they can have an idea of the topics the farmers would like to discuss and can be better prepared to answer any specific questions or can suggest specific resources for the farmer to connect to. Because all the farmers we work with have diversified growing operations, it is not expected from the researcher to know everything there is to know in terms of specific crop diseases, pest control, best varieties, etc., but communicating the right resources to the farmers is a first step towards improving the collaborative relationship. It is important to develop this project as an empowering tool for farmers and not as a knowledge extractive research.

More guidance on tomato breeding for farmers

Some of the farmers have voices their interest in learning further about tomato breeding, including, but not limited to, the technical aspect of making the crosses, understanding the genetic and biological processes, seed saving, and how to evaluate the breeding lines being trialed. Multiple resources on this topics are available online, such as the ‘Organic Tomato Seed Production’ training video, published by eOrganic (2020) and developed by the Tomato Organic Management and Improvement Project (TOMI). The Organic Seed Alliance also has several publications, including “How to breed tomatoes for organic agriculture” (McKenzie, 2014), and “Tomato Seed Production Guide” (McKenzie & Zystro, 2021). When communicating with the farmers, it would be recommended to share these resources, either the links to access them, or printed versions of the guides. This, accompanied with an on-site training activity would benefit the learning experience for both the farmer and the researcher.

Fruit quality is still a priority

As the farmers returned their on-farm evaluations, it was made clear that overall fruit quality is still a priority when thinking about tomato improvement. There were a couple of varieties that had great flavor but had considerable stem side cracking, which made the harvest unmarketable or lowered the selling price. Those lines should be further evaluated, an improving the irrigation system might have a positive effect on the amount of this issue, as discussed in Chapter 2. Because all the varieties are trialed on-station in a randomized block design, it is not possible to adjust watering for each specific breeding line. Other varieties were positively evaluated on flavor and yield; thus, those should be further trialed on-station and on-farm to continue selection.

CONCLUSIONS

Crop improvement decentralization is key to developing cultivars that are adapted to the local environmental conditions and farmers’ specific needs, in terms of production traits and their objective market. Organic farming can specifically benefit greatly from participatory plant breeding efforts where all the stakeholders partake of the variety development process to guide it towards the local and regional needs of the farmers. Organic farming has been historically neglected by big seed companies that prioritize developing varieties that area adapted to overall uniform conventional production systems that can be replicated in a wide range of environments, leaving aside the improvement of crops that are locally adapted to organic systems. This is true to many crops, including organic tomato production in the Upper Midwest. The collaboration between organic farmers and plant breeders that has driven this project has made it possible to identify the key traits that our breeding program has focused on, including yield, disease resistance, and flavor improvement. Farmers have had an essential role in this project by trialing, evaluating, and selecting tomato varieties according to their own farm and market needs. A continuous interaction and collaboration between the researcher and the farmers were challenged by the novel 2020 coronavirus pandemic that, besides completely changing the way people could communicate, it also highlighted the need to have strong and diversified food systems that can provide to all. As the situation "normalizes" it will be high priority to the project to re-connect with farmers by visiting their farms and having them visit our on-station trials as well. As the breeding program progresses and new crosses are made, early selection and evaluation will be done on-farm and on-station, as the farmer's time and space availability permit it.

Reaching a more diversified farmer population, specially the BIPOC and Latinx communities, has been identified as a challenge that can be further explored and subsided by being more intentional on reaching out to and involving them in this collaborative effort. Fostering further communication and collaboration with a diversified group of farmers will benefit not only the tomato variety improvement itself but will also help strengthen farmers' and researchers' networks and future partnerships that could be initiated. In addition, extension and teaching material will be made available in Spanish, so more people can access it regardless of their mother language.

Overall, the participatory aspect of the project has shown to be beneficial to the tomato variety improvement process, by including all the stakeholders involved in tomato production and consumption. The qualitative data that farmers and chefs provide from their trials in combination with the quantitative data obtained from the on-station trials have facilitated the analysis and selections of the best breeding lines.

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CHAPTER 3. QUANTITATIVE AND QUALITATIVE EVALUATION OF TOMATO BREEDING LINES ADAPTED TO ORGANIC FARMING SYSTEMS IN THE UPPER MIDWEST

INTRODUCTION

The tomato is an important crop worldwide and in the Midwest of the US. It is a key crop for diversified farmers in the North Central Region, due to its high value compared to other crops. Farmers seek tomato varieties that perform well under local growing conditions while satisfying the needs of their market, and it has been especially hard for organic farmers to find such varieties. According to the State of Organic Seed report (Hubbard & Zystro, 2016), as of 2016, 82% of the respondent vegetable growers still relied on conventional seed for some part of their production system, with an average of 70% of their acreage under production using organic seed. The top three vegetable crops planted by the same farmers were tomatoes, lettuce and greens, and squash. One of the reasons listed as to why they did not use organic seed was the lack of desirable traits available in an organic variety. Conditions among conventional farms are relatively similar due to higher and more uniform usage of synthetic fertilizers and pesticides, whereas more variable management practices in organic systems leads to field conditions that differ greatly 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. Hoagland et al. (2015) found that flavor was the top priority in a breeding program for organic tomato growers in the Midwest, followed by disease resistance, crack resistance, and nutritional value. In terms of disease, 67% of the organic farmers said that early blight (*Alternaria solani*) (EB) was difficult to control, 72% said *Septoria* leaf spot (*S. lycopersici*), and 73% said *Fusarium* wilt (*F. oxysporum* f. sp. l*ycopersici*). 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 et al., 2011). Decentralizing the breeding process and involving farmers with on-farm trials can result in improved organic breeding outcomes (Casals et al., 2019; Dawson et al., 2011).

This project emerged as an initial step towards wider collaborative organic breeding efforts that can meet the overall demand for reliable organic varieties while at the same time 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) which we chose as parental varieties for our participatory breeding project in which organic farmers host production trials of our breeding lines, chefs evaluate their culinary qualities, and research trials assess yield, production traits, and response to plant diseases. This paper 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

Location and area

The advanced lines were evaluated in the summer of 2020. Both field and high tunnel tomato trials were located at West Madison Agricultural Research Station (WMARS) near Madison, Wisconsin. The high tunnel dimensions were 32 feet x 88 feet and covered approximately 2,816 feet. The field covered an identically sized area. The high tunnel and the field were oriented with their length running north-south, and the long ends facing east and west. The rows within each management system were oriented east-west. Both systems were certified organic by Midwest Organic Services Association (MOSA).

Experimental design

The experiment was designed as a randomized block design. The experimental unit was comprised of three individual plants. The experimental units were replicated twice in the high tunnel.

A total of six individual plants per breeding line were present in the high tunnel. The check varieties for this experiment were ‘Big Beef’, ‘Pruden’s Purple’, ‘Defiant’, ‘Caiman’, ‘Damsel’, ‘JTO-1021’, ‘Japanese Black Trifele’, and ‘Paul Robeson’.

Parental varieties

The varieties chosen as parental lines were selected based on their characteristics and their breeding importance. Following is a short description of each variety or breeding line, and a summary of their characteristics is presented in Appendix A.

- Defiant: F1 hybrid that has high resistance to late blight (resistance genes Ph-2 and Ph-3) and intermediate resistance to early blight. It also has high resistance to *Fusarium* wilt races 1, 2, late blight, and *Verticillium* wilt (Johnny’s Selected Seed, 2021)
- OSA404: a cross between WI 55 and a disease-resistant North Carolina State inbred, selected by the Organic Seed Alliance for disease tolerance and flavor over several years. Received in 2014 as an advanced line and maintained by selfing.
- A6: an Amish heirloom selected for Midwest adaptation by Craig Grau, a retired plant pathologist at UW Madison.
- Japanese Black Trifele (JBT): crack-resistance heirloom with a smoky flavor maintained by Ken Greene at the Hudson Valley Seed Company
- Crimson Sprinter: an heirloom from Ontario, CA with partial Septoria leaf spot resistance, earliness and good flavor.
- Summer Sunrise: a cross between ‘Golden Dwarf Champion’ and ‘Green Giant’ in 2005 by Patrina Nuske Small. Released from the "*Dwarf Tomato Project*" in 2012 as a selection made by David, Susan, Neil Lockhart and Justin Morse, with additional help from Ted Maiden and Craig LeHoullier. Tart, sweet, intense flavor.

- P321: breeding line from Jim Myers at Oregon State University as a cross between Indigo Rose and Ananas Noir, selected for larger size and excellent flavor. Contains the anthocyanin trait from Indigo Rose giving purple shoulders.

Management

Tomato management

During the summer of 2017, the parent lines were planted and crossed following a diallel scheme. During the summers of 2018 and 2019, the crosses were self-pollinated and evaluated in the field and high tunnel. The plants were grown in three-plant plots (three plants per experimental unit). The management of the tomato plants followed the methods practiced by small to mid-size organic farmers in the North Central Region.

Planting procedure

For the summer seasons, seeds were started by West Star Organics in Cottage Grove, WI, a USDA-certified organic grower of starter plants. Seeds were sown into plug trays in early spring, between March and April, using West Star Organics all-purpose growing mix media. After eight weeks, seedlings were moved to an acclimation room to prepare for high tunnel and field transplanting. Both high tunnel and field beds were distanced 1.2 m apart, from center to center. In the high tunnel and field, beds were covered with black landscape fabric and black-colored plastic mulch, respectively. Transplants were planted in the beds with an in-row spacing of 1 m. Aisles and borders were mulched with straw in both the high tunnel and field systems. During winter, the tomatoes were grown in 18.9 L pots using the PROMIX HP growing media. Pots were spaced 30 cm apart.

Fertilizer and Soil Amendments

In 2018 and 2019, the soils of both systems were amended according to soil nutrient analysis results using feather meal, an organic approved source. In 2019, each plant in the field received two doses of 2% N fish emulsion solution, four and eight weeks after transplant. During winter, the pots were fertilized once a week after transplant using a 10-20-10 fertigation solution.

Growing system

The plants in the field were trellised using the Florida weave system, while the high tunnel followed the hanging-string system. In both systems, plants were trained to two main leaders. Both trellising techniques are commonly used by tomato growers in the North Central region of the United States of America. During the winter season, the plants grown in pots were trellised using bamboo stakes and trellising tape to attach the branches to the stakes.

Pruning

Pruning of axillary branches, also called “suckering”, of the indeterminate varieties was done weekly until plants were approximately 1.2 m tall. After this, pruning was done as required. When the plants reached 1.5 m tall, the bottom two leaves were pruned to increase the airflow of the canopy and to increase the distance between the first bottom leaf and the soil. This was done to decrease the potential of disease development and spread. As the season moved forward, more bottom leaves were pruned as deemed necessary to keep them off the ground. For determinate plants, axillary branches were pruned only at the first stage of development.

Watering

During the summer, both field and high tunnel tomatoes were watered using drip irrigation. The high tunnel was watered consistently three days a week, ensuring that watering was not done the day before or day of harvest to prevent splitting. The field irrigation followed the same dosage and frequency as the high tunnel when the natural precipitation was not enough. During winter, the plants were hand-watered daily.

Temperature

The high tunnel temperature was closely monitored to decide when to open and close the sides. Sides vents and doors were opened both to maintain good ventilation and to maintain temperatures below 95 F. Higher temperatures could cause negative effects on pollen quality, pollination, flower abortion, fruit set, and others. During winter, the daily temperature was kept between 73 F and 78 F, and the night temperature between 62 F and 64 F.

Crosses and self-pollination

For crosses between different parents, we performed manual pollination following the guidelines published by the University of California – Davis (R. Chetelat & Peacock, 2013). Flowers of the parental lines used as females were emasculated in the lime green stage, early in the morning. The next day, pollen was harvested using the VegiBee sonic pollinator (Riverstone, Dover) and collected in a plastic spoon. The pollen was used to pollinate the flowers emasculated the day before. Each hand-pollinated flower was tagged with the date, parental lines name, and initials of the person pollinating. For seed advancement, flowers were left to self-pollinate without intervention in the greenhouse. In the open field and high tunnel systems, flowers were bagged using organza fabric bags (Nashville Wraps, Nashville). Even though the tomato self-pollinates, the presence of insects and wind can cause unintended cross-pollination, thus the need to use a physical barrier. The crossing scheme can be found in Appendix F.

Seed harvest and cleaning

Seed harvest, cleaning, and storage was done following the protocol published by Seed Savers Exchange (Colley & Zystro, 2015). Selected fruits were harvested when fully ripe and ready to eat. After harvest, the fruits where immediately processed for seed extraction and cleaning. To extract the seeds, fruits where cut in quarters and squeezed into a jar, where the seeds and placenta gel fell. After this, the jars were covered with a cheese cloth and sealed with an elastic band and left in a warm location for 48 to 72 hours to let fermentation occur. Once fermentation is complete, the seeds where decanted, rinsed and dried. After this, the seeds were treated with Trisodium Phosphate (TSP), used to prevent the Tabacco Mosaic Virus (TSV). This was done following the protocol shared by Jim Myers (OSU) and Emily Haga (Johnny's Selected Seeds) (direct communication, 2019).

Data collection

Production traits

Production and fruit quality data were collected in the summer of 2018 and 2019. Production data included:

a) Marketable weight(kg/plant): weight of the fruit considered sellable.
b) The number of marketable fruits per plot: sellable fruit that does not show any physical or disease damage, and that has the expected size, shape, and color for the specific breeding line or variety.
c) Average fruit weight (g/fruit).
d) Unmarketable weight (kg/plant): weight of fruit that shows physical or disease damage, or that does not have adequate size.
e) Reasons for un-marketability: blossom end rot, radial splitting, cracking, insect damage, physical damage, small, catface, worm, bacterial speck, sunscald, anthracnose, bacterial spot, desiccation, interior blemish, misshapen, rodent, scar, zippering, and windowing.
f) Proportion unmarketable (%): Unmarketable weight/ (Marketable weight + Unmarketable weight)

Each week, each plot (experimental unit) was harvested from the "turning" stage up to the "red" stage. The "turning" stage can be described as "…pink or red color shows on over 10% but no more than 30% of the tomato surface", and the "red" stage can be understood as "…more than 90% of the tomato surface, in aggregate, is red" (USDA, 2005) From each plot, fruit weight was recorded in grams, in addition to the marketable and unmarketable fruit number and the marketability reasons.

