CHAPTER

Genetically modified bananas for communities of the great lakes region of Africa

6.2

Namanya Priver, PhD, Tindamanyire Jimmy, PhD, Buah Stephen, PhD, Namaganda Josephine, PhD, Kubiriba Jerome, PhD, Tushemereirwe Wilberforce, PhD

National Agricultural Research Laboratories-Kawanda, National Agricultural Research Organisation, Kampala, Uganda

Introduction

Over 50 million people in the East and Central African region including Uganda, Tanzania, Rwanda, Burundi, DR Congo, and Kenya depend on the East African Highland banana (EAHB, AAA-EA, Musa spp.), a unique type of cooking bananas, as a staple food and for income. East and Central Africa are considered a secondary center of diversity for the EAHBs, also called Matooke. Annual regional production is worth US$ 4.3 billion, which is about 5% of the East and Central African (ECA) region's gross domestic product (FAOSTAT, 2014). Banana has the unique advantage of producing acceptable yields amid erratic rainfall, coupled with an all-year-round fruiting characteristic. It is therefore, not surprising, that there is relatively less poverty and food insecurity incidences among the banana-dependent communities. The banana's extensive root system and leaf canopy have environmental benefits in terms of reduced soil erosion and stabilizing agroecologies (Karamura et al., 2016). Furthermore, the banana forest—like plantations capture significant amounts of carbon dioxide from the atmosphere, which is quickly recycled into soil organic matter (Kamusingize et al., 2017).

The average yield of the banana crop on-farm is estimated at 10 ton/ha/year in Uganda, yet the crop's potential is over 60—70 ton/ha/year; while the commercial systems of India and Ecuador are reported to produce bananas up to 120 ton/ha/year. This yield gap is attributable to a complex of biotic constraints such as banana bacterial wilt (also called banana *Xanthomonas* wilt, BXW), weevils, fusarium wilt, nematodes, black Sigatoka; and abiotic stresses (nutrient deficiencies and moisture/

Genetically Modified and Irradiated Food. https://doi.org/10.1016/B978-0-12-817240-7.00007-3

drought stress) (Wairegi et al., 2010). There have been attempts to increase production by increasing the land area under banana cultivation over the past 50 years in order to meet the escalating food demands from a high population growth rate of about 9.1 million people per year (AfrDB, 2012). However, this is not sustainable since the available arable land area is limited. Uganda's National Banana Research team with its global network of research for development continuously generates and deploys technologies that aim at adequately addressing the above problems to increase production per unit area per unit labor force. The yield losses from some of the most important biotic and abiotic constraints of banana are summarized in Table 6.2.1.

The key pests and diseases can be feasibly managed using sanitation practices and clean planting materials. However, sanitation practices are labor intensive and therefore not sustainable. For instance cultural control for the destructive BXW has very high implementation costs arising from the need for whole communities to act in order to manage the disease. This involves engaging not only many farmers but also their supportive and massive administrative and political machinery (Kubiriba and Tushemereirwe, 2014). As is the case for all crops, the most effective way of addressing the problems is through use of host plant resistance. Using conventional breeding to develop the resistant bananas is the preferred approach in Uganda. However, most preferred varieties are sterile and so cannot be improved through conventional breeding. Furthermore, the few that have minimal fertility have to date yielded hybrids that are less acceptable to consumers than the land races largely because the resistance is obtained from inedible wild sources. The only option available to address these challenges is to use genetic engineering to insert the lacking traits.

Using biotechnology for development of banana with pest and disease resistance

Banana researchers have made great strides in crop improvement through conventional breeding leading to the release of high-yielding banana hybrids. However, consumer acceptability is vital for products with resistance to major pests and diseases such as weevils, nematodes, banana bacterial wilt (BXW)m and *Fusarium*

Table 6.2.1 Yield Loss due to abiotic and biotic constraints of banana.

