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Emerging Agricultural Biotechnologies for Sustainable Agriculture and Food Security

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ABSTRACT: As global populations continue to increase, agricultural productivity will be challenged to keep pace without overtaxing important environmental resources. A dynamic and integrated approach will be required to solve global food insecurity and position agriculture on a trajectory toward sustainability. Genetically modified (GM) crops enhanced through modern biotechnology represent an important set of tools that can promote sustainable agriculture and improve food security. Several emerging biotechnology approaches were discussed in a recent symposium organized at the 13th IUPAC International Congress of Pesticide Chemistry meeting in San Francisco, CA, USA. This paper summarizes the innovative research and several of the new and emerging technologies within the field of agricultural biotechnology that were presented during the symposium. This discussion highlights how agricultural biotechnology fits within the context of sustainable agriculture and improved food security and can be used in support of further development and adoption of beneficial GM crops.

KEYWORDS: agricultural biotechnology, genetically modified crops, sustainability, food security, semiochemicals

INTRODUCTION

Producing enough food, feed, fiber, and biofuel to meet society's needs has been the goal of agriculture for thousands of years. Sustainable agriculture attempts to fulfill these same basic societal demands with an emphasis on preserving environmental quality, decreasing agricultural inputs, reducing environmental effects, and sustaining economic viability.¹ Over the next 30 years, the world population is projected to increase to approximately 9 billion people, and the increased demand for food, feed, fiber, and biofuel will challenge the agricultural sector to keep pace.¹ In addition to increasing population pressure, climate change and associated extreme weather events, as well as limitations on the amount of arable land and water that is devoted to agriculture, will affect agricultural productivity. Without the development of crops that can outperform current varieties, more land will need to be converted to cultivated acres, and more inputs will be needed to support increasing societal demands. For example, Tilman et al. estimated that an additional ~1 billion hectares (ha) of cultivated land will be needed by 2050 if current agricultural trends continue.² Achieving food security in the face of these challenges, without increasing the environmental footprint, will require an integrated and diversified approach.

Food security exists "when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy

life".³ Although food security in a global context is complex and hinges on many socioeconomic, infrastructural, and political factors, the agricultural sector's ability to maximize crop yield and production without compromising the environmental resource base will also be important. Agricultural biotechnology offers great potential for promoting sustainable agriculture and improving food security; however, the role and potential contributions of agricultural biotechnology in the context of sustainability and food security are only beginning to be realized.

The Convention on Biological Diversity (CBD) defines biotechnology as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use".⁴ Under this broad definition, agriculture has been using biotechnology to select for desired traits, improve germplasm, and enhance crop genetics through selective breeding for thousands of years. More modern genetic and molecular tools, including tissue culture, embryo rescue, double haploids, and marker-assisted breeding, also fall under the umbrella of agricultural biotechnology and give additional context and refinement to this definition. These biotechnology tools help facilitate the development of pure genetic lines and

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Figure 1. Golden mosaic disease symptom in field trials comparing genetically modified dry bean “carioca” seeded advanced lines developed by Embrapa to be resistant to bean golden mosaic virus (BGMV) (right panels) with their respective conventional recurrent parents (left panels).

have increased the speed and efficiency of selecting desired traits, relative to traditional breeding. Additionally, genetic and molecular tools are being used to produce transgenic (also referred to as genetically modified (GM)) crops, which are central to the field of modern agricultural biotechnology.

In 2014, over 181 million hectares of GM crops were grown globally.⁵ On the basis of current trends, the adoption rate of GM crops with biotech traits is expected to continue to increase, especially in developing countries.⁶ To date, most of the advancements in genetically modified crops have been focused on a few key row crops (e.g., cotton, maize, and soybean) that express insect-resistant proteins from *Bacillus thuringiensis* or that are tolerant to glyphosate and glufosinate–ammonium herbicides. Alternative technologies offering tolerance to different herbicides (e.g., dicamba or 2,4-D), as well as a variety of stacked traits offering herbicide tolerance in combination with multiple modes of action for insect resistance (e.g., Agrisure or SmartStax) are also becoming readily available to farmers. Further advancements in the field are increasing not only the variety of crops being modified (e.g., vegetable crops, wheat, rice, cowpea, and banana) but also the range of genetic modifications being advanced (e.g., enhanced nutrition, modified composition, improved digestibility, abiotic stress tolerance, virus, bacterial, and fungal resistance).

Several emerging biotechnology approaches were discussed in a recent symposium organized at the 13th IUPAC International Congress of Pesticide Chemistry meeting in San Francisco, CA, USA. The goal of this paper is to highlight advancements in the field of agricultural biotechnology within the context of sustainable agriculture and food security. Examples of emerging traits developed to help improve yield, reduce inputs, and provide protection from viral, bacterial, and fungal pathogens and insect pests, as well as enhance crop performance and productivity under abiotic stress conditions, are discussed. Additionally, the utility of using a life cycle assessment model to assess the benefits and potential impacts of agricultural biotechnology is also highlighted.

