ALERT!

RNA interference GMOs to enter South Africa and Nigeria

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Introduction

In July 2017, the South African government received an application for the commodity clearance (import for food, feed and processing) of a 'multi-stacked variety' of genetically modified (GM) maize – MON87427 × MON89034 × MIR162 × MON87411.

This GM maize represents the entry of the second generation of genetically modified organisms (GMOs) in South Africa. Unlike standard first-generation GMOs, this GM maize variety utilises what is termed the RNA interference (RNAi) pathway. This pathway, a natural mechanism that regulates gene activity, is being used to exert lethal effects on the target pest, Western corn rootworm, by interfering with its genes.

As detailed below, such second-generation GM crops are associated with novel biosafety risks, which need to be addressed by updating risk assessment protocols to incorporate appropriate experiments. Biosafety testing of MON87411 has been woefully inadequate to date, and has relied on assumptions of safety, while ignoring the latest scientific understanding of the far-reaching effects of RNA (ribonucleic acid) interference, which is now thought to cross species and even kingdom barriers.

Such GMOs are the latest in the GM push on the wider African continent. Indeed, Nigeria has recently received an application for the field trials of a GM cassava variety that uses RNAi to reduce the amount of starch in cassava, with the purported aim of preventing starch breakdown during storage.

Background to MON87427 x MON89034 x MIR162 x MON87411

This GM maize variety is a 'stacked' trait developed by conventional breeding of four separate genetically modified maize varieties:

MON87427 is a glyphosate-tolerant trait, carrying the *cp4 epsps* transgene, and has not been approved for commodity clearance as a single event trait in South Africa.

MON89034 is a stacked Bt trait, containing two Bt toxins, Cry2Ab2 and Cry1A.105. Cry1A.105 (also known as CS-cry1A.105 3.53) is not one single Bt toxin, but a protein comprised of naturally occurring Cry1Ab, Cry1F, and Cry1Ac proteins. The gene Cry1A.105 is a chimeric gene comprising of four domains from other Cry genes previously used in transgenic plants. Bt insecticidal toxins were isolated from the bacteria *Bacillus thuringiensis* subsp. *kurstaki Strain HD-1* and *Bacillus thuringiensis* subsp. *kumamotoensis*.

MIR162 is an insecticidal trait, containing a modified version of the native Vip3Aa1 gene from *Bacillus thuringiensis*, which confers resistance to certain lepidopteran pest species, and a phosphomannose isomerase (PMI) protein, used as a selectable marker.

MON87411 is also an insecticidal trait termed an RNAi GM trait, the first of its kind introduced into South Africa. It carries a gene that encodes for an RNA molecule that disrupts gene function. The RNA molecule introduced into MON87411 is designed to silence a gene, Snf7, in Western corn rootworm *Diabrotica spp* species to induce mortality. MON87411 also carries the Cry3Bb1 Bt toxin that also targets the Western corn rootworm, as well as a gene conferring glyphosate herbicide tolerance, *cp4 epsps*. As far as we can ascertain, this trait as a single event has not been approved by the South African government for commodity clearance. Though the application received is for commodity release only, this can often lay the ground for applications for general release. MO87411 however, appears to be completely unsuitable for South Africa where there is no Western corn rootworm, and further, no evidence has been presented by the applicant to show efficacy of the RNAi pathway against South African pests.

Sneaking unapproved single-gene GM crops into stacked events

Neither MON87411 nor MON87427 varieties appear on the list of crops approved for commodity clearance for South Africa, on the ISAAA.org website. This raises significant concern surrounding the lack of regulatory review and authorisation of these varieties. To date, approval of stacked events/varieties has been based on prior approval of the parental single event varieties, but this practice appears to have been abandoned as the growing tide of stacked events enters the country. Clarification of the regulatory procedure is urgently required. This is of particular concern with regards to MON87411, which is the first genetically modified RNAi trait to enter South Africa, showing complete lack of oversight in addressing the novel biosafety risks associated with this type of GMO.

Further, the inclusion of MON87427 is highly dubious, considering that MON87411 is also a glyphosate-tolerant variety. MON87427 therefore adds no novel trait to the stacked event that is not already present.

It appears that the trend towards stacked varieties has overtaken any desire for best biosafety practices by the South African government.

What is RNA interference and what are RNAi crops?

RNAi is a natural biological process that can turn off the activity of a gene, known as gene silencing.

In all organisms, when genes are expressed, many of them are copied (transcribed) into what are called messenger RNA molecules, which are then translated into proteins that go on to perform specific functions in a cell. RNAi interferes with gene expression by targeting particular messenger RNAs that are destined to be translated into proteins. The RNAi machinery, once it has recognised which messenger RNA to target, acts as molecular scissors, cutting up the messenger RNA, thus preventing the production of the protein. It targets particular messenger RNAs through the use of a particular type of RNA species, called double-stranded RNAs (dsRNAs), which have a sequence that is complementary to the target messenger RNA and that guides the molecular scissors of the RNAi machinery to their target messenger RNA. In RNAi GM varieties, a transgene that encodes a particular dsRNA is introduced into the plant.

