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Safety Evaluation of Specific Traits in Transgenic Crops under Development in Africa

Introduction

Biotechnology research and development activities on important African food crops currently at the experimental phase on the continent attest to a slow but gradual awakening to its possibilities in addressing long standing food and nutrition security challenges in Africa. This progress is however hampered by the perceived safety risks associated with modern biotechnology. This policy brief discusses the safety evaluation of some important traits that have been transferred into existing commercialized genetically modified (GM) crops, and that are currently being introduced into staple African food crops. As outlined in Policy Briefs 1 and 2 in this series, there are a variety of studies necessary to establish the acceptable safety of transgenic food crops including compositional analysis of key nutrients, antinutrients and toxins in comparison to non-transgenic analogs. This brief focuses on an additional step, the safety evaluation of the traits themselves, particularly the proteins which are coded by the transgenes and their potential health effects on consuming organisms. Other aspects of these GM crops relevant to food safety will be discussed in future briefs.

Genetically Modified Food Crops in Africa

Table 1 summarizes some of the GM crops, staple to Africa, that are at various experimental stages – laboratory, greenhouse or confined field trials – in different countries and the different genetic traits that are being introduced.

Considerations and steps for safety assessment

Food is not always safe. Some foods are intrinsically hazardous, such as cassava, some mushrooms, or sprouted potatoes. In addition, any food can become hazardous when contaminated with natural toxins (e.g. aflatoxins), man-made chemicals (e.g. residual pesticides), or biological agents (e.g. salmonella). However, over time humans have learnt to handle and process foods to minimize known safety risks. For foods developed by the more recent biotechnological techniques however, where a history of safe use is still being established, a rigorous safety assessment process is currently a prerequisite to commercialization and marketing of these crops. The safety evaluation of GM food is conducted on a case-by-case basis and generally follows a comparative approach involving a stepwise process. One step in this process is to address any safety risks due to the expected effects of the gene(s) that have been transferred into the food crop. Compositional analysis of key components for which the crop is a significant contributor is the other major area for safety assessment. The potential of these risks occurring is evaluated and the risks characterized. The safety evaluation of the expressed protein(s) considers:

- o History of safe use of or previous dietary exposure to the protein
- o Bio-informatics analysis for potential allergenic and toxic effects
- o Mode of action and expected effects of the expressed protein in the recipient plant or following consumption
- o In vitro digestibility of the expressed protein(s) as a measure of the novel protein(s) potential to be an allergen

Additional risk characterization is determined on a case-by-case basis and may involve a toxicological assessment in a rodent model, depending on the outcomes of the previous risk evaluation steps. Additional studies may also be required to assess the potential for allergenicity if the gene is derived from a donor organism known to cause human allergies.

Table 1. On-going Biotech Research on Important Food Crops in Africa (ISAAA, 2011)

Trait	GM food crop	Country
Nutritional enhancement	Sorghum,[3,4,5] Cassava,[3,4] Banana[6]	1. Burkina Faso 2. Egypt 3. Kenya 4. Nigeria 5. South Africa 6. Uganda
Herbicide tolerance	Maize[5]	
Drought tolerance	Maize[3,5,6]	
Viral resistance	Cassava,[3,6] Sweet potato,[3] Tomato[2]	
Bacterial resistance	Banana[6]	
Insect resistance	Maize,[2,5] Cowpea,[1,4] Sweet potato,[3,6] Pigeon pea,[3] Potato[5]	

Photo credit: Bio-Innovate Program

A sweet potato harvest from the fields

Insect Resistance Traits

These traits currently involve the introduction of one or more genes derived from a bacteria called Bacillus thuringiesis (Bt), that confers resistance to some common insect pests through production of an insect-specific toxic protein (Cry protein). There are several different Cry proteins currently introduced into transgenic crops which can confer resistance to different types of insects. In Africa, crops including maize, cowpea, sweet potato, pigeon pea and potatoes are currently at different stages of development to confer resistance to pests such as stem borers, weevils, and *Maruca*. Evaluation of the safety of the transgenic crops with this trait entails:

1.Potential risk identification and evaluation

(a) History of safe use – Microbial Bt-based pesticide products have been used commercially in sprays for more than 50 years by farmers including organic growers without known adverse effects to the environment or to human health. Crops treated with microbial-based Bt products can be harvested and consumed immediately after application, thus demonstrating further the dietary safety of the Bt proteins.