Constraint	Yield loss (%)	Source
Black Sigatoka	30–50	Tushemereirwe et al. (2003)
Banana *Xanthomonas* Wilt (BXW)	80–100	Kubiriba et al. (2014)
Banana weevil	60	Okech et al. (2004)
Fusarium wilt	60%–100%	Kangire, 1998.
Soil Nutrient deficiency (K and N)	28–68	Nyombi et al. (2010)
Drought stress	20–65	van Asten et al. (2011)

oxysporum cubense (FOC). For example, the FHIA banana hybrids—developed by Fundación Hondureña de Investigación Agrícola (FHIA) of Honduras, with resistance to FOC, have had acceptability limitations in East and Central Africa due to changes in the preferred taste for a dessert banana (Karamura et al., 2016). For a more complex disease, because of its rapid spreading and very destructive nature, banana bacterial wilt has no known source of resistance in the *Musa* germplasm.

Recently, the banana researchers started to employ biotechnology approaches to complement conventional breeding thereby offering opportunities to introgress genes that are outside the Musa species domain for improved banana products. For example, out of successful efforts of conventional breeding, National Agricultural Research Organisation (NARO) developed and released a number of EAHB hybrids, primarily resistant to black Sigatoka, including KABANA 6H and KABANA 7H, released in 2010 and 2011 and NAROBAN banana Hybrids 1, 2, 3, and 4 released in 2017 (Nowakunda et al., 2015; Tumuhimbise et al., 2017). Because of this NARO does not use biotechnology tools to develop resistance to black Sigatoka in bananas. However, in order to develop varieties with multiple resistance, biotechnology approaches are being explored to add traits such as bacterial wilt resistance into officially released elite conventional EAHB hybrids.

Using biotechnology to address human nutritional deficiencies

Vitamin A deficiency (VAD) and iron-deficiency anemia (IDA) are major global public health problems. In Uganda, VAD is estimated at 33% among children less than 5 years and 35% among reproductive women (UDHS, 2011). Options for addressing micronutrient deficiencies including supplements and food fortification have not been able to reach some poor community members who rely on staple starch foods like bananas and those who do not visit health facilities where the supplements are supplied. There are some banana varieties with high levels of provitamin A (pVA) such as Fe'i bananas that exist in Micronesia (Englberger et al., 2006). However, these Fe'i bananas do not have the acceptable taste attributes to consider for direct adoption for Eastern and Central African banana consumers. GM bananas enhanced with pVA are considered among the sustainable options for addressing VAD in hard to reach banana-dependent communities. When adopted, it is expected that pVA-enhanced GM bananas will significantly contribute to reducing the number of people with VAD in Uganda.

In order to harness the potential of biotechnology for enhancement of pVA, a banana-derived gene, phytoene synthase (PSY2a), was isolated from Fe'i cultivar ASUPINA bananas (Mlalazi et al., 2012). The proof-of-concept studies were first conducted in Cavedish bananas in Australia (Paul et al., 2018, 2017). pVA—rich EAHBs have been developed using the banana PSY2a genes and are in confined field trials in Uganda. Phytoene synthase is the enzyme that catalyzes the first committed step or the turning point in the biochemical process in plants that leads to the formation of pVA carotenoids.

The genetically modified development pipeline

The process of producing GM bananas involves several stages starting from establishing the first building blocks of life which are banana cells, growing them into a fully grown plant (Namanya et al., 2014), Fig. 6.2.1. The cells are the units of the plant that have the natural ability to receive genetic material and reproduce it. Similarly, there is a naturally occurring soil bacterium *Agrobacterium tumefaciens,* which interacts with plants and has the ability to transfer its genetic material. Scientists have studied the gene transfer system of *Agrobacterium* so that the disease-causing genes have been removed and replaced with "preferred" genes leaving its transfer system intact (Gelvin, 2003). Under optimum conditions, the bacterium is conditioned to transfer the "preferred good attributes" (such as pVA-enhancing genes, disease-resistance genes) when brought in contact with the plant cells. The good attributes are then introduced into any plant cell utilizing the natural ability of a "tricked/disarmed" *Agrobacterium* to transfer its genes into plant cells. The process of introducing such attributes into plant cells is what is referred to as genetic engineering. Plants regenerated from such banana cells are genetically modified (GM). Therefore genetic engineering utilizes natural systems of plant–bacteria interaction, whereby GM banana plants are developed from single cells of known banana varieties and not injected with chemicals as perceived by some stakeholders.

