Portable DNA sequencer helps farmers stymie devastating viruses

Leah Shaffer, *Science Writer*

Armed with a battery-operated minicomputer, a handheld DNA sequencer, and portable DNA-extraction machine, researchers gathered in a cassava field in Tanzania last August to chase down a major plant pest. Their plan was unprecedented: sequence the whole genome of the plant material to detect all potential viruses—and do so in a single day on a farm. "We called it tree lab, we were sitting under a tree," says Laura Boykin, a computational biologist at the University of Western Australia.

Because plant samples are typically sent overseas to test for viruses, there's a big distance between the farm and the laboratory—thousands of miles if you're in eastern Africa. But within a few hours, a group of researchers, part of the Cassava Virus Action Project, determined what viruses infected the cassava crops on the farm and, more importantly, alerted farmers they would need to plant new crops that are resistant to the viruses they found.

The researchers call themselves an "action group" because they're "taking the level of knowledge we get as scientists, down to the farmer," says Peter Sseruwagi, a research scientist at Mikocheni Agricultural Research Institute (MARI) in Tanzania. "We're able to show the farmer in a single day, what their crops are infected with."

This is a big deal, especially in Africa, where cassava, a sweet potato–like starch, is intricately tied to lives and livelihoods for many of the people on the continent. The two main cassava diseases, cassava brown streak virus and cassava mosaic disease (CMD), are estimated to cause a loss of $2 billion to $3 billion annually (1). CMD alone can cause loss of up to 40% of a crop (2). The work of the cassava action project, however, doesn't only have an impact on African farmers. It can then pave the way to better crop science around the world.

A group of farmers looks over a field of cassava in Mbinga, Tanzania, in 2016; unearthed cassava root is pictured (*Inset*). Researchers in the Cassava Virus Action Group have been able to increase yield by using portable DNA sequencers and extractors that facilitate the early detection of diseased plants. Image credit: Laura Boykin (photographer).

Cassava diseases are often spread by the whitefly, *B. tabaci*, a notorious plant virus vector. Image credit: Monica Kehoe (photographer).

Diagnosing Sick Plants

Cassava diseases are often spread by the whitefly, *Bemisia tabaci*, a notorious vector for more than 100 plant viruses worldwide. In Florida for instance, researchers are grappling with a type of whitefly called *B. tabaci* biotype Q that is resistant to insecticides and can spread diseases to ornamental and vegetable crops. Once plants have these diseases, they may or may not be symptomatic, so they can easily spread as farmers share what appear to be healthy plants.

To accurately detect a virus, plant virologists have a couple tools: If it's a well-known virus, they can use ELISA (enzyme-linked immunosorbent assay), which detects virus antigens using manufactured antibodies. But viruses often mutate in a way that the antibodies in ELISA cannot detect. And disease is often not just from one stable source: Cassava diseases have nine different viruses (3) associated with them, explains Alana Jacobson, a plant pathologist at Auburn University.

Jacobson, who has studied African cassava viruses with Sseruwagi, says they are still trying to understand the distribution of cassava viruses, the different virus abundance in different locations, and what causes it to mutate. Detection tools such as ELISA are not equipped to identify a rapidly changing virus.

Researchers can also look for the DNA or RNA signatures of the virus using PCR, which makes use of molecular primers that amplify certain sections of nucleotides. The primers serve as bookends and allow researchers to cut out the section of DNA or RNA they want to amplify or copy to identify a virus. But to select the correct primers, the researchers need some idea of what sequence they're looking for. If you have a rapidly mutating virus or new viruses with a vastly different sequence, that primer isn't going to bind with the DNA, which means you can't detect it, explains Jacobson. With PCR, it's a shorter segment and not the "long read" of the full sequence, which means they could miss something. Also, researchers around east Africa can't always easily get to PCR equipment. And they need answers fast.

Taking Action

The Cassava Virus Action Project is a partnership among primarily Ugandan, Tanzanian, and Kenyan researchers, including Sseruwagi, and MARI director Joseph Ndunguru. They ended up partnering with Boykin in 2013. But up until last year, they were reliant on laboratories outside of the country to do much of the genetic sequencing work, says Sseruwagi.

Typically, they'd have to send plant samples to a lab in the United Kingdom or Asia. Then it would take 2 to 3 weeks, sometimes even a month or more, before they would get results—and that doesn't include the time it takes to analyze those results. By the time the findings get back to the farmer, 6 months or even a year could pass, he says.

That changed in 2016, with news of a new sequencing device used to track Ebola viruses (4). The handheld device, called MinION, made by Oxford Nanopore Technologies, sequences DNA without the use of primers. MinION identifies molecules using a microscopic tube, called a nanopore, on a synthetic membrane that is charged with an electric current. Drop a molecule of DNA through that tube, and the current changes in a way that identifies the sequence of nucleotides. It's small, fast, and gives researchers the code for the whole genome, not just one amplified segment. MinION's rival, Pacific Biosciences' Sequel device also provides these "long-reads" of DNA sequences with a reportedly higher degree of accuracy (5), but its $350,000 price tag keeps it out of reach for many researchers.