RNAi was first discovered as a mechanism in bacteria, to target and kill invading viruses. When a virus invades the bacterial cell, it produces RNA molecules with a specific sequence that targets particular viral RNA molecules. Without viral RNA, no viral proteins are produced, and thus the virus cannot replicate. Since this initial discovery, RNAi has been found in many species, including humans, and is part of the growing field of epigenetics – the regulation of gene activity, without altering the genetic sequence itself. The fine-tuning of gene regulation is a vital function of any living organism, and epigenetic mechanisms, such as RNAi, are now recognised as major players in this process.

A major focus of RNAi research is to understand the ability of such molecules to regulate genes across different species and even kingdoms, opening up the possibility of our genes being regulated by RNAs found in the vegetables we eat, for example. A recent study found that RNAs from rice regulate metabolic genes in the livers of mice. Cross-kingdom regulation by RNAi has also been documented, including between hosts and eukaryotic pathogens, pests, parasites or symbiotic micro-organisms.

RNA interference crops on the market

MON87411 received deregulation status in the United States (US) in 2015. The DvSnf7 transgene introduced encodes a dsRNA molecule that targets the messenger RNA of the Snf7 gene in the Western corn rootworm (*Diabrotica virgifera virgifera*). The RNA molecule introduced blocks the ability of the corn rootworm to translate the Snf7 gene into the protein required for carrying out a particular vital function in transporting molecules and viruses out of cells, thus killing the rootworm.

Several other crops that utilise RNAi have been commercialised, including the non-browning Arctic® apple commercialised in Canada by Okanagan Specialty Fruits, as well as several potatoes by J.R. Simplot that confer blight resistance, reduced spot bruising and reduced acrylamide, now approved for planting in the US. In order to generate RNAi crops, classic transgenic techniques are used to insert genetic material, therefore all the hazards associated with current GMOs apply to RNAi GM crops.

The GM cassava varieties awaiting approval for field trials in Nigeria, called the AMY3 RNAi lines, carry a gene encoding a dsRNA molecule that silences the gene encoding for the protein α-amylase, an enzyme that breaks down starches. This is the first trial on these crop varieties anywhere in the world. The application was submitted by the International Institute of Tropical Agriculture, in collaboration with the ETH Zurich, while Syngenta hold patents on the use of RNAi in corn to target α-amylase.

RNAi GM crops raise novel biosafety concerns

This use of RNAi in GMOs is not one of the six novel GM techniques under global discussion for GM legislation as such GMOs are generated via classic transgenic approaches, with the permanent introduction of genetic material. Nonetheless, these crops deserve specific attention, due to the alternate risks that come with employing epigenetic mechanisms in the plant. In addition to the biosafety risks of standard GMO crops commercialised to date, RNAi crops come with novel biosafety concerns, based on two main issues:

1. The potential risks of exposure following consumption

Unlike messenger RNAs, dsRNAs are highly stable in the environment and have been detected in nearly all bodily fluids, such as blood, breast milk and saliva. Regulators have often dismissed this stability; in fact industry has assumed that dsRNAs were unstable, like other RNA species, but this has been shown to not be the case.

Plant-derived dsRNAs from various foods, such as rice, barley, corn, tomato, soybean, wheat, cabbage, grapes and carrots have been detected in humans. Their bioactivity has been controversial, though a recent study showed that dsRNA from rice regulated a mammalian liver gene involved in metabolism. This goes against industry claims that RNA molecules are highly unstable and do not survive mammalian digestion. Such studies have been aggressively attacked by the GMO industry, as they open up novel biosafety hazards surrounding the potential for dsRNAs in GMOs to regulate the genes of those consuming them, including people. However, in recent years, evidence accumulating in the scientific literature shows this possibility cannot simply be ruled out. Testing of the potential for such cross-species effects should, therefore, be incorporated into any risk assessment prior to regulatory approval of these types of crops.

2. The potential for off-target regulation of unintended genetic pathways

Potential for RNA molecules to regulate the activity of genes that have a similar sequence to those being targeted is another novel biosafety risk, since the whole mechanism of RNAi relies on sequence complementarity of the RNAi molecule to its target sequence. Many RNAi pathways have been shown to regulate many genes. Further, certain dsRNA molecules do not require complete sequence complementarity to exert their effects. As such, the introduction of a dsRNA molecule designed to regulate one desired target gene may also go on to regulate additional unintended genes in exposed non-target organisms.

While it has been shown that incomplete complementarity can result in activation of the RNAi pathway, the mechanisms and extent of complementarity that is needed for the RNAi machinery to be activated are not yet fully understood. However, Monsanto's risk assessment for DvSnf7 dsRNA relied solely on bio-informatics computer algorithms to predict potential unintended off-target effects, only assessing for unintended gene targets that have complete sequence complementarity. This method cannot reliably predict and excludes off target effects. Further, Monsanto only analysed potential effects on mice, rats and humans, thereby excluding the wider food web that may be exposed to MON87411.

To conclude

The efficacy of RNAi in MON87411 remains impossible to determine, considering that it also carries the Cry3Bb1 Bt toxin that targets the same animal. Further, approving the importation of such a crop should not be allowed to go ahead in South Africa, given the incomplete scientific understanding of RNAi mechanisms, as well as in the light of outdated regulatory principles. Regulators in Nigeria should also disallow the application for field trials for the GM cassava featuring the untested and wholly risky RNA technology.