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Developments in Agricultural Biotechnology in Sub-Saharan Africa

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A number of crops with a variety of traits are being developed by public-private partnerships in sub-Saharan Africa. These include maize that is resistant to the parasitic weed, *Striga*; tolerant to drought, and resistant to the African endemic maize streak virus. Others in the pipeline are insect-resistant cowpea and potato, as well as nutritionally-enriched sorghum. Some of these are undergoing field trials in various parts of the sub-continent. However, the South African regulatory authorities denied permission for release of the genetically modified potato and for glasshouse trials of the sorghum. After an appeal against the latter decision, permission was eventually given after two years. It remains to be seen how regulatory authorities respond to requests regarding the other crops.

Key words: cowpea, drought, genetically modified crops, insects, maize, potato, sorghum, viruses.

Introduction

Many laboratories in public and private institutions in sub-Saharan Africa are in the process of developing improved crops, particularly for use by resource-poor farmers. This article describes some of these crops being produced both by advanced breeding techniques and by genetic engineering. It is by no means a complete account and includes only those presented at the 14th annual conference of the International Consortium on Applied Bioeconomy Research (ICABR) held in Ravello, Italy in 2010.

Striga-Resistant Maize

Striga spp, commonly known as witchweed, is a genus of 28 species of parasitic plant that occurs naturally in parts of Africa and Asia. Three species cause the most damage: *S. asiatica*, *S. gesnerioides*, and *S. hermonthica*. *Striga* affects 20-40 million hectares of cereal farmland in sub-Saharan Africa. Yield losses range from 20 to 40% but can reach 100%. Continuous mono-cropping and declining soil fertility intensify the *Striga* problem (Emechebe et al., 2004). The plants give rise to thousands of tiny seeds, which can remain dormant in the soil for many years until a cereal host seed germinates. This sends signals to the *Striga* seed to germinate and as the weed sprouts, it attaches directly to roots of cereal seedlings, sucking away nutrients and causing 50-100% of grain yield loss by harvest time (International Maize and Wheat Improvement Centre [CIMMYT], 2005). Resource-poor farmers faced with this problem often simply leave the infected fields unplanted, or planted to non-host plants.

Herbicide-resistant maize was the first product deployed under the auspices of the African Agricultural Technology Foundation (AATF). It is not a genetically modified (GM) crop but was developed by BASF during their breeding program for maize resistant to the herbicide Imazapyr. Farmers in Kenya are able to buy Imazapyr-resistant maize seed. Further commercialization efforts are underway in Tanzania, Uganda, and Nigeria to facilitate farmers' access to the Imazapyr-resistant maize technology.

The technology is enabling farmers in the region to plant maize in fields that for many years have been infested with *Striga* seeds, and even abandoned at times. Through its stewardship program, the AATF has empowered farmers on proper handling and use of the technology. These include developing user guidelines on issues such as the importance of washing their hands after planting the coated seeds, before inter-planting with companion crops such as legumes, which are sensitive to—and thus can be killed by—Imazapyr. This flagship project has provided the AATF invaluable lessons and opportunity to develop working public-private partnerships, including non-governmental (NGOs) and community-based organizations (CBOs), who deal regularly with resource-poor farmers.

Insect-Resistant Cowpea

Cowpeas—rich in protein—are consumed by approximately 200 million people in Africa. However, it is often infested by the borer *Maruca vitrata*, which decreases the yield from a potential 2-2.5 to 0.05-0.5 tons per hectare.

The AATF is in partnership with the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia; CSIRO has transformed cowpea with the Bt *cry1Ab* gene for *Maruca* resistance. Initial confined field trials (CFTs) carried out in Puerto Rico were sufficiently promising for similar trials, conducted in Nigeria for two consecutive years, 2009 and 2010.

Water-Efficient Maize for Africa (WEMA)

Africa is a drought-prone continent and maize is very susceptible to drought stress. Since maize is the most widely grown staple crop in Africa, drought conditions inevitably lead to food shortages. In 2003, the World Food Program spent $0.57 billion on food aid for Africa (Doering, 2005). This problem will only be exacerbated in the years to come by global warming.

