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How can we protect bananas?

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Abstract

A fungal disease, called Black Sigatoka, is threatening banana crops worldwide. The reason for this is the lack of *genetic diversity* in commercial bananas - the Cavendish - as all of these bananas are *clones.* So any disease that can kill one of them can kill them all. And this is the case with Black Sigatoka. Farmers can protect their crops by applying chemicals that kill fungi but this raises production costs and is bad for the environment. To better understand the problem, we explored the relationship between bananas and its *pathogen*. We found that due to continuous chemical spraying, most of the tested strains of the fungus that causes Black Sigatoka have developed resistance to the chemicals that are supposed to control them. We showed that wild resistant bananas carry *resistance* genes to Black Sigatoka that work by detecting a specific protein in the fungus. This is a very exciting discovery: disease resistance genes could be used to save the banana industry.

Introduction

Have you ever wondered why all bananas look and taste the same? It's because almost every banana we eat, which is the Cavendish variety, is genetically the same – they are all clones of one single banana (Figure 1). The banana fruits are also all *sterile*, so they can only grow as clones, not reproduce naturally. This genetic uniformity is quite dangerous – if one banana is susceptible to a disease, they all are and it can spread really quickly. It's a familiar scenario: in the 1950s the bananas in the stores were not Cavendish but Gros Michel. A different fungal disease wiped these bananas out so farmers had to turn to the Cavendish variety which were resistant to that disease, but now again are on the verge of collapse.

Contemporary bananas are threatened by two major fungal diseases. One of them is the Black Sigatoka or "black leaf streak disease" (BLSD). The fungus which causes it is *Pseudocercospora fijiensis*.

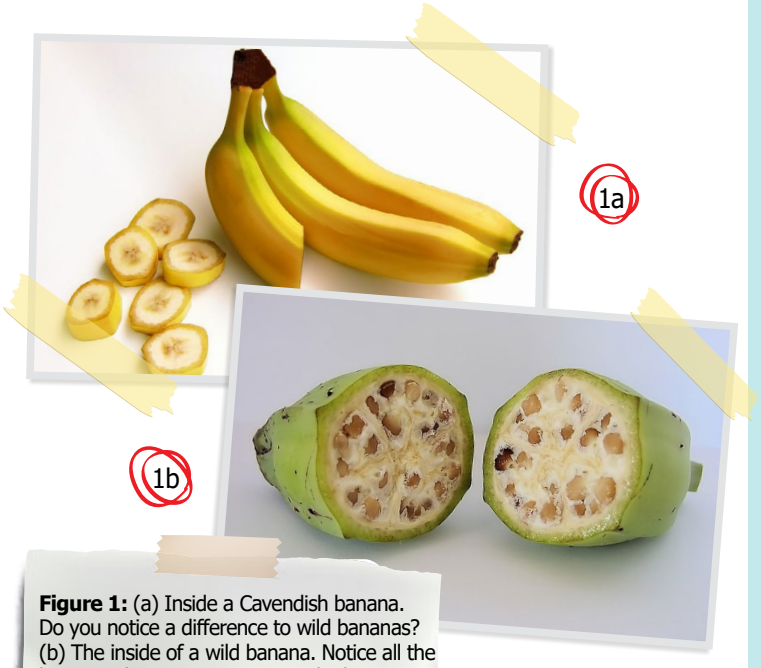

Figure 1: (a) Inside a Cavendish banana. Do you notice a difference to wild bananas? (b) The inside of a wild banana. Notice all the large seeds! Can you imagine why banana breeders have (successfully!) tried to get rid of them? (Photo credit: Lycaon)

When bananas are affected by BLSD, the fungus destroys the leaf tissue which reduces photosynthesis (and thus, crop yields) greatly (Figure 2).
Since bananas are genetically identical and sterile (they don't transfer genes), they all lack effective resistance against this disease.

Farmers can control BLSD only through *fungicides*, and this greatly increases the economic and environmental costs to produce bananas. Moreover, the frequent use of fungicides leads to strains that are resistant to these chemicals.

