Breeding Disease-Resistant Heirloom-Quality Tomatoes

Carol Deppe

Project Summary:

During the 2020 growing season, I was successful in developing nine lots of breeding materials designed to allow farmers to breed disease-resistant heirloom-quality tomatoes of several colors and types—the heirloom tomatoes of tomorrow.

Introduction:

Right now we organic growers have a choice of growing heirloom and other open-pollinated tomato varieties that taste great but carry little disease resistance or commercial tomatoes with disease resistance but inferior flavor. Full-flavored heirloom tomatoes are major sellers for organic farmers in local markets. But many organic farmers can't grow heirloom tomatoes because of disease vulnerability.

Worse yet, there are new lines of late blight spreading throughout the country that make it increasingly difficult to grow heirloom tomatoes outdoors in some regions. I predict late blight will make it completely impossible to grow all heirloom tomatoes outdoors in most regions within the next ten years. None of the heirlooms have more than token resistance to the new lines of late blight. (It is already impossible to grow heirloom tomatoes outdoors in much of Europe.)

There are many university and large seed company breeders working on late-blight-resistant tomatoes, and late-blight-resistant tomatoes are a major focus of the Northern Organic Vegetable Improvement Collaborative (NOVIC). However, they are breeding commercial style tomatoes that include genes for uniform ripening and long shelf-life; these genes also cause grossly inferior flavor. I've tasted the available late blight resistant tomatoes as well as many as yet unreleased. (I'm a participant in NOVIC, so annually taste-test unreleased varieties from university and seed company breeders.) So far, none of these meet the flavor standard expected of organically grown tomatoes bought in local markets.

A second problem is the university and corporate late-blight-resistant tomatoes are generally being released as proprietary hybrids. I believe organic farmers' interests are best served by *non-proprietary* open-pollinated varieties they can save seed of as well as *non-proprietary* inbred lines they can use to make their own hybrids, if desired. I will release all my material as OSSI-Pledged breeding material. This means I will transfer it to those interested accompanied by the Open Source Seed Initiative Pledge: ***You have the freedom to use these OSSI-Pledged seeds in any way you choose. In return, you Pledge not to restrict others' use of these seeds or their derivatives by patents or other means, and to include this Pledge with any transfer of these seeds or their derivatives.***

I propose, with the help of organic farmers throughout the land, to create an entire new generation of heirloom-quality tomato varieties that have serious resistance to modern diseases, focusing primarily on resistance to late blight, early blight, and Verticillium and Fusarium wilts and secondarily on other diseases. The goal of this project is to produce the breeding materials from which these varieties may be selected.

Objectives:

Prior work: I started by crossing each of ten beloved heirloom tomato varieties to a commercial hybrid that carries two major genes for late blight resistance (*Ph-2* and *Ph-3*) and a good package of other disease resistances. The heirlooms included red, pink, black, orange, and paste-tomato varieties. The second season I developed second generation (F_2) seed from this material.

The objective for the grant year (2020) was to develop a number of lots of breeding materials from which heirloom quality tomato varieties with resistance to late blight and other critical diseases could subsequently be selected. I had two major approaches in mind initially, only one of which was described in the proposal because of space limitations. The first was to grow 200 F3 plants (the third generation of seed saving after the initial cross), evaluate some of the disease resistance genes each carried using marker assisted selection, and grow and save seed from the plants that carried the most promising package of genes for disease resistance.

The second approach follows my general plant breeding principle of always using multiple approaches when possible. Plant breeding is an unpredictable process. The hybrid I used to cross to the heirloom varieties (Iron Lady F1), is full of useful disease resistance genes. But the hybrid itself doesn't taste good. Nor was it as early as I would like. And the size is pretty small too. (Other options were even worse.) What if none of the 200 plants for which I did marker assisted selection was the slightest bit appealing? If there are many genes that the hybrid is lacking that are required to do well under organic growing conditions and/or in my region, or to have the superb flavor expected of heirloom tomatoes, this is quite possible. So the second approach was to grow out about two thousand tomato plants (fairly crowded in the field), and just save seed from the plants that produced the earlier bigger more appealing tomatoes without knowing anything about the disease resistant genes they carried. The statistics of the situation would give a high probability that any one of the plants would contain multiple useful disease resistance genes. Saving the seed from each of these best plants would be selecting for performance under organic and regional conditions first, then for disease resistance subsequently.

The "measurable objectives" are the seeds themselves, ready to be used for the next steps in breeding the heirloom tomatoes of tomorrow.