Flavor was evaluated qualitatively through tasting events involving both our lab crew and the public. The form included an evaluation of texture, sweetness, acidity, bitterness, umami, and intensity (Appendix A).

Disease scoring

Eight weeks after transplanting, disease scores were collected from the field and the high tunnel tomatoes. Disease scoring was done every other week and was recorded using a 0% - 100% scale. A 0% score referred to a plot that had no symptoms of diseases and was otherwise healthy. A 100% score referred to a plot where the plants were completely dead. The evaluation included the most common tomato diseases in the Upper Midwest, such as early blight (*Alternaria solani)*, leaf mold (*Passalora fulva)*, powdery mildew (*Oidium neolycopersici*), and Septoria leaf spot (*Septoria lycopersici*). These diseases were identified to be of interest for farmers in the survey carried out by Hoagland et al. (2015).

The check varieties were scored by Juan Astroza, another graduate student part of the Dawson lab, that focused in comparing high tunnel, caterpillar tunnel, and open field management systems. The author of this thesis scored all the breeding lines. Both graduate students carried out a disease scoring calibration at the beginning of the season where they identified the specific symptoms of each disease and how to score each one of them.

The diseases scores were analyzed using the area under the disease score (AUDPC) calculation, which is a quantitative summary of the disease intensity over time, and is useful to compare values across plant varieties, years, locations, or managements. We followed the trapezoidal method to calculate the AUDPC values for each disease for each breeding line, by discretizing the time variable (weeks) and calculating the average disease intensity between each pai of adjacent time point (Jeger, 2004).

Flavor evaluation

Flavor evaluation was done by the research team and summer field workers. The group participated in a calibration exercise at the beginning of the season. This exercise included recognition of the basic flavor components – sweet, acid, salty, bitter and umami – at varying concentrations in both water and tomato juice. Varieties were divided into different tasting groups depending on the parental and market similarities. For example, the breeding lines with Defiant as a parent were tasted the same day, when possible. When a breeding line did not have enough fruit for tasting in the designated group, it was tasted later in the season. Only completely ripe fruit were used, and samples were prepared by slicing tomatoes into wedges so that each sample included both stem and blossom end. Fruit from each plot of each variety were bulked in a composite sample from each management system.

Tasters rated each sample on a 1-5 scale for sweetness, acidity, saltiness, bitterness and umami where 1 was very low perception and 5 was very high perception of that flavor component. Flavor intensity was also rated on a 1-5 scale with 1 being low and 5 being high intensity of ‘tomato’ flavor. Samples were rated from 1-5 for appearance and texture with 1 being not preferred and 5 being very preferred. 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. The rating of sweetness and acidity along with intensity and preference allows a comparison between tasters’ perceptions and easily measurable components from flavor such as sugar and acidity. An example of the flavor evaluation form can be found in Appendix G.

Usually, farmers and chefs are invited to a field day at WMARS to see the research and also to carry out tastings of the different breeding lines. Due to the COVID-19 global pandemic that hit the world at the beginning of 2020, no visits where possible to the research station from general public.

On-farm evaluation

Families of crosses were sent to farmers in 2019 and 2020 for on-farm evaluation. The farmers were asked to fill two forms, one related to the system management, and another where they evaluated the breeding lines they received. The forms were returned electronically at the end of the season. Each farmer received six breeding lines and were asked to integrate into their farming in form of 4 plant plots that were randomly distributed into two rows that where ideally situated not in the edge of their system, but in the middle, so that their regular variety rows could act as borders. The instructions sent to the farmers, the suggested plot maps, and the management form, can be found in Appendix B, C, and D, respectively.

Statistical analysis

The information collected was analyzed using a mixed model Analysis of Variance (ANOVA) and calculating least squares (LS) means for management, variety, and the interaction between management and variety. The variety model was defined as:

$$D_{ijk} = U + M_i + V_j + MV_{ij} + e_{ijk}$$

Where U represents the grand mean, M_i represents the main effect management at the *i*th management, V_j represents the main effect variety at the *jth* variety, MV_{ij} represents the interaction effect and e_{ij} represents the error term. Dependent variables were marketable fruit count, average fruit weight, marketable yield, percent unmarketable yield.

The disease model was defined as:

$$D_{ijkl} = U + M_i + C_j + V(C)_{jk} + MV(C)_{ijk} + e_{ijkl}$$

Where U represents the grand mean, M_i represents the main effect management at the *i*th management, C_j represents the category effect, $V(C)_{jk}$ represents the main effect variety nested in the category effect, $MV(C)_{ijk}$ represents the interaction effect between management and variety, nested in category, and e_{ij} represents the error term.

Selection

After gathering all the traits data, the breeding lines were scored from 1 to 3, 1 being a high priority to advance a generation, and 3 a low priority to advance a generation. The scoring process considered all the traits mentioned in data collection and disease scoring. After selection, the breeding lines with high priority were grown in the greenhouse facilities and self-pollinated to move forward with the advanced generations. Breeding lines with score 2 were included depending on the space limitations and the notes that were taken throughout the summer trial season. The selected breeding lines that are evaluated in this study are presented in Table 1. A photo of each of the breeding lines can be found in Appendix H.

Table 1. Parental, market, and generation information of the breeding lines evaluated in this project.

Family name	Female parental line	Male parental line	Generation	Market
A6JB-F5-34	A6	Japanese Black Trifele	F5	Slicer
A6JB-F5-35	A6	Japanese Black Trifele	F5	Slicer
JBDE-F5-28	Japanese Black Trifele	Defiant	F5	Slicer
JBDE-F5-31	Japanese Black Trifele	Defiant	F5	Slicer
JBDE-F5-32	Japanese Black Trifele	Defiant	F5	Slicer
O4JB-F5-MV1-115	OSA 404	Japanese Black Trifele	F5	Heirloom
O4JB-F5-MV1-116	OSA 404	Japanese Black Trifele	F5	Heirloom
O4JB-F6-5	OSA 404	Japanese Black Trifele	F6	Slicer
CSDE-F6-46	Crimson Sprinter	Defiant	F6	Slicer
CSDE-F6-47	Crimson Sprinter	Defiant	F6	Slicer
O4DE-F5-43 [3]	OSA 404	Defiant	F5	Slicer
O4DE-F5-44 [3]	OSA 404	Defiant	F5	Slicer
P3SS-F4-61	P321	Summer Sunrise	F4	Slicer
O4A6-F4-MV1-109	OSA 404	A6	F4	Slicer

Heritability (H^2)

For our analysis we calculated **broad-sense heritability** (*H*), which estimates the proportion of phenotypic variance (V_P) that is due to genetic causes (V_G) (Bernardo, 2002):

$$H = \frac{V_G}{V_P}$$

V_P is calculated as:

$$V_P = V_{G+} \frac{V_e}{n_{rep}}$$

Where n_{rep} is the number of replications. In this case, the management variance and the interaction between management and variety is not included in the calculation of the phenotypic variance, because the heritability analysis only includes one management (high tunnel system).

To understand the family effect on the heritability of the traits, heritability was also calculated using the family variance as:

$$H = \frac{V_{Family}}{V_{Family} + \frac{V_G}{m} + \frac{V_e}{n_{rep} * m}}$$

Where V_{Family} is the family variance, n_{rep} is the number of reps, and *m* is the number of genotypes per family.

Expected response to selection (R)

The response to selection is the change in the population mean due to selection: The selection differential (*S*) is the difference between the mean of the selected individuals ($\mu_{C0\ selected}$) and the overall mean of the population from which they were selected (μ_{C0}):

$$S = \mu_{C0\ selected} - \mu_{C0}$$

When epistasis is assumed absent, the relationship between *S* and *R* is (Bernardo, 2002):

$$R = h^2 S$$

Correlated response to selection

The correlated response to selection refers to the response in one trait when selecting for a different trait if there is a genetic correlation between the two traits. The genetic correlation between traits X and Y is:

$$r_G = \frac{COV_G}{\sigma_{G(X)}\sigma_{G(Y)}}$$

Where COV_G is the genetic covariance between X and Y, $\sigma_{G(X)}$ the genetic standard deviation of X, and $\sigma_{G(Y)}$ the genetic standard deviation of Y. With this, it is possible to calculate the change in breeding value for trait Y per unit change in the breeding value for X trait as:

$$b_{G(Y\ X)} = r_G \frac{\sigma_{G(Y)}}{\sigma_{G(x)}}$$

Then, the correlated response in trait Y due to selection in X is:

$$R_Y^C = b_{G(Y\ x)} R_x$$

Where R_x is the direct response to selection for trait X. The efficiency of indirect selection is equal to the ratio between the correlated response and the direct response to selection for Y is (Bernardo, 2002):

$$\frac{R_Y^C}{R_y} = \frac{|r_A| h_X}{h_Y}$$

RESULTS

The following results are from the data obtained from the 2020 trial carried out at WMARS.

Production traits analysis

Table 2 shows p-values of the F-tests of significance from the analysis of variance (ANOVA) for the different sources of variation and the interaction between them across the production traits studied in the experiment. The traits are described in more detail below, followed by disease assessment and quality tasting.

Table 2. ANOVA p-values of F-tests of the significance of production traits evaluated from 22 tomato varieties grown under high tunnel and open field management systems in 2020.

Source	Marketable fruit count	Marketable weight	Average fruit weight	Proportion unmarketable
Variety (V)	<0.0001	<0.0001	<0.0001	<0.0001
Management (M)	0.0012	<0.0001	0.0573	0.0006
V*M	<0.0001	<0.0001	0.0009	<0.0001

Table 3 shows the means by trait for each management. Significance groupings for pairwise comparisons are given. The high tunnel had a higher marketable weight and marketable count than the open field. The proportion of unmarketable weight was significantly higher in the open field than the high tunnel.

Table 3. Least squared means by trait for each management system where 22 tomato varieties were evaluated in 2020. Values with the same letter within a trait are not significantly different at the p<0.05 level,

Variable	High Tunnel	Open Field
Marketable weight (kg/plant)	6.44 a	1.23 b
Marketable fruit count (fruits/plant)	32.63 a	6.75 b
Average fruit weight (g/fruit)	227.73 a	240.54 a
Proportion unmarketable by weight	0.32 b	0.73 a

Table 4 shows the means for each variety for each production trait, only from the high tunnel management.

Table 4. Least squared means of 22 tomato varieties for each production trait for the high tunnel management system evaluated in 2020.

Category	Variety	Marketable count (fruits/plant)	Marketable weight (kg/plant)	Fruit average weight (g/fruit)	Proportional unmarketable weight
Crosses	A6JB-F5-34	15.4	5	313.5	0.4
	A6JB-F5-35	17.2	4.6	298.7	0.4
	JBDE-F5-28	48.9	5.3	109.9	0.1
	JBDE-F5-31	60.9	5.3	87	0.1
	JBDE-F5-32	21.2	5.3	263.6	0.4
	O4JB-F5-MV1-115	4.2	1.6	143.7	0.7
	O4JB-F5-MV1-116	14.9	3.5	241	0.6
	O4JB-F6-5	20	6.5	320.7	0.3
	CSDE-F6-46	53.4	7.4	139.2	0.1
	CSDE-F6-47	46.4	6.3	136.1	0.2
	O4DE-F5-43	20.2	7	372.3	0.4
	O4DE-F5-44	19.5	7.1	380.8	0.3
	P3SS-F4-61	33.7	3.2	99	0.6
	O4A6-F4-MV1-109	28.5	5.6	197.7	0.4
Check varieties	Big Beef	40.6	10.7	257.7	0.2
	Caiman	46.4	11.4	245.5	0.1
	Damsel	30.4	6.8	218.9	0.3
	Defiant	76.8	8.6	107.9	0.1
	Japanese Black Trifele	50.7	8.1	150.9	0.3
	JTO-1021	32.8	11.3	342.7	0.2
	Paul Robeson	13.4	3.4	249.5	0.6
	Pruden's Purple	22.7	7.7	333.9	0.4
	Fishers LSD	25	12.0635	38.86	30.35
	p-value	<0.001	<0.001	<0.001	<0.001

Green cells indicate higher values, white cells indicate lower values. For proportion unmarketable weight, green cells indicate lower values, and white cells higher values.

Table 5 shows the means for each breeding family for each production and disease trait of the plants grown under the high tunnel management system. A6JB corresponds to the A6 by Japanese Black Trifele cross, CSDE to Crimson Sprinter by Defiant, JBDE to Japanese Black Trifele by Defiant, O4DE to OSA 404 by Defiant, O4JB to OSA 4040 by Japanese Black Trifele, and P3SS to P321 by Summer Sunrise.

Table 5. Family means of the production and disease traits of 22 tomato lines grown in 2020 under high tunnel management system at the West Madison Research Station.

Trait	A6JB	CSDE	JBDE	O4A6	O4DE	O4JB	P3SS
Marketable weight	4.83	6.86	5.31	5.58	7.05	3.85	3.17
Marketable count	16	50	44	29	20	13	34
Average fruit count	306	138	153	198	377	235	99
Prop Unmarketable weight	0.37	0.13	0.16	0.40	0.30	0.52	0.57
Brix	4.55	5.05	5.02	4.40	4.40	5.33	7.15
CA	0.30	0.40	0.42	0.32	0.26	0.38	0.51
Early blight AUDPC	614	751	553	700	516	973	641
Leaf mold AUDPC	490	739	463	364	298	525	455
Powdery mildew AUDPC	158	690	272	140	105	506	70
Septoria leaf mold AUDPC	79	79	42	0	53	96	0
Total AUDPC	1341	2258	1330	1204	971	2100	1166

AUDPC= Area Under the Disease Curve

Marketable weight

For marketable weight, the ANOVA shows strong evidence for the main effect of management (p-value < 0.0001, Table 2). Both management systems were significantly different as seen in Table 2. The overall mean for marketable weight in the high tunnel was 6.44 kg/plant and in the open field is 1.23 kg/plant. There was strong evidence for variety main effect (p-value < 0.001, Table 2). Overall, the check varieties had a higher marketable yield than the breeding lines. There was strong evidence for interaction between variety and management (p-value < 0.001, Table 2). The nature of the interaction was scalar. For the high tunnel, Caiman was the variety with the highest marketable yield, followed by the rest of the check varieties (Table 4). The best performing breeding line was CSDE-F6-46. For the open field management, Defiant had the highest marketable yield, followed by the JBDE-F5-28 breeding line. The breeding line O4JB-F5-MV-115 was the worst performing variety in both management systems. It is important to note that harvest data was lost in weeks 34 and 36, for the O4JB-F5-MV1-116 breeding line.