GM banana plants go through various stages of rigorous screening to select those that will have taken up the good trait into the DNA in the nucleus of the cell before taking them to subsequent stages of development. Through the plant development cycle, the DNA of the transformed individual banana plant is screened using PCR (polymerase chain reaction)—a molecular method that confirms that the plants have taken up the introduced genetic material. In order to check the function of

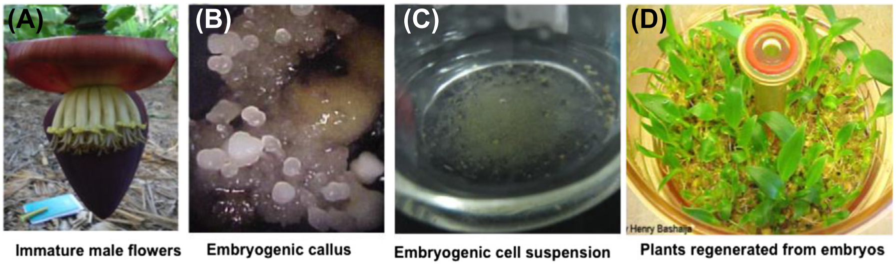

FIGURE 6.2.1

Banana cells suspensions are developed from meristematic tissues in immature male flowers of a choice cultivar. (A) Immature male flower, (B) Embryogenic callus, (C) Embryogenic cell suspension, (D) Plants regenerated from embryos.

the incorporated trait, the GM plants that consistently test positive for the trait are weaned, potted into soil, and subjected to specific stresses under screenhouse conditions (providing a netted shield, decreased humidity, natural lighting, and room temperature for the potted plants). For example, GM banana plants transformed with bacterial wilt resistance genes are inoculated with the bacteria in the screenhouse to select resistant lines (Fig. 6.2.2).

Selected GM banana lines from the screenhouse are progressed to confined fields and further subjected to specific stresses under natural field conditions to select resilient lines. An example for selection of GM bananas under confined field conditions has been demonstrated for resistance to banana bacterial wilt disease (Tripathi et al., 2014). GM bananas that have pVA-enhancing genes incorporated are only evaluated under field conditions where the fruit is assessed after harvest. In addition to the disease resistance or enhanced pVA content, GM bananas are selected for normal growth characteristics including their ability to produce suckers, plant height, flowering time, maturity period, and fruit characteristics to ascertain conformity to the original identity of the variety, with no difference from the untransformed variety. Yield data are collected to ensure that there is no yield penalty associated with the desired attribute.

Once the selected lines have the attribute (gene) of interest that is functional, with normal growth and yield, the selected GM lines go through advanced molecular checks to ascertain that the genetic makeup of the new variety has not changed except for the intended improvement. This includes gene function, interaction with the existing genetic composition of the plant, and ensuring that the attribute of interest is properly incorporated in the banana genome such that no undesirable gene products are created.

Bananas grow in different agroecological conditions. Therefore, GM bananas need to be selected for adoption in different agroecologies of banana-growing communities of varying social economic setups. Consequently, the performance of the selected GM banana lead lines is further assessed in multilocation-confined field trials (MLTs) in different agroecologies to ascertain stability of the trait and their

FIGURE 6.2.2

Selection of bacterial wilt resistant lines in potted GM bananas in screenhouse. (A) Susceptible nontransgenic control, (B) Only the infected leaf of GM banana wilts, (C) No wilting in infected GM banana plant.

performance, food safety, and nutritional integrity. All the data collected from the beginning up to field selection stage contribute to a dossier for release of GM banana product by the national regulator as guided by the national laws.

Genes and their safety

The choices made from inception of GM development cycle take into consideration the concerns about the safety of GM bananas under development. Initially, the choice of attributes (genes) incorporated into the banana is carefully sourced from edible plants or naturally occurring organisms that already interact with plants. For example, the genes that were constitutively expressed to confer resistance to banana bacterial wilt disease, *HRAP* (hypersensitive response-assisting protein) or *PFLP* (plant ferredoxin—like protein), originated from sweet pepper (Capsicum annuum) (Chen et al., 2000). Similarly, the genes for enhancement of pVA in banana were sourced from another type of banana variety, Asupina (Fe'i), an edible banana found in the Pacific Islands (Mlalazi et al., 2012). There is therefore a long history of safe use for the HRAP, PFLP, and pVA proteins incorporated into banana. Furthermore, for many generations in the history of mankind, pVA carotenoids have been safely consumed from a wide range of vegetables and fruits by humans and animals.