■ GENETICALLY MODIFIED VIRUS-RESISTANT COMMON BEAN

Common bean (*Phaseolus vulgaris* L.) is an economically, nutritionally, and socially important crop, especially in developing countries in Latin America and eastern and southern Africa. Brazil is the main producer and consumer country of *P. vulgaris*. The dry bean is a very popular and relevant crop, representing the major source of dietary protein. The Brazilian per capita consumption can be as high as 17 kg/person/year. The total growing area in 2013 was 1.89 million hectares, with a mean productivity of 1350 kg/ha.⁷ One of several factors that compromise common bean yield worldwide is the high number of destructive pathogens that attack the crop and cause serious damage. Among them is the bean golden mosaic virus (BGMV), a geminivirus transmitted by the whitefly (*Bemisia tabaci*). BGMV causes severe foliar yellow mosaic symptoms, stunted growth, deformation of pods and grains, and abortion of flowers. In Brazil, losses in grain yield may range from 40 to 100%, and at least 200,000 ha of Brazilian farmland is unusable for bean growth due to BGMV. Annual losses ranging between 90,000 and 280,000 tons of grain would be enough to feed 6–20 million adult Brazilians.⁸

Effective resistance to BGMV controlled by major genes has not been identified in *P. vulgaris* germplasm, despite over 40 years of conventional breeding efforts in Brazil. Insecticide spraying to control the whitefly and prevent virus incidence has been overused, with limited success and serious environmental concerns. For this reason, the Brazilian Agricultural Research Corporation (Embrapa) developed a GM dry bean resistant to the BGMV using a pathogen-derived resistance approach, based on RNAi technology.^{9,10} Biosafety assessment assays managed by Embrapa, but with the support of more than 10 different partner institutions, have already been performed according to the rules established by the National Technical Commission on Biosafety (CTNBio, Brazilian Government). The resulting biosafety dossier was submitted for evaluation in December 2010 and the GM virus resistant bean was approved for commercial growth in Brazil in September 2011.


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MsDef1      RTCENLADKYRGPCFSG--CDTHCTTKENAVSGRCRDD--FRCWCTKNC
MtDef4      RTCESQSHKFKGPCASDHNCASVCQTERFSG--GRCRGFRRRCFCTTHC
γ-core motif                                GHCXXXXXXC

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Figure 2. Amino acid sequences of MsDef1 and MtDef4. The consensus γ -core motif is shown. Eight conserved cysteines are shown in red.

“Carioca” seeded GM common bean advanced lines derived from Pérola and BRS Pontal cultivars have been developed.¹¹ Although not grown in other parts of the world, “carioca” seeded beans are the most consumed market class in Brazil, representing around 70% of the internal market. Field trials to evaluate the agronomic performance of the GM virus-resistant advanced lines, which are required for the registration of new cultivars in Brazil, have been conducted since 2012 in a national assay network coordinated by Embrapa. The seed production process (breeder seed and prebasic seed) also began in 2012. All of these aforementioned activities have been developed following a Stewardship Program specifically created for the Embrapa GM virus-resistant bean.

Partial results of the final field trials have demonstrated that no grain yield penalty has been observed when the GM virus-resistant advanced lines are compared with their respective recurrent parents. In addition, GM lines are resistant to BGMV under field conditions, presenting no visible symptoms, whereas BGMV severity ranging from 50 to 70% has been observed in the recurrent parents and control cultivars (Figure 1). At the moment, there is no technical concern about agronomic performance or BGMV resistance effectiveness of the GM advanced lines. Other modern “carioca” seeded dry bean cultivars developed by Embrapa are also being converted with the event conferring resistance to BGMV. After the conclusion of all final field trials, the superior virus-resistant line will be commercially released in the Brazilian domestic market, representing the first GM common bean cultivar in the world. The development of common bean GM cultivars resistant to BGMV will play an important role for the integrated management of the viruses transmitted by the whitefly and for food security in Brazil.

MECHANISMS OF ACTION OF ANTIFUNGAL PLANT DEFENSINS AND GENETIC ENGINEERING FOR DISEASE RESISTANCE

Fungal and oomycete pathogens impose major constraints globally on agricultural production and food safety. An estimated 10–15% of crop yields is lost due to these pathogens, despite the continued release of resistant cultivars and chemical fungicides. Safe and effective antifungal agents with novel fungus-specific mechanisms of action are urgently needed in agriculture. Plants express various cationic antimicrobial peptides that play a major role in plant defense against fungal and oomycete pathogens. Plant defensins are cysteine-rich antimicrobial peptides of 45–54 amino acids. They are constitutively expressed or induced in response to various biotic or abiotic stresses and are targeted to the apoplast or vacuole. They display a conserved tertiary structure characterized by the presence of one α -helix and three antiparallel β -strands. The α -helix is connected to the second β -strand through a cysteine-stabilized α -helix/ β -sheet (α/β) motif.^{12–15} The amino acid sequences of these defensins contain a signature γ -core motif GXCX_{3–9}C (where X is any amino acid) that is conserved among all antimicrobial peptides with disulfide bonds.¹⁶ Despite their structural similarity, primary amino acid sequences of plant defensins exhibit very low sequence homology. For example, apoplast-localized defensins, MsDef1

and MtDef4, from *Medicago sativa* and *Medicago truncatula*, respectively, are structurally similar, but share only 41% amino acid identity (Figure 2).

