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Genetic associations of sugarcane mosaic virus resistance and endosperm carbohydrate composition in sweet corn (*Zea mays.*)

by

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Explanation of research for a more general audience

My research at the University of Wisconsin-Madison (UW) has been a study of the genetics of diverse sweet corn inbreds, focused on two areas: first, endosperm carbohydrate composition; second, resistance to sugar cane mosaic virus (SCMV). The purpose of this chapter is to explain my research to a broad, non-scientific audience. To me, conducting research is a humble act of service for humanity, adding to our understanding of the truth. In order to fulfil my scientific duty, I need to communicate my research so that it can be understood and utilized by others.

Thanks to the Wisconsin Initiative for Science Literacy at UW-Madison for providing this platform, and for sponsoring and supporting the creation of this chapter.

Carbohydrates!

Background

The carbohydrates I studied were starches and sugars inside the corn kernel. In the kernel, most of the carbohydrates are stored in the endosperm, the part of the seed that serves as an energy storage compartment. If the kernel germinates and develops into a new corn plant, the starch in the endosperm can provide energy for the growing plant. Most of the corn we grow in the United States is called **field corn** and has kernels mostly composed of starch with little to no sugar. The starch in field corn kernels is used for animal feed, human food, and other industrial uses. **Sweet corn**, developed for fresh human

consumption as corn on the cob or the yellow kernels we see on our dinner plates, is higher

in sugar and lower in starch than field corn.

Figure 0.1 Fresh eating stage sweet corn. This sweet corn has a *shrunken2* mutation, though you can't see it with your eyes at this stage of development. You can, however, easily taste the higher sugar levels.

The biological difference between field corn and sweet corn boils down to the sugar

content of the kernels. In field corn, **sucrose** (table sugar) made by photosynthesis in the

leaves enters the kernel. Once it's in the endosperm of the kernel, the sucrose turns into

starch through a complex biochemical pathway with about two dozen steps. Each step is

controlled by a gene. If the gene responsible for a step doesn't work, the pathway halts and

sugar builds up with nowhere to go. If the pathway breaks down at different points, there

can be different kinds of sugars and ratios of sugars to starches in the kernel. One

mutation (a broken gene), *sugary1*, breaks the pathway late in the process. This mutation results in the creation of big carbohydrate orbs and higher levels of sucrose than in field corn. The orbs are soluble in water and give this type of corn a pleasant creamy texture. Another mutation is *shrunken2.* Most of the sweet corn available for sale today has *shrunken2*, which breaks the pathway really early in the process, resulting in LOTS of sucrose buildup. Theoretically, almost anywhere the pathway breaks could result in sweet corn. For the most part, though, corn breeders use the *shrunken2* and *sugary1* mutations because they work consistently to break the pathway and the breeders have plenty of tools and experience breeding with these mutations.

The experiment

We know plenty about the *shrunken2* and *sugary1* mutations and we know that, generally, *shrunken2* corn is sweeter than *sugary1* corn (counterintuitively, given their names)*. We also know that there are different amounts of sugar among varieties of *shrunken2* corn and among varieties of *sugary1* corn, but we don't know why.

To explore this question, we created a **diversity panel,** a collection of sweet corns from around the world, focusing on inbreds that are genetically diverse from different backgrounds.

Figure 0.2 Ears of different inbreds from the diversity panel at mature stage. Notice the differences in size, shape, and color

Inbreds are a kind of corn that is uniform between parent and offspring. The panel focuses on publicly available inbreds, meaning inbreds that are available for anyone to grow without any patents attached. We planted the diversity panel in two different places (at Cornell University and UW), in two different years (2014 and 2015). We harvested the sweet corn at the point in its growth called fresh eating stage, when the corn is ready to be eaten on-the-cob. If the corn is harvested after that stage, it loses its moisture and gets tough. We flash froze the corn immediately after harvest to preserve it at that stage.

Figure 0.3 Flash freezing corn that was just harvested at fresh eating stage. We use liquid nitrogen to freeze the ears and then remove the frozen kernels for testing. Freezing done by my colleague Carl Branch in this photo.