The strategy for safety assessment of GM bananas follows provisions of internationally recognized principles and practises such as those provided for through Allergen Online and Codex Alimentarius. The first level of safety considers that the choice of gene has no potential to cause allergens. Bioinformatic searches for the HRAP and PFLP proteins against the Allergen Online database (http:www.allergenonline.org/databasefasta.shtml) did not show any significant alignments with any allergens. The HRAP and PFLP protein had less than 35% cross-reactivity below the threshold recommended by Codex Alimentarius, above which the gene would have been rejected. Before release, strategies are already in place to pretest GM banana food products for allergenicity and toxicity, following FAO/WHO's Codex Alimentarius standards relating to foods, food production, and food safety.

Biodiversity and environmental safety

There is a perception that farmers will loose their traditional banana varieties when superior GM crops are introduced. On the contrary, GM bananas will add to the diversity of varieties in cultivation. For example, banana bacterial wilt decimates all banana cultivars in cultivation. In specific cases where there is no known resistance to such biotic stresses, GM has the potential to introduce improved varieties with desired resistance. Therefore, introducing genes for resistance into such cultivars would preserve them. However, with or without biotechnology, biodiversity continues to be lost due to climate stresses and known and unknown outbreaks of pests and diseases. Unlike conventionally bred hybrids where many characteristics of the variety change, picking some from the male parent and others from the female parent during the conventional development process, the change in the GM banana is only specific to the target attribute, thereby preserving all other characteristics for the target variety. This provides an advantage to cultivar preservation. For example, in the pVA-biofortified GM Nakitembe (AAA-EA), the banana product remains an EAHB cultivar Nakitembe in all characteristics except for enhanced pVA levels.

No unintended effects on nontarget organisms

Scientists are aware of potential introgression of the introduced genetic material into naturally occurring flora and fauna, either through cross-pollination or lateral gene flow. In the case of banana, all cultivated EAHB are naturally sterile. They do not cross-pollinate by themselves, even assisted seed production during hybrid development is difficult and consequently they reproduce vegetatively. Therefore there are no biosafety concerns of gene flow from any of the GM banana cultivars to wild species or other traditional banana varieties.

Furthermore, studies have been conducted to determine the effect of GM bananas on nontarget microorganisms in the soil and plant environment. Nimusiima et al. (2015) studied the effect of transgenes for BXW resistance, HRAP, and PFLP, in confined field trials of transgenic bananas 3 years after establishment. Transgenic and nontransgenic lines and their associated microbiome were investigated by molecular fingerprinting. There were no significant differences found in the profiles of the bacterial communities associated with transgenic and nontransgenic banana plants. In a separate confined field trial conducted at NARO (National Agricultural Research Organisation) for transgenic banana plants transformed with genes conferring nematode resistance, faunal analysis was conducted after 3 years. Again, these studies showed no significant differences in nematode diversity for nontarget nematode species including the bacterial, fungal, and carnivorous feeders, between plots of transgenic and nontransgenic banana plants (Fig. 6.2.3, NARO-ABSPII end of project report, 2016—unpublished).

The choice of genes incorporated into banana varieties for traits such as enhancement of pVA or resistance to BXW was derived from plant species with a long history of safe use. In future, when varieties with resistance to pest and diseases have been deployed into cultivation, they will require minimal or no use of pesticides, an invaluable addition to environmental safety. There are reports showing that use of biotechnology crops has reduced carbon footprints of active ingredients of chemicals. An example report by Brookes and Barfoot (2017) shows reduction in pesticide use and environmental impact quotient, a measure of pesticide effect on the environment, by 17.6% and 18.5% in 2014 and 2015 translating into 6.4% and 6.1% pesticide saving, respectively. Such contributions to the environment must be recognized while taking keen consideration for regulatory regimes that harness strict adherence to safety.