Several plant defensins exhibit potent antifungal activity in vitro at micromolar concentrations (Figure 3)¹⁶ but display different modes of action (MOA). Because of their cationic character, defensins are able to interact with negatively charged molecules present at the fungal cell membrane causing an increase in membrane permeability that leads to cell leakage and death.^{16–18} They also induce Ca²⁺ influx and K⁺ efflux and disrupt a Ca²⁺ gradient essential for polar growth of hyphal tips.^{19,20} Some defensins bind with high affinity to specific sphingolipids present in the fungal cell wall and/or plasma membrane of their target fungi.^{21,22} Alternatively, other defensins are internalized by fungal cells and target key components of fungal cell processes.^{23–25} The Shah Laboratory at the Danforth Center has been investigating structure–activity relationships and MOA of MsDef1 and MtDef4. Although both defensins permeabilize the plasma membrane of *Fusarium graminearum*,¹⁷ they exhibit different MOA. MsDef1 interacts with the cell wall associated sphingolipid glucosylceramide, and this interaction is required for its antifungal activity. MtDef4, on the other hand, is internalized into fungal cells and binds strongly to phosphatidic acid (PA), a key phospholipid second messenger in the cell.²⁵ The γ -core motif is a major determinant of the antifungal activity in vitro of MtDef4 (Figure 3) and plays a major role in enabling PA binding and fungal cell entry of MtDef4.²⁵ Studies are underway to understand the role this interaction plays in the MOA of MtDef4.

Because of their potent antifungal activity, defensins are being exploited in agricultural biotechnology applications to generate disease-resistant crops. Constitutive overexpression of several plant defensins significantly enhances resistance to fungal and oomycete pathogens in various transgenic crops.^{16,18,26–29} For example, expression of the apoplast-targeted MtDef4 in transgenic wheat confers strong resistance to an obligate biotroph, *Puccinia tritici*, which causes leaf rust disease (Kaur and Shah, manuscript in preparation). Nonetheless, the challenge is to achieve durable and robust resistance to fungal and oomycete pathogens in transgenic plants through expression of these proteins without compromising important agronomic traits and crop yields.³⁰ Several strategies have emerged to overcome that challenge. Precise temporal and spatial expression of defensins in plants using pathogen-inducible or tissue-specific promoters can minimize any potential deleterious effects of constitutive expression. Tailoring the expression of antifungal defensins to match the unique lifestyle of each fungal or oomycete pathogen can result in a more effective control of the disease.³¹ Moreover, coexpression of defensins and other antifungal proteins can provide better protection from fungal and oomycete infection.³²

In conclusion, with a better understanding of the structure–activity relationships and MOA of plant defensins, and the availability of adequate gene expression tools, robust and durable resistance to fungal and oomycete pathogens in transgenic crops is achievable. This technology offers the means to begin development of durable disease-resistant crops for increased

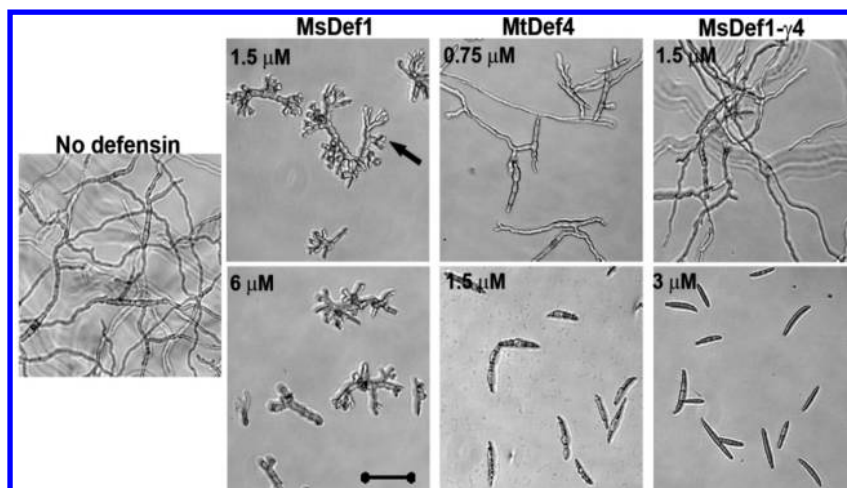


Figure 3. Inhibition of *F. graminearum* PH-1 conidial germination and hyphal growth at different concentrations of MsDef1 (1.5 and 6 μ M), MtDef4 (0.75 and 1.5 μ M), and the MsDef1 variant, MsDef1- γ 4, containing the γ -core motif of MtDef4 (1.5 and 3 μ M). The γ -core motif of MtDef4 determines the antifungal properties of MsDef1. Images were taken after 16 h of incubation of conidia with defensins. Bar = 50 μ m. Hyperbranching of hyphae in the presence of MsDef1 is indicated with a black arrow.

yields while substantially minimizing or eliminating the use of environmentally harmful chemical fungicides. Successful deployment of this technology will significantly contribute to food security and environmental sustainability.

■ SEMIOCHEMICALS AS NEW TARGETS FOR GM CROPS

The main currently registered insecticides, fungicides, and herbicides comprise small lipophilic molecules (SLMs) often developed from, or inspired by, natural product leads.³³ For example, the recently introduced butenolide insecticides, typified by flupyradifurone, are structurally related to the plant secondary metabolite stemofoline.³⁴ These SLMs can also be obtained directly from natural-based systems, particularly fermentation organisms (e.g., spinosad comprising spinosyns obtained from the yeast *Saccharopolyspora spinosa*). Thus, in nature there exist the genes for the biosynthesis of SLM insecticides, and other pesticides, that could be used to furnish a new generation of GM crops resistant to pests. GM crops expressing these SLMs as pesticides could have the same efficacy as current pesticides but with the added advantage that they could be delivered, as with earlier GM pest-resistant crops, sustainably via the seed or other planting material.³⁵

There are many natural SLMs showing promise as targets for new GM pest-resistant crops, and these include compounds that because of instability and potential nontarget effects could be developed only by GM routes (e.g., the benzoxazinoids or hydroxamic acids).³³ In addition to directly toxic MOA, GM pest-resistant crops expressing SLMs that act through sophisticated signaling mechanisms (e.g., involving pheromones and other semiochemicals (signals acting between species)) also have great promise for emerging MOA.³⁵ For example, the aphid alarm pheromone, (*E*)- β -farnesene, has a negative impact on aphid pests and increases foraging by antagonistic organisms such as aphid parasitoids. Genetic engineering in an elite wheat variety has produced stable expression of highly pure (*E*)- β -farnesene, giving excellent results in the laboratory against cereal aphids and in increasing foraging by braconid parasitoids of aphids. Field trial results³³ are currently being analyzed.

