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Paul Nampala and Arthur Makara, Editors

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We are also grateful to the authors who responded to our after-the-conference call and submitted papers for these proceedings and responded to the thorough peer reviews and editing recommended during the review process. Your support to this process will help expose research and critical thinking into the use of agricultural biotechnology in shaping the future of development in developing countries.

PREFACE

The International Conference on Agro-Biotechnology, Biosafety and Seed Systems in Developing Countries was held in Kampala during March 8-11 2010 at the Imperial Royale Hotel and attracted over 150 participants form various countries in Africa, Europe and North America. Over 50 presentations were made on various aspects of biotechnology including governance, biosafety, genetic engineering for crops, seed systems, communication, and industrial applications, among others. Thirteen papers were submitted for publication after conference and these covered nearly all themes of biotech above.

The potential role of biotechnology, specifically modern biotechnology in contributing to development has been the subject of debate for more than fifteen years in developing countries. With the exception of newly industrialised countries such as India, Brazil and China, many developing countries have not fully tapped the potential of using modern biotech in agriculture, only South Africa, Burkina Faso, and Egypt have to date commercialised products of modern biotech in agriculture on the African continent. Papers here show the potential, challenges, options, and the need for an integrated approach covering communication, biosafety, and development of relevant biotechnologies if developing countries, particularly in Africa, are optimise biotech tools in national and regional development.

For the first time ever, this conference also addressed the closely intertwined areas of biotechnology research and development, biosafety, regulation and seed development and delivery in the context of genetic engineering revolution. After the conference, the resolutions the stakeholders were presented in a communiqué (*see page 85*) that was widely circulated in different media outlets and direclty among stakeholders through variuos communication channels. The general recommendation from the conference was the call for African governments to take bold steps and fast track decisions geared at establishing feasible regulatory regimes for development of biotechnology while at the same time ensuring biosafety for the benefit of their citizens.

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Biotechnology regulation in a developing country context: the role of scientists

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ABSTRACT

Scientists for a long time have been associated with the role of generating the evidence-base and reliable knowledge that ultimately informs public policy with a view to ensure evidence-based and/or research-informed policy decisions. However, recent demands for accountability in management of controversies associated with biotechnology have created a new platform for experts in biotechnology research and regulation. This has challenged the previously undisputed knowledge production role with the public demanding to be part of the biotechnology governance and policy decision-making process. The role of scientists in biotechnology regulation in practice is investigated using Kenya Biosafety Act formulation process and implementation as a case study. Based on interview data solicited from different stakeholders who participated in the process, this paper exposes challenges that exist when scientists get entangled in public policy formulation process due to the underlying value based practices. It appeals for reflexivity in order for the process to accommodate different values and interests towards a biotechnology development for the benefit of the poor.

Keywords: *Biosafety Act Kenya, reflexivity*

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Introduction

New advanced biotechnology applications involving genetically engineered (GE) technology particularly in agriculture are poised to revolutionalize the sector through transformation of specific traits to increase productivity, manage pests and weeds as well as enhance nutritional value of products (FAO, 2004; 2010).). Despite this progress, the focus on ensuring effective technology transfer pathways has generated scepticism regarding the process of technology transfer to contribute to significant social and economic impact, especially considering the fact that process of developing transgenic crops and subsequent adoption has been very slow (FAO, 2010). Some of the factors that have contributed to this slow progress are linked to the political economy of biotechnology governance and particularly biosafety regulation (Paarlberg, 2008). Governance is a contested subject both in theory and practice. It has been applied in public policy-making to reconcile the role of multiple actors in debating, defining and achieving policy goals where the role of the respective governments becomes that of coordinating and steering (Lyall *et al.*, 2009a: 4). Indeed in new biotechnologies, there is a clear call to engage a wide range of stakeholders in regulatory policy-making (Tait *et al.*, 2006). Analysis of governance is thus heavily anchored in the decision-making approaches that broadly define governance based on the rules, institutions, practices and power that shape the behaviour of different actors (Harsh and Smith, 2007:252).

Biotechnology regulation has been debated widely and it is now understood that regulation is a key device available to governments interested in shaping governance of technology to promote the public interest. At the global level, this regulation is provided through the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD secretariat, 2000). Regulation of biotechnology allows consumers' health and environmental protection and at the same time leaves room for harnessing the potential benefits (FAO, 2004). However, regulation implementation is multifaceted involving very many players at different levels (Fukuda-Parr, 2006). This complicates the process of arriving at a consensus since these multiple actors have different views around how this process should be advanced. The scientific communities are caught up in this and their viewpoints have become a subject of debate in public policy making.