Materials and Methods:

All the tomatoes were grown and evaluated on Harcombe Farm, which is Oregon Tilth certified organic.
Organic certification of Harcombe Farm:

Stellar Certification Services: NOP Effective Date—August 26, 2016 to March 14, 2019.
Oregon Tilth: NOP Effective Date—March 14, 2019 to present.
(When Stellar went out of business; we switched to Oregon Tilth.)
(Land has been organically managed since Paul Harcombe bought it more than ten years ago. Just not certified until there were enough crops to justify the cost.)

Marker Assisted Selection analysis was performed by AgBioTec. (See reference section.)

See information in my book *The Tao of Vegetable Gardening* as well as information at the Tomato Genetics Resource Center for information on how to grow tomatoes, do tomato crosses, etc. (See References.) *The Tao of Vegetable Gardening* has a section that gives information on how many of the major gens that effect tomato growth patterns and fruit color and shape are inherited. *Breed Your Own Vegetable Varieties* goes into the theory a bit more.

The main tomato materials used were various lots of F3 (third generation) seeds I developed by crossing a hybrid late-blight resistant commercial tomato variety to a number of heirloom tomato varieties especially noted for their flavor, earliness, and ability to perform well in organic conditions. The hybrid is Iron Lady F1, sold by Johnny's Selected Seeds. Cornell vegetable breeder Martha A. Mutschler bred one of the inbred lines used to create the hybrid Iron Lady, and generously shared information about its genetics with me. The information about the genetics of Iron Lady F1 should be cited as a personal communication from Martha A. Mutschler to Carol Deppe, 2020.

Iron Lady F1 is homozygous (pure-breeding) for the two major late blight resistance genes *Ph-2* and *Ph-3*. It is also homozygous for resistances to Verticilium, Fusarium 1, and Fusarium 2. It is heterozygous for three different QTLs (quantitative trail loci) for Early Blight resistance. ('Heterozygous' means the plant has one copy of the gene rather than two so is not pure-breeding for that gene/locus.) Iron Lady is also heterozygous for *SLS-1* and *SLS-2*, two different loci associated with Septoria Leaf Spot resistance (essential for organic production in the Northeast). These disease resistance genes all act quantitatively. That is, two doses of the gene give you more resistance than one dose which gives you more resistance than none.

Dr. Mutschler says that there is a recessive gene associated with extreme sensitivity linked closely to *SLS-1*. This water-stress-sensitivity gene is very detrimental when homozygous. So she recommends when we select for open pollinated varieties, that we eliminate the gene for water stress sensitivity, even though that will also eliminate one of the genes for Septorial Leaf Spot resistance. She says one gene for Septorial Leaf Spot will probably be enough once it is homozygous.

Iron Lady F1 is a determinate variety. (It is homozygous for the *cut* gene, so is *ct ct*.)

Iron Lady F1 is also homozygous for *uniform* (shoulders), *uu*. This recessive gene gives uniform instead of differently colored shoulders. This gene is in nearly all commercial tomato varieties for the beautiful even color of the fruit. Unfortunately, this gene lowers the sugar, dry matter content, and aromatic components in the fruit and ruins the flavor. I suggest we freelance breeders select against any plants that produce uniform-shoulder fruits and teach our tomato customers that uniform shoulders means poor flavor. Iron Lady F1 probably also contains genes intended to extend its shelf life; these genes, too, usually come at the expense of good flavor.

I started my project by crossing Iron Lady F1 to a number of well-known heirlooms. Here are the heirloom tomato varieties involved as one of the parent. I particularly focused on the black/brown/purple tomatoes for their great flavor—Black Krim, Cherokee Purple, and Paul Robeson. One variety was the pink tomato Pruden's Purple, which has a flavor very similar to Brandywine but which is earlier. Also included was a good-flavored orange tomato, Orange King. (Orange King is determinate.) Also included are two paste tomatoes, Grandma Mary's and Gilbertie. Both these had somewhat elongated fruits in the F1, so you can actually identify the exact genotypes with respect to these shape genes. These tomatoes are all large, but early for their size, and all do well under organic management. I also included two smaller (two inch) tomatoes that are very early but still excellent flavor—Stupice, which is indeterminate, and Glacier, which is determinate.