To support the sustainable production of bananas, we wanted to:

- find out more about the genetic basis of banana disease resistance and the genetics of the fungus
- figure out how we could manage fungicide treatment

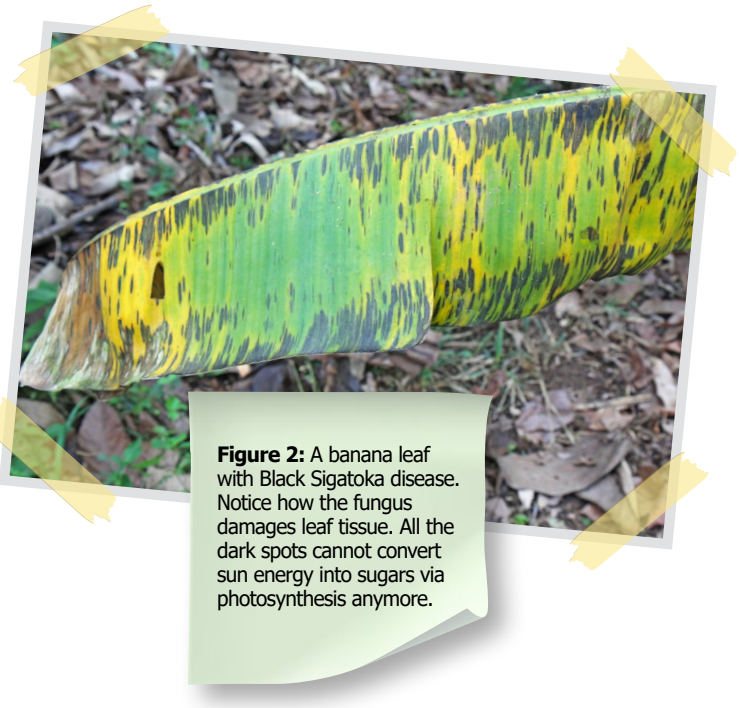

Figure 2: A banana leaf with Black Sigatoka disease. Notice how the fungus damages leaf tissue. All the dark spots cannot convert sun energy into sugars via photosynthesis anymore.

Methods

To better understand the relationship between Cavendish bananas and the fungus that attacks it *(P. fijiensis)*, we isolated DNA from the fungus and *sequenced* its whole *genome*. We then determine the genome's structure with a computer program: its size and content such as the amount of *coding and non-coding sequences*, including *repeats*.

We built a genetic map to complete the genome picture. It shows the location of the genes in the *chromosome*, much like a geographic map shows us different components of the landscape such as a river, roads or buildings. We also determined the length and number of chromosomes in different individuals by conducting a type of *electrophoresis* (separating DNA fragments according to their molecular size).

We found a gene that is coding a protein really similar to a tomato pathogen protein, which is recognized by the plant and hence makes the resistance process easier. To test if our newly found protein (called an *effector protein*) helps the banana's defence, we conducted an experiment: we grew Cavendish bananas (not resistant to *P. fijiensis*) and another type of resistant bananas. We then injected the effector protein (which we previously isolated) in every plant's leaves and monitored the effect.

Finally, we wanted to determine whether *P. fijiensis* is resistant or sensitive to fungicides. We collected banana leaves from three plantations in Costa Rica which apply fungicides regularly. As a *control group* we collected banana leaves from another plantation which is never sprayed with fungicides. We then isolated fungal spores from the leaves and extracted their DNA. Via *Polymerase-chain reaction* we searched for genes that are responsible for resistance to fungicides.

Results

Here is what we found out:

- The genome of *P. fijiensis* is large and greatly expanded compared to those of closely related fungi. It also contained a lot more repetitive DNA.
- The electrophoresis showed a large variety of chromosome length and number among the different individuals of the fungal populations in these farms.
- We detected a decreased number of genes related to infection and a gene coding a protein very similar to the effector protein of a tomato pathogen.
- The functional test (Figure 3) of our newly found protein showed that it is in fact an effector protein, which triggers the *resistance mechanisms* of the plant.

- Almost all *P. fijiensis* strains collected from plantations sprayed with pesticides were resistant to them. However, all individuals collected from the control group were still sensitive to fungicides.

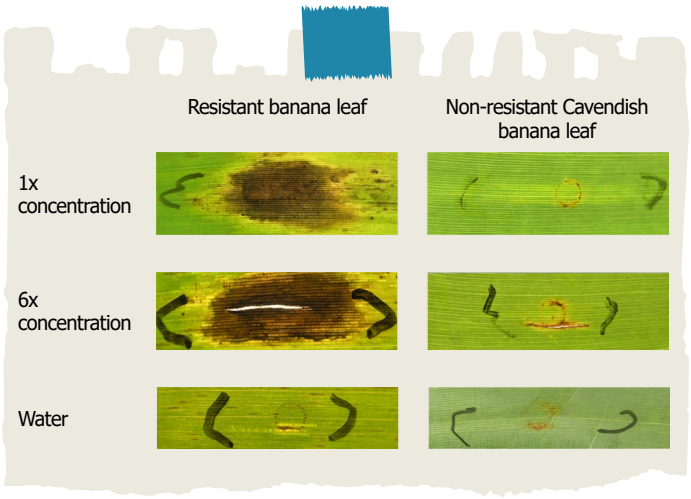

Figure 3: Testing the ability of our isolated effector protein to induce a response to *P. fijiensis* in resistant (left) and non-resistant bananas (right). We did this by injecting different concentrations (1x and 6x) of the effector protein into the leaves of both plants and used (injected) water as a negative control and monitored the effect.