MinION, at $1,000, is much more affordable, and its portability is useful for researchers in Africa looking to track human viruses. Why not, thought Boykin, try tracking plant viruses as well?

Fast Acting

In their first tests of the MinION device in 2017, the team visited farms in Uganda, Tanzania, and Kenya to see how the device, compared with PCR, detected viruses. They confirmed plants that tested positive through MinION for cassava viruses were also found to be positive through PCR. In a few cases, MinION detected the virus when PCR did not. The researchers confirmed there was a virus because those plants developed symptoms 3 months later, says Boykin. The MinION test took about 48 hours to get results, but the researchers wanted it to be even faster. The missing tool was the ability to extract DNA in the field, a solution Boykin first encountered when she gave a talk in New Zealand earlier last year. There, she met Jo Stanton, a researcher in the department of anatomy at the University of Otago, who has partnered with a tech firm to produce what she calls the PDQeX DNA extraction system ("pretty damn quick extraction").

It all came together last August and early September as the team visited farms in Tanzania, Uganda, and Kenya to test their devices in the field. They started in Tanzania, and by the time they finished in Kenya, they had whittled the process down to 2 to 3 hours. Conventional means would have taken months to get results, says Boykin.

And the farmers were "incredibly happy to see results," she adds. "It's a big trust-building thing with them as well." It also builds enthusiasm among African researchers keen on tools that don't rely on lots of power and fast Internet, both of which can be hard to find in parts of Africa. The data analysis of the genome is conducted by using another Oxford Nanopore Technologies device, a book-sized computer called MinIT, a module that plugs into MinION and can be controlled via laptop or tablet. With battery-operated gear, scientists can plug MinIT into their laptops instead of finding reliable Internet to download enormous data files from remote laboratories.

Whole sequencing can even help spot viruses before they affect crop yields. Last summer, the researchers visited a region of Tanzania where a group of farmers tended supposedly virus-resistant plants. First, the researchers checked the status of those healthy plants. "We were hoping that we wouldn't find virus, but we did," says Boykin. Whether the plant has symptoms or not, if the virus is present, even in low levels, farmers will need to replant with fully virus-free cuttings. Farmers can pick up virus-resistant plants at regional distribution centers, according to Boykin.

Disease-resistant varieties are going to lose that resistance eventually, adds Boykin. That's one of the advantages of doing the whole-genome sequencing: They can characterize the viruses over time before the plant has symptoms. The more that researchers—and a given country's agriculture agency—understand how the virus changes and reacts to different management strategies, the better equipped they'll be to save crops. "Being able to predict when the variety is going to break down is crucial," says Boykin. Catch the virus early, and they can then share virus-free plants to prevent the spread.

Sseruwagi says that many farmers in Africa are unaware of the diseases or pathogens they're dealing with. The first method of management is chemical pesticides and insecticides, neither of which would fix a plant infected with a virus. "Cassava is a low-income crop and many farmers cannot afford to spray," he says, adding that "ensuring the health of planting materials is of paramount importance."

Other means of chasing down plant viruses are in development as well. Jane Polston, a plant pathologist at the University of Florida, is working on developing faster virus detection tests using recombinase polymerase amplification (RPA), which functions at room temperature and can work off crude DNA extracts, unlike PCR. In laboratories already equipped with PCR gear, techniques such as RPA or other forms of next-generation sequencing may do the trick. Companies such as Illumina (which recently purchased Pacific Biosciences) and 10X Genomics are providing technology to allow PCR equipment to produce long reads of genetic sequences that provide a more complete picture of a genome. According to an article in *Trends in Genetics*, researchers found the main downside of MinION is its degree of error (approximately 15% in a 2017 test) compared with other equipment. However, authors of that article note that the company's newest technology lowers that error rate to 3% (5).

Scarce Resources

Funding for such approaches is a challenge, says Sseruwagi. They would like to roll out more training for studying not only cassava but also other crops. "Our next steps [are] to look for funding that will allow us to use this on a routine basis," he says.

"It was the best day of my life. . . The woman was building a house for her family because she had so much cassava that she was able to start selling it."
—Laura Boykin

"It's frustrating to me because I think a lot of people are just scared of what they don't know," says Boykin, speaking of the funding challenges of getting support for a new type of sequencing. But she has all the motivation she needs when she sees farmers.

A little over a year ago, they visited Asha Mohammad's farm in Tanzania where there was almost no yield because of the cassava diseases. Boykin and the other CVAP researchers tested Mohammad's farm and found her crop infected with CMD, and the farmer and her community replanted with new resistant varieties. The team visited again recently, and Mohammad's yield had increased dramatically.

"It was the best day of my life. . . The woman was building a house for her family because she had so much cassava that she was able to start selling it," says Boykin. "We just need to do this, over and over again."

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