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How can we save bananas from a deadly disease?

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Abstract

Have you ever wondered why all bananas in supermarkets look alike and taste exactly the same? Because almost all exported bananas *(Musa spp)* are of a single *cultivar*, the Cavendish. Every single Cavendish is genetically identical; basically they are all *clones!* So what?

Cavendish bananas are under a threat from a disease called *Panama disease*, also known as *Fusarium wilt Tropical Race 4* (TR4) that is caused by a fungus. It is spreading very fast and has already destroyed many *plantations* in Asia and Australia and has recently spread to the Middle East and Africa.

This is because Cavendish bananas do not have an active *gene* to fight off TR4. However, a wild banana from Indonesia does - the RGA2 gene.

Here, we added the RGA2 gene to Cavendish plants and tested them in fields infested with TR4. Three years later, some of these *genetically modified* (GM) plants resisted the fungus and stayed disease free. That means we may have found a solution to control TR4 and help millions of people who rely on bananas for food and income.

Introduction

Bananas are an important crop worldwide – a major export business and a *staple food* in many developing countries (Fig. 1). But they are dangerously close to disappearing- at least from grocery stores.

Nearly all of the bananas for the export trade are from one cultivar – called Cavendish. Since commercial bananas are seedless, farmers grow new plants from existing plants. That makes every single Cavendish banana in the world genetically identical. Lack of genetic diversity poses a big threat to the Cavendish population: a *pathogen* that could kill one plant could kill them all! That is exactly why the fungus named *Fusarium oxysporum f.sp cubense* or 'Foc' that is causing TR4 could wipe out all Cavendish plants worldwide.

The Foc fungus infests the soil where it waits until banana plants are grown – then it infects the plants through the roots. *Spores* of the fungus can survive in the soil for decades, which makes controlling the disease very difficult. There are no suitable chemical controls, and no Cavendish banana plants are known to be resistant to TR4. However, scientists recently identified an active resistance gene called RGA2 in a wild banana from Indonesia. But this wild banana has small, seedy fruits which cannot be eaten.

Figure 1: Banana is a staple food and major income for millions of people. 40% of world production, and all export trade are based on the Cavendish variety. 1a shows bananas for sale in an industrialized country, 1b in a more rural setting in Malawi, Africa.

In the lab, we transferred the RGA2 gene into the *DNA* of Cavendish banana plants (Fig. 2). Then, we grew these *transgenic* (contains genes from another organism) plants in TR4 infested fields.

Can the gene from a wild relative save the Cavendish? To answer this question, we conducted a three year long field trial with the transgenic bananas and identified lines with resistance to TR4.

Figure 2: Professor James Dale with a little seedling of the modified Cavendish in the lab.

Methods

Preparation of transgenic plants:

We modified the DNA of Grand Nain bananas (a Cavendish cultivar) with the TR4 resistance gene (RGA2) derived from a wild Indonesian banana. This process generated five RGA2 transgenic banana lines: RGA2-2, 3, 4, 5, and 7.

Control group (non-transgenic banana plants):

- Grand Nain and Williams- cultivars of Cavendish, susceptible to TR4
- GCTCV 218 - Giant Cavendish from Taiwan, some tolerance to TR4
- DPM25 - Dwarf Parfitt, resistant to another type of Foc called subtropical race 4 (STR4)

Field trial:

Over a three year period (2012 -2015), we conducted a field trial on a commercial banana plantation in Northern Australia on soil that had TR4 infestations in the past. We planted the transgenic banana plants, along with the non-transgenic control group. To increase the exposure to the fungus, we buried TR4-infected banana plant material between each plant and reinfested the soil heavily. We regularly inspected plants for TR4 symptoms, such as wilting and leaf yellowing. For the final assessment, we tested surviving plants for TR4 and checked whether the plants tissues turned brown inside (another characteristic symptom of the disease) (Fig. 3).