This paper looks at the renewed role of scientists as experts in the context of the political environment under which they operate and disseminate policy relevant knowledge in biosafety regulation and implementation. The term 'expert' is understood from the perspective of expertise that denotes the mechanism by which problems are framed whereby experts are called upon to respond to these problems. In the process, they incorporate scientific judgments and basic social, political and cultural predispositions and commitments (Nowotny *et al.*, 2001: p. 215). The expertise advanced in the process therefore, captures technical knowledge in both scientific and non-scientific domains (Nowotny, 2003). The paper seeks to bring to the limelight the dynamics around biotechnology regulation and how this can be brought to bear on productive practice for biotechnology development. It is informed by experiences of Kenya in developing requisite regulatory structures for management of biotechnology research and development (Kingiri, 2010).

Research context and methodology

Kenya presents an excellent case to investigate the dynamics associated with modern biotechnology in terms of regulatory policy environment and context. This is because the initiation of biotechnology research activities that commenced in 1990's paralleled the establishment of the requisite regulatory process providing an exemplary context to investigate the dynamics around knowledge production with both technological and regulatory orientations. This parallel process engaged communities in research, policy and public arenas in an iterative manner bringing about interesting biotechnology and institutional innovations. Secondly, policy initiatives like the strategy for revitalising agriculture (RoK, 2005) and the Vision 2030 embraces an integrated approach to innovation towards economic development.

This context created a conducive environment to undertake qualitative in-depth semi-structured interviews with over 50 individual knowledge actors who had (or claimed to have) a stake in decisions pertaining to biotechnology as researchers, policy makers, employees of nongovernmental organisations (NGOs) and members of the public (mainly consumers and farmers). The research period was between 2006 and 2010. This was complemented by observation carried out during different scientific and public workshops in biosafety and biotechnology held during this period, and analysis of relevant secondary documents. Interviewees' points of engagement in the regulatory activities and decision processes are seen in the context of effort to provide knowledge (e.g. information, expertise and other resources) to influence policy outcomes. Consequently, the data analysis captured the different ways knowledge is used in the regulatory processes and what factors come into play.

In some cases, codes are used to report information cited in this paper in order to guarantee anonymity of some of the interviewees as requested.

Milestones in Kenya's biotechnology sector

Modern biotechnology has revolutionised many sectors including agriculture and embraces a wide range of applications including tissue culture, markers assisted selection and genetic engineering (GE) also referred elsewhere in this paper as modern biotechnology. All these are being applied in Kenya, but the latter is the focus of this paper. Just like many African countries, GE is relatively new, but GE products have been handled indirectly through trade in form of food aid (Kagundu, 2008).

Actual work involving advanced GE commenced in 1991 when Kenyan scientists went to USA and in collaboration with scientists there, engineered a virus resistant sweet potato (Odame *et al.*, 2003). Thereafter in 1998, the transformed plants required regulatory approval for this research to continue in Kenya. However, actual process of regulatory process and implementation had commenced prior to 1998.

To date, over six GE research initiatives have been evaluated in public institutions in conjunction with local and international partners (see Kingiri and Ayele, 2009). These activities include Bt maize and Bt cotton engineered for resistance to insect pests, cassava for resistance to viruses and sorghum for resistance to striga weed. The recombinant rinderpest vaccine initiative targeted control of rinderpest disease in cattle and other viruses in small ruminants. Other initiatives are in the pipeline for example the sorghum fortified with nutrients funded by the Bills and Melinda gates foundation through the Africa Harvest Biotechnology Foundation International (see www.africaharvest.org). Since the approval of the first transgenic crop- the sweet potato in 1998, no product has reached the farmers and the furthest the biotechnology activities have gone towards a product is the confined field testing. It is hoped that with the establishment of a functional biosafety framework, the situation will change.

Biosafety regulatory mechanism

Biosafety encompasses the regulatory mechanisms that the government has put in place for the governance of GE activities. Article (8g) of the Convention on Biological Biodiversity (CBD, 2000) and Article (16) of the Cartagena Protocol provide for establishment of appropriate mechanisms to regulate, manage and control risks associated with Living Modified Organisms (LMOs). The protocol emphasises on risk assessment (RA) and risk management, and provides guidelines to achieve this (Annex III).

At the early stages of biotechnology research activities, Kenya opted to use the existing infrastructure, the Science & Technology Act (RoK, 1980) to institute regulatory mechanisms through the drafting and adoption of the *Regulations and Guidelines for Biosafety in Biotechnology in Kenya* (RoK, 1998). Thereafter, in an effort to legalise the regulations as well as the biotechnology activities, the *National Biotechnology Development Policy* was drafted and later approved in 2006 (RoK, 2006). This was followed by different versions of the biosafety bill which became Biosafety Law in Feb. 2009 (RoK, 2009).