In addition to pheromones, new targets for other semiochemicals, including a series of defense-related isoprenoid

oxidation products, are being studied. A method termed the “push–pull” utilizes companion crops to release semiochemicals, including these oxidized isoprenoids, to “push” away pests from the food producing crop and to “pull” them into trap crops with other uses.³⁶ This is exemplified in a push–pull system for protecting cereals (e.g., maize and sorghum) produced for small-holder sub-Saharan African farmers, who are not normally able to purchase insecticides.³⁷ Under this system, the trap crops comprise valuable ruminant forage grasses, and the intercrops comprise the cattle forage grass molasses grass (*Melinis minutiflora*) or various species of the forage legumes in the genus *Desmodium*, which release oxidized isoprenoids termed homoterpenes, such as (*E*)-4,8-dimethyl-1,3,7-nonatriene (I). This approach is already being taken up by tens of thousands of farmers in East Africa,³⁷ thereby demonstrating the value of these homoterpenes in insect pest management. Thus, the homoterpenes present potentially valuable new targets for using defense semiochemicals in GM crops, and associated biosynthesis genes are being characterized.

For the new approaches described herein, nonconstitutive expression of the associated genes represents another innovative aspect. Thus, gene promoter sequences are employed that are activated via use of a further group of SLMs active as defense elicitors. These include *cis*-jasmones, which, although related to the plant stress-induced and defense-related hormone jasmonic acid (JA), signals differently³⁸ and elicits defense in a more consistent way³⁹ and without phytotoxicity³⁵ as is mostly found with JA. *cis*-Jasmones can also elicit priming of defense in which the effect is manifested once the insect attack occurs.⁴⁰ New elicitors are being characterized that, as with *cis*-jasmones, do not require introduction to the plant by feeding damage, for example, from eggs of the pest⁴¹ and via mycorrhizal mycelial networks.^{42–45}

By exploiting the approach of deploying semiochemicals via GM crops, a sustainable system for seed delivery of pest management in food crops is created. Development of new MOA based on SLMs has great potential for controlling insect pests and providing alternatives for managing insect resistance. The additional benefits of yield stability and reduced pesticide use will be important considerations for food security and agricultural sustainability.

■ ENGINEERING ABIOTIC STRESS TOLERANCE IN CROP PLANTS

Fertilizer, water availability, and water quality are critical for crop productivity, and their availability directly affects food security in both developed and developing nations. Salinity and water deficit stress alone account for >35% loss in agricultural yield potential, and the efficiency of applied nitrogen fertilizers is $\leq 50\%$ due to losses attributable to leaching, runoff, soil fixation, and gaseous emissions.⁴⁶ Working with commercial and humanitarian development partners, Arcadia has seen positive yield results in the field for technologies that improve nitrogen use efficiency (NUE), water deficit stress tolerance, and salinity tolerance. These technologies have also shown positive results in our first field tests with combinations of nitrogen and water deficit stress.

Nitrogen Use Efficiency. Engineering of plants to be more nitrogen efficient would both increase farmer productivity and decrease the environmental impact of nitrogen applications. The NUE technology developed at Arcadia is based on modulating the expression of an aminotransferase gene. Whereas the GS/GOGAT cycle is the major route of nitrogen (N) assimilation in plants, altering the expression of enzymes directly involved in this cycle has not led to reproducible, field-demonstrated NUE. Aminotransferases are integral to N assimilation for the production of amino acids and N allocation in plants. Alanine aminotransferase enzymes catalyze the reversible formation of alanine and 2-oxoglutarate from glutamate and pyruvate. Increased NUE in transgenic plants expressing an alanine aminotransferase (AlaAT) from *Hordeum vulgare* under the control of a stress-inducible promoter from the *Brassica napus* turgor-responsive gene (btg26) was first demonstrated in canola.⁴⁷ Arcadia's NUE technology enables plants to absorb and utilize nitrogen fertilizer much more efficiently than their nontransgenic controls. This results in the same high yields as conventional crops, while using half as much nitrogen fertilizer, or higher yields if using the same amount of fertilizer (unpublished results). In either case, less nitrogen escapes into the water and air.

Salt Tolerance Technology. Arcadia's salinity-tolerance technology is based on the overexpression of plant vacuolar Na^+/H^+ antiporter(s) (NHXs).^{48,49} Vacuolar NHXs catalyze the electroneutral exchange of cytoplasmic sodium (and potassium) with vacuolar protons. NHX overexpression promotes the sequestering of sodium ions into the vacuoles of the cells, where it is not toxic and contributes favorably to the osmotic balance of the cells and plant tissues. This strategy, which is based on the characteristic high activity of vacuolar NHX activity observed in salt-tolerant halophytes, promotes the tolerance of shoot tissues to sodium. There is also evidence that the overexpression of NHX in roots promotes K^+ homeostasis under saline conditions.⁵⁰ It permits the growth and production of seed under salinity stress levels that would otherwise have a negative impact on yield. The technology is applicable to a wide range of crops, including maize, rice, soybeans, wheat, and vegetables. Arcadia is also currently evaluating complementary salinity tolerance genes for rice in collaboration with the United States Agency for International Development (USAID) and the Bangladesh Rice Research Institute (BRRI), with the aim of providing robust yields on cropland under chronic salinity stress. Arcadia's salt-tolerance technology can improve farming efficiencies and reduce the need to expand agricultural activities into new areas. In addition, this technology can reduce the need

for fresh water by allowing increased use of lower quality (brackish) water.