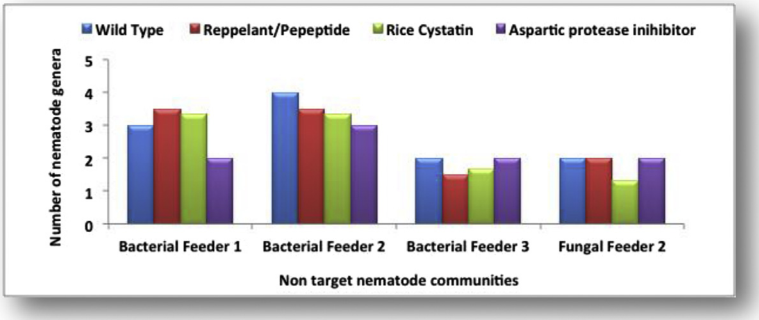

FIGURE 6.2.3

Shows nontarget nematode communities identified from a confined field trial for nematode resistance under plots cultivated with transgenic banana plants.

Economics and trade implications

Just like most countries in the world, biotechnology and its products are not without controversy in Uganda. Some stakeholders share concerns that introducing GM crops could disrupt Uganda's trade with the EU markets who have a stand against GM crops. Although global trade in agricultural commodities especially to the EU constitutes a significant 24% share of Uganda's GDP (gross domestic product), a negligible amount of bananas are exported from Uganda. Currently Ugandan scientists are focusing on applying modern technologies primarily to meet the domestic needs first, especially the food security of an increasing population. Besides, the skepticism toward production and use of GM crops in EU has been gradually changing. For example, EU annually imports 32M tonnes of soybean of which 90% is GM, 2.5 Mton maize (25% is GM), and 2 Mton (20% is GM) of rapeseed (USDA-GAIN, 2016). By 2016, some of the EU countries such as Portugal, Spain, Czech Republic, and Slovakia had increased their acreage of biotechnology crops (James, 2016). While the short-term targets are for local needs, the prediction for the future global markets is positive.

GM bananas are expected to have significant social economic and health benefits on communities translating to national and regional impacts. Studies by Ainembabazi et al. (2015) show that 65% of the farmers (Fig. 6.2.4) were ready to adopt GM banana with BXW resistance immediately. The expected rate of adoption was up to 74% farmers covering 40% acreage allocated to banana production with a yield gain of 54% per ha. Overall, it was estimated that Ugandan farmers would, over 25 years, earn up to $953m worth of bananas at present value. In earlier related studies, Kikulwe et al. (2013) predicted willingness among banana end users to adopt GM banana resistant to the fungal disease black Sigatoka after assessing potential benefits, costs, consumer perceptions, and related policy implications.

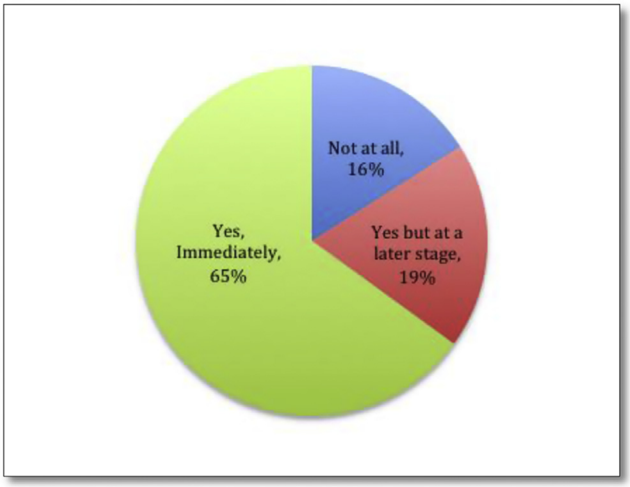

FIGURE 6.2.4

Farmers response and willingness to adopt genetically modified banana with BXW resistance in Uganda.

