Science Societies

Strawberry breeders tap heirloom and wild genotypes to fight fungal pathogens

By DJ McCauley

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Backdrop image: UCD Moxie is a recent University of California cultivar release that is resistant to Verticillium wilt. Photo by H. Amezcua, College of Agricultural and Environmental Sciences, University of California–Davis.

- The fungal pathogen *Verticillium dahliae* causes Verticillium wilt in a variety of horticultural crops, including cultivated strawberry.
- University of California–Davis researchers recently published a screening study in *The Plant Genome* examining Verticillium wilt resistance in nearly 1,000 strawberry genotypes.
- Their findings are an important step in the fight to increase strawberry resistance to fungal pathogens.

I will admit: the greatest threat to the patch of strawberries in my backyard is a 13-year-old Pekingese dog with a sweet tooth. Far more threatening on a global scale is the fungal pathogen *Verticillium dahliae.*

The fungus is a soilborne pest that attacks more than 400 plant species, from cotton to potatoes and maple trees to strawberries. It causes a disease called Verticillium wilt, in which infected plants show symptoms like wilting leaves, stunted growth, and eventual necrosis. The pest causes persistent yield losses without proper management.

Since the 1960s, farmers managed the spread of the pathogen in horticultural crops using a highly effective chemical fumigant: methyl bromide (MeBr). But MeBr is a potent ozone-depleting greenhouse gas. Even in agricultural use, where producers tarp soils during fumigation, the USEPA estimates that 50 to 95% of MeBr eventually enters the atmosphere (**https://bit.ly/2KwDOyj**).

In 2005, the USEPA banned the use of the fumigant, and it was phased out by 2016 (**https://bit.ly/3mleDvT**). There are both chemical and non-chemical alternatives, but none manage fungal populations of *Verticillium dahliae* as effectively as MeBr. Now, growers are seeing a resurgence of diseases that haven't been a problem for years in many crops, including strawberries.

A team at the University of California–Davis (UC-Davis) is looking for new ways to protect these juicy berries from soilborne diseases. In a recent article published in *The Plant Genome*, the team at the Strawberry Breeding and Research Lab screened nearly 1,000 elite, heirloom, and wild varieties for Verticillium wilt resistance (**http://doi.org/10.1002/tpg2.20054**).

Crown of a Verticillium-infected strawberry. Unlike some other soilborne pathogens such as Fusarium wilt (Fusarium oxysporum f. sp. fragariae) or charcoal rot (Macrophomina phaseolina), Verticillium-infected crowns do not show internal discoloration or browning. Photo by S. Koike, TriCal Diagnostics.

A Fungal Menace

California's "Strawberry Belt" stretches from the picturesque valleys of Santa Cruz, just south of San Francisco, all the way down to Ventura, just north of Los Angeles. In 2018, California growers produced 88% of all strawberries in the United States, harvesting more than 1.8 billion pounds of fruit (**https://bit.ly/34eXTjH**).

But the threat of Verticillium wilt looms. It's not a new disease—growers first spotted it in California soils in the early 1900s—but breeding for resistance became far less critical with the widespread adoption of MeBr fumigation.

Verticillium dahliae lives in the soil in the form of microsclerotia. These tiny capsules are hardened masses of hyphal threads that lie dormant for up to 14 years. When the roots of a suitable plant host bump into one of these capsules, the fungus attacks, colonizes, and multiplies in plant tissue. When the plant dies, the newly formed microsclerotia return to the soil to lie in wait for another host to attack (**https://bit.ly/2Kaj7sn**).

In this manner, even a small amount of unmanaged *Verticillium* can cause big problems for a farmer over time, especially if that farmer primarily grows horticultural crops that all serve as pathogen hosts. Plus, the rise of organic farming (coupled with MeBr bans) means that more producers need resistant plants.

“Finding the source of resistance is really the first line of defense,” Dominique Pincot says. “If you have a resistant cultivar, you can always pair that with management practices on farm.”

Over the course of two seasons, Pincot undertook a massive screening study on the hunt for sources of resistance as part of her doctoral dissertation with the guidance of Steven Knapp, a strawberry breeder and Director of the UC-Davis Strawberry Breeding Program.

Wild, Heirloom, and Elite Cultivars

“We started by casting a really wide net,” Knapp says. “We created a genome-wide association panel with about 1,000 genotypes to test against the major soilborne diseases in California. These were our building blocks.” Despite its presence year-round on grocery store shelves, commercial strawberry was “behind the curve,” as Knapp put it.

“What’s really amazing to me is how little progress has been made with these disease problems in this plant,” Knapp says.

The team collected cultivars from far and wide. They used wild varieties from their natural ranges in North and South America, elite germplasm from the UC–Davis collection, and heirloom varieties from breeding programs across the world.

Under the direction of Pincot, the team obtained and grew clones of 961 strawberry genotypes in the greenhouse. Clones, in strawberry plants, are daughter plants that grow on the runners of mother plants (**https://bit.ly/2Wwf3Fa**). They inoculated all of the clones with *Verticillium dahliae* and transferred them to fields in the Armstrong Plant Pathology complex. These specialized fields are testing grounds for pathology studies, with strict protocols to prevent disease spread.

“We’re so lucky to have those fields as a resource,” Pincot says. “I don’t think we’d ever be able to match the scale of that project without those fields.”