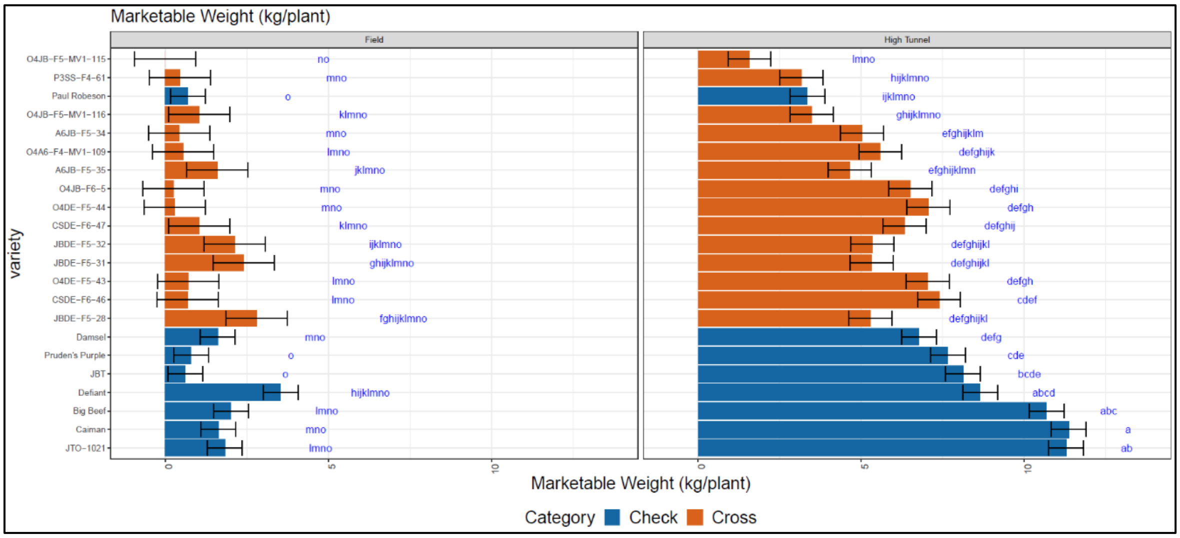

Figure 6. Marketable weight by variety and management. Significance groupings are within management. Error bars are the standard error of the mean. Varieties with the same letter are not significantly different at the p<0.05 level.

Marketable count

There was evidence of the main effect of management (p-value <0.05), Table 2). Marketable count was significantly higher in the high tunnel (32.63) than the field (6.75) (Table 3). There was also strong evidence for the main effect of variety (p-value < 0.001). Overall, the check varieties had a higher marketable count than the breeding lines. In the high tunnel, Defiant had the highest marketable count (51.22) and O4JB-F5-MV1-115 had the lowest (2.13) (Table 4). There was strong evidence for interaction between variety and management (p-value < 0.001). However, the nature of the interaction was primarily scalar rather than crossover.

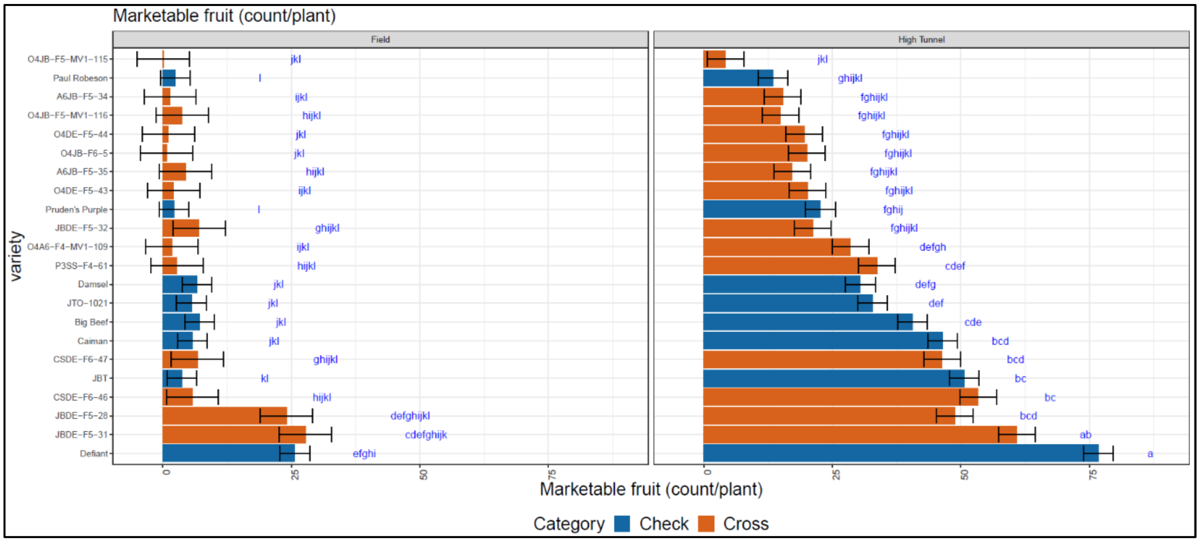

Figure 7. Marketable fruit count by variety and management. Error bars are the standard error of the mean. Varieties with the same letter are not significantly different at the p<0.05 level.

Average weight

There was no evidence for management main effect (p-value >0.05, Table 2). Overall, the average weight was higher in the open field than the high tunnel, but there was no significant difference between both management systems. There was strong evidence for variety main effect (p-value < 0.001). Overall, O4DE-F5-443 had the highest average weight (grams/fruit) and O4JB-F5-43-MV1-115 had the worst performance in this trait (Table 4). There was strong evidence for interaction between variety and management (p-value <0.05).

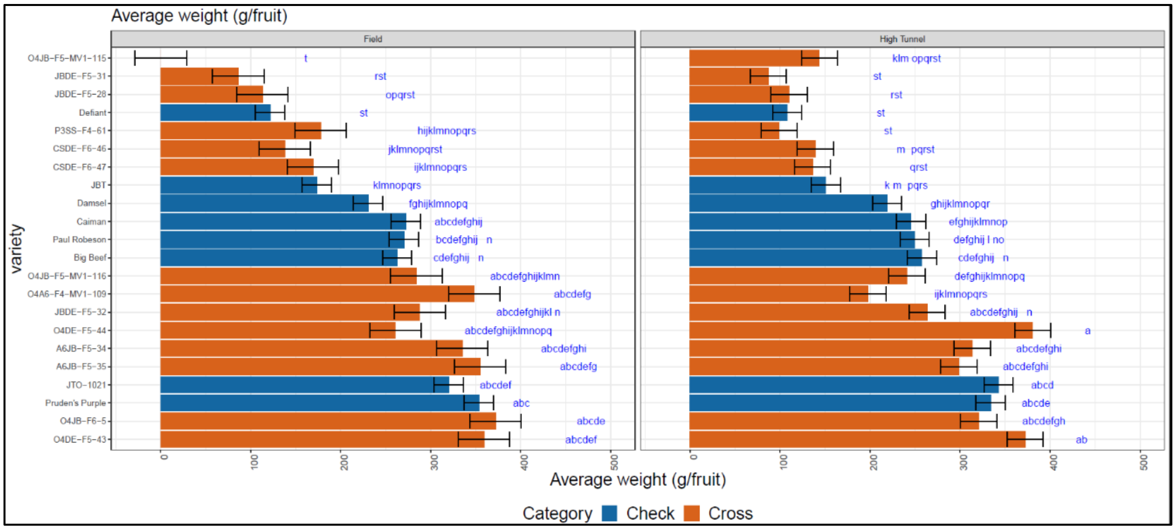

Figure 8. Average fruit weight (g/fruit) per variety for each system. Error bars are the standard error of the mean. Varieties with the same letter are not significantly different at the p<0.05 level.

Unmarketable proportion

The main effect of management on the proportion of unmarketable weight was significant (p-value <0.001), showing significant differences between management systems. The open field had a higher proportion of unmarketable weight (0.73), while the high tunnel had a much lower value (0.32) (Table 3). The main effect of genotype on the proportion of unmarketable weight was significant (p-value <0.001). Overall, the breeding line O4JB-F5-MV1-115 had the highest value for this trait, while Defiant had the lowest (Figure 9). There was strong evidence of interaction between management and genotype for the unmarketable proportion (p-value <0.001, Table 2). For the high tunnel, stem side cracking was the main reason for the primary cause of fruit unmarketability (47%), followed by radial splitting (16%), and hornworm damage (6%) (Figure 10). Similarly for the field, stem side cracking accounted for 64% of the counts, followed by radial splitting (10%), and rodent damage (10%) (Figure 11). For secondary cause, radial split was the main reason for the high tunnel (18%) and the field (21%). Blossom end rot accounted for 1% of the unmarketable causes in the field, and 5% in the high tunnel.

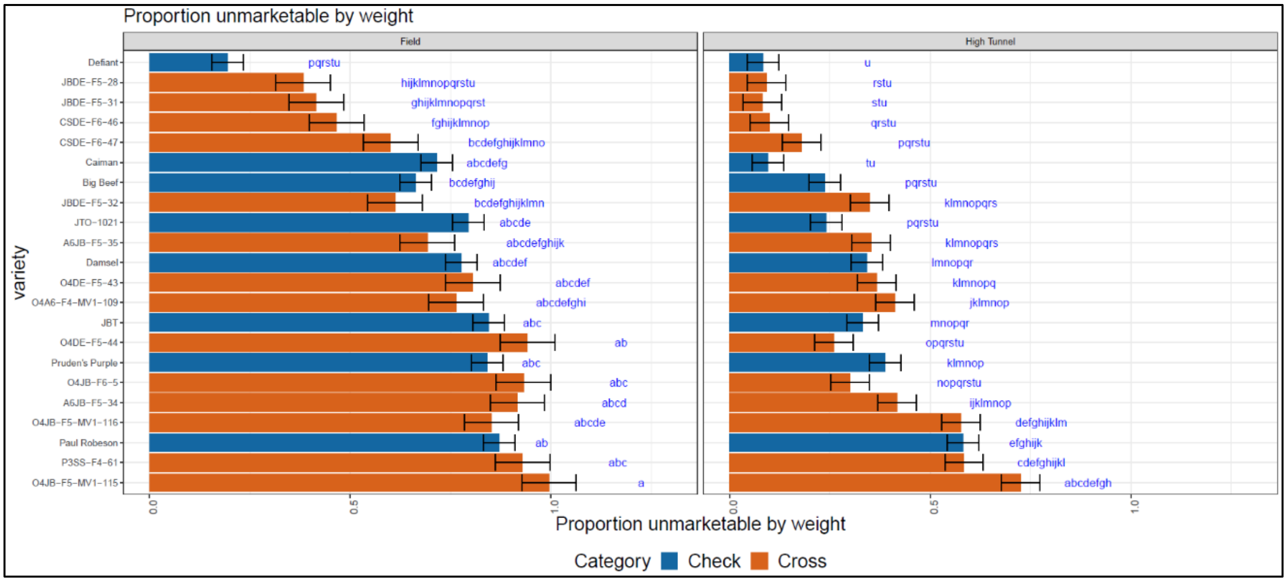

Figure 9. Proportion of unmarketable weight by variety and management. Error bars are the standard error of the mean. Varieties with the same letter are not significantly different at the p<0.05 level.

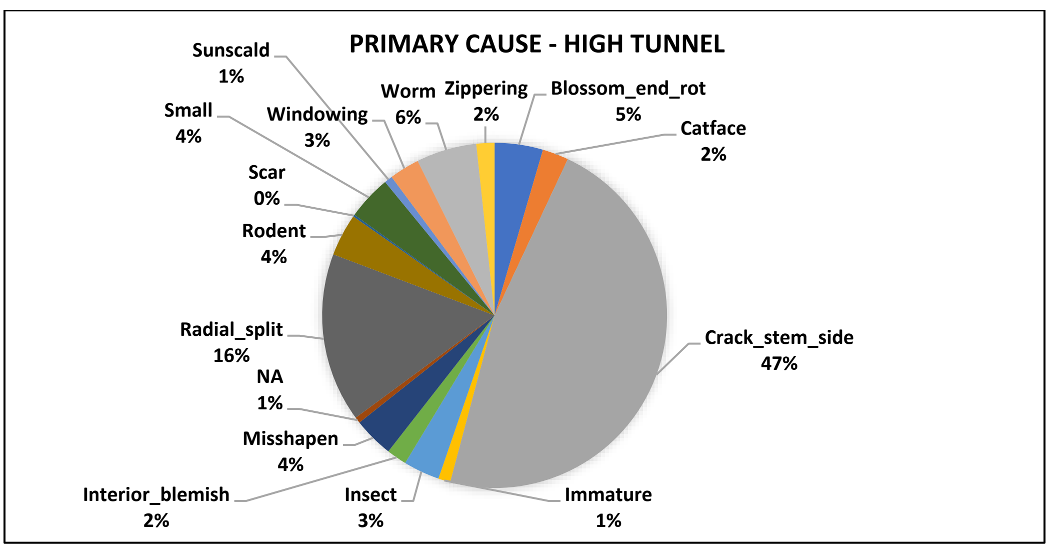

Figure 10. Primary cause reasons of unmarketability for high tunnel system.

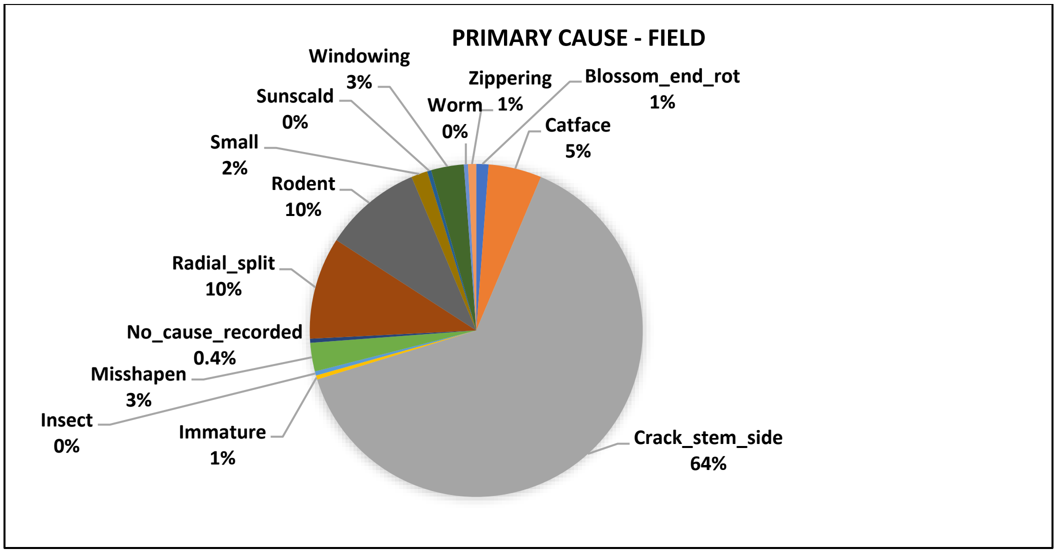

Figure 11. Primary cause reasons of unmarketability for field system.