Source: Ainembabazi, J.H., Tripathi, L., Rusike, J., Abdoulaye, T., Manyong, V., 2015. Ex-ante economic impact assessment of genetically modified banana resistant to Xanthomonas wilt *in the great lakes region of Africa. PLoS One 10, e0138998. https://doi.org/10.1371/journal.pone.0138998.*

Partnerships, ownership, patents, and access

The GM development cycle may be viewed as a six-step process from discovery of genes to release of a product. Ugandan scientists have for the last 15 years acquired capacity and expertise to develop products for the Ugandan consumers covering all aspects of the GM product development cycle from tissue and cell culture, genetic engineering, laboratory and field testing, and recent regulatory trials (Fig. 6.2.5, Table 6.2.2). The regulatory system and policy framework has grown and evolved along with the science and will finally bring the GM banana products through to release. In the process, National Agricultural Research Organisation (NARO) has established strong national and international partnerships in training, technology access, as well as technical backstopping with institutions such as the Queensland University of Technology (QUT)—Australia, Cornell University—USA, Makerere University—Uganda, University of Pretoria—South Africa, AATF—Kenya, IITA—Nigeria, University of Ghent—Belgium, University of Leeds—UK.

However, the NARO drives the agenda for GM research on banana in Uganda. Technical partnerships are governed by agreements that ensure the developed products addressing public needs as public goods. For example, genes incorporated into GM bananas for development of pVA enhancement were accessed directly from Queensland University of Technology, Australia, who are technology partners under the Grand Challenges in Global Health Initiative. The intellectual property for the

FIGURE 6.2.5

Shows the pipeline for GM development from gene discovery through to commercialization.

Table 6.2.2 Skills developed at Masters and Doctoral level for GM banana development.

Number of scientists	Skills acquired	Level of deployment
7	Gene isolation and bioinformatics	Medium
5	Cell culture and tissue culture	High
16	Genetic engineering	High
2	Biosafety, food standards, and regulation	Medium

pVA-enhanced banana is governed by the global access agreements whereby products are developed for charitable markets without encumbrances (Gates Foundation, n.d). As a result, GM bananas developed with enhanced pVA will be accessed and owned by small holding farmers. Upon release, farmers will be able to access initial planting materials from NARO and Ugandan private tissue culture laboratories. Consequently the recipient farmers have full control of the banana seed system requiring no on-going costs to access new planting material, no restrictions to sharing suckers or replanting them, because farmers can reproduce their own suckers since the banana is clonally propagated.

Regulatory aspects

The regulatory framework for GM research in Uganda is gradually evolving with the progressing research for different products. While the National Banana Research Program has released conventionally bred banana varieties in the past, there are no GM bananas on-farmers fields yet. All GM bananas are still going through the selection process in experimental-confined field trials restricted on research stations only and none on farmers' fields. The confined field experiments were approved by National Biosafety Committee (NBC) comprised of independent representative experts who evaluate, guide, and approve the research applications under the biosafety framework and mandate of the Uganda National Council for Science and Technology (UNCST) as the overall regulator of all science and technology research in Uganda (UNCST statute 1990). All banana GM research is regulated by NBC-UNCST in accordance with national and international biosafety practices and considerations.

Conclusion

Millions of people in East and Central Africa still eke their livelihoods out of bananas. The problems that affect banana production are still largely unresolved by available management approaches including conventional breeding. Biotechnology, therefore, as a complementary tool, has potential to provide the products that will address food production and nutrition constraints. Most of the concerns toward genetic engineering technology whether regulatory or safety are addressed along the development cycle, and the benefits of biotech crops in terms of financial, environmental, and humanitarian gain are phenomenal. The future of GM products in Uganda is very bright with up to 65% Ugandan farmers ready to accept GM bananas immediately. The number of people willing to embrace the technology with a positive attitude is increasing, the stakeholders' trust in the regulatory regime to address their fears is high, and the process of building functional structures for implementing the necessary policies has finally and gradually gained momentum. And it is becoming increasingly clear that the challenges of increasing food production and nutrition will be meaningfully addressed if all options available to scientists are harnessed.

Acknowledgments

The authors acknowledge Uganda Government, the United States Agency for International Development (USAID) through the ABSPII (Agricultural Biotechnology Support Project) for financial support. We acknowledge technical collaboration with Queensland University of Technology (QUT), Australia, in training, technology, and product development for pVA-enhanced bananas. We acknowledge International Institute of Tropical Agriculture (IITA) as technical partners in development of BXW resistance. We also acknowledge Academia Sinica, Taiwan, for the genes for BXW resistance and African Agricultural Technology Foundation (AATF) for their role in accessing technologies for BXW resistance from Academia Sinica.

References

African Development Report, 2012. Towards Green Growth in Africa. African Development Bank (AfDB) Group, ISBN 978-9938-882-00-1.