As a result, the Bill and Melinda Gates Foundation and Howard G. Buffett Foundation are funding a multi-partner project—managed by the AATF—to develop water-efficient maize for the continent. The *Bacillus* cold-stress protein gene, *cspB*, (Castiglioni et al., 2008) was donated royalty-free by Monsanto and is being transferred into African maize varieties by the CIMMYT, Monsanto, and the National Agricultural Research Institutes of Kenya, Uganda, Tanzania, Mozambique, and South Africa. The Monsanto lead commercial event, MON 87460, is being subjected to CFTs in South Africa by the Agricultural Research Council (ARC). Similar CFTs are planned for other member countries including Kenya, Uganda, Tanzania, and Mozambique through their National Agricultural Research Systems (NARS).

Maize Resistant to Maize Streak Virus

Maize streak virus (MSV) is endemic to Africa and is the major viral pathogenic constraint on maize production (Martin & Shepherd, 2009), making resistance a key target for crop improvement. For a number of years, scientists at the University of Cape Town and Pannar Seed have been developing maize resistant to MSV. For many reasons, the pathogen-derived-resistance strategy using the viral coat protein that was used to protect papaya from papaya ringspot virus (which effectively saved the Hawaiian papaya industry) is not effective against MSV.

A member of the *Geminiviridae* family, MSV has a small (2.7 kb), single-stranded circular DNA genome encoding the coat protein, movement protein, and two replication-associated proteins, Rep and RepA. As an alternative pathogen-derived-resistance strategy, maize was transformed with a truncated and mutated version of the gene encoding Rep, cloned downstream of the constitutive maize ubiquitin promoter. This gene is called $rep^{1\text{-}219Rb\text{-}}$ to denote the amino acids it encompasses (amino acids 1 to 219 of the 360-amino acid Rep), and a mutation (Rb^-) in a motif that is important in the viral lifecycle. Rep functions as a multimer to initiate viral DNA replication by the rolling circle method. Therefore, the rationale behind this approach is that upon viral infection of a plant cell constitutively expressing $rep^{1\text{-}219Rb\text{-}}$, the over-expressed non-functional Reps would bind to any newly-synthesized viral Reps and prevent the complex from binding to the viral origin of replication. Thus, the replication of the incoming viral DNA would be inhibited and consequently, the maize plant would be resistant to MSV.

Hi-II maize plants transformed with the $rep^{1\text{-}219Rb\text{-}}$ construct (which was in a pUC8 background) were crossed with an elite inbred line at Pannar. The resultant plants showed resistance to MSV when challenged (Shepherd et al., 2007). Subsequently, new transgenics were generated containing $rep^{1\text{-}219Rb\text{-}}$ in a minimal transgene cassette background. These lack selectable markers and antibiotic resistance genes. Challenges on T_1 and T_2 generation transgenics showed a reproducible reduction in infection percentages as well as a delay in the onset of symptoms, compared with a sensitive genotype (Golden Bantam) and a conventionally-bred MSV-tolerant hybrid, Pan77 (Figure 1).

The next step in the development of this crop will be CFTs planned for the 2012/2013 season.

Drought-tolerant Maize

Scientists at the University of Cape Town are also working on a project to develop maize tolerant to abiotic stresses such as dehydration, salinity, and temperature. The origin of the genes is the desiccation-tolerant resurrection plant *Xerophyta viscosa*. This species can withstand the loss of 95% relative water content (RWC) for many months. Upon watering, it can rehydrate to 100% RWC within a period of about 72 hours. A number of genes have been identified as being involved in the dehydration process. These include

- *XvSap1*, encoding a stress-associated protein, which contains seven trans-membrane motifs (Garwe, Thomson, & Mundree, 2006);
- *XvAld1*, encoding an aldose reductase, which converts glucose to sorbitol;