Varieties Used in Crosses

(Disease resistant commercial hybrid Iron Lady F1 was crossed with each of the non-disease-resistant heirloom tomatoes subsequently listed.)

		size/color/shape/type				Selected F4 seed obtained
Iron Lady F1	det	small	red	stan	mid	+
Black Krim	ind	large	black	stan	mid	+
Cherokee Purple	ind	large	black	stan	mid	+
Paul Robeson	ind	large	black	stan	mid	+
Pruden's Purple	ind	large	pink	stan	mid	+
Orange King	det	large	orange	stan	mid	+
Grandma Mary's	ind	large	red	paste	mid	+
Gilbertie	ind	large	red	paste	mid	+
Stupice	ind	small	red	stan	early	-
Glacier	det	small	red	stan	early	+

abbreviations: det= determinate; ind=indeterminate; stan=round slicing tomato; paste=elongated paste type tomato; mid=mid-season; early=early season

Project Results:

The overwhelming majority of the F3 plants had late small tomatoes that were still green during the ordinary harvest season. That is, only a very small proportion of the plants developed from the cross with the commercial disease resistant hybrid could perform at all acceptable under organic conditions in my region. None of the genetically evaluated plants for which I had ordered genetic marker analysis were early, big, or productive enough to be good candidates from which to collect seeds for breeding lines. (It would have been prohibitively expensive to order marker analysis on all the plants.) Fortunately, I had anticipated this possibility and had a

Plan B—that is, to just harvest seed from the earliest F3 plants with the biggest tomatoes from each of the original crosses. So I saved seed from the best dozen or so of the two thousand plants that were not genetically analyzed, but that were early enough and big enough to be appealing to use as breeding material. Note that had I pursued just my Plan A, the entire project would have failed.

Other problems were caused by the fact that the timing of payment for the grant didn't match tomato growing needs very well. By the time I received the funds that allowed me to subcontract for growing 2,000 transplants, it was late to be planting tomatoes. In addition, fires in the regions that caused the air to be unbreathable outdoors for two weeks during the harvesting season didn't help. (But some friends of mine had to evacuate their farms and worry about their houses burning down. So merely being unable to do any field work for a couple of weeks was getting off easy.) However, I ended up harvesting even the best of the tomatoes as green tomatoes that had to be ripened indoors. Nevertheless I did end up with good though small lots of seed from each of the crosses I started with. In addition, it was impossible to get photographs, as the tomatoes were still green and unphotogenic when harvested, and the period when photography could have been done, the air was too toxic with smoke and no one could be outside working in field at that point.

The marker assisted selection presented problems too. One of the markers for which AgBiotech tested clearly didn't work at all. Fortunately I had enough controls to identify the problems. (It's important to have control samples submitted that are from varieties known to include and not include the given marker.) And some of the genes for persistent chlorophyll that give the black/brown color and major flavor of varieties such as Black Krim, Cherokee Purple, and Paul Robeson were actually misidentified in the literature. I had, fortunately, taken a cheerfully cynical approach and tested all offspring involving those varieties against two different markers instead of just the one they were supposed to contain. I plan to describe the use of marker assisted selection in this project in a paper for those who participate in further breeding with the new breeding materials.

A major take home lesson is that the F3 (third generation) of the cross of the highly disease resistant hybrid to the heirlooms produces such a large proportion of inferior plants that marker assisted selection to help identify disease resistant genes so that only those with the best disease resistance genetics would require thousands of plants analysed and way more funding than can be done in this sort of project. It's far more workable for the first four generations to simply select in the field the plants that are early enough, and have big enough enough fruits, and have the desired color or shape. (The color genes determine the major flavor class of the tomatoes.) Then evaluate the disease resistance genetics using marker assisted selection, if at all, only at or after the fourth generation. There is, by the way, not too much value in selecting for specific flavors at these early stages in a project such as this one, as the plants will not ordinarily be stable for flavor at this stage. That was no problem in this case, since other problems with that year meant I was harvesting green tomatoes and letting them ripen indoors instead of under standard conditions.

To summarize my advice to others who want to repeat my approach using different heirloom parents: Make the cross. Then just save seed from what are the most interesting plants under organic conditions until at least the fourth generation. And marker assisted selection can be skipped completely in varieties bred for use on just one farm or delayed until the fourth generation or beyond for varieties or inbred lines to be used more widely.

The object of this project was to develop several lots of breeding material which can be used to develop new tomato varieties that combine the spectacular flavor of the best heirloom tomatoes with the disease resistances of modern hybrid varieties. I was successful in developing nine selected F4 lines of breeding materials designed to breed disease resistant versions of tomato varieties of the following classes: red, black, pink, orange, paste, and very early. Both determinate and indeterminate plant forms are included.