All in all, I ended up with about 4000 samples to analyze. For each of these samples, I measured seven different carbohydrates in the kernels. Measuring carbohydrates takes a great deal of time, effort, and complicated equations that I won't get into here. This data allowed me to look at the carbohydrate levels across all the inbreds in the panel. I looked at how much carbohydrate variation there was between mutant-type groups (i.e., the *shrunken2* group vs. the *sugary1* group), as well as variation within each group. Across the panel, inbreds ranged from about 10% to 40% of the kernel being sugars. The *shrunken2* inbreds' kernels were 32% sugar on average, ranging from 30% to 40%. The *sugary1* inbreds' kernels were 18% sugar on average, ranging between 10% and 30%. So, the *sugary1* inbreds had less sugar than the *shrunken2* types, but they also had a wider range of variability. Our *shrunken2* inbreds had more sugar than *sugary1,* which agrees with

previous research about these two mutants. And it makes sense that *sugary1* had more variability within its group because *sugary1* sweet corn has been bred for thousands of years, so there's much more genetic diversity among those inbreds. The *shrunken2* mutation was developed in the 1960's and hasn't had as much time to become as genetically diverse.

A Sugar Content by Endosperm Type

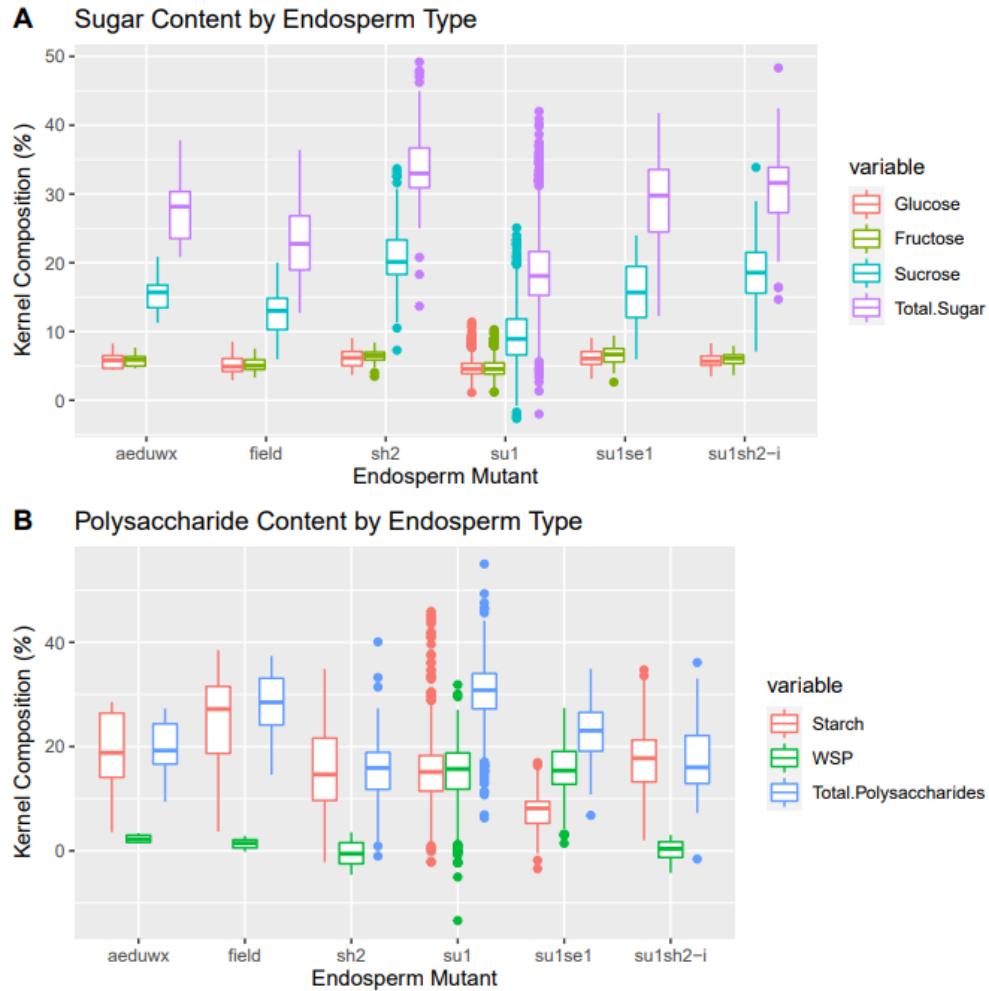**B** Polysaccharide Content by Endosperm Type

Figure 0.4 Carbohydrate levels of all the inbreds, grouped by their sweet corn mutant type. aeduwx, sh2, su1, su1se1, and su1sh2-i are all different types of sweet corn. WSP stands for water-soluble polysaccharides, those carbohydrate orbs.