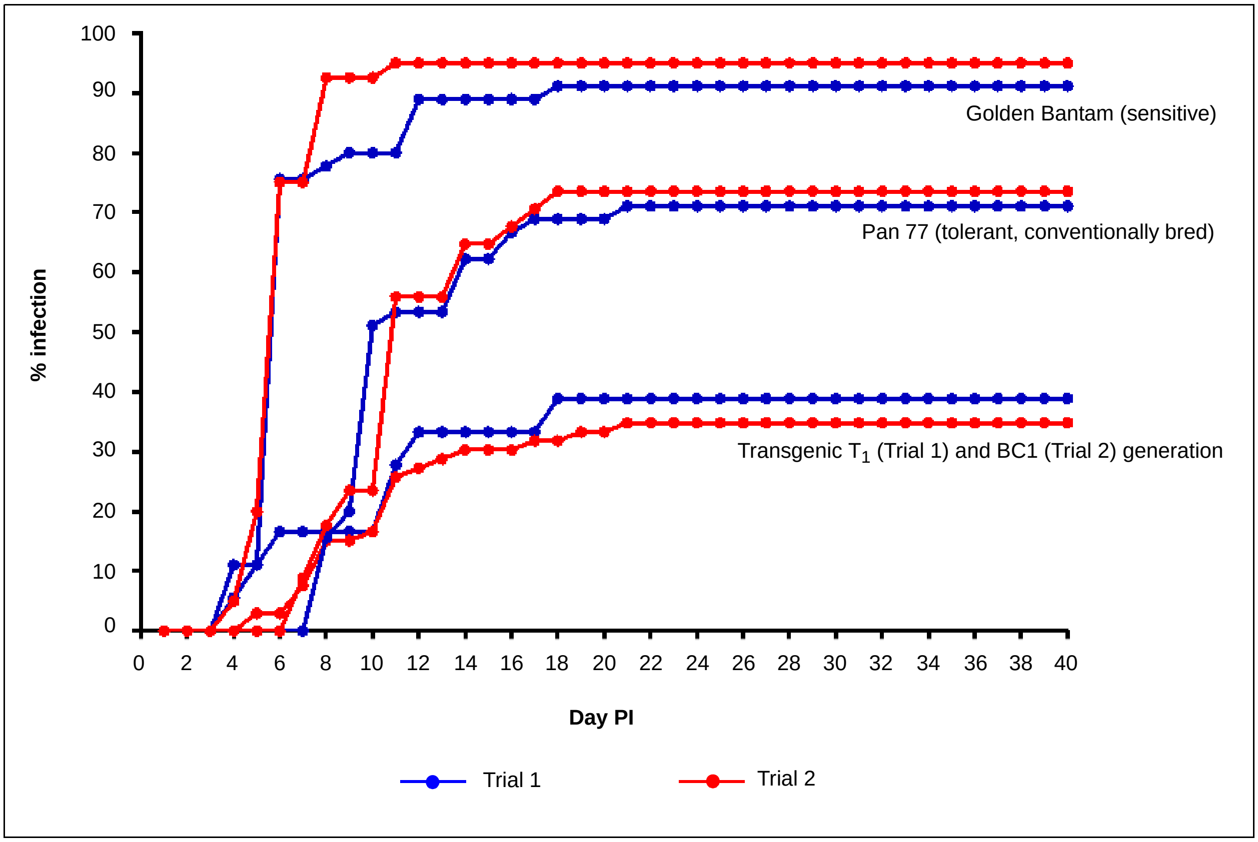

Figure 1. Percent infection of three genotypes of maize over two generations (first generation, Trial 1; second generation, Trial 2).

For each trial, 48 three-day old seedlings were challenged with MSV via three separate agroinoculations. The percent infection data represent the number of plants that were infected out of the total number challenged. For the transgenic maize, T_1 = transgenic offspring from initially transformed maize (T_0) crossed with an elite inbred line. BC1 = a backcross of T_1 generation transgenic maize with the recurrent parent. Pan77 is a conventionally-bred hybrid with tolerance to MSV. Golden Bantam is a variety of sweetcorn that is very sensitive to MSV. Note that percentages of transgenic plants infected are much lower than both the sensitive and tolerant genotypes, and that infection rates are reproducible for successive generations over two trials (each trial of which consisted of three separate agroinfections). Day PI = Day Post-Inoculation.

- *XvPrx2*, encoding a peroxiredoxin, an antioxidant involved in dehydration stress response; and
- *XvG6*, encoding a dehydration stress response protein of unknown function.