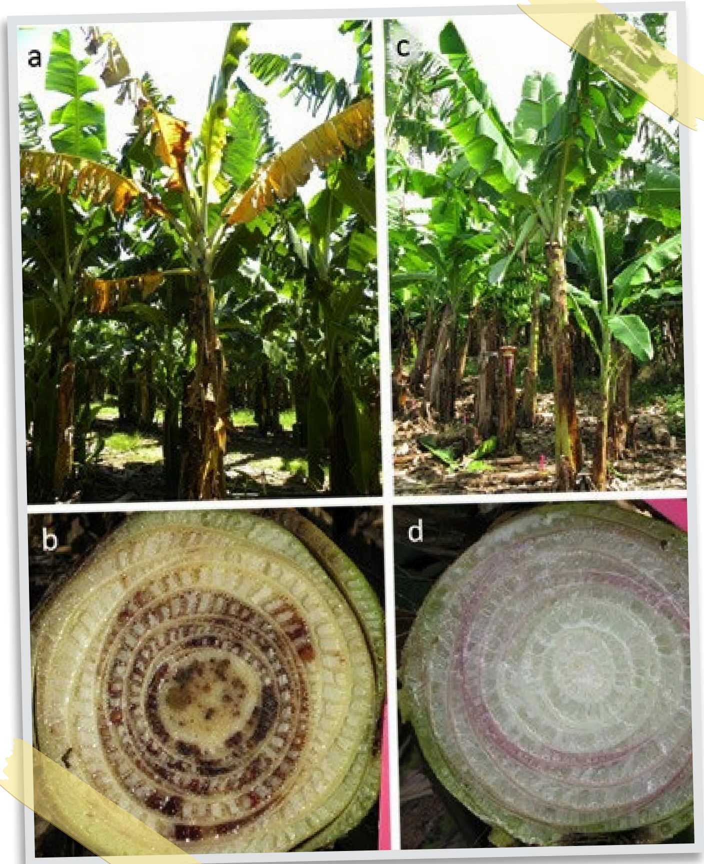

Figure 3: Foc is a soil-borne fungus. It infects the plants' vascular (transportation) tissue blocking its ability the take up nutrients and water. We observed wilting and yellowing in Foc infected plants. At the end of the trial, all plants were visually inspected for browning of the vascular tissue by cutting across the stem.

a & b: Foc-infected plant showing yellowing of the leaves and browning of vascular tissue

c & d: healthy RGA2-3 transgenic plant

Results

1. The results from the field trial of transgenic bananas (Fig. 4) were very promising:
2. Throughout the study, one modified Cavendish line (RGA2-3) remained completely TR4-free. We didn't observe any internal (color change inside the plant) or external symptoms.
3. Three other transgenic lines (RGA2-2, 4, and 5) had strong resistance to TR4. Only 20% or less of these plants were infected or dead.
4. On the contrary, the non-transgenic control group didn't do so well. Between 67-100% of all control plants were either dead or infected. All of them had browning of the vascular tissue.
5. The more active the RGA2 gene was (produced more proteins), the more resistant the banana plants were.

   The RGA2-3 was the most resistant banana; its RGA2 gene was about 10 times more active than some other cultivars we tested.
6. Genetic modification did not affect the number or size of bananas the transgenic plants produced.
7. We found that the RGA2 gene is also present in Cavendish bananas but it has extremely low activity. Increasing the activity level of this gene could be an alternative way to combat TR4.

In which of these transgenic plants is the RGA2 gene most active? How do you know?

Answer: RGA2-3 has the highest RGA2 activity level. There is a positive correlation between the activity level of the gene and TR4 resistance. Since it has the highest resistance, RGA2-3 has the most active RGA2 gene.

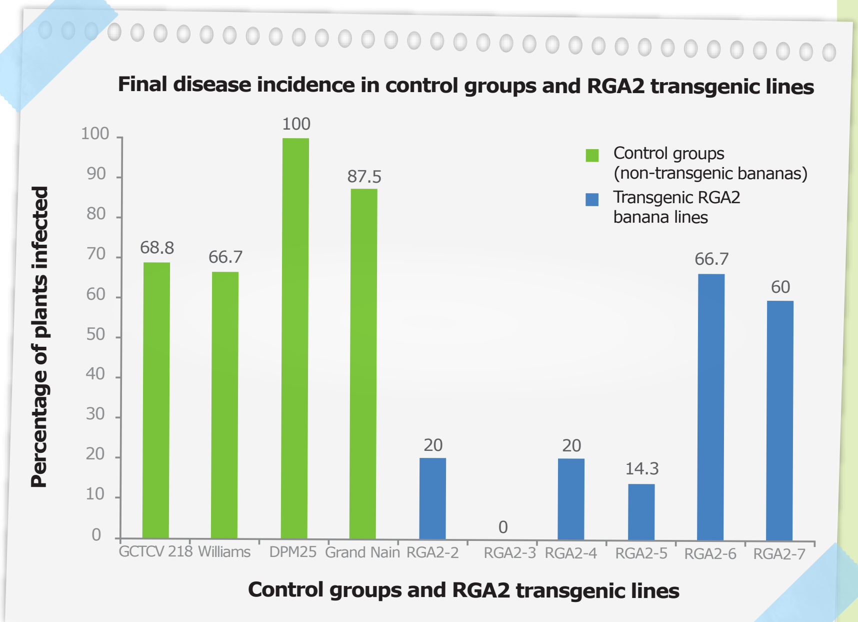

Figure 4: At the end of the 3 years, 4 out of 6 transgenic banana lines showed strong resistance to TR4. RGA2-3 was completely TR4 free throughout the trial. All non-transgenic plants (control group) showed symptoms of TR4.