Drought Tolerance and Water Use Efficiency Technology. The technology being developed at Arcadia to address water deficit stress is based on the production of cytokinins under stress conditions. This approach has been shown in tobacco⁵¹ to be effective in preserving yields under chronic deficit irrigation and at mitigating yield loss under extended periods of soil drying. The transgenic construct (pSARK-IPT) contains a maturation-induced promoter (SARK) that controls the expression of isopentenyltransferase (IPT), which is the rate-limiting enzyme in cytokinin biosynthesis. Plants typically respond to water stress by reducing transpiration. Initially this will induce stomatal closing. Senescence and abscission of leaves for the recovery of nitrogen and photoassimilates and the reduction of canopy size are typical adaptive responses that allow plants to set seed under prolonged or severe stress. However, the yield is greatly reduced. In crop plants, a severe yield reduction is considered crop failure. Better control over senescence initiation via cytokinin production provides protection against yield losses in pSARK-IPT transgenic plants subjected to limiting water conditions.

Combined Technologies. Crop plants are often affected by different abiotic stresses during a single growing season. Therefore, combining NUE and drought- and salinity-tolerance technologies into a single construct is technically and strategically advantageous even without considering the potential synergies between the technologies. Field trials of rice incorporating the "triple-stack" technology, which includes NUE, water use efficiency (WUE), and salt tolerance (ST), have shown substantial yield increases over conventional rice. In two years of field trials, Arcadia's triple-stack rice produced yield increases of 13–18% under various nitrogen application rates, 12–17% under water stress conditions, and 15% under combined stress. Parallel trials under salt stress in greenhouse conditions showed yield increases as high as 42%. These results demonstrate that these traits can work in combination to improve crop performance under a number of stress conditions.

Both NUE alone and stacked technologies in the NERICA (New Rice for Africa, an interspecific hybrid rice developed specifically for dryland cultivation) rice background are being developed in collaboration with the African Agricultural Technology Foundation (AATF). Field trials have demonstrated NUE efficacy at The International Center for Tropical Agriculture (CIAT) in Colombia, and trials are ongoing in Ghana, Uganda, and Nigeria. Arcadia is developing similar trait stacks in other crucial food and feed crops, including wheat and soybeans. Developing and developed nations will be affected by the need to support a growing global population while utilizing limited agricultural resources such as land, fertilizer, and water. With traits such as NUE, WUE, and ST, alone or in combination, we expect a reduction in demand on these limited resources. This, combined with improvements in yield traits, pest resistances, and sustainable farming practices, will be a contributor in overall improved global food security.

■ GENETICALLY MODIFIED (GM) BANANAS RESISTANT TO XANTHOMONAS WILT (BXW) DISEASE

Banana and plantain (*Musa* sp.), the eighth most important staple food crop in the tropics and subtropics, is grown in more than 140 countries, with an annual world production of around 144 million tons.⁵² Approximately one-third of total bananas are produced in Africa, mainly in the Great Lakes region of East

Africa, including Burundi, Rwanda, Democratic Republic of Congo, Uganda, Kenya, and Tanzania.⁵² However, banana production is affected by many diseases, such as black Sigatoka, *Fusarium* wilt, banana *Xanthomonas* wilt (BXW), banana bunchy-top virus (BBTV), and banana streak virus (BSV), and pests, such as weevils and nematodes.^{53,54}

BXW, caused by *Xanthomonas campestris* pv. *musacearum* (*Xcm*), is considered to be one of the most devastating diseases of banana and the biggest threat to banana production in the Great Lakes region of eastern and central Africa.⁵⁵ BXW was first reported in Ethiopia in *Ensete* species and then in banana.^{56,57} Outside Ethiopia, BXW was first identified in Uganda in 2001⁵⁴ and subsequently in the Democratic Republic of Congo,⁵⁸ Rwanda,⁵⁹ Kenya, Tanzania, and Burundi.⁶⁰ Once BXW is established in fields, it is difficult to manage due to lack of availability of chemicals, biocontrol agents, or resistant varieties.⁵⁵ Currently, the disease is managed by cultural practices, including cutting and burying of infected plants, restricting the movement of BXW-infected banana suckers (planting materials) from affected fields to disease-free areas, debudding, and the use of sterilized farming tools. However, the adoption of such practices has been inconsistent among farming communities as they are very labor intensive.⁶¹

The lack of known genetic resistance in banana germplasm against *Xcm*, the difficulties associated with conventional breeding of this vegetatively propagated crop, and low adoption of labor-intensive cultural practices favor biotechnological approaches to develop BXW-resistant varieties. Transgenic technology has opened new horizons in banana improvement, particularly for varieties that are not amenable to conventional breeding. Due to lack of cross-fertile wild parents in many banana-producing areas and the male and female sterility of most edible cultivars and clonal propagation, there is a low risk of gene flow, which makes a transgenic approach even more attractive.