Tobacco plants transformed with some of these genes have been shown to be resistant to a variety of stresses including dehydration, salinity, and high temperatures (Garwe et al., 2006).

Given that some researchers have found that the constitutive expression of anti-stress genes in transgenic plants inhibits their growth in the absence of stresses, maize is currently being transformed with constructs in which these genes have been cloned downstream of the stress-inducible promoter *XvPsap1*.

African Biofortified Sorghum

More than half a billion people around the world rely on sorghum as a dietary staple. Its tolerance for drought and heat make it an important food crop in Africa (it is indigenous to Ethiopia and Sudan). However, it lacks certain essential nutrients. In order to give it added nutritional value, the Bill and Melinda Gates Foundation are funding the African Biofortified Sorghum project, run by an international consortium under the leadership of Africa Harvest, an African-based international non-profit organization. African biofortified sorghum contains the gene for a high-lysine storage protein from barley and has increased levels of Vitamin A, iron, and zinc.

In 2006, scientists from the Council for Scientific and Industrial Research (CSIR) in South Africa applied to the Registrar of the Directorate for Genetic Resources Management in the National Department of Agriculture, the body that administers the GMO Act of 1997 (as amended in 2010), to undertake greenhouse trials. This application was denied by the Executive Council (EC) on the following grounds.

- In view of the potential risks pertaining to environmental impact (as a result of gene flow), the Council recommended that this experiment be conducted on a non-indigenous species with no wild relatives in South Africa.
- Taking into consideration the Council's concerns about gene flow, the applicant should take note that the possibility of obtaining a trial release or general release authorization with this species—as with any other indigenous species—would be extremely low.
- The Council expressed concerns regarding the current containment levels of the facilities that would be involved in the proposed activities and indicated that such activities should be conducted in at least a Level 3 containment facility.

In its appeal, the CSIR pointed out that the South African Biotechnology Strategy stresses the importance of value-addition to indigenous crops. However, the decision by the EC could be interpreted to mean that no research on indigenous crops should be allowed.

The CSIR also noted that the EC was prejudging future applications for trials and/or general release and was taking into account irrelevant considerations.

Finally, the appeal noted that the CSIR did, indeed, have a Level 3 containment facility that had been approved by the Directorate for Genetic Resources.

Two appeals were turned down, but finally, in 2009, permission was granted. However, the damage done by this slow and complicated process has had many negative outcomes. One of these is that most of the R&D for this project has been moved to Kenya, where approval for GM sorghum greenhouse trials was obtained within three months, and trials began within five months. This outcome also shows that South Africa, which has the greatest expertise and capacity in plant biotechnology in Africa, is likely to lose the advantage for carrying out projects that involve applications for permits under the GMO Act. This is due to the uncertainty of the regulatory goals and the lengthy process each application requires. These types of projects will in the future most likely be funded and initiated in other African countries, such as Kenya, Nigeria, Uganda, and Malawi.

Insect-resistant Potatoes

The larvae of the potato tuber moth (PTM), *Phthorimaea operculella*, bore into potato leaves, stems, and tubers, causing extensive damage. In addition, fungi and mites can grow in the galleries formed by the PTM's burrowing, resulting in the decomposition of the tuber. The impact of the PTM fluctuates from season to season in response to climate, but reoccurs regularly at high levels and can cause up to R 40 million in losses per annum (Visser & Schoeman, 2004).

In July 2008, an application was submitted by the Agricultural Research Council (ARC) of South Africa to the Directorate of Biosafety of the Department of Agriculture, Forestry, and Fisheries for a general release of GM potato event SPUNTAG2. This event had been developed by Michigan State University and carried the Bt *Cry1la1* gene (Douches et al., 2002). The required information was submitted, including socio-economic impact data and a stewardship plan. However, on August 25, 2009, the application was rejected. The reasons for this refusal included socio-economic issues as follows (taken verbatim from the letter to the ARC from the Directorate of Biosafety).