Disease analysis

Table 6 shows p-values of the F-tests of significance from the analysis of variance (ANOVA) for the different sources of variation and the interaction between them across the diseases studied in the experiment. The “Category” source is the category that was assigned to each variety (check variety or breeding line). Because the check varieties were scored by a different person than the breeding lines, it was necessary to control for human subjectivity of visually scoring each plot.

Table 6. ANOVA p-values of F-tests of significance for disease scores (AUDPC) for Total foliar disease, Early Blight, Leaf Mold, Powdery Mildew, and Septoria Leaf Spot evaluated in 22 tomato varieties grown under high tunnel and open field management systems in 2020.

Source	Total	Early Blight	Leaf Mold	Powdery Mildew	Septoria Leaf Spot
Management (M)	0.731	0.393	<0.001***	0.786	0.004**
Category (C)	0.009**	0.302	<0.001***	0.351	0.001***
M*C	0.563	0.486	<0.001***	0.475	<0.001***
Variety(within C)	0.446	0.151	<0.001***	0.751	0.996
M:V(within C)	0.043*	0.066+	<0.001***	0.402	0.991

P-value≤ 0.10=+, *P-value≤ 0.05, **P-value≤ 0.01, ***P-value≤ 0.001.

Table 7 shows the means by Area Under the Disease Progress Curve (AUDPC) by disease for each management. Significance groupings for pairwise comparisons are given.

Table 7. Least squared means for each disease scored for each management system from the evaluation of 22 tomato varieties grown in WMARS in 2020. AUDPC = Area Under the Disease Progress Curve. Values within a trait that share the same letter are not significantly different at the p<0.05 level.

Variable	High Tunnel	Open Field
Total AUDPC	1979 a	2132 a
Early Blight AUDPC	539 a	435 a
Leaf Mold AUDPC	734 a	24 b
Powdery Mildew AUDPC	704 a	807 a
Septoria Leaf Spot AUDPC	30 b	892 a

Total AUDPC

Total AUDPC corresponds to the addition of the score of each disease for each of the 22 tomato varieties evaluated in this project. There is strong evidence for the category main effect and the interaction of management and variety nested within category. There was no evidence for the other main effects. Overall, Big Beef had the highest AUDPC (3186), while the breeding line O4A6-F4-MV1-109 had the lowest (1350).

Early blight (EB) AUDPC

There was no evidence of the main effects on the EB AUDPC (p-values >0.05). Overall, the breeding line O4JB-F5-MV1-115 had the highest Early blight AUDPC (920), while Defiant had the lowest (180). EB symptoms started showing in week 36 in the field, and week 34 in the high tunnel. O4JB-F6-5 had the highest EB percentage by week 38, with an average of 50% severity in the field and 35% in the high tunnel. CSDE-F5-46 followed with an average of 40% severity in the field and 35% in the high tunnel by the same week.

Leaf mold AUDPC

There was strong evidence for the genotype, category, and management main effects (p-value <0.001, Table 6), as well as the interactions between effects. Leaf mold AUDPC was significantly higher in the high tunnel than in the field (Table 7). The nature of the interaction between both effects was scalar. Overall, the severity of leaf mold in the field was very low, reaching a maximum average of 7% by week 36. In the high tunnel, on the other hand, Big Beef, Damsel, and Pruden's Purple had the highest severity by week 40, with an average of 50%. Overall, the check varieties had the highest average severity percentage in the high tunnel, whereas the breeding lines had a severity lower than 10% throughout the whole season.

Powdery Mildew AUDPC

For the powdery mildew AUDPC, there was no significant effect of genotype, category, management, or the interaction between them (p-value >0.05, Table 6). There was also no significant difference in yields between the high tunnel and field management systems. Caiman had the highest average severity by week 40, with 57% in the field and 42% in the high tunnel. Paul Robeson and Pruden's Purple averaged 50% severity in the field by the same week.

Septoria Leaf Spot AUDPC

There was strong evidence for the management and category main effects, and the interaction between them (Table 6). Variety did not show evidence of effect (p-value >0.05). Overall, Caiman had the highest score (870), and the breeding line CSDE-F6-47 had the lowest (92). The open field had a significantly higher score than the high tunnel (Figure 11). The nature of the interaction between management and variety for the commercial varieties was scalar and for the breeding lines was cross-over.

Fruit quality

°Brix and Citric acid

In the fruit quality analysis, both °Brix and Citric Acid (%) showed significant evidence for the genotype effect (p-value <0.05) (Table 8).

Table 8. ANOVA p-values of f-tests of significance for °Brix and Citric Acid (%) of 22 tomato varieties grown in high tunnel management system in 2020.

Source	°Brix	Citric Acid (%)
Variety (V)	0.016	0.0001

The breeding line P3SS-F4-61 had the highest °Brix value (7.15), and the highest CA% (0.51). Pruden's Purple had the lowest °Brix value (4.13) and A6JB-F5-35 had the lowest CA% (0.23).

Sensory Analysis

When evaluating all the varieties together, the genotype component of variation was significant to the model for Acidity, Sweetness, and Texture (p-value<0.001, Table 9). When evaluating only the Slicer varieties, the genotype component of variation was significant to all the attributes but Bitterness. When evaluating only the Heirloom varieties, the genotype component of variation had a significant effect on all the attributes but Umami. ANOVA p-values of F-tests of significance of the variety effect of the tasting attributes including all the varieties, only the Slicer varieties, and only the Heirloom varieties.

Table 9. ANOVA p-values of F-tests of significance of the Variety effect of the Tasting attributes evaluated in 22 tomato varieties grown under a high tunnel management system in WMARS in 2020.

Attribute	Variety P-value All varieties	Variety P-value Slicer
Overall	0.31	<0.001***
Intensity	0.06	<0.001***
Umami	0.31	<0.001***
Bitterness	0.23	0.42
Acidity	<0.001***	<0.001***
Sweetness	<0.001***	<0.001***
Texture	<0.001***	<0.001***
Appearance	0.05*	<0.001***

Within the Heirloom breeding lines, O4JB-F5-MV1-115 had the highest score for Appearance (3.6), Intensity (3.5), Sweetness (3.1), Bitterness (1.8), and Umami (3.4) (Table 10). Japanese Black Trifele had the best Overall score, followed by Pruden's Purple (Figure 12). Within the Slicer breeding lines, P3SS-F4-61 had the highest score for Appearance (4.2), Sweetness (3.5), Umami (3.2), and Overall (4.2). For the Overall evaluation, P3SS-F4-61 had the highest score, followed by Damsel, and CSDE-F6-47.

Table 10. Least squared means for each Tasting attribute of the 22 tomato varieties grown under high tunnel management system. Scores range from 1 to 5 (See Appendix A for more details).

Variety	Appearance	Texture	Sweetness	Acidity	Bitterness	Umami	Intensity	Overall
A6JB-F5-34	3.2	3.5	2.6	2.9	1.6	2.8	3	2.9
A6JB-F5-35	3.3	2.9	2.6	2.2	1.7	2.5	2.4	2.7
Big Beef	4.3	3.2	3.3	2.8	1.7	2.6	3	3
Caiman	3.8	3.8	2.9	3.1	1.7	2.6	3	3.2
CSDE-F6-46	4.1	3.5	2.3	2.1	1.8	2.2	2.4	2.6
CSDE-F6-47	4.1	3.8	3.2	3.2	1.7	2.9	3.4	3.4
Damsel	4.4	3.9	3.6	3.1	1.8	3.3	3.9	3.9
Defiant	3.8	3.3	2.6	4.2	1.6	2.7	4	3.2
Japanese Black T.	3	3.4	2.9	2.6	1.6	2.8	3	3.6
JBDE-F5-28	3.5	3.3	3.1	2.4	1.8	2.7	2.9	3.2
JBDE-F5-31	3.9	3.3	2.7	2.8	1.8	2.7	3.1	2.9
JBDE-F5-32	3.1	3.3	3	2.5	1.6	2.7	2.8	2.9
JTO-1021	3.6	3.1	2.9	2.8	1.6	2.6	3.1	3.3
O4A6-F4-MV1-109	2.8	3	2.8	2.6	1.7	2.7	3	3
O4DE-F5-44`	3	2.4	2.2	2	1.7	1.9	2	2.5
O4JB-F5-MV1-115	3.6	3	3.1	3.3	1.8	3.4	3.5	3.2
O4JB-F5-MV1-116	3.6	2.2	2.3	2.7	1.9	2.7	2.8	2.4
O4JB-F6-5	4.2	2.8	3	2.5	1.5	2.6	2.7	3.1
P3SS-F4-61	4.2	3.8	3.5	2.8	1.5	3.2	3.6	4.2
Paul Robeson	3	2.7	2.8	2.7	1.8	2.8	3	2.8
Pruden's Purple	3	3	3	3.4	1.5	3	3.4	3.3

Blue cells indicate higher values, white cells indicate lower values, with a range from 1 to 5.

Figure 9 shows the overall rating of the varieties evaluated only from the high tunnel management system.

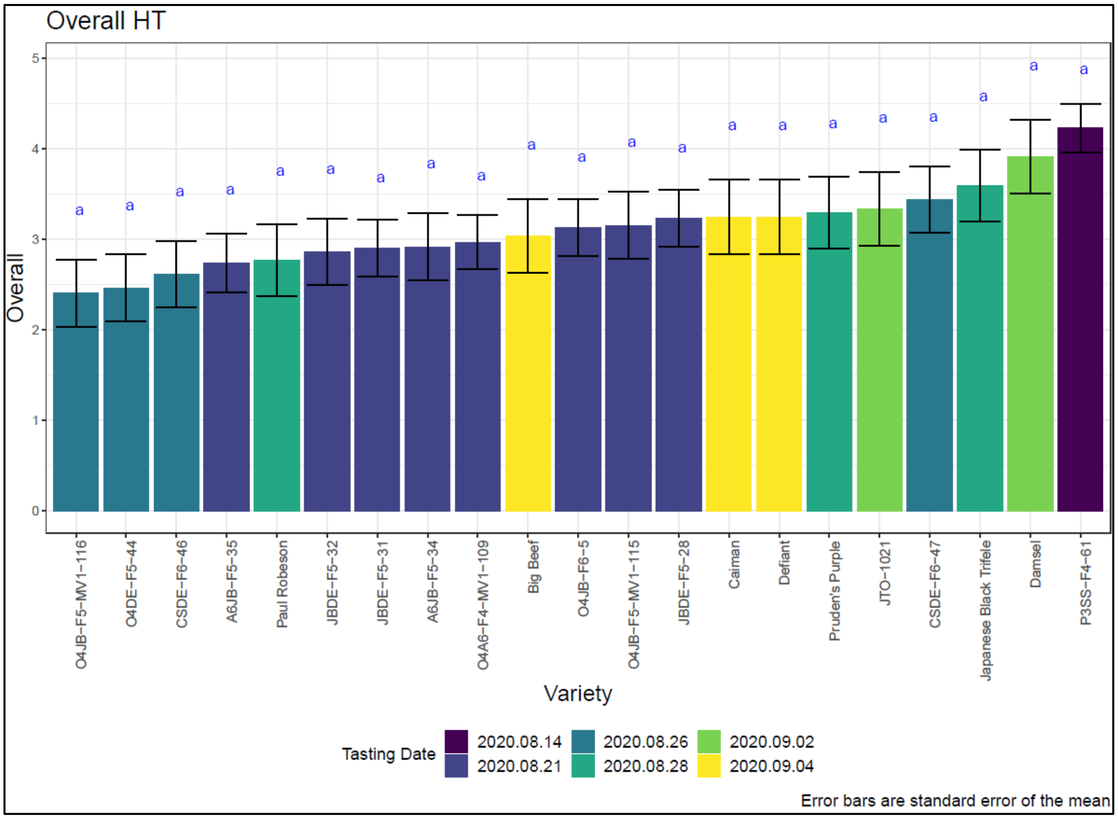

Figure 12. Overall flavor rating of 22 tomato varieties. Colors group different tasting dates. Varieties that share the same letter are not significantly different at the $p<0.05$ level.

Heritability

Overall, all production traits had a heritability higher than 0.8 (Table 11). In terms of disease, powdery mildew had the highest heritability (0.78). Leaf mold had the lowest heritability (0.48). All tasting traits had a low heritability (<0.4).

Table 11. Genetic variance (Vg), environmental variance (Ve), and heritability (H^2) by production, disease, and fruit quality traits from 22 tomato varieties grown under high tunnel management system in 2020.

Trait	Vg	Ve	H^2
Marketable weight	2.27	0.881	0.84
Marketable count	269.2	42.38	0.93
Average fruit weight	10420	814	0.96
Prop unmarketable weight	0.036	<0.001	0.94
Early blight	48,460	22953	0.81
Leaf mold	11,980	25874	0.48
Powdery mildew	57,540	33286	0.78

Septoria leaf spot	2,796	3741	0.60
Total disease	232,900	163486	0.74
°Brix	0.45	0.28	0.77
Citric acid	0.0039	0.00	0.65
Tasting: Appearance	0.19	0.78	0.32
Tasting: Texture	0.19	0.86	0.31
Tasting: Sweetness	0.12	0.77	0.24
Tasting: Acidity	0.19	0.65	0.37
Tasting: Bitterness	0.0068	0.43	0.03
Tasting: Umami	0.078	0.73	0.18
Tasting: Intensity	0.15	0.59	0.34
Tasting: Overall	0.039	1.03	0.07

Table 12 shows the heritability calculated using the family variance instead of the genotype variance. All the traits show a lower value than when heritability is calculated using the genotype variance.