In the absence of known host plant resistance against *Xcm* in banana germplasm, scientists at the International Institute of Tropical Agriculture (IITA) and the National Agriculture Research Organization (NARO)—Uganda have been investigating the potential of defense genes, hypersensitive response-assisting protein (*Hrap*) and plant ferredoxin-like protein (*Pflp*) from sweet pepper, for BXW disease resistance in banana. The *Hrap* is a plant protein that enhances the harpinPSS-mediated hypersensitive response (HR), a common plant defense mechanism to protect plants against invading pathogens.⁶² *Hrap* dissociates harpinPSS multimeric forms into dimers and monomers, which induces a stronger hypersensitive cell death (HCD) necrosis. There are studies demonstrating enhanced resistance against virulent pathogens in transgenic tobacco and *Arabidopsis* overexpressing the *Hrap* gene.^{63,64} Similarly, the overexpression of sweet pepper *Pflp* gene in transgenic tobacco, tomato, orchids, calla lily, and rice has shown resistance against a broad range of bacterial pathogens such as *Erwinia*, *Pseudomonas*, *Ralstonia*, and *Xanthomonas* spp.^{65–69} The disease resistance provided by overexpression of the sweet pepper *Pflp* gene in transgenic plants is reported to be due to induction of defense responses through enhanced production of active oxygen species (AOS) and activation of the HR in resistant plants during infection with bacterial pathogens.^{66,70}

The wilt-resistance genes, *Pflp* and *Hrap*, were licensed by the AATF on a royalty basis from the patent holder Academia Sinica in Taiwan. IITA and NARO—Uganda have developed hundreds of transgenic lines of banana using *Hrap* and *Pflp* genes. These lines were characterized for the presence and copy number of

gene by PCR and Southern blot analysis and gene expression by qRT-PCR. The transgenic lines were evaluated for resistance against *Xcm* using rapid in vitro bioassays and artificial inoculation of potted plants under glasshouse conditions. The transgenic banana plants conferred strong resistance to BXW, in both the in vitro assay and glasshouse screening test.^{71,72} The 65 resistant lines, selected on the basis of enhanced resistance to BXW using potted plants in the glasshouse, the presence of low copies of the transgene, and detectable gene expression, were further evaluated in a confined field trial at the National Agricultural Research Laboratory (NARL), Kawanda, Uganda. The majority of transgenic lines had significantly higher resistance in comparison to control nontransgenic plants.⁷³ Eleven of these transgenic lines (7 *Hrap* lines and 4 *Pflp* lines) were highly resistant, demonstrating 100% disease resistance for two successive crops cycles (mother and ratoon) in comparison to control nontransgenic plants. Approximately 85–93% resistance with mother plants and 100% resistance with ratoon plants was also observed in an additional five lines. The field trial results also confirmed the transfer of the disease resistance trait from mother to progeny in several lines. Aside from enhanced resistance to BXW, the transgenic lines also showed flowering and yields (bunch weight and fruit size) similar to those of nontransgenic plants, indicating there were no observable unintended impacts of the transgenes on crop performance.⁷³ The best 10 lines were further planted in a second confined trial with more replicates to test the durability of disease resistance and agronomic performance. These lines will be grown in multilocation trials to test them in different environmental and climate conditions. It is well-known that pathogens can evolve and single gene-based disease resistance can break down. To avoid this, we are stacking these two genes together in the same line to enhance the durability of the resistance trait.

All transgenic plants are required to undergo thorough and rigorous safety and risk assessments before commercialization. The *Hrap* and *Pflp* proteins are not listed as being potential allergens in AllergenOnline, predicting that these proteins are safe for human consumption.⁷³ These proteins are present in several plant species, such as tobacco, *Arabidopsis*, rice, and vegetable crops such as pepper, which are even eaten raw as salads. The banana transgenic lines will be analyzed for food and environmental safety in compliance with biosafety regulations before the varieties are released to farmers. Currently, we are studying environmental impacts, such as nontarget effects of disease-resistant bananas on soil microorganisms in the banana rhizosphere.

The transgenic bananas expressing *Hrap* and *Pflp* may also provide resistance to other bacterial diseases, such as moko, bugtok, or blood disease, which are affecting banana production in Latin America, Caribbean, Philippines, and Indonesia. Bananas are vitally important for sub-Saharan Africa, not only for food security, but also as an important local cash crop for small-scale farmers. Food security studies revealed that bananas constitute 30–60% of the daily per capita caloric intake in Uganda, Rwanda, and Burundi.⁷⁴ BXW-resistant varieties would boost the available arsenal to fight the BXW disease epidemic and save the livelihoods of African farmers, who depend on bananas as a staple food crop.

■ VIRUS-RESISTANT CASSAVA FOR AFRICA (VIRCA)

Among the crops being grown by small-holder farmers in sub-Saharan Africa, cassava (*Manihot esculenta* Crantz), a root crop, is an important staple food crop. It is estimated to be a major source



Figure 4. Cassava brown streak disease (CBSD) resistance using RNAi technology in cassava: (left) non-transgenic control; (right) transgenic event expressing the siRNA cassette.

of food and income for more than 250 million people in Africa and nearly a billion people globally.^{75,76} Cassava is a hardy and drought-tolerant crop that can be grown on marginal lands with very little input costs for farmers. Cassava is also an ideal food security crop for small-holder farmers because the tuberous roots can be stored in the soil for up to 3 years after planting without deterioration in quality and can be harvested as needed.^{77,78} In addition to being a staple food crop, cassava roots and flour offer a huge potential for use in feed, starch, brewery, biofuel, and other industrial applications. Despite substantial efforts being made by agronomists and breeders for crop improvement, the yield of cassava in Africa remains very low compared to other parts of the world and substantially lower than those of other food crops grown with intensive crop management practices.^{75,79}