- "The applicant provides studies undertaken with both commercial and subsistence producers. Within the commercial group it was evident that such farmers do not anticipate this event to present a significant lowering of inputs, as the same spraying regime is required to manage other pests which this event does not target. For the smallholder group it is clear that planting is dependent on a number of factors such as well-prepared fertile soil, sufficient rain, seed availability (certified, if possible), and access to pesticides, if required.
- There is no evidence that other pest management strategies against PTM have been considered or compared with the release of GM Spunta.
- The applicant makes several arguments of the value of this event for small-scale farmers, but it remains a concern of the entry of these GM potatoes into the formal trade. Segregation of the GM from non-GM would require an identity preservation system which is currently not in place.

- The capacity of small-scale farmers to implement risk-management measures could potentially be onerous.
- Considering the biology of potatoes, vegetative material (tubers) may be used for propagation, which may complicate risk management.
- PTM is not a major pest for stored potatoes but rather rodents."

There were also some technical issues, including that

- no data were presented of the expression levels of the Cry1la1 protein in tubers;
- concerns were expressed that the toxicity testing was conducted with boiled potatoes, as raw, freeze-dried potatoes were deemed to have been better suited;
- no evidence was presented that the known potato allergen, Sol t1 (patatin), was not over expressed in the GM potato; and
- no actual toxicity data of the cry protein on the target organism, PTM, was presented.

These issues were addressed in a reply from the ARC dated September 21, 2009, requesting to appeal the decision.

1. Information on many of the socio-economic issues can only be collected if the application is approved. This approval is needed to enable the farmer participatory evaluation, which must precede any decision on whether the ARC will use this trait for the improvement of South African potato varieties. Indeed, farmer participatory trials will help to answer many of the questions regarding the impact of the trait on potato production and farmers posed by the EC in its decision.
2. A general release approval for SpuntaG2 is essential for farmers to undertake assessments regarding productivity, production constraints, appearance, taste, storages, and marketability.
3. No identity preservation system has been required for transgenic maize, cotton, soybean, or canola used in South African formal markets for more than 10 years. Therefore, it is unlikely that an identity preservation system will be needed for marketing transgenic potatoes in formal markets. However, if this were to become necessary, a system would be possible based on existing systems for conventional potatoes that already segregate and identify specific cultivars in South African markets.
4. The use of vegetative planting material requires no additional effort compared to the use of true seed with other crops.
5. The levels of all potato pests vary from season to season, but PTM remains the primary storage pest. "The Prokonnuus 2006/2007 data collected at fresh produce markets in South Africa clearly indicate that tuber moth was the third major cause of spoilage in potatoes, after greening and mechanical damage, and caused million of Rands in losses."
6. The EC, in turning down the application, cited technical issues that needed to be addressed in order to approve the safety of the SpuntaG2 event. However the EC did not request the applicant to supply additional information in order to address these concerns. The process in the GMO Act gives the applicant the opportunity to provide additional safety information if this is identified as necessary by the regulatory authorities.
7. Regarding the technical issues:
   - Sound scientific reasoning (based on published literature) and conservative calculation were used to extrapolate the level of expression of Cry1la1 in tubers.
   - The EC incorrectly views the main objective of the animal feeding study with potatoes as a toxicology study. The main objective of the study was to assess the nutritional adequacy of the GM potato, which is consistent with the Codex Alimentarius guidelines (CAC/GL 45-2003).
   - Patatin is the major protein found in potato tubers, comprising 30-40% of the total soluble protein. Therefore, this protein is already expressed at a high level, and any "over expression" will not change the allergenicity profile of the modified potato. Neither the levels of protein nor individual amino acids showed differences between the Spunta and SpuntaG2 varieties.
   - Toxicity of the protein on the target organism was repeatedly demonstrated by the experiments showing that PTM larvae were killed when feeding on potato plants and tubers, both in the field and in the laboratory.

From the above, it could be argued that the EC overstepped its mandate when they determined that smallholder farmers would not need this technology. It is the mandate of the ARC and farmers themselves to assess whether this GM technology is appropriate for local use. Weak decision-making processes could jeopardize the ongoing funding for this and other public sector projects.

In conclusion, there are a number of very promising GM crops in the pipeline in sub-Saharan Africa. Their deployment—in many cases to aid smallholder, subsistence farmers—depends, however, on the approval by the relevant regulatory authorities. It is hoped that such authorities base their decisions on sound scientific reasoning.

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