Table 12. Heritability values of production and disease traits calculated using the family variance of the breeding lines grown under high tunnel management system.

Trait	Heritability with family variance
Marketable weight	0.57
Marketable count	0.58
Average fruit weight	0.53
Prop unmarketable weight	0.58
Early blight	0.59
Leaf mold	0.51
Powdery mildew	0.53
Septoria leaf spot	0.29
Total disease	0.48
°Brix	0.46
Citric acid	0.54

Correlation

Table 13 shows the phenotypic correlations among 4 production traits and 5 disease traits evaluated in tomatoes grown in a high tunnel. Marketable weight shows a strong positive correlation with the marketable count, resistance to early blight and proportion unmarketable.

Table 13. Pearson's correlation of production traits of 22 tomato varieties grown in a high tunnel system in 2020.

Trait	Marketable weight	Marketable count	Average weight	Proportional unmarketable	Early blight	Leaf mold	Powdery mildew	Septoria leaf spot
Marketable count	0.58+							
Average weight	0.24	-0.59*						
propUMwt	-0.88***	-0.85***	0.18					
Early blight	-0.67**	-0.5+	-0.11	0.67*				
Leaf mold	0.18	0.03	-0.07	-0.15	0.19			
Powdery mildew	-0.19	-0.04	-0.26	0.02	0.37	0.25		
Septoria leaf spot	0.25	0.02	0.26	-0.13	0.02	0.24	-0.32	
Total disease	-0.37	-0.28	-0.18	0.31	0.79	0.58	0.74***	0.07

propUMwt, proportional unmarketable weight
P-value≤ 0.10=+, *P-value≤ 0.05, **P-value≤ 0.01, ***P-value≤ 0.001.

Table 14 shows the phenotypic correlations between the fruit quality traits and marketable weight. Marketable weight shows a high negative correlation with fruit acidity, umami, and intensity. Titratable acidity is positively correlated with fruit acidity (tasting), and °Brix is also highly positively correlated with the sweetness perceived by the tasters. CA and °Brix have a high positive correlation (0.87). CA is also positively correlated to Texture, Sweetness, Acidity, Umami, Intensity, and Overall. Marketable weight has a negative correlation with all the flavor components.

Table 14. Pearson's correlation fruit quality of tomato varieties grown in a high tunnel system.

Trait	Mkt wt	CA	°Brix	Appear	Texture	Sweetness	Acidity	Bitterness	Umami	Intensity
CA	-0.36									
°Brix	-0.59*	0.87***								
Appearance[1]	0.06	0.60*	0.55+							
Texture[1]	0.11	0.59*	0.38	0.40						
Sweetness[1]	-0.37	0.66*	0.68*	0.38	0.56+					
Acidity[1]	-0.63*	0.72**	0.59*	0.28	0.43	0.63*				
Bitterness[1]	-0.02	-0.09	-0.26	-0.16	-0.27	-0.41	-0.05			
Umami[1]	-0.8***	0.66*	0.68**	0.18	0.39	0.75***	0.88***	-0.13		
Intensity[1]	-0.6*	0.83***	0.72**	0.33	0.57**	0.80***	0.92***	-0.14	0.93***	
Overall[1]	-0.35	0.71**	0.78***	0.42	0.66**	0.93***	0.55***	-0.55+	0.70**	0.76***

Mkt wt, marketable weight. Appear, appearance.
[1]Traits evaluated through tastings.
P-value≤ 0.10=+, *P-value≤ 0.05, **P-value≤ 0.01, ***P-value≤ 0.001.

Expected response to selection (r)

The expected response to selection was calculated for the production and disease traits. This was calculated using the data only from the high tunnel system. The 30% best breeding lines of the population for each trait were selected (4 out of 14 breeding lines) and their mean was used to calculate the selection differential (*S*). Table 15 shows the selected lines for marketable weight, proportional unmarketable weight, EB, and Septoria leaf spot. The lines CSDE-F6-46, CSDE-F6-47, JBDE-F5-28, and JBDE-F5-31 are listed as the best lines for multiple traits.

Table 15. Selected lines in the high tunnel for marketable weight, proportional unmarketable weight, Early blight, and Septoria leaf spot.

Marketable weight	Proportional unmarketable weight	Early blight	Septoria leaf spot
CSDE-F6-46	CSDE-F6-47	JBDE-F5-28	JBDE-F5-28
O4DE-F5-44	CSDE-F6-46	O4DE-F5-43	P3SS-F4-61
O4DE-F5-43	JBDE-F5-28	O4DE-F5-44	O4A6-F4-MV1-109
O4JB-F6-5	JBDE-F5-31	A6JB-F5-35	O4JB-F5-MV1-116

Table 16 shows the correlated response of the tasting attributes when selecting solely for Intensity, °Brix and CA. The correlated response for Overall is higher when selecting for Intensity than the direct response. The correlated response for Overall and Intensity are much higher when selecting for °Brix or CA than direct response. Table 17 shows the correlated response to selection when selecting solely for marketable weight, early blight, leaf mold, and Septoria leaf spot. When selecting for marketable weight, the marketable fruit count correlated response is lower than the direct response, and this is also true for the correlated response in average weight and proportion of unmarketable weight. When selecting for EB resistance, the correlated marketable weight is a 17% increase, compared to a 28% increase with direct selection.

Table 16. Expected correlated response expressed in the percent change in the overall mean of fruit quality attributes when direct selection is practiced solely for °Brix and CA (%)[1].

Trait	Direct Response to selection (R)	Intensity correlated response	°Brix correlated response	CA correlated response
Tasting: Appearance	5%	3%	7%	6%
Tasting: Texture	5%	5%	7%	10%
Tasting: Sweetness	4%	6%	11%	11%
Tasting: Acidity	6%	7%	9%	11%
Tasting: Bitterness	0%	0%	-1%	-1%
Tasting: Umami	3%	8%	11%	11%
Tasting: Intensity	6%	9%	14%	15%
Tasting: Overall	1%	7%	14%	12%

[1]Assuming upper 30% individuals are selected. Predicted response per generation.

Table 17. Expected correlated response expressed in the percent change in the overall mean of production and disease traits when direct selection is practiced solely for marketable weight, Early blight, Leaf mold, and Septoria leaf spot[1].

Trait	Direct response	Marketable weight correlated response	Early blight correlated response	Leaf mold correlated response	Septoria correlated response
Marketable weight (kg/plant)	28%	---	17%	-2%	-9%
Marketable count (fruits/plant)	75%	25%	25%	-9%	-2%
Average fruit weight (g/fruit)	54%	20%	9%	11%	-7%
Proportional unmarketable weight	-65%	-48%	-38%	7%	16%
Early blight (AUDPC)	-27%	-19%	---	-12%	1%
Leaf mold (AUDPC)	-17%	2%	-11%	---	-7%
Powdery mildew (AUDPC)	-31%	-8%	-28%	-25%	0%
Septoria leaf spot (AUDPC)	-60%	43%	5%	-33%	---
Total disease (AUDPC)	-24%	-8%	-25%	-23%	-5%
CA	12%	-9%	-4%	0%	5%
°Brix	14%	-8%	-5%	-3%	8%
Tasting: Appearance	5%	1%	-1%	-4%	-3%
Tasting: Texture	5%	1%	4%	-5%	3%
Tasting: Sweetness	4%	-6%	1%	1%	4%
Tasting: Acidity	6%	-10%	-9%	2%	5%
Tasting: Bitterness	0%	-1%	-1%	0%	1%
Tasting: Umami	3%	-13%	-7%	1%	5%
Tasting: Intensity	6%	-11%	-6%	2%	7%
Tasting: Overall	1%	-6%	2%	2%	4%

[1]Assuming upper 30% individuals are selected. Predicted response per generation.

On-farm evaluation

Participating farms (Chapter 1, *Participating farmers*) received 6 breeding lines from different families to try in their farming systems. All the varieties evaluated on-farm were grown in high tunnels. At the end of the season, each farmer submitted a qualitative evaluation form that indicated which varieties they preferred with respect to productivity and flavor along with any other relevant observations. Table 18 presents a summary of all the evaluations received from 2019 and 2020. Is important to note that not all participants received the same breeding families, and overall, the summary includes the evaluation of 2 or 3 farms per cross.

The CSDE family had high productivity with good flavor, for some farmers it was the "winner" in terms of flavor compared to the rest of the lines. One farmer mentioned that ripening started late compared to their commercial varieties but did not specify how late (days or weeks). O4JB had high yields and great flavor and was categorized as the best overall. In terms of earliness, it was between Damsel (earliest) and Caiman (a little later than Damsel). JBDE had great flavor, and productivity varied from medium to high. A6JB was identified as a typical heirloom with a very sweet and good flavor. It had low disease resistance and low productivity. O4A6 had medium disease resistance, good flavor, and productivity ranged from low to high. Farmers also indicated that higher amounts of radial splitting and cracking, and it must be sold soon after harvesting due to its soft skin.

Table 18. Summary of on-farm evaluations of breeding lines grown in a hoop house or high tunnel during 2019 and 2020. JBT: Japanese Black Trifele.

Family	**General characteristics**	**Flavor**	**Productivity**	**Fruit size**	**Other comments**
Crimson x Defiant (CSDE)	Competitive with weeds, healthy	Good acid/sweet balance, tough skin. Best of all.	High - moderate	Medium	Late
OSA404 x JBT (O4JB)	Not as big as CSDE, but still a good size. Great disease resistance to Early blight. Later than Damsel and later than Caiman.	Good, sweet flavor. Heirloom look. Nice and tasty.	High - good	Some medium, some Big Beef size	Short harvest. Best overall. Yields higher at the beginning of the season
JBT x Defiant (JBDE)	Healthy plants	Very tasty, sweet flavor. Tough skin.	Moderate, not a lot. Very productive for another farm.	Medium	
A6 x JBT (A6JB)	Typical heirloom. Not good disease resistance. Prone to rot.	Sweet, good flavor	Not a lot of fruit	Big, heirloom type	A lot of variability in fruits, some nice purples. Soft fruit.
OSA404 x A6 (O4A6)	Decent leaf disease resistance.	Very sweet	Low – high (varied from farm to farm and year)	Medium	Took a long time to ripen. Soft fruit. Radial cracking and green shoulders.

Discussion

Marketable weight

In this study, we present marketable weight as the average marketable weight per plant. This trait is of great importance to farmers, who seek to boost production of marketable produce. The genetic component of variation had a significant effect, concluding that there is important variability in the marketable weight within the varieties. The interaction between genotype and management was also significant to the model, and most of the varieties showed a scalar-interaction type, with significantly higher marketable weight in the high tunnel than the field. This aligns with results of previous research (Healy et al., 2017; Hodge et al., 2019). The highest yielding breeding lines in the high tunnel were, in decreasing order, CSDE-F6-46 (7.4 kg/plant), O4DE-F5-44 (7.1 kg/plant), and

O4DE-F5-43 (7.0 kg/plant). The lowest yielding breeding lines in the high tunnel were, in increasing order, O4JB-F5-MV1-115 (1.6 kg/plant), P3SS-F4-61 (3.2 kg/plant), and O4JB-F5-MV1-116 (3.5 kg/plant). Interestingly, the top three varieties are descendants of Defiant, and two of the crosses are with OSA404. At the same time, two of the lowest yielding varieties are descendants of OSA404.

Overall, our results show that all the varieties and crosses had a significantly different marketable weight between the two management systems. The yield in the high tunnel was more than 5 times greater than the open field (Table 3). This can be attributed to the season extension effect of the high tunnel and the significantly reduced disease pressure and incidence. The high tunnel was planted 3 weeks earlier than the field and provided two more weeks of additional harvest at the end of the season. This translated to earlier fruit harvest in the high tunnel compared to the field, resulting, in part, in a higher marketable weight. O'Connell et al. (2012) found similar results when evaluating the production of organic heirloom varieties, where the high tunnel yielded 33% more than the open field system. Healy et al. (2017) also found analogous results when comparing tomato production under hoop house and open field systems, seeing a significant effect of management on yield, with increased production in the hoop house. In addition, the high tunnel protects against early season frosts and rainfall. This can reduce the spread of diseases, especially those that require free water, such as early blight and Septoria leaf spot. The lack of splashing means that disease spreads far more slowly in the high tunnel than in the field. Management had a significant effect on the severity of the disease, and the high tunnel had significantly lower severity than the field.

Marketable count

Marketable count corresponds to the total fruit number per plant throughout the season. It is closely related to marketable weight, and it can also be an important production trait for farmers. In this study, all the factors in the model had a significant effect on marketable count. This was expected, as fruit number has been shown to be controlled by multiple quantitative trait loci (QTLs)

(Bretó et al., 1994; Monforte et al., 1996). The breeding lines with the highest marketable count in the high tunnel, in descending order, were JBDE-F5-31 (61 fruits/plant), and Defiant descendants CSDE-F6-46 (53 fruits/plant), and JBDE-F5-28 (49 fruits/plant). The breeding lines with the lowest marketable count in the high tunnel were O4JB-F5-MV1-115 (4 fruits/plant), O4JB-F5-MV1-116 (15 fruits/plant), and A6JB-F5-34 (15 fruits/plant). Management was the largest component in the ANOVA table, and marketable count was significantly higher in the high tunnel system than in the field system. As discussed previously, the high tunnel provides benefits that contribute to a higher marketable yield, which is usually positively correlated to a higher yield count, as was found in this study (marketable weight and fruit count correlation = 0.58, p-value<0.1).

Average fruit weight

Average fruit weight is also an important trait for farmers, especially those who sell in direct markets. The genotype main effect proved to be significant to the model (p-value <0.001), as well as the interaction between genotype and management. Management did not show a significant effect on the variation of fruit weight, with no significant difference between systems. Average fruit weight ranged from 87g/fruit (JBDE-F5-31) to 365g/fruit (O4DE-F5-43). Interestingly, both are descendants of Defiant, the first being a cross with the smaller-fruited Japanese Black Trifele, and the second with larger-fruited OSA 404. The breeding lines with the highest average fruit weight in the high tunnel were O4DE-F5-44 (381 g/fruit), O4DE-F5-43 (372 g/fruit), and O4JB-F6-5 (321 g/fruit). The breeding lines with the lowest fruit weight in the high tunnel were JBDE-F5-31 (87 g/fruit), P3SS-F4-61 (99 g/fruit), and JBDE-F5-28 (110 g/fruit).