Ainembabazi, J.H., Tripathi, L., Rusike, J., Abdoulaye, T., Manyong, V., 2015. Ex-ante economic impact assessment of genetically modified banana resistant to *Xanthomonas wilt* in the great lakes region of Africa. PLoS One 10, e0138998. https://doi.org/10.1371/journal.pone.0138998.

van Asten, P.J., Fermont, A.M., Taulya, G., 2011. Drought is a major yield loss factor for rainfed East African highland banana. Agricultural Water Management 98 (4), 541—552.

Brookes, G., Barfoot, P., 2017. Environmental impacts of genetically modified (GM) crop use 1996—2015: impacts on pesticide use and carbon emissions. GM Crops and Food 8, 117—147. https://doi.org/10.1080/21645698.2017.1309490.

Chen, C., Lin, H., Ger, M., Chow, D., Feng, T., 2000. cDNA cloning and characterization of a plant protein that may be associated with the harpinPSS-mediated hypersensitive response. Plant Molecular Biology 43, 429—438. https://doi.org/10.1023/A:1006448611432.

Englberger, L., Schierle, J., Aalbersberg, W., Hofmann, P., Humphries, J., Huang, A., Lorens, A., Levendusky, A., Daniells, J., Marks, G.C., Fitzgerald, M.H., 2006. Carotenoid and vitamin content of Karat and other Micronesian banana cultivars. International Journal of Food Sciences and Nutrition 57, 399—418. https://doi.org/10.1080/09637480600872010.

FAO, 2014. Statistical Yearbook. http://www.fao.org/3/a-i3590e.pdf.

Gelvin, S.B., 2003. Agrobacterium-mediated plant transformation: the biology behind the "Gene-Jockeying" tool. Microbiology and Molecular Biology Reviews 67, 16—37. https://doi.org/10.1128/MMBR.67.1.16-37.2003.

Gates Foundation, n.d. Global Acess [WWW Document]. The Role of IP in Global Access. URL (http://globalaccess.gatesfoundation.org/).

James, C., 2016. Global Status of Commercialized Biotech/GM Crops (No. Brief, 52), ISAAA Briefs. ISAAA.

Kamusingize, D., Mwanjalolo Majaliwa, J., Komutunga, E., Tumwebaze, S., Nowakunda, K., Namanya, P., Kubiriba, J., 2017. Carbon sequestration potential of East African highland banana cultivars (*Musa* spp. AAA-EAHB) cv. Kibuzi, Nakitembe, Enyeru and Nakinyika in Uganda. Journal of Soil Science and Environmental Management 8, 44—51. https://doi.org/10.5897/JSSEM2016.0608.

Kangire, A., 1998. Fusarium wilt (Panama disease) of exotic bananas and wilt of East African highland bananas (Musa, AAA-EA) in Uganda. Thesis submitted for the degree of Doctor of Philosophy. Department of Agriculture, Reading University, p. 304.

Karamura, E.B., Tinzaara, W., Kikulwe, E., Ochola, D., Ocimati, W., Karamura, D., 2016. Introduced banana hybrids in Africa: seed systems, farmers' experiences and consumers' perspectives. Acta Horticulturae 239–244. https://doi.org/10.17660/ActaHortic.2016.1114.33.

Kikulwe, E., Birol, E., Wesseler, J., Falck-Zepeda, J.B., 2013. Benefits, costs, and consumer perceptions of the potential introduction of a fungus-resistant banana in Uganda and policy implications. In: Genetically Modified Crops in Africa: Economic and Policy Lessons from Countries South of the Sahara. IFPRI, Washington, DC.

Kubiriba, J., Tushemereirwe, W., 2014. Approaches for the control of banana *Xanthomonas wilt* in East and central Africa. African Journal of Plant Science 8, 398–404. https://doi.org/10.5897/AJPS2013.1106.

Mlalazi, B., Welsch, R., Namanya, P., Khanna, H., Geijskes, R.J., Harrison, M.D., Harding, R., Dale, J.L., Bateson, M., 2012. Isolation and functional characterisation of banana phytoene synthase genes as potential cisgenes. Planta 236, 1585–1598. https://doi.org/10.1007/s00425-012-1717-8.