In addition to lack of resources available to small-holder farmers for input costs, biotic factors impose severe limitations on the yield and productivity of cassava. In particular, two viral diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD), cause major yield losses.^{80,81} CMD is a foliar disease that has resulted in devastating yield losses in sub-Saharan Africa. Recently, several tolerant varieties have been developed through conventional breeding to combat CMD using the source of resistance available within the cassava germplasm. CBSD affects cassava by causing brown necrotic lesions on roots, resulting in complete spoilage of edible roots and up to 100% yield loss. CBSD is spreading in East Africa and is considered to be a major threat to cassava production in this region.⁸⁰ CBSD has been recognized as one of the seven most dangerous crop diseases in the world, capable of severely affecting global food security.⁸² CBSD is caused by two viruses, cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV), belonging to the Potyviridae family. CBSD shows subtle and hardly noticeable foliar symptoms but results in complete spoilage of edible roots. At present, for CBSD there is no reliable source of resistance available in cassava germplasm for use in conventional breeding programs.

The VIRCA project is focused on developing pathogen-based RNAi technology to combat viral diseases for increasing yield and production of cassava. Using this technology, researchers at the Institute for International Crop Improvement (IICI) at Danforth Center, USA, in collaboration with the National Crops Resources Research Institute (NaCRRI) in Uganda, the Kenyan Agricultural and Livestock Research Organization (KALRO), and the International Institute of Tropical Agriculture (IITA) in Kenya, are developing improved virus-resistant varieties of cassava. VIRCA is a public and private sector partnership supported by the Bill and Melinda Gates Foundation, the Howard Buffett Foundation, USAID, and the Monsanto Fund.

VIRCA was initiated in 2005 and is currently in the second phase of funding for technology development and confined field testing for the release of improved varieties to small-holder farmers.⁸¹ The primary focus of the project in this phase is to characterize and test the transgenic CBSD resistance lines developed using the RNAi technology. For CBSD resistance, an expression cassette designed to generate siRNAs against the coat protein (CP) sequences of two viruses (CBSV and UCBSV) that cause CBSD was inserted into the transgenic lines of farmer-preferred cassava varieties. Confined field trials, approved by the appropriate national regulatory authorities, with the transgenic lines are currently in progress for elite event selection in both Uganda and Kenya. To date, the results obtained from these trials demonstrate conclusively the efficacy of RNAi technology for sustained, season-long, and durable control of CBSD necrotic root rot (Figure 4). The elite events selected from these trials will be tested in multilocation trials in both countries to generate data for full regulatory dossiers.

The VIRCA project will provide the improved virus-resistant varieties to small-holder farmers in Uganda and Kenya through existing distribution channels and with no technology cost to the farmer. However, approval from in-country regulatory agencies is required before improved transgenic varieties can be distributed to farmers. Following international guidelines, the VIRCA team is working with national government regulatory authorities in Kenya and Uganda to define the specific data requirements for food, feed, and environmental safety evaluation of virus-resistant cassava varieties for eventual general release. In Uganda and Kenya, excellent infrastructure exists for confined field testing of transgenic events. Confined field trials are, therefore, progressing smoothly in compliance with national regulatory authorities' guidelines and regulations. Each successful field trial generates data for an eventual dossier for safety assessments that would enable approval of improved cassava varieties for small farmers to grow, resulting in increased amount of available food and income. Even so, the situation in each country may present a challenge in the final stages of regulatory approvals. For example, in Kenya, the Biosafety Bill was passed in 2009, but there is no precedence for farm release of a GM crop. In Uganda, the Biosafety Bill has been advanced to the Ugandan Parliament and is likely to be enacted soon. Although there is a very strong commitment and willingness on the part of technology developers, partner organizations, and government agencies for science-based approval and rapid release of virus-resistant cassava varieties, considering the current regulatory environment in both target countries, a much more concerted effort will be needed for general release of such varieties.

Sustainable agricultural production for food security is a global issue, but the impact of food insecurity is likely to be more pronounced in the developing world, especially in the sub-Saharan African region. In this region, a large proportion of farmers are small land holders who are primarily dependent on subsistence farming of crops, such as cassava, on marginal land for food and income. The availability of virus-resistant cassava varieties to small-holder farmers, therefore, will have a substantial impact on improving the yield and productivity of cassava and will eventually play a major role in enhancing the food security and well-being of resource-poor farmers.

■ USING LIFE CYCLE ASSESSMENT (LCA) TO ASSESS ENVIRONMENTAL AND SOCIOECONOMIC IMPACTS OF GM CROPS

The benefits of the current agricultural biotechnology traits (specifically insect-resistant and herbicide-tolerant GM crops) are well-documented. They have been shown to reduce the use of pesticides, labor, and machinery and at the same time have helped to increase yields or provide greater yield stability. Nevertheless, the potential contributions of agricultural biotechnology to support sustainable development in agriculture remain controversial. A holistic and comprehensive framework for the assessment of the environmental and socioeconomic impacts of different production systems may help to bring transparency to this discussion.