Proportion of unmarketable weight

The genotypic main effect was a significant source of variation for the proportion of unmarketable weight. In the high tunnel, Defiant had the lowest proportion of unmarketable weight, followed by the breeding lines JBDE-F5-28, JBDE-F5-31, CSDE-F6-46, and CSDE-F6-47. The first two are a cross of Defiant with JBT and the last two a cross of Defiant with Crimson Sprinter. The primary causes of unmarketability in the high tunnel were blossom end rot (28%) and interior blemish (28%) for JBDE-F5-28, side stem crack (36%) and insect (27%) for JBDE-F5-31, stem crack (38%) and insect (19%) for CSDE-F6-46, and radial splitting (32%) and side stem crack (16%) for CSDE-F6-47.

Management also had a significant effect on the proportion of unmarketable weight, with the field having more than double the proportion of unmarketable harvest than the high tunnel. Soil moisture levels fluctuate greatly in the field due to the lack of rain protection, which leads to fruit development issues including blossom end rot, side cracking, and splitting (Hunter et al., 2010; Peet & Willits, 1995). Disease pressure is higher in the open field, which also contributes to the increase of the unmarketable weight (Blomgren & Frisch, 2007; Rogers & Wszelaki, 2012).

Overall, cracking was the most common cause for unmarketability, causing 47% of the unmarketable fruit in the high tunnel, and 64% in the open field. Japanese Black Trifele and Damsel were the most affected by stem side cracking, and the breeding lines O4JB-F5-MV1-115, O4A6-F4-MV1-109, and O4JB-F5-MV1-116 also had a high percentage of unmarketability for the same reason (>70%). Radial splitting was the second most common reason for unmarketability. Side stem cracking and radial splitting are physiological disorders that occur as a result of erratic moisture conditions (Peet & Willits, 1995). Both disorders can cause important problems for producers, lowering the marketable yield and, therefore, net profit. There is also a genetic component to cracking susceptibility, where the thickness of the skin, fruit size, and the number of fruits per plant also plays an important role (Peet, 1992). Considering these factors, high percentages of cracking could be attributed to a combination of genetic components and unpredictable irrigation. Management practices such as maintaining more consistent soil moisture levels and harvesting fruits before the pink stage may help mitigate this problem (Peet, 1992).

Early blight

Early blight can be a fatal disease in tomatoes if no preventive and control measures are taken. For organic farmers, prevention is especially important due to the limited number of products approved for use in organic certified farms. Prevention measures include rotating solanaceous crops for 3-4 years, controlling solanaceous weeds, and using varieties with some degree of resistance, among other cultural managements strategies (Delahaut & Stevenson, 2004). The breeding lines O4JB-F5-MV1-115 and O4JB-F5-MV1-115 had the highest EB AUDPC in both systems. EB symptoms started showing two weeks earlier in the high tunnel than the field, but the varieties had the highest average percentage of severity in the field by week 40. The high tunnel was planted on week 20 and the field on week 23 of the year 2020. This 3-week planting gap could explain the earlier symptoms in the high tunnel, but then the average of EB incidence percentage is higher later in the season in the field than in the high tunnel. The high tunnel protects against rain and soil splashing, which can decrease the early blight severity by keeping the foliage clean and dry (Rogers & Wszelaki, 2012). The lowest AUDPC scores were 140 in the field (Defiant) and 107 (Paul Robeson) in the high tunnel. Defiant's low Early blight score in the field is consistent with its history of breeding for intermediate EB resistance and high late blight resistance (Johnny’s Selected Seed, 2021). The breeding lines with the lowest EB scores in the high tunnel were JBDE-F5-28 (235), O4DE-F5-43 (568), and O4DE-F5-44 (585). For the field, O4JB-F6-5 (758), P3SS-F4-61 (660), and JBDE-F5-31 (660) had the lowest scores.

Leaf Mold

There was strong evidence for the category main effect (p-value <0.001), and the check varieties had significantly higher scores than the breeding lines in the high tunnel. Visual disease scoring is error-prone and dependent on the experience of the evaluator, so we can assume differences in assessment style between the two people scoring. This issue might may be mitigated by having the same person score throughout all the plots in the trial or ensuring that all scorers receive the same visual calibration training. This was difficult due to the COVID pandemic in the 2020 field season. The breeding lines with the lowest leaf mold score in the high tunnel were O4DE-F5-43 (280), O4DE-F5-44 (315), and JBDE F5-31 (315). The severity of leaf mold was significantly higher in the high tunnel than in the field throughout the season. this may be due to the fact that the fungal pathogen requires a highly humid environment to be infectious (39 – 90 F) (McGrath, n.d.) and relative humidity was often higher in the tunnels when kept closed during bad weather.

Powdery Mildew

Overall, the severity of powdery mildew was mild and did not seem to negatively affect yields. This fungal disease benefits from dry conditions, which is why its severity can vary from one year to another (Vallad et al., 2018). It appears that conditions during the 2020 growing season did not support the spread of powdery mildew in the study areas.

Septoria Leaf Spot

Check varieties had significantly higher scores than the breeding lines. As discussed in the previous Leaf mold results, the fact that a different scorer evaluated the check varieties could have influenced the overall result. Septoria leaf spot severity was more severe in the field which may be due to factors not present in the high tunnel such as rain splashing spores from plant to plant (Fealko et al., 2020). This can explain why the severity was higher in the field, compared to the protection that the high tunnel offers. In the field, the breeding lines with the lowest score were CSDE-F6-47, CSDE-F6-46, and O4JB-F5-MV1-115 (average AUDPC of 167 for all).

Fruit Quality

Fruit quality was evaluated only in the high tunnel. The genotype had a significant effect on variation for °Brix and citric acid (%). The correlation between fruit weight and °Brix was -0.47 (p-value 0.04) and -0.63 with citric acid (%) (p-value <0.01). Zörb et al. (2020) found a highly negative correlation between fruit weight and citric acid (-0.565) and with sugars like glucose and fructose considered together (-0.897). Similarly, Saliba-Colombani et al. (2001) found a correlation of -0.56 between fruit weight and °Brix, and -0.58 with titratable acidity. The breeding line P3SS-F4-61 had the highest value for both traits. At the same time, this variety had the lowest average fruit weight. This demonstrates the negative correlation between °Brix and citric acid (%) with fruit weight.

In terms of tasting evaluation, when comparing the slicer and heirloom varieties separately, the genotype had a significant effect on the Overall, Intensity, Acidity, Sweetness, Texture, and Appearance scores. There were significant differences among varieties for these traits. Among the heirloom varieties, Japanese Black Trifele had the highest Overall score. O4JB-F5-MV1-115 had the highest score for Intensity, while P3SS-F4-61 had the highest score among the slicer varieties. O4DE-F-44 had the lowest Intensity score among all varieties. These varieties also had low citric acid (%) and low °Brix.

Heritability, correlations, and Response to selection

The heritability of the productivity traits was very high (all ≥0.84, Table 11). This indicates that with the selection and breeding of improved genotypes, these traits may be adequately evaluated across relatively few growing environments. The same true for the EB, Septoria leaf spot, and leaf mold heritability. This indicates that the resistance to EB is highly controlled by genetic factors. The caveat with these heritability values is that we only evaluated the lines in one location and one year, which will result in heritability values higher than what they might otherwise be. In addition, we had a wide range of genetic backgrounds in the trial which increases genetic variance and broad sense heritability. As we make selections and drop some families, the broad sense heritability is likely to decrease. A replicated trial will be carried out in 2021 and heritability will be calculated again. Marketable weight means of the breeding lines overall were not as high as the check varieties, but genetic variance was high, which tells us that there is potential for future gains for this trait. The same happens for the marketable count and average fruit weight. For EB, the scores were not significantly different between breeding lines and check varieties. The EB AUDPC mean is lower than expected, which indicates that this disease might not be a concern in the high tunnel management system. The scores were higher in the open field, but not significantly different than the high tunnel. As the project continues, this could mean that future selections will focus on improving yields and flavor rather than early blight resistance.

Looking at the heritability calculated using the family variances (Table 12) also provides interesting insights into the breeding population. The family variance was lower than the genotype variance for all the traits, and this is to be expected since the breeding lines evaluated are in advanced generations (4^{th}, 5^{th}, and 6^{th} depending on the breeding line), where selection has already decreased the variance of the traits selected for (mainly yield, disease resistance, and flavor).

When selecting only for marketable weight, the correlated response of marketable count is lower than the direct response but is still an improvement from the overall mean (25% and 75% increase respectively). Following the same trend, the correlated response of average fruit weight is lower than the direct response, when selecting for marketable weight (20% and 54% increase respectively). Marketable weight was negatively correlated with the proportion of unmarketable weight, meaning that the higher the yields, the lower the proportion of unmarketable weight. The correlated response of proportional unmarketable weight was -48% and the direct response -65%. Selecting only for higher marketable weight would also increase the marketable count and average fruit weight, while decreasing the proportion of unmarketable weight.

Traits evaluated through the tasting process had a low heritability. Our methods of flavor evaluation likely do not have an adequate level of precision when dealing with more variable populations. Selection based on this type of sensory evaluations would be inefficient to significantly improve the overall flavor of the varieties. It may be more effective to do individual plant flavor evaluations with a ranking method for flavor evaluation within families, while still using the current flavor evaluation method to determine differences among families or to compare breeding populations to check varieties. Using laboratory-based methods for evaluating ˚Brix and CA and only asking tasters to evaluate flavor intensity may be another avenue to pursue. The correlation between Overall flavor and Intensity was 0.76 (p-value<0.001). Baldwin et al. (1998) found similar results, obtaining an $r = 0.71$ correlation between the same traits. This suggests that consumers prefer more intensely flavored fruit. Merscher (2020) also found a significant correlation between Overall and Intensity (0.74), and Overall and Sweetness (0.7), when evaluating pink and red slicer tomato varieties. The correlation between marketable weight and CA and °Brix is negative (-0.39 and -0.59 respectively). In parallel, CA is highly correlated with Acidity (0.72), Intensity (0.83), and Overall (0.71), and °Brix is highly correlated with Sweetness (0.68), Intensity (0.72), and Overall (0.78). This suggests that °Brix and CA could be good measures to anticipate flavor. The expected correlated response of Overall when selecting solely for °Brix is a 14% increase, and 12% when selecting for CA, both higher than direct selection for Overall (1%). These results are explained because of the low heritability of the tasting traits, and higher heritability of °Brix and CA. An analysis of specific sugars, like glucose and sucrose, could give better estimates of flavor. Baldwin et al. (1998) found Sucrose/TA (titratable acidity) to be more useful in predicting the overall acceptability of a tomato, rather than °Brix/TA. Such analyses require more labor and increases the cost of each breeding line evaluation. The correlation found between °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.

Marketable weight had a high negative correlation with Intensity (-0.6) and an intermediate negative correlation with Overall flavor (-0.35). and this was also reflected on the expected correlated response when selecting for marketable weight. The direct responses for Overall flavor and Intensity are low 1% and 6% increase respectively), and they would both decrease if selecting for marketable weight (-6% and -11% respectively).

We can see that overall, fruit flavor and marketable weight are negatively correlated, making it hard to improve both traits at the same time. Whole-genome sequencing and genome-wide association 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 ever larger fruits produced the loss of specific alleles that contribute to higher soluble content, making current selection for higher weights and higher soluble contents at the same time a difficult challenge to overcome. It could be possible to significantly increase the content of favorable aromas by replacing alleles that are associated with unpleasant volatiles, like guaiacol and methyl salicylate (Zhao et al., 2019). De Souza et al. (2012) evaluated the yield, °Brix, and TA of the F1s obtained from a diallelic cross between 5 parental lines. They obtained a correlation of -0.18 between plant yield and soluble solids (°Brix), and -0.13 between plant yield and titratable acidity. Selecting for higher yields can only negatively affect the overall flavor perception by the consumers. This can make the future selection process difficult because the main objectives of this breeding program are to generate a variety that has high yields and excellent flavor, adapted for organic systems. From verbal communication with the tasters, overall, the breeding lines and check varieties tasted much better than tomatoes purchased at the supermarket. We received similar feedback from the farmers that tried some of the breeding lines in their production systems: the flavor was outstanding in some of the breeding lines. This information, along with the flavor evaluations, showcase a promising starting point in terms of flavor. With the current data, and the 2021 trial evaluations, we can develop a selection index formula that includes plant yield, CA, °Brix, and disease scoring.

Septoria leaf spot scores were very low in the high tunnel and did not seem to affect production traits. The field had significantly higher scores. So far, the high tunnel production has outperformed the field, both because of the benefits that the system provides, like earlier harvest, protection against rainfall, and others, and due to a genetic component. The increase of Septoria leaf spot AUDPC could be cause of precaution when selecting for marketable weight. The correlated response was 43%, while the direct response was -60%, meaning that selection for marketable weight could cause an increase of Septoria incidence. Even though the overall incidence of Septoria in the breeding lines was low in the high tunnel, further selection should consider a per plant disease scoring to select for the lower AUDPC possible within the higher marketable weight breeding lines. In the case of early blight, the correlated response when selecting for marketable weight showed a decrease in the overall mean. Even though the Early blight incidence was low and did not differ significantly from the field values, it is still positive to predict a decrease in the AUDPC, as the disease pressure could change from year to year.

Overall, the breeding lines perform better in the high tunnel, so we will guide future selection for high tunnel production improvement. For high tunnel production Septoria leaf spot is not as much of a priority given the low impact on overall plant health and fruit production. For field production, much stronger disease resistance is needed, and we are working with a national group to select disease resistant and flavorful varieties with new crosses using the genetic background of WI55, Crimson Sprinter and additional North Carolina State varieties in addition to disease resistant parents from Oregon State University, Cornell and the private sector.

Promising breeding lines

Based on the results of our evaluations for production, disease, and fruit quality traits, we have chosen several breeding lines for further field evaluation.

CSDE-F6-46 had the highest marketable weight (7.4 kg/plant) of all the breeding lines. The average weight of the medium-sized, round fruit was 139g. It had a low proportion of unmarketable weight (0.1), and the primary cause of unmarketability was stem side crack. Its early blight AUDPC was close to the overall mean of only the breeding lines in the high tunnel (791 and 751 respectively). It was not particularly affected by any of the rest of the diseases evaluated. In terms of the sensory evaluation, it had a low Overall score (2.6), low Intensity (2.4), and high Appearance (4.1).