(b) Bioinformatics analysis – Insecticidal proteins derived from Bacillus thuringiensis exhibit no amino acid sequence similarity to known mammalian protein toxins or allergens, which reinforces the conclusion that there is a very low possibility of causing toxic or allergenic effects when consumed by humans.

(c) Mode of action of expressed protein – The toxicity of these proteins to susceptible insects depends on the presence of specific receptors on cells of the midgut. These are not found in vertebrate intestinal cells, and so livestock, humans, birds, and fish are not susceptible to these proteins. Most other invertebrates, including many other insects, are similarly insensitive since they lack the necessary receptors. Furthermore, cry proteins are only activated to bind to those specific receptors by specific cleavage in the alkaline midgut of susceptible insect larvae. This cleavage does not occur in organisms with acidic intestinal tracts, such as humans and other animals and this would be an additional protection if the native protein is the form expressed in the crop.

(d) In vitro digestibility – All commercialized insecticidal proteins derived from Bacillus thuringiensis, such as the Cry1Ab protein are rapidly degraded under conditions that simulate the mammalian gastric environment. However, on one occassion a cry protein (Cry9C) exhibited biochemical properties that differentiate it from previously studied ones, for example having enhanced resistance to in-vitro digestive enzymes and stability at high temperatures. These properties are often associated with proteins that are known food allergens. In this case the transgenic product was restricted in use to animal feed as a precautionary step. There is, however, no direct evidence that any of the forms of insect toxins from Bt cause human allergies.

2. Additional safety evaluation

Since the cry proteins are insect toxins, acute toxicity studies in a rodent model have historically been conducted as a confirmatory test for evaluation of potential toxicity. To date, there are no indications of toxicity as measured by these tests.

Viral Resistance Trait

This particular trait gives crops the ability to resist destructive diseases caused by viruses; two important examples are the cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). In Africa, important crops under development to possess this trait are cassava, sweet potato, tomatoes and potatoes. One introgression approach involves the transgenic expression of a viral coat protein that protects the crop from infection by a particular virus. The mechanism may be complex but probably involves the stimulation of the plant's existing viral defense processes that silence the expression of the coat protein genes (both transgenic and viral). Expression of coat protein in plant cells thus prevents infection by additional virus. Evaluation of the safety of transgenic crops with this trait entails:

1. Potential risk identification and evaluation

(a) History of safe use – The expression level of the potato virus Y (PVY) coat protein for example – a coat protein derived from potato virus Y and known to protect potato from PVY – in transgenic lines is 0.01% of the total found in "naturally" infected tubers. This PVY coat protein is identical to the Y coat protein found in the diet when infected potatoes are consumed. Based on data gathered through surveys of tuber infection, and widely available in the public domain, calculations show that the consumption of the Y coat protein from transgenic potato would be less than that currently in the diet. In this particular case, there is a very strong history of safely consuming the same potato virus Y coat protein that has been introduced to protect the potato from new viruses. Since there is no significant new dietary exposure, no further safety assessment of the potato virus coat protein is warranted. This is the basis for assessing the safety of virus resistance trait using the virus coat protein method. Usually bioinformatics approaches and digestibility studies have been sufficient to establish safety. Nevertheless, additional safety tests on toxicity, allergenicity and nutritional assessment have been conducted and revealed no safety concern.

However, it is important to note that in most of the crops currently under development, the viral coat protein genes might be used but they are intentionally engineered so that there is no expression of the protein and the viral gene is silenced more directly by production of sequence-specific small interfering RNAs (siRNAs). Therefore, the question of protein safety in siRNA plants is negated.