After I measured the carbohydrates, then I could analyze the genetics of the corn. We genetically mapped every inbred. With the map I could run a **genome-wide association study** or GWAS. This technique compared the genetics of the various inbreds relative to their carbohydrate compositions. I did this one carbohydrate at a time, so let's use sucrose as an example. I grouped all inbreds with high sucrose separately from all

inbreds with low sucrose. The computer can look at the genetic map and determine where the genetic code looks different between the high and low sucrose groups. If the maps look consistently different at one location in the high sucrose group from the same location in the low sucrose group, it's a **significant locus** (spot on the map). That locus might be significant because it's connected to a gene that makes sucrose build up in the kernel. However, that locus might also be significant simply because all the high sucrose inbreds are related to one another! You share lots of genes in common with your family members. To take that into account, we also look at the **relatedness** of the inbreds, and whether they share common ancestors. I can factor in the relatedness information into the GWAS and eliminate the similarities that are only due to being related. That means that any significant genes I find are going to be connected with the trait I'm interested in. In this experiment I found strong associations between carbohydrate content and those two important mutations, *shrunken2* and *sugary1*. Additionally, I found hundreds of other significant genes all across the 10 chromosomes. (For context, humans have 23 pairs of chromosomes. Corn has only 10 pairs.) This confirms that *shrunken2* and *sugary1* are important for the different levels of carbohydrates in corn. My GWAS results agree with the conclusion I drew by looking at the average sugar differences between the two groups. This also means that there are hundreds of other places in corn's genetic code that can lead to different levels of carbohydrates in the kernels. This is good news for people who like sweet corn because plant breeders can continue to improve the flavor of sweet corn by breeding with these many less-explored genes.

Disease resistance!

Background

Healthy vegetables grow better and produce better harvests. That's why plant breeders care about controlling diseases in their plants. One of the diseases that impacts sweet corn is sugarcane mosaic virus (SCMV). It's a virus that's carried by aphids and infects corn all across the world. Aphids are everywhere and growers can't keep them out of their fields completely.

Figure 0.5 Close up of aphids on a corn leaf

When SCMV infects a sweet corn plant, the plant grows poorly, and it doesn't produce healthy, tasty ears. We know a plant has SCMV because it makes the leaf look like it's pixelated, with a mosaic-like pattern.

Figure 0.6 Left: a healthy corn leaf with dark green color. This plant has been inoculated with sugarcane mosaic virus and is resistant. The plant shows no sign of infection. Right: a leaf from a plant and infected with sugarcane mosaic virus. The leaf is mottled with light and dark green "pixelation".

Some kinds of corn are immune to SCMV. This immunity is genetic, and offspring of the immune corn can also be immune. Lots of research has been done about SCMV resistance in field corn, but very little has been done with sweet corn. For this experiment, I wanted to see if the same immunity genes in field corn were also at work in sweet corn. Also, no one has gone through and classified which sweet corn inbreds are resistant and which aren't.

The experiment

To test which sweet corns are immune and why, I conducted [an experiment](#) similar to the carbohydrate experiment. I took the diversity panel of sweet corn inbreds and planted them in the greenhouse. When the inbreds were a few weeks old, I infected them with SCMV. This process is called **inoculation**. To inoculate the plants, I took some leaves from corn infected with SCMV and I ground them up with water and an abrasive powder, carborundum. I then applied this slurry to each corn seedling by hand. The abrasive powder makes tiny holes in the leaves like an aphid bite, and the virus can get inside the plant. If an inbred is susceptible to SCMV, it will get sick and develop symptoms that we can see on the leaves. If an inbred is immune to SCMV, the virus can't successfully infect the plant and the plant is normal and healthy. After the inbreds were inoculated, my helper and I counted how many seedlings in each pot were sick and could calculate the percentage of infection per inbred. If an inbred didn't show any symptoms at all, we tested it again to make sure we hadn't made a mistake. Overall, out of the 563 inbreds I planted, I found 46 inbreds completely resistant to SCMV. That's more inbreds than I expected to be resistant!

Figure 0.7 Corn inbreds in the greenhouse inoculated with sugarcane mosaic virus ready to be checked for signs of infection. Each pot contains a different inbred.

Once I had all the data about inbreds' resistance ratios, I was able to conduct a GWAS on the inbreds I had genetic information about. My GWAS found that the primary difference between resistant and susceptible inbreds was a single locus near the gene *Scm1*. This is a gene that's been identified in field corn. Basically, this gene makes an antioxidant that keeps the virus from gathering inside the corn cells. So, it looks like SCMV resistant sweet corn does use the same gene that resistant field corn uses!

Conclusion!

These studies have given us new information about the science of sweet corn. Knowing which sweet corn inbreds have the most sugar or are resistant to SCMV can help sweet corn plant breeders make more informed choices. It also tells us more about how kernel carbohydrates and disease resistance work.