In the same breeding family, the line CSDE-F6-46 had a lower marketable weight (6.3 kg/plant), a slightly higher unmarketable proportional weight (0.18). It had a similar Total disease AUDPC to CSDE-F6-46 (no significant difference). On the other hand, this variety did well in the sensory evaluation. It had the second-best score in the Overall category (3.4), a high intensity (3.4), and high Acidity and Sweetness (3.2 both). It also had high CA (%) (0.45), and intermediate °Brix (5.4). This line could be further selected, considering that the expected response to selection increases yields to 1.45 kg/plant keeping close attention to the flavor evolution, since yield and flavor are negatively correlated. It is also important to note that farmers liked this family and found it to be productive with good flavor.

JBDE-F5-31 and JBDE-F5-28 both had intermediate marketable weight (5.3 kg/plant for both, no significant difference). The average fruit weight was low (87 and 108 g/fruit respectively), resulting in a medium-sized fruit. Both had a low unmarketable proportion (0.08 and 0.09), with the primary cause for unmarketability being stem side cracking, which is common in Japanese Black Trifele, one of the parental lines. In terms of flavor, JBDE-F5-28 did better Overall (3.2), with high Intensity (3.1), Sweetness (3.1), and low Acidity (2.4). This family was liked by farmers because of the medium to high yields, the sweet flavor, and the overall good health of the plants. However, testers commented that the skin of both lines was too thick.

P3SS-F4-61 had the best Overall flavor score, with high acidity, sweetness, intensity, and umami. In terms of production, it had a low marketable weight in the high tunnel (3.1 kg/plant) compared to the rest of the slicer breeding lines (overall mean 5.3 kg/plant). The marketable weight variance was high, and this trait also had a high heritability, so there is room for yield increase when selecting future generations. Average fruit weight had a high variability within plants, with a confidence interval from 58 to 140 g. Because this is an F4, uniformity has not yet been achieved, and that has been made clear with fruit size variation. The proportion of unmarketable weight was high (0.58), and the primary cause of unmarketability was radial splitting (85%). Better irrigation management at the time of fruit ripening could help decrease the amount of unmarketable fruit, and at the same time increase the marketable weight. In terms of disease, it had scores close to the mean in all the diseases evaluated and did not show high susceptibility or resistance to any of them.

CONCLUSIONS

The objective of this project is to develop tomato varieties that produce high-yielding varieties with excellent flavor, good disease resistance, and that are suitable for organic farming systems. We evaluated production, disease, and fruit quality traits in 22 tomato breeding lines and commercial varieties. The genotype component had a significant effect on all the production traits, early blight, leaf mold, and Septoria leaf spot (from the genotype by environment effect). It was also significant in all the fruit quality traits besides bitterness, including CA and °Brix, when evaluating the slicer and heirloom varieties separately. The management component had a significant effect on marketable weight, marketable fruit weight, and the proportion of unmarketable weight. All the varieties performed better in the high tunnel system. As detailed in the discussed section, the high tunnel provides benefits like season extension (earlier and longer harvest), a more controlled climate, and plant disease mitigation. All the breeding lines performed better in the high tunnel, and total disease AUDPC was lower in this system. Early blight resistance was one of the main traits of interest for this project, but in general, scores were low in the high tunnel and did not seem to affect the overall yields.

The productivity (marketable weight, fruit count, average fruit weight) of the breeding lines was lower than the check varieties, but the genetic variance and heritability were both high. This indicates room for improvement in these traits, although with the caveat that these calculations are based on a single location and a single year. Incorporating the data from the 2021 trial will provide a more reliable value for genetic variance and heritability.

The negative correlation between marketable weight and the fruit quality traits will be a limitation for future selections. Selecting for higher yields could cause a decrease in fruit quality because the correlated response for Acidity and °Brix were negative. Similarly, the correlated response for Overall flavor, Intensity, Sweetness, and Acidity, are all negative. °Brix and CA (%) can be good predictors of flavor since both traits are highly correlated with Intensity and the Overall perception of the fruit.

P3SS-F4-61 shows great promise, with great flavor, medium yields, and low disease incidence. The fruit size variation between plants made it clear that uniformity has not been achieved, so future selections should consider optimal size, individual productivity, unmarketable proportion, and actual marketability (likelihood of the farmer selling harvest). Yield could be improved, though not at the expense of fruit quality.

The CSDE family was also promising. It has high yields and low unmarketable proportion yet did not have an outstanding flavor. Further selection could evaluate the yield effects of fruit quality improvements.

Next steps for this project include a replication of the trial in 2021 to provide data that will solidify the genetic and phenotypic variances, heritability, expected response to selection, and expected correlated response to selection. To decide which varieties should be selected for further advancement, a Selection index will be developed, where the yield traits, flavor, and disease resistance will be assigned a proper weight to give a score to each breeding line. The selection index will be adapted specifically for the needs of the Upper Midwest farmers and will account for the nuances of their production systems and customer bases. After selecting the best lines, we will conduct broader on-farm trials, the results of which will inform the potential release of a line as a variety. Depending on 2021 data, promising lines could become parental line material for future breeding programs. Because on-farm trials will play an essential role in adapting these breeding lines to organic systems, we will expand our network of on-farm collaborations while working more closely with currently participating farms to further refines the participatory process.

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CHAPTER 4: FINAL CONCLUSIONS

The objective of this project is to develop tomato varieties that are adapted for organic conditions in the Upper Midwest. For this, we followed a participatory plant breeding approach where we collaborated with farmers and researchers to identify the key traits to focus on and to select the best breeding lines of the crosses initially made. Disease resistance showed to be high priority for farmers, because they rely on it to prevent spread disease in their systems. Even though there are a few diseases resistant tomato varieties on the market, it has been noted by farmers that those varieties usually lack equally important traits such as high yields and good flavor, so they lean towards varieties that stand out on those traits rather than disease resistance. Selecting for disease resistance is difficult, and farmers usually don’t have the time or resources to have a detailed analysis and weekly evaluation that selection for disease selection requires. For this breeding program, we selected parental varieties that had shown moderate disease resistance or tolerance in previous trials, making them promising genetic material. After making crosses and evaluating the early generations on-station, we sent seed from different breeding lines to organic farmers to evaluate on their systems.

The analysis of the 2020 on-station breeding lines evaluation showed that diseases like early blight and Septoria were not significant and did not cause a major decrease in yields in the high tunnels. On the other side, the tomatoes grown in open field management showed higher disease incidence. Overall, all the breeding lines performed significantly better in the high tunnel, and this can be attributed to the season extension that the structure provides, the protection against rainfall and extreme cold temperatures, and consequently a reduction in disease pressure due to the lower amount of free water that can help propagate fungal spores. In terms of production, the marketable yield significantly varied among breeding lines, with a couple very productive lines, and other less productive with higher percentage of unmarketable fruit. Fruit quality was evaluated through sensory analysis with crew and chefs' tastings, and in parallel the °Brix and titratable acidity were measured in the laboratory. A couple of breeding lines stood out in terms of fruit quality and productivity, and where also highly evaluated by farmers from their own on-farm evaluations. Another year of data (2021) will help provide a more reliable evaluation of all the production, disease, and fruit quality traits, but so far there are outstanding lines that show promise for further selections and possible release as commercial varieties or to use as germplasm for future new crosses.

This project will continue to further select and evaluate the breeding lines presented in this work as well as the new crosses that will be made, and the next three years will be focused on increasing on-farm evaluation to have a multi-location analysis. We expect to maintain and develop new partnerships with different community members as well as small plant breeders, more local farmers, and chefs.

Appendix A. Parental lines characteristics table

Table 1. Characteristics of the parental lines used in this breeding program.

Founders	**Market**	**Size and shape**	**Fruit weight (oz)**	**DTM**	**Fruit color**	**Disease resistance**
Defiant	Slicer	Mid-sized, round	6-8	65	Red	Yes
OSA 404	Slicer	Mid-sized, round	8-10	70	Red	Moderate
Japanese Black Trifele	Heirloom	Mid-sized, pear-shaped	4-6	85	Mahogany red	No
A6	Heirloom	Big, round	10-12	75-80	Red	No
Crimson Sprinter	Slicer	Mid-sized, round	5-7	65	Red	Some
Summer sunrise	Saladette	Medium, round	2-3	65	Yellow	No

*DTF: days to maturity

Appendix B. Instructions sheet for the tomato breeding lines sent to farmers to evaluate on-farm in 2020.

SKC Tomato Breeding trials

Grower evaluations of tomato breeding populations on farms in the Upper Midwest

SKC
Seed to Kitchen Collaborative

Thank you for being part of our tomato trials!

Based on previous Seed to Kitchen trials, we crossed some of the best performing varieties for both field and high tunnel production. After growing out the first few generations at the research station, we've selected the most promising families to send to growers this year. We are interested in your opinions on these families and the possibility of you making on-farm selections and saving seed.

For these on-farm trials, we are asking for one replicate of each family in either the field or high tunnel (or both): 1 plot x 6 families = 6 plots x 6 plants/plot = 36 plants in either system

Please follow the plot map for planting. Call or email with any questions.

Data to be collected is similar to Seed to Kitchen trial evaluation, with a little additional information requested. Some of these families are more advanced than others. In earlier generations, individual plants in the same family can be quite different from one another. We are asking for your feedback on the family as a whole, but we would also like to know if there are any individual plants that stand out to you.

Please flag or otherwise mark the plants you think are exceptional, so we can collect seed during our visit.

The information we would like is:

- Opinions on flavor, marketability, yield, and disease resistance for whole families
- Identification of superior individuals within a family and reasons why
- Major strengths and weaknesses of each family
- Your thoughts and interests on the future of each family

Management sheets is enclosed. It follows the same format as the Seed to Kitchen trials. Please contact if you have any questions.

If possible, we would like to visit once during the season to walk the trial and collect seed from the best individuals you flagged. If you are interested, we can also work with you to save your own seed.

Have a great season!

Ambar Carvallo
Agroecology MS student
UW-Madison
+1 608 421 8509 – carvallolope@wisc.edu

Appendix C. Suggested plot maps for on-farm tomato breeding trials.

Bed 1	Your variety							
Bed 2	Your variety - 3 plants	Family 1 - 6 plants	Family 2 - 6 plants	Family 3 - 6 plants	Family 4 - 6 plants	Family 5 - 6 plants	Family 6 - 6 plants	Your variety - 3 plants
Bed 3	Your variety							

OR

Field Plots - short

Bed 1	Your variety				
Bed 2	Your variety - 3 plants	Family 1 - 6 plants	Family 2 - 6 plants	Family 3 - 6 plants	Your variety - 3 plants
Bed 3	Your variety - 3 plants	Family 6 - 6 plants	Family 5 - 6 plants	Family 4 - 6 plants	Your variety - 3 plants
Bed 4	Your variety				

High Tunnel -- rows parallel to sides

	Side wall				
Bed 1	Your variety				
Bed 2	Your variety - 3 plants	Family 1 - 6 plants	Family 2 - 6 plants	Family 3 - 6 plants	Your variety - 3 plants
Bed 3	Your variety - 3 plants	Family 6 - 6 plants	Family 5 - 6 plants	Family 4 - 6 plants	Your variety - 3 plants
Bed 4	Your variety				
	Side wall				

OR

High Tunnel -- rows parallel to ends

	End wall			
Bed 1	Your variety			
Bed 2	Your variety - 2 plants	Family 1 - 6 plants	Family 2 - 6 plants	Your variety - 2 plants
Bed 3	Your variety - 2 plants	Family 4 - 6 plants	Family 3 - 6 plants	Your variety - 2 plants
Bed 4	Your variety - 2 plants	Family 5 - 6 plants	Family 6 - 6 plants	Your variety - 2 plants
Bed 5	Your variety			

Appendix D. Trial management Information form sent to farmers that received tomato breeding lines seed in 2020.

CROP SPECIES				
soil type				
prior crop				
cover crop				
bed preparation				
planting method				
seed date				
transplant date				
between row spacing				
in row spacing				
fertilizer				
mulch				
irrigation				
pest or disease treatments				
trellising method				
pruning method				
standard varieties				
Unusual weather events or problems				

Appendix E. Received On-farm evaluation forms

Table 2. Luna Circle evaluation Summer 2019 evaluation		
Family	A6JB-F2	CSDE-F3
What do you think of the family as a whole?	ok	good
Are their individual plants that stand out? Which ones and why?		
What did you think of the flavor?	good	ok
What did you think of the productivity?	low	good
What did you think of the marketability?	eye catching shape	lots of cracking
Any disease/ insect/ stress problems?	lots of disease problems	lots of disease problems
Strong points	shape	under better weather conditions may have fewer cracks so more marketable fruits
Major flaws	low productivity	cracking
Are you interested in growing the family again and making selections?	yes	yes
General Notes or Comments		

Table 3. Voss Organics Summer 2019 evaluation		
Family	O4JB-F3 - High Tunnel	O4A6-F3- High Tunnel
What do you think of the family as a whole?	Loved this variety	Loved this variety
Are their individual plants that stand out? Which ones and why?	relatively consistent	relatively consistent as well
What did you think of the flavor?	superior - saved seed	superior - saved seed
What did you think of the productivity?	excellent	excellent
What did you think of the marketability?	very marketable,	very marketable,
Any disease/ insect/ stress problems?	No, in the high tunnel there were no problems AT ALL	No, in the high tunnel there were no problems AT ALL
Strong points	Strong plant, appearance of fruit was superior with lobing and subtle striping.	very large leaves, intoxicating color
Major flaws	No flaws	No flaws
Are you interested in growing the family again and making selections?	Yes, I saved seed in 2019 and would be happy to again.	Yes, I saved seed in 2019 and would be happy to again.
General Notes or Comments		

Table 4. Luna Circle Summer 2020 evaluation			
Family	CSDE-F6	O4JB-F6	JBDE-F5
What do you think of the family as a whole	good	ok - didn't seem to have a lot of the JBT traits	I liked this one a lot. Some of the best traits of the JBT without some of the flaws like green shoulders.
Are there stand-out individuals? Which ones and why?			
What do you think of the flavor?	ok	ok	flavor is excellent
What do you think of the productivity	good	good	very productive and marketable