Kenya signed and ratified the Cartagena Protocol in May 2000 and January 2002 respectively. This further obligated the government to put up regulatory structures to operationalise it. This Biosafety Act therefore primarily seeks to operationalise the Protocol. The controversial developments surrounding its formulation over the years are at the centre of this paper where different actors were proactively engaged particularly between 2002 and 2009.

Previously, all the involved government actors and other nongovernmental players involved in biotechnology governance were brought together as a Committee (NBC) under the umbrella and coordination of the National Council for Science and Technology (NCST) that acted as a boundary organisation overseeing the management of biotechnology research through regulation. This role has since been taken over by the National Biosafety Authority (NBA) formed under the provision of the Biosafety Act.

Theoretical framework

To analytically situate the discussion in sound theoretical debates, this paper draws upon insights from science policy literature in particular governance debates on the policy formulation process (see for instance Lyall *et al.*, 2009a; Tait and Lyall, 2005). These scholars try to explain the changing role of science in policy deliberations and the changing integrated knowledge production architecture prompted by new technological developments (Gibbons *et al*., 1994). In the case of biotechnologies, this brings about governance challenges linked to biosafety regulation imposed to promote technological competitiveness and encourage public acceptance of these new technologies (Lyall, 2007).

Dynamics associated with biotechnology regulation: implications for knowledge production

In this section, practical reasons why and how scientists got entangled in Kenya's regulatory process is explored and the kind of reactions this generated. This helps us understand the controversies and challenges associated with biotechnology and how this may hamper a productive regulatory process that may lead to pro-poor biotechnology development.

Scientists' proactive role in regulatory process

The section tracks empirically the Kenya's regulatory trajectory paying attention to the involvement of scientists in this process, and exposes the tensions that this generated. It is important to note that many interviewees desired a regulatory environment that would enhance deployment of products of GE science. Biosafety bill was a gateway towards achieving that goal. Media reports analysed during field work confirm some activism by the scientific and non scientific communities in support or against the biosafety bill. Biosafety formulation process as a pertinent step in legalising the regulatory regime engaged the scientific community intensely. Scientists collectively educated policy makers and journalists, sensitizing them on GE thus making *"a case for biotechnology"* as well as persuading them to support it (interview with RSIn-GP2, Dec. 2007). This was however viewed with suspicion by some interviewees, who were concerned with what they viewed as biotechnology promotional agenda and associated politics. Several documents obtained during field work and numerous media reportage by both proponents and opponents seemed to confirm this pro-activeness.

Scientists as experts

Empirical data revealed different roles played by the scientific communities in the policy, academic and NGOs arena under the umbrella of experts and advisors. The scientists' early involvement in drafting and steering the regulatory process was not disputed because as argued by one of the members, they had the needed technical capacity to understand the purportedly technical and complex science:

> *"The constitution of the first team that wrote the guidelines was predominantly scientists. It was historical in that capacity of other groups such as consumers and other groups was limited in understanding the science behind the development of biotechnology."* (Interview with a technological & biosafety policy advisor, Public University, Nov. 2007)

Research scientists from Kenya Agricultural Research Institute (KARI) were instrumental in shaping the Kenyan regulatory process and were widely mentioned by both scientist and non scientist respondents as having pushed for the drafting of the first regulations and guidelines. KARI's role actually revolutionized the government operations and priorities. Consequently, the NCST actually shifted its focus from general science and technology to the establishment of a regulatory regime in order to support GE research (Sander, 2007). A respondent undertaking biotechnology research explained how this occurred:

> *"If there was no KARI or research institution trying to push, the priorities of NCST would have been different because their work is not exclusively GE. What they [KARI scientists] were doing created need for regulations to be developed. It was a need-based initiative. KARI as a research institute was vital in defining the priorities of NCST with regards to GM research."* (Interview with a research scientist, international intermediary organisation, Nov. 2007)

The key policy scientists interviewed in this study claimed that they were relied upon extensively to advise the Ministry of Agriculture and regulatory agencies on both biotechnology and regulatory issues. Consequently, this advisory role impacted upon the regulatory process trajectory.

Additionally, in the formulation of the Biosafety Act (2009), the scientific communities were actively involved in various capacities namely; technology experts, regulatory experts and advisors as well as lobbyists (this is clearly demonstrated in Karembu *et al.*, 2010). The nature and role of the ensuing

relationships formed around the formulation of the bill were consequently interpreted in different ways as collaborations, facilitation or activism, lobbying and advocacy, spurred by different factors.

