THE Science BREAKER
SCIENCE MEETS SOCIETY

May 3, 2018

Plant Biology

Will gene therapy save the banana?

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This Break was edited by Carlos Rivera-Rivera - TheScienceBreaker

It's got to be one of the most popular fruits around: the banana. Although there are over a 1000 varieties, few people realize that almost the whole banana export is dependent on one type of banana, the Cavendish. This banana - cultivated by the personal gardener of William Cavendish in the early 19th century - is propagated by means of vegetative cloning, as it has no seeds and therefore cannot reproduce sexually. The Cavendish banana acquired its prominent position because it was resistant to the Panama disease, which threatened to devastate the banana industry in the early 20th century. This disease, whose devastating effects were first observed in Panama, turned out to be caused by a fungus affecting the roots of banana trees. History seems to repeat itself now as a slightly different strain of the same fungus, the tropical race 4 (TR4) of *F. oxysporum f. sp. cubense*, has popped up which *does* affect the growth of the Cavendish.

Because we are so reliant on one banana clone, having effectively created a monoculture of bananas, the new Panama disease (referred to as TR4) will hit the banana industry hard unless we act fast. The authors of a recent paper published in *Nature Communications* propose a possible solution to avoid a potential banana crisis. Their solution relies on gene therapy, which in this case means introducing additional genes into the banana plant, whose expression might confer resistance to the TR4 Panama Disease. In their paper, the authors report different genes that do precisely this.

The TR4 fungus affects banana crops by causing extensive cell death in the corm (underground stem) and pseudostem (bottom part of the trunk). One of the genes tested in the study was *Ced9*, which prevents cell death in the roundworm *C. elegans.* The second gene was the banana-derived *Rga2*, for which closely related genes exist that confer resistance to TR4 in other fruits, such as melons. Both the *Ced-9* gene from worms and the banana-derived *Rga2* gene were injected into cells that can give rise to a whole Cavendish banana tree. As a result, they obtained five lineages of banana trees that were "transformed", meaning that they now expressed either the *Ced9* or the *Rga2* gene. These plant lineages were then compared with five others that were not transformed at all: the control lines.

These different banana trees were then planted on an Australian commercial banana plantation where the TR4 fungus had infected previous crops. Once infested, the fungus can remain in the soil for over 40 years. To be absolutely certain that all the lines were exposed to the fungus, infected plant material was buried between each plant. They were then left to grow for three years during which signs of TR4 infection were studied. The appearance of reddish-brown spots in the pseudostem of the banana tree seemed to be a very precise (≈99%) marker of TR4 infection and at the end of the study all control lines (the ones that had no resistance genes inserted) showed this phenotype. Importantly, in all transformed lines - expressing the *Ced-9* or *Rga-2* gene - symptom development was significantly lower compared to the untransformed controls. In fact, for both the *Ced-9* and *Rga-2* transformants there was one line (out of 5) that was completely resistant to infection.

The transgenic lines were shown to indeed express the *Ced-9* or *Rga-2* gene. For the *Rga-2*

lines, a distinction had to be made between expression of the *Rga-2* gene already present in the Cavendish genome and the artificially introduced *Rga-2* gene. The most resistant line turned out to be the one with the highest total expression of *Rga-2*, originating from both the introduced copy of the gene, and the one that was already there.

From this, the authors propose that the best strategy for protecting the Cavendish against the TR4 Panama Disease is to boost *Rga-2* expression, as the three-year field trial showed that this increases resistance against the Panama Disease. Although this gene is already present in Cavendish clone, its expression is insufficient to protect the tree from infection. Therefore, gene therapy aiming at delivering extra copies of the gene will be required.

While this solution does offer a way out of our current banana crisis, it is unfortunately only a temporary solution. We might end up in a vicious circle of gaining and losing resistance against constantly evolving pathogens. The only real way out of this situation is bringing back natural variation in banana cultivation. Fortunately, thousands of different wild banana species can be found in nature, which represent a precious genetic resource to keep the banana alive and in our stores; though perhaps looking slightly different from the ones we know so well.