Discussion

TR4 is the deadliest disease bananas have ever seen. It kills Cavendish and many other banana cultivars. It is probably only a matter of time before TR4 becomes widespread in Africa, and moves on to devastate global export industries in Central and South America. There is no other resistant commercial variety that could replace Cavendish. Therefore, a worldwide TR4 epidemic would have a huge impact on the price and availability of bananas. But more importantly, it would be a life changing event for millions of people who rely on bananas as a major source of nourishment and income. But what can we do? TR4 is unstoppable by conventional methods. Chemicals can't kill it and efforts to contain the disease are ineffective (*quarantine*, destroying infected plants, disinfecting machinery, etc). However, our results show that modifying Cavendish DNA with the RGA2 resistance gene is a highly effective method. After three years of field trials, we identified the modified Cavendish lines that stayed disease free or showed strong resistance against TR4. Our results are very exciting because they offer a solution to control this horrible banana disease. That is a major step towards protecting the global export of Cavendish bananas and maintaining food security in developing tropical countries.

Conclusion

The heated debate on genetically modified organisms (GMO) has been going on for some time now - groups argue whether GM crops should or shouldn't be allowed. Are GM crops the only solution to world hunger? Do the risks outweigh the benefits?

As future decision makers, it is important for us to understand both sides of the debate before taking a position.

Here are some tips:

- Educate yourself about the science behind GMOs.
- Follow unbiased scientific publications (such as Science Journal for Kids) to learn about improvements in genetic engineering.

Glossary of Key Terms

Clone - an organism or cell, or group of organisms or cells, produced asexually from one ancestor or stock, to which they are genetically identical.

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 scientists confirm that the experimental treatment actually makes a difference. Every good experimental study must have a control group if it wants to reach valid results.

Cultivar - a variety of a plant created by plant breeders who combine different varieties together to develop a variety with the best features of each.

DNA - (short for DeoxyriboNucleic Acid): a molecule that carries the genetic information used in the growth, development, functioning and reproduction of all known living organisms, including humans.

Field trial - a scientific experiment that takes place in the real world (usually outside) instead of the laboratory.

Fungus - a group of spore-producing, simple organisms that are not plants, animals or bacteria. They feed on organic matter, including molds, yeast, mushrooms, and toadstools.

Food security - ability for people to have reliable access to enough affordable and nutritious food to keep them healthy.

Gene - a small section of DNA with the instructions for characteristics of an organism.

Genetically modified organism (GMO) - any organism whose genetic material has been altered.

Genetic engineering - the direct manipulation of an organism's genes using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms.

Nourishment - the food or other substances necessary for growth and health.

Pathogen - a virus, bacterium or other microorganism that can cause disease in another organism.

Plantation - an area in which trees have been planted, especially for commercial purposes.

Resistance gene - a gene in plant genetic structure that makes it resistant (immune) to a pathogen.

Quarantine - a state, period, or place of isolation in which people or animals that have arrived from elsewhere or been exposed to infectious or contagious disease are placed.

Spore - a reproductive cell capable of developing into a new individual without fusion with another reproductive cell (asexual reproduction).

Staple food - a food that is eaten routinely and in such quantities that it constitutes a dominant portion of a standard diet for a given people, supplying a large fraction of energy needs and generally forming a significant proportion of the intake of other nutrients as well.

Transgenic organism - an organism that contains a gene or genes which have been artificially inserted. A transgenic plant, for instance, would be a plant that underwent genetic engineering. It often contains material from at least one unrelated organism, e.g. from another plant, animal, or virus.

REFERENCES

James Dale, Anthony James, Jean-Yves Paul, Harjeet Khanna, Mark Smith, Santy Peraza-Echeverria, Fernando Garcia-Bastidas, Gert Kema, Peter Waterhouse, Kerrie Mengersen, Robert Harding (2017). *Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race* 4. Nature Communications
https://www.nature.com/articles/s41467-017-01670-6

More info on Panama Disease
http://www.fusariumwilt.org/index.php/en/about-fusarium-wilt/

Green Facts: Genetically modified crops
https://www.greenfacts.org/en/gmo/index.htm#5

Check your understanding

1 Commercial bananas are seedless. Farmers generate new plants from an existing plant through cloning. Why is that a problem?

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2 Why is saving Cavendish bananas such an important task?

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3 Why is genetic modification of banana plants the most effective way to control TR4?

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4 What genetically modified crops are currently approved to be grown in your country? Are they labeled?

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5 Shall countries have mandatory labeling of GM? What do you think? Discuss the pros and cons

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6 Even "The Science Guy" can change his position on an issue. Watch this video where Bill Nye explains the 3 reasons that changed his mind about GM crops: **http://bigthink.com/videos/bill-nye-frankenfoods-are-ok**
What is your stance? Take a position and explain your reasoning.