LCA is a useful tool for quantitative sustainability assessment along value chains and across industry sectors, and it is increasingly being used to assess the environmental impacts of agriculture (see, for example, refs 83–85). LCA is based on a “cradle-to-grave” approach, which begins with the gathering of raw materials from the earth to create an input needed in the production system (in the case of agriculture, for example, fertilizer or crop protection products) and ends at the point when all materials are returned to the earth (through emissions or disposal). LCA enables the estimation of the cumulative environmental impacts resulting from all stages in the product life cycle, often including impacts not considered in more traditional analyses (e.g., raw material extraction, resource depletion, energy consumption). By including the impacts throughout the product life cycle, LCA provides a comprehensive view of the environmental aspects of the product or process and a more accurate picture of the true environmental trade-offs. An international framework for LCA exists and gives guidance for standardized procedures for assessment.^{86,87}

On the basis of this framework, a holistic methodology called AgBalance has been developed that integrates the results of up to 69 indicators covering the environmental, economic, and social aspects of agricultural production.⁸⁸ In addition to considering energy use, emissions, ecotoxicity, and resource efficiency, other environmental indicators relevant to agriculture are also included (e.g., land use, water use, soil health, and biodiversity). In terms of economic assessment, both production costs and economic performance are taken into account, with the functional unit being defined relative to quantity and quality (e.g., 1 ton of maize grain). Production costs are grouped into variable and fixed costs and are quantified using an overall total cost of ownership for the defined functional unit.⁸⁹ Economic performance is assessed using farm profitability as the central criterion for economic sustainability. The social assessment in AgBalance is based on the UNEP-SETAC guidelines for social LCA of products⁹⁰ and defines five stakeholder categories (farmer, consumer, local

community, internal community, and future generations) for which appropriate indicators are included in the method.

The AgBalance model was applied in a case study to assess the environmental and socioeconomic impacts of herbicide-tolerant and insect-resistant (*Bacillus thuringiensis*, Bt) GM maize varieties. In this case study, the production of non-GM maize was compared to (1) herbicide-tolerant GM maize (specifically tolerant to glyphosate (Roundup Ready, RR)); (2) GM maize containing both herbicide tolerance and insect resistance (RR&Bt); and (3) a GM maize variety having multiple modes of both herbicide tolerance and insect resistance (SmartStax).⁹¹ Results from this study have been described previously.⁹¹ Briefly, the LCA of this case study showed a positive effect from the GM products in six of eight categories. These categories included land use, soil impact, ecotoxicity potential, emission, energy consumption, and resource consumption. Of the remaining two categories, biodiversity and water use, positive results were observed for RR&Bt and SmartStax but not for RR. The negative result for RR on biodiversity was potentially due to the decreased weed abundance in the maize field and other indirect effects of the herbicide. The increased herbicide usage was also associated with increased water usage, thus causing a negative result for water use.

More specifically, the case study showed that land use was reduced by up to 26% compared to non-GM maize due to higher yields, and soil erosion was decreased by 72% by the adoption of no-till cultivation practices, which are favored by herbicide-tolerant corn varieties. The positive effect on ecotoxicity potential was related to reduced intensity of insecticide use. The life cycle perspective showed that the production and use of fertilizer was the main driver in the energy consumption, resource consumption, and greenhouse gas emissions categories. For example, energy consumption was dominated by the production of fertilizer, which constituted roughly $\geq 75\%$ of all energy required in all four production systems. Similarly, the emissions impact category was highly determined by emissions related to the production and use of nitrogen fertilizer. For example, greenhouse gas emissions were dominated by 89% in the case of non-GM to up to 95% in the case of SmartStax by production of fertilizer and field emissions from nitrification processes in soils. This LCA showed that GM traits contributed to increased productivity and increased sustainability from an environmental as well as socioeconomic perspective. In the aggregated sustainability score across all three dimensions, the RR, RR&BT, and SmartStax products support approximately 7, 15, and 17% better overall sustainability scores than the non-GMO alternative, respectively. Particularly, the RR&BT and SmartStax products supported higher yield, reduced cost, and reduced environmental burden from insecticide use. Herbicide-tolerant maize may also have a positive overall impact if the trait provides yield stability and no-till cultivation practices are used, which may reduce soil erosion and fuel use associated with field operations. Although the data used in this case study do not represent all agricultural biotechnology traits, they highlight the value of LCA for identifying the main drivers of agricultural sustainability and inform our understanding of how agricultural biotechnology can complement a portfolio of agricultural tools for maximizing sustainability and food security in the future.

■ DISCUSSION

Total arable land that is devoted to agriculture is limited and in some regions of the world may be of poor quality for intensive agricultural production. Responsible stewardship of environ-

mental resources, such as water, topsoil, renewable and nonrenewable energy, and nutrient inputs, is central to sustainable agriculture. In the past, traditional breeding has been used to select for crop varieties with improved characteristics (e.g., increased yield, improved stress tolerance, enhanced nutrition). However, the advent of modern biotechnology and the development of GM crops have enhanced the breeder's toolbox and allowed agriculture to move much more rapidly on a trajectory toward sustainability. These technologies have a broad and proven track record of improving yield and prosperity, with small-holder farmers in developing regions benefiting more than those in developed regions.⁹² The innovations discussed here are just a few examples of what is possible for improved food security and environmental stewardship. Although these new technologies hold much promise, it is important to recognize that agricultural biotechnology represents only one set of tools for improving agricultural sustainability and food security. An integrated approach including precision agriculture for input applications, conservation tillage, cover cropping, increased crop diversity, and other best management practices will all be important for enhancing sustainability and managing agriculture's environmental burden.⁹³

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Notes

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