Table 5. Voss Organics Summer 2020 evaluation

Family	O4DE-F5	A6JB-F5	O4JB-F5	O4A6-F4	JBDE-F5
What do you think of the family as a whole	Liked the plant size	Liked A6 family best for color, flavor, productivity, and size. clear winner	Most blossom end rot. Not preferred	Liked A6 family best for color, flavor and size	Liked the plant size
Are there stand-out individuals? Which ones and why?		Best: striking color and shape		Best: manageble plant habit, great flavor, large size, striking color	
What do you think of the flavor?	good	Best	good	Best	good
What do you think of the productivity	strong	productive in number and size	tendency toward end rot reduced marketable fruit	strong	Good

Table 6. Voss Organics Summer 2020 evaluation					
Family	O4DE-F5	A6JB-F5	O4JB-F5	O4A6-F4	JBDE-F5
Would you grow this variety again?	yes	YES!	no	YES	yes
Germination? 1=very low, 5=very high	5	5	5	5	5
Vigor? 1=poor, 5=very high	5	5	5	5	5
Any disease/ insect/ stress problems? 1=very susceptible, 5=very resistant	4	4	2	4	4
What did you think of the productivity? 1=poor, 5=excellent yield	3	3	3	4	3
How marketable / useable is it? 1=not marketable, 5=very marketable	4	5	blossom end rot decreased usability 2	5	4
Appearance? 1=poor, 5=excellent	5	5	5	5	5
Flavor? 1=very poor flavor, 5=excellent flavor	4	5	4	5	5
Overall rating? 1=poor, 5=excellent	4	5	2	5	4
General Notes best/worst attribute	Likes the semi determinate size	Love the color here	blossom end rot susceptibility		

Table 7. Riverbend Farm Summer 2020 evaluation

1: CSDE-F5; 2:O4JB-F5; 3:JBDE-F5; 4: A6JB-F5; 5: GGO4-F4; O4A6-F4

Name: RIVERBEND FARM

Crop: Tomato breeding lines

GREG

Family	What do you think of the family as a whole	Are there stand-out individuals? Which ones and why?	What do you think of the flavor?	What do you think of the productivity
1 *Write here the name of the lines that you received*	COMPETITIVE WITH WEEDS, HEALTHY	NO	GOOD ACID/SWEET BALANCE, TOUGH SKIN	LOTS OF MEDIUM SIZED TOMATOES * LATE
2	MAYBE NOT QUITE AS BIG AS #1, STILL GOOD SIZE	NO	GOOD SWEET FLAVOR HEIRLOOM LOOK	LOTS OF BIG BEEFSTEAK SIZED TOMATOES. SOME PLANTS DIED OUT EARLY
3	FEWER TOMATOES MID SIZE, PLANTS LOOK GREAT	NO	VERY TASTY, SWEET FLAVOR, TOUGH SKIN	OKAY, NOT A LOT OF RIPE TOMATOES
4	FEW BIG TOMATOES -TYPICAL HEIRLOOM	NO	SWEET, GOOD FLAVOR	PLANTS LOOK GOOD TEND TO ROT WHEN RIPE. NOT A LOT OF TOMATOES
5	SMALL PLANTS, LOTS OF SMALL FRUIT	NO	UNINSPIRED, SWEET FLAVOR	ORANGISH COLOR WITH STRIPES
6	BIGGER PLANTS MIDSIZED FRUIT	NO	SO-SO SWEET FLAVOR	MAYBE SLOWEST TO RIPEN

* ALL TOMATOES WERE SLOW TO RIPEN THIS YEAR

#1 HAD BEST FLAVOR

#2 BEST VARIETY

SEE FAMILY NOTES ON BACK

Table 8. Riverbend Farm Summer 2020 evaluation

Name: Riverbend Farm

Crop: Tomato breeding lines — LOGAN

Family	What do you think of the family as a whole	Are there stand-out individuals? Which ones and why?	What do you think of the flavor?	What do you think of the productivity	
1	Seems good, few blemishes, varying ripenesses overall, some rotting	Mostly uniform in size, productivity	sweet, low acid	Very high	Favorite ✓
2	Plants appear to be drying up, yellowing leaves	Towards beginning of row, yields seem to be higher	Sweet, low acid, "smooth"	Moderate-high	2nd favorite
3	Plants are smaller, thinner branches, leaves still green	seem to hold up well without becoming soft	Moderate acid, "earthy" taste, slightly sour — not my favorite!	Moderate	
4	seem prone to rot, plants still appear healthy		Mild, moderate acid, slightly tangy	Moderate-Low	
5	Prone to rot Plants are yellowed, speckled, small fruit		Moderate acid, Mild sweetness Mild tang	Very high	
6	Very few blemishes, rot, plants look healthy		Low acid, low sweetness "bland"	Moderate - low	

Write here the name of the lines that you received

1: CSDE-F5; 2:O4JB-F5; 3:JBDE-F5; 4: A6JB-F5; 5: GGO4-F4; O4A6-F4

Table 9. Riverbend Farm Summer 2020 evaluation

Name: Riverbend Farm

Crop: Tomato breeding lines

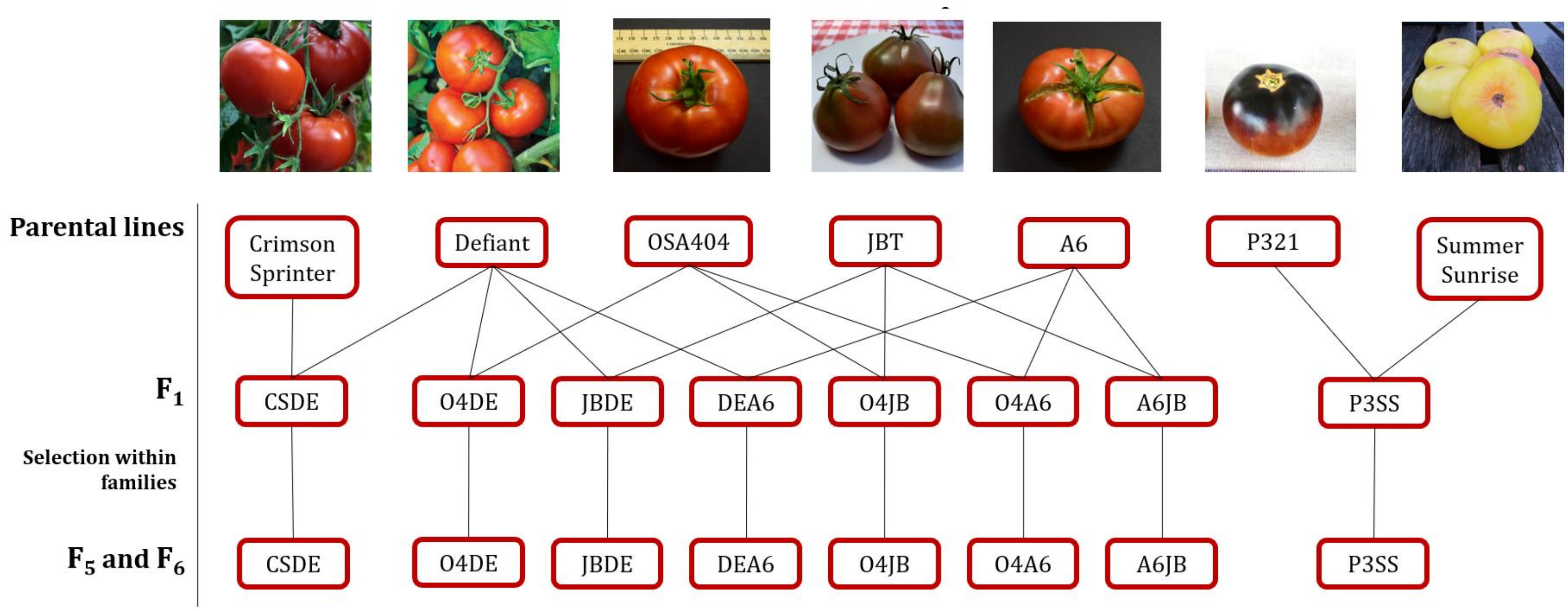
IAN

Family	What do you think of the family as a whole	Are there stand-out individuals? Which ones and why?	What do you think of the flavor?	What do you think of the productivity
1	HELD UP W/ WEEDS/ROUND RIPE AROUND 15% SMALL SIZE	ALL SEEM ROUGHLY EQUAL	VERY MEATY, GOOD AMOUNT OF FLAVOR SWEET-SLIGHT ACID	SEEM TO YIELD A LOT, RIPENESS DEPENDANT ON WEATHER
2	PRODUCTIVE LARGE SIZE ODD SHAPES	THEY ALL GENERATED ABOUT THE SAME, SOME SEEM TO HAVE MORE RIPE THAN OTHERS	SWEET, MORE SEEDS WALLS ARE TOUGHER	GOOD PRODUCTIVITY -DECENT CONSISTENCY IN SIZE
3	SMALLER SIZE ROUND LESS RIPE THAN OTHERS	SOME GENERATED LARGER FRUIT	SWEET, SLIGHT SOUR, VERY RIPE THICK SKIN	LESS PRODUCTIVE THAN (1/2)
4	VARIOUS SIZES - MEDIUM-LARGE SEEM BOTH OVER AND UNDER RIPE HEIRLOOM	~~[illegible]~~ THE RIPE ONES LOOK GREAT	GREAT SWEET FLAVOR	NOT MUCH FOR GOOD RIPE ONES NOT MUCH YIELD
5	SMALL AND ROUND EVENLY RIPENED	ALL PLANTS SEEM TO BE AT THE SAME PACE	A LITTLE BLAND SOFT SKIN THICK/DENSE WALLS	GOOD PRODUCERS SEEMS TO HAVE GOOD YIELD
6	NOT MUCH RIPE	NOTHING SIGNIFICANT	GOOD FLAVOR, SWEET GOOD TEXTURE	NOT GREAT

Write here the name of the lines that you received

1: CSDE-F5; 2:O4JB-F5; 3:JBDE-F5; 4: A6JB-F5; 5: GGO4-F4; O4A6-F4

Appendix F. Crossing scheme of the tomato breeding project developed in the University of Wisconsin-Madison



Appendix G. Tomato tasting evaluation form, paper version.

Date ___________________
CROP ___________________
Taster ___________________

Instructions
Use a 1-5 score for each category below

1=low 2=moderately low 3=moderate 4=moderately high 5=high

1) For appearance, rate how appealing each variety looks: what is the likelihood you would purchase this variety at a market?
2) For each flavor category, note the strength of that particular flavor component.
3) For overall category, give your global appreciation (1-5) of the flavor of each variety, excluding the appearance category.
4) For unusual flavors, note any particularly strong flavors or anything that tastes “off”.

Variety Code:	**NVM**	**PSJ**	**IDP**	**JXE**	**HKJ**	**XQM**
Appearance						
Texture						
Sweetness						
Acidity						
Bitterness						
Umami						
Intensity						
Overall flavor						
Unusual flavors						

Comments: ____
____
____

Appendix H. Photos of each of the tomato breeding lines evaluated in this project, grouped by family.

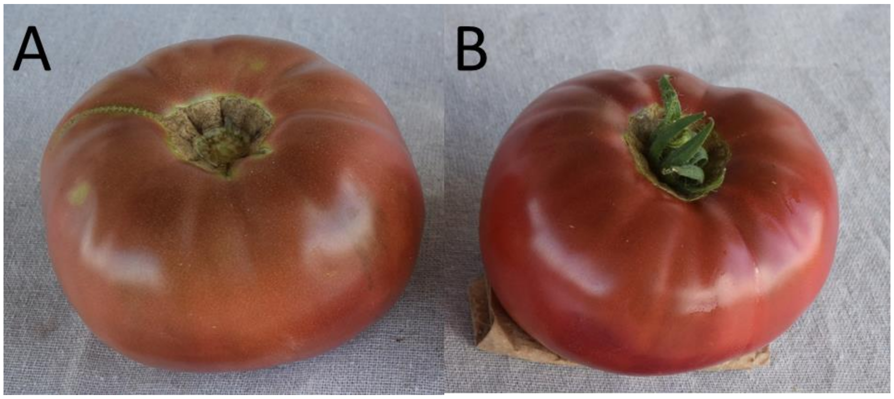


Figure 13. Photos of the A6 by Japanese Black Trifele family. A: A6JB-F5-34. B: A6JB-F5-35.

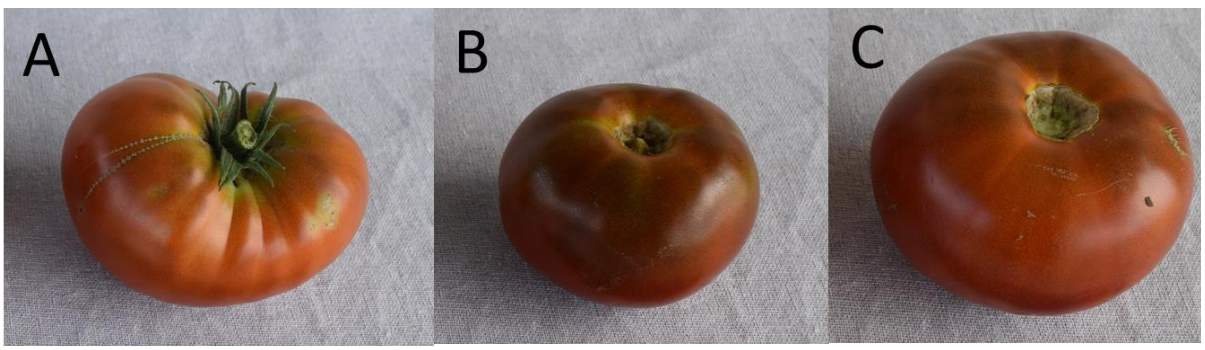


Figure 14. Photos of the Japanese Black Trifele by Defiant family. A: JBDE-F5-28. B: JBDE-F5-31. C: JBDE-F5-32.

Figure 15. Photos of the OSA404 by Japanese Black Trifele family. A: O4JB-F5-115. B: O4JB-F5-116. C: O4JB-F5-6.

Figure 16. Photos of the Crimson Sprinter by Defiant family. A: CSDE-F6-46. B: CSDE-F6-47.

Figure 17. Photos of the OSA404 by Defiant family. A: O4DE-F5-43. B: O4DE-F5-44.


Figure 18. Photo of the P3-2-1 by Summer sunrise cross, P3SS-F4-61.


Figure 19. Photo of the OSA404 by A6 cross, O4A6-F4-MV1-109