Namanya, P., Mutumba, G., Magambo, S.M., Tushemereirwe, W., 2014. Developing a cell suspension system for Musa-AAA-EA cv. 'Nakyetengu': a critical step for genetic improvement of Matooke East African Highland bananas. In Vitro Cellular and Developmental Biology – Plant 50, 442–450. https://doi.org/10.1007/s11627-014-9598-0.

Nimusiima, J., Köberl, M., Tumuhairwe, J.B., Kubiriba, J., Staver, C., Berg, G., 2015. Transgenic banana plants expressing *Xanthomonas wilt* resistance genes revealed a stable non-target bacterial colonization structure. Scientific Reports 5, 18078.

Nowakunda, K., Barekye, A., Ssali, R.T., Namaganda, J., Tushemereirwe, W.K., Nabulya, G., Erima, R., Akankwasa, K., Hilman, E., Batte, M., Karamura, D., 2015. 'Kiwangaazi' (syn 'KABANA 6H') black Sigatoka nematode and banana weevil tolerant 'Matooke' hybrid banana released in Uganda. HortScience 50, 621–623.

Nyombi, K., Van Asten, P.J., Corbeels, M., Taulya, G., Leffelaar, P.A., Giller, K.E., 2010. Mineral fertilizer response and nutrient use efficiencies of East African highland banana (Musa spp., AAA-EAHB, cv. Kisansa). Field Crops Research 117 (1), 38–50.

Okech, S.H., Van Asten, P.J.A., Gold, C.S., Ssali, H., 2004. Effects of potassium deficiency, drought and weevils on banana yield and economic performance in Mbarara, Uganda. Uganda Journal of Agricultural Sciences 9 (1), 511–519.

Paul, J.-Y., Harding, R., Tushemereirwe, W., Dale, J., 2018. Banana21: from gene discovery to deregulated golden bananas. Frontiers of Plant Science 9. https://doi.org/10.3389/fpls.2018.00558.

Paul, J.-Y., Khanna, H., Kleidon, J., Hoang, P., Geijskes, J., Daniells, J., Zaplin, E., Rosenberg, Y., James, A., Mlalazi, B., Deo, P., Arinaitwe, G., Namanya, P., Becker, D., Tindamanyire, J., Tushemereirwe, W., Harding, R., Dale, J., 2017. Golden bananas in the field: elevated fruit pro-vitamin A from the expression of a single banana transgene. Plant Biotechnology Journal 15, 520–532. https://doi.org/10.1111/pbi.12650.

Tripathi, L., Tripathi, J.N., Kiggundu, A., Korie, S., Shotkoski, F., Tushemereirwe, W.K., 2014. Field trial of *Xanthomonas wilt* disease-resistant bananas in East Africa. Nature Biotechnology 32, 868–870. https://doi.org/10.1038/nbt.3007.

Tumuhimbise, R., Barekye, A., Kubiriba, J., Tushemereirwe, W., Ssali, R.T., Akankwasa, K., Oyesigye, N., Arinaitwe, J., Arinaitwe, I., Kabita, N.,B., Wasswa, W., Buregyeya, H., Namanya, P., Talengera, D., Batte, M., Nyiine, M., Nabulya, G., Lugolobi, N., Namaganda, J., 2017. A Submission to the Variety Release Committee for the Release of Four Cooking Banana Varieties M19, M20, M25 and M27. NARL-NARO.

Tushemereirwe, W.K., Kashaija, I.N., Tinzaara, W., Nankinga, C., New, S., 2003. A Guide to Successful Banana Production in Uganda: Banana Production Manual. New.

UDHS, 2011. Uganda Demographic and Health Survey. Kampala Uganda 2011,. Uganda Bureau of Statistics (UBOS) and ICF International Inc.. Uganda Bureau of Statistics, Kampala.

USDA-GAIN, 2016. Agricultural Biotechnology Annual EU-28 (No. GAIN Report Number FR1624).

Wairegi, L.W.I., van Asten, P.J.A., Tenywa, M.M., Bekunda, M.A., 2010. Abiotic constraints override biotic constraints in East African highland banana systems. Field Crops Research 117, 146–153. https://doi.org/10.1016/j.fcr.2010.02.010.