Does the sensitive, non-resistant, Cavendish banana detect the effector protein of *P. fijiensis* and react somehow?

Discussion

Researchers have observed genome extension due to the addition of repetitive DNA in other fungal plant pathogens. Moreover, the decreased number of genes related to pathogenicity (the ability of an organism to cause a disease) is another known feature of these pathogens. But why is that? Most probably, *P. fijiensis* (and other similar fungi) has evolved and developed a mechanism to go undetected by a plant's defence system, since products of these genes are exactly what the plant's immunity is looking for to protect itself from infection.

The variety in chromosome length and numbers among different *P. fijiensis* individuals, on the other hand, is another adaptation mechanism. This time it may be at least partly due to changes in the environment, like fungicide applications.

And indeed, almost all fungi sprayed regularly with fungicides were resistant to them. This had not been the case 10 years ago! This rapid evolution of fungicide resistance poses a great threat to the sustainable production of bananas.

So is there a way out of this threat for Cavendish bananas? Perhaps our newly found effector protein is the key for BLSD disease control. This is the first effector protein we know of that triggers a response in resistant bananas: the banana recognizes it and kills its infected cells, thereby stopping the spread of the infection. If any bananas recognize this protein, then it's likely they possess a resistance gene. This could greatly help banana breeding and production.

Conclusion

BLSD is an increasing threat for Cavendish bananas because of their genetic uniformity, which makes them vulnerable to this and many other diseases. Our results may help breeders produce new and better, more resistant bananas, which provides the ultimate solution: genetic diversity! Why should we be happy with just one banana: the Cavendish?

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Glossary of Key Terms

Chromosome - structure inside the nucleus of a cell, which packages DNA with the help of proteins.

Clone - genetically identical copy of an organism. For example, when bacteria reproduce (asexually) they create offspring which are genetically identical to the parent.

Control group - one of the tested groups in a scientific experiment, which is NOT exposed to any experimental treatment but is tested under the same conditions as all other groups. This helps the scientists confirm that the experimental treatment actually makes a difference.

DNA repeats - DNA that occurs in multiple copies throughout the genome. It's a source for genetic variation and gene regulation. There are probably many functions which are still not known.

Effector protein - a pathogen molecule, which is involved in the infection by changing host cells. For example, the effector can help the fungus to avoid detection by the host organism or can trigger host resistance responses.

Electrophoresis - a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size or charge.

Fungicide - a chemical which destroys fungi or their spores.

Genetic diversity - diversity of the genetic material in a population or species. The higher the genetic diversity of a species is, the better are its chances of survival.

Genome - all genetic material in a cell or organism. The genome consists of DNA - both coding (it carries the instructions to make proteins) and non-coding DNA (see below).

Non-coding DNA - the larger part of the genome, which doesn't carry information for the production of proteins. Non-coding DNA sometimes has a regulatory function, or it produces non-coding RNA molecules. Non-coding DNA includes DNA repeats as well.

Pathogen – a virus, fungus, bacterium or other microorganism that can cause disease in another organism. For example HIV, which causes AIDS; or Vibrio cholerae, that causes cholera.

Pathogenicity - the ability of an organism to cause a disease.

Polymerase-chain reaction - a technique used in molecular biology to multiply DNA, generating thousands to millions of copies of a particular DNA sequence.

Resistance mechanisms - mechanism that help organisms (including plants) to defend themselves. This can help bananas and other plants sense pathogens' molecules and trigger antimicrobial responses.

Sequencing and Whole Genome Sequencing - DNA sequencing is the process of determining the precise order of the building blocks of DNA. Whole genome sequencing is the process of determining the complete DNA sequence of an organism's genome at a single time.

Sterile - not able to reproduce sexually. Cavendish banana don't have any seeds. This makes them easier to eat, but they cannot reproduce sexually and mix their genes. This reduces their genetic diversity.

Strain - a genetic variant or subtype of a microorganism (in our case, a fungus).

Check your understanding

1. Why are Cavendish bananas sterile?
2. What is the problem with banana sterility?
3. Why aren't fungicides working against black leaf streak disease (BLSD)?
4. How do resistant bananas react to *P. fijiensis*, which causes BLSD?