Photo credit: Wikipedia

Cassava tubers

Herbicide Tolerance Trait

A herbicide tolerance trait confers resistance to specific commonly used herbicides. A common example is the glyphosate-based herbicide, Roundup. This herbicide specifically inhibits the enzyme EPSP (5-enolpyruvylshikimate-3-phosphate) synthase that is critical for the biosyntheses of aromatic amino acids in plants. Glyphosate-tolerant transgenic crops express a version of this enzyme that is derived from Agrobacterium species – strain CP4. This particular enzyme is insensitive to glyphosate and thus confers tolerance to glyphosate in the GM crops while maintaining aromatic amino acid biosynthesis. In Africa this trait is currently being introduced into maize. Evaluation of the safety of this trait entails:

1. Potential risk identification and evaluation

(a) History of safe use – The CP4 EPSPS protein (enzyme), derived from a common soil bacterium (Agrobacterium), is a member of a class of proteins found ubiquitously in plants and microorganisms. Therefore this is a type of protein that human beings are exposed to all the time in consuming plant material. There are no indications of any adverse effects to consumers.

(b) Bioinformatics analysis – No similarity has been found when comparing the CP4 EPSPS protein to known protein toxins and allergens based on amino acid sequence homology searches.

(c) Mode of action of expressed protein – All plants, bacteria and fungi contain the EPSPS protein but it is not present in mammals including humans who do not synthesize their own aromatic amino acids. This is an important differentiation because it means that mammals do not have specific substrates or receptors with which the EPSPS proteins can interact. Since EPSPS is already active in plants, it does not affect plant biochemistry in new or unexpected ways. It is therefore not likely that it will cause harm to humans and animals based on the knowledge of its mode of action.

(d) In vitro digestibility – The CP4 EPSPS protein has been shown to degrade rapidly in simulated digestive fluid experiments and therefore loss of activity will occur.

2. Additional Safety evaluation

Because no safety issue has been identified based on the aforementioned discussion there would be no reason to perform further tests. However, toxicological tests have been conducted to provide more evidence of safety. Acute oral toxicity studies of the CP4 EPSPS protein in mice have shown no adverse effects at high dosage.

Other, currently less common, mechanisms of herbicide tolerance involve enhanced degradation of the herbicide in the plant. These genetic mechanisms would need independent evaluation but are unlikely to adversely affect the consumer on the basis of current knowledge.

Photo credit: Wikipedia

From laboratory to plantations: disease-free and biofortified bananas

Nutritional Enhancement Trait

In Africa, a number of nutritionally enhanced crops are in development including biofortified cassava, sorghum and banana with enhanced pro-vitamin A levels (beta carotene); increased zinc and iron and improved protein digestibility. For crops nutritionally enhanced using modern biotechnology, the same principles for safety assessment of the proteins as the agronomic traits aforementioned are followed. The guiding principle is the understanding of the possible adverse effect of the introduced protein and how these changes affect the nature and amount of expression products and metabolites. Because nutritionally enhanced varieties may be expected to have major changes in the amounts of one or more nutrients, an additional important step is assessing the human and animal exposure to these products, particularly if the exposure is significant. Nutritional assessment should be conducted in order to assess the consequences of the changes and whether the nutrient intakes are likely to be altered by the introduction of such foods into the food supply.

Conclusion

Focusing specifically on the safety evaluation of gene products and their expected effects on plant metabolism, a variety of tools are available for assessing potential impacts on human and animal health in food crops. So far, considering the first transgenic crops that emphasize input traits such as insect and herbicide resistance, it seems quite clear that these traits themselves present no appreciable risk to consumers. However, as plants with output traits such as altered nutritional content or other traits like drought tolerance approach commercialization, continued care will be needed to evaluate their potential health effects using appropriate methodologies on a case-by-case basis. The ability of African regulators to evaluate and provide scientific opinion to decision makers on the safety of GM crops, will in-part determine the rate of adoption of this technology and therefore the impact it will have in contributing towards addressing food and nutrition security challenges afflicting the continent.

This is the third in a series of policy briefs published by the African Union/NEPAD - African Biosafety Network of Expertise (ABNE) addressing food safety aspects of modern biotechnology. This policy brief is targeted for regulators and decision makers.

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