The project's results are the seeds, not information. Because of problems of various sorts, however, I ended up with only very small amounts of each of the nine new lines of breeding material. I will need to grow these lines another year or get volunteers to help do so and sorted out into 18 or more lines so that those who are interested in particular types of tomatoes will have lines that are mostly the desired type and there will be enough tomato seed to distribute.

Conclusions and Discussion:

A plant breeding project fits poorly into the "Conclusions and Discussion" format. The object is to develop the seed, not to evaluate some particular method.

I was successful in developing nine lines of breeding materials from which it should be possible for organic farmers throughout the nation develop disease-resistant heirloom-quality tomatoes—the heirloom tomatoes of tomorrow, as indicated in the table. However, I ended up with only tiny lots of seed, and will need to do a further growout of each lot before I have enough seed to distribute. (Usually when you distribute seed many people do nothing with it; so you need a lot of seed in order to have enough to distribute useful amounts to each would-be participant as well as have enough held as backup.)

Outreach:

There's no outreach as yet. I need to expand the supply of seed before I undertake seducing farmers into wanting to work with and develop it further. I hope to do some of the additional growouts needed during the 2022 season and recruit certain trusted friends to do some of the seed increases as well.

Leveraged Resources:

I have not acquired additional funding myself. However, Bill Braun, of the Freed Seed Foundation, has already acquired funding to work with the breeding materials I've developed to select varieties appropriate for the Northeast.

References:

The Tao of Vegetable Gardening (Carol Deppe) will likely be the major resource for farmers and gardeners to use to develop the breeding material I provide with the help of this grant into finished varieties. It has extensive chapters on growing and breeding tomatoes. The genetics is "soft."

Breed Your Own Vegetable Varieties: The Gardener's and Farmer's Guide to Plant Breeding and Seed Saving (Carol Deppe) goes into more technical detail on plant breeding than *Tao*.

Tomato Genetics Resource Center http://tgrc.ucdavis.edu. Includes a list of known tomato genes and their effects. Excellent information and photos on how to do tomato crosses.

Martha A. Mutschler and Margaret T. McGrath. "Creating New York Adapted Tomatoes with Resistance to Multiple Fungal and Bacterial Diseases," 2019. https://cdn.sare.org/wp-content/uploads/20191202200337/Cornell-Disease-Resistant-Tomatoes-2019-full.pdf This download gives a summary of the late blight resistant varieties developed using Cornell inbred lines. (All hybrids.)

The information about the genetics of Iron Lady F1 given in the Materials and Methods section should be cited as a personal communication from Martha A. Mutschler to Carol Deppe, 2020.

AgBioTech.net. Company I used for molecular analysis of DNA markers in transplants before planting. This page shows the genes for which they have molecular markers and for which they can analyze. https://agbiotech.net/dna-markers/

Photos and other addenda:

The season was too hectic to do photography, the late-planted crowded Plan B plants were not photogenic, and the air was orange-brown from the regional fires and was unbreathable just when the photography would have needed to be done.

With a little luck, my 2022 season's plants will be photogenic and the air will be breathable. I'm expecting to develop articles and instructions for participants including a podcast, an article for a major gardening magazine I've worked with before, and instruction sheets for those who plan to use the breeding material.

Financial Accounting:

PI Name: Carol Deppe
Project Name: Breeding Disease-Resistant Heirloom-Quality Tomatoes

Item	EXPENSES Budgeted Amount	Actual Spent
Personnel Costs/Fringe	$2,566	$0,000.00
Farmer Stipend	1,850	5,302.00
Researcher/PI Stipend	8,320	6,957.40
Ag Biotech DNA analysis	5,000	2,490.60
Cost of seeding and growing 2,000 transplants.	500	650.00
TOTAL EXPENSES:	18,236	15.400.00

FIRST PAYMENT AMOUNT RECEIVED: $15,400.00
TOTAL OWED FOR FINAL PAYMENT: 1,540.00

Justication for Variances

1. We initially thought that my farmer/collaborator would do some of the labor and his field hand would do more. As it turned out, the field hand was unavailable, and my farmer/collaborator did all the work we initially thought would be his plus that of the field hand too.
2. The amount of DNA marker analysis had to be truncated to accommodate the budget of $15,400 vs the originally proposed $18,236.
3. The project took the me, the Researcher/PI, more time than she thought, and more than is reflected by that line item. Which is par for the course.
4. Otherwise the advance estimates of what things would cost was pretty close to the original estimates.