A low-elevation strawberry nursery in Winters, CA utilized by the University of California–Davis (UC-Davis) strawberry-breeding program. Strict phytosanitation and disease-free production *environments are critical for breeding and safely preserving germplasm collections. Photo by S.J. Knapp, UC-Davis.*

Over the course of two growing seasons, Pincot and other members of the team rated the severity of Verticillium wilt symptoms on a scale from 1 to 9 with 1 being no symptoms whatsoever and 9 being total dieback.

In the first study year of 2017, the team also collected a new leaf off each plant, isolated its DNA, and then completed single-nucleotide polymorphism (SNP) marker genotyping. The goal was to document any variations in the genome that correspond with increased resistance to Verticillium wilt.

Despite the massive number of genotypes the team screened, there were very few that showed outstanding resistance. The team took the top 10 most resistant lines and crossed them after the 2017 season to get a head start on the breeding process, which can take years to produce varieties with both resistance and all the traits that make strawberries commercially valuable.

“We’ve had really great success with those lines so far,” Knapp says. “We’ve selected parents from that study to drive four more years of breeding work.”

The team did find a couple interesting correlations. Accessions developed before 1960 tended to show higher levels of resistance as did those from breeding programs at higher latitudes.

But what of the major resistance gene? Is there a “silver bullet” marker that confers resistance?

"Unfortunately, we didn't see a major resistance gene pop out," Knapp says. "It's a funny thing: for Fusarium wilt, it popped up right away. But we've done follow-up studies, continuing our search for major resistance genes."

The Octoploid Strawberry Genome

The second major obstacle the team faced was the lack of a reference genome for cultivated strawberry. Without a reference genome and the genomic resources that come along with it, integrating sources of resistance into elite strawberry genotypes is much more time consuming and difficult.

The reason nobody had created this reference genome is because of the octoploid genome of the hybrid cultivated strawberry.

Three University of California (UC)–Davis accessions assessed for Verticillium wilt resistance. Tahoe (left) and Wiltguard (right) are Verticillium wilt resistant heirloom cultivars developed by UC in 1937 and 1952, respectively. A susceptible UC genotype from 2012 is shown in the middle for comparison. Photo by D.D.A.

Pincot, UC-Davis.

Wild strawberries are diploid—one set of chromosomes from mom, one set from dad. But their berries are tiny, seedy, and sour. Since its introduction from the woods into French home gardens sometime in the 1300s, breeders have made magnificent progress, turning the seedy little berry into big, juicy, shippable strawberries on plants with crowns that are massive and bountiful compared with their wild counterparts (**https://bit.ly/2LA1JgS**).

“The problem is, when those horticultural traits are your priorities, you might lose a little ground on flavor, aroma, and disease resistance,” says Kevin Folta, a strawberry breeder and professor in the University of Florida Horticultural Sciences Department. Folta was not an author of this study.

He was, however, part of the team that sequenced the very first diploid strawberry genome back in 2010 (**https://go.nature.com/2Kq8FN6**).

“After the wild strawberry, that was enough sequencing for me,” Folta says. “Sequencing the octoploid genome would be like trying to untangle a single spaghetti noodle from a whole plate of spaghetti.”

The cultivated strawberry’s octoploid genome features eight sets of chromosomes—two sets of seven chromosomes from each of four ancestral parents. In spite of this complexity, it still shows a Mendelian-type inheritance pattern. That is, the four ancestral subgenomes behave like diploids with limited crossing over. This means that it *is* possible for breeders to integrate novel genes into elite cultivars.

Undaunted by the task of untangling a single noodle from the spaghetti plate, the UC-Davis team partnered with Patrick Edger and other researchers from Michigan State

University. They published the full-blown octoploid strawberry reference genome—along with information about the origin and evolution of the cultivated species—in *Nature Genetics* in 2019 (**https://go.nature.com/2WiaQVI**).

Knapp insists that the octoploid genome isn't so tough to handle.

"It's like having quadruplets, but they're not identical," Knapp says. "You look at them and you can see, okay, that's Sally, that's Sarah. Distinguishing among them allows you to navigate the genome, and that's a really powerful thing."

This reference genome gives breeders a huge leg up when integrating resistance markers into cultivated strawberry. With the genome in hand, the team can now track those markers within the complex octoploid genome, speeding up the breeding process using high-throughput methods.

"Before, we might take 10 parents and cross them, and have 1,000 plants to screen for resistance, and maybe 90% of them would die," Pincot says. "Now, we can screen for resistance beforehand and really narrow it down. So instead, maybe 90% survive and you have so much more material to select for other traits. It biases your seedlings for resistance from the get-go."

The work completed by the UC-Davis team demonstrates the power of finding novel sources of resistance for plants that face biotic stresses like pathogens. As environmental concerns change management practices, and growers and consumers choose alternative production methods, it's important to have really strong genetic sources of resistance for the plants we love to eat.

"It's really great to recognize this study—here's federal money that's being used to identify genes that will enhance breeding and production of new varieties so that consumers can continue to get high quality berries with less environmental impact," Folta says.

Knapp, Pincot, and the rest of the UC-Davis Strawberry Breeding Program already have big plans to integrate and release new varieties that keep us stocked up with bright red berries.

It's a good thing, too, because I haven't found any sources of genetic resistance against Pekingese attacks. In the meantime, I'll pick up a pack of market strawberries and put up a picket fence.

Dig Deeper

Check out the original open access article, "Accuracy of Genomic Selection and Long-Term Genetic Gain for Resistance to Verticillium Wilt in Strawberry," published in *The Plant Genome* at **https://doi.org/10.1002/tpg2.20054**

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