Scientists' role and implications

National efforts to establish a legally binding regulatory regime in compliance with Cartagena Protocol engaged stakeholders in various ways. One of the roles of the NBC according to RoK (1998) was to draw policies and procedures to govern biotechnology. In this regard, this gave NBC the legal powers to spearhead the policy-making process. However, NBC coordination role in the biosafety bill formulation process was perceived to be blurred by the activism of other actors, a view shared by both scientists and non scientists. Arguably, the scientists and their allies became the main drivers of the bill formulation process:

> *"The main players were the biotechnology industry, and the scientists make much of the industry. The whole process was supposed to be an initiative of the government but the interest was with people from the biotechnology industry than what we would call the broader section of Kenyan society."* (Interview with JO-NS6, journalist, local daily, Apr. 2008)

NBC was also largely made up of scientists representing different organisations with two representatives from the civil society. This being the case, it can be concluded that scientists and their affiliated institutions played vital roles as technical experts. This role is however threatened by perceived motivations and interests likely to bring about conflicts of interest. It was a concern of non-scientists from the civil society that technical information used in risk assessments (RA) and consequent decision making pertaining to GE trials was solicited by scientists from technology developers who are interested parties. The relationships established around the regulatory process in the Kenyan context were mutual in that the participating players expected to benefit. Scientists and the government were for instance receiving financial support from non-state actors and donors. These relationships and partnerships were perceived by many interviewees to have positively enhanced the regulatory process. Further, some interviewees were in agreement that the government has inadequate capacity to support the regulatory process, so these other supporting parties were filling in that gap. From these accounts, resources and in particular financial support was a key incentive cementing these relationships (for a detailed account of the role of scientists and controversies this generates, see Kingiri, 2010).

Despite the potential conflict of interests, scientists were perceived to be key players in regulatory process as facilitators, and through their active involvement provided a regulatory mechanism through which the biosafety institutional regime could operate.

Conclusion and recommendations

From the foregoing, it is clear that the roles played by the scientists directed the regulatory process without any contestation. The paper suggests that scientists are not disinterested actors in the regulatory instruments formulation process, and are inspired by different motivations and interests. This has an impact on the ensuing regulatory practice prompted by the unprecedented biosafety revolution. This leads to a compelling urge to reconsider how policy and regulatory formulation processes are conceptualised and articulated. Biotechnology regulation, if it is to achieve greater effect in reconciling the governance agenda of modern biotechnology on the one hand, and role of actors in providing evidence-based expertise into the process; it must factor into the process the different inspirations.

In addition, effective policy and regulatory processes must first acknowledge the potential of experts to influence policy directions. Consequently, strategies should be devised that encourage a reflexive and responsive behaviour (Lyall, et al., 2009b: 261). This may enrich how policies are implemented considering that cultural practices in biotechnology are linked to values and interests (Laurie et al., 2009).

In conclusion, the paper appeals to the policy, public and scientific communities to adopt a reflexive approach to biotechnology regulation in order to enhance convergence of knowledge for sustainable pro poor biotechnology development.

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A Social Audit Model for Agro-biotechnology Public-Private Partnerships

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The Challenge of Trust-building in Agro-biotechnology Public-Private Partnerships (PPPs)

Projects in agro-biotechnology, led by public-private partnerships (PPPs) are advantageous due to their integrated approach to innovation and delivery of agro-biotechnologies (World Bank, 2007), but face public resistance due to issues of trust around genetically engineered crops and the complex nature of the public and private partners' varied interests and priorities. Building trust among project partners, and between the project and the community they aim to serve, can help to mitigate the risks threatening projects' success. The purpose of this brief communication is to explain our Social Audit Model and show its utility in agricultural biotechnology public private partnerships.

Keywords: *Trust, public private partnership, social audit, accountability, stakeholder engagement*

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The Ethical, Social, Cultural, and Commercialization (ESC[2]) team at the McLaughlin-Rotman Centre for Global Health, of University Health Network and University of Toronto, have developed a Social Audit Model for agro-biotechnology PPPs with humanitarian goals (Ezezika, *et al.*, 2009). The model includes an assessment of project needs and goals, development of social audit tools, engagement of internal and external stakeholders, social auditing service[1], a communications strategy, and provision of feedback to project management, governance and funders, and the public. Implementation of the model can facilitate accountability and transparency in the project, and improve project management, which, in turn, can help build public trust and mitigate risk for the project (Figure 1).

How the Model Works

This Social Audit Model has been applied to the Water-Efficient Maize for Africa (WEMA) project, a PPP aimed at developing and delivering conventionally bred and genetically modified drought-tolerant maize varieties to small-scale farmers, royalty-free, in five countries in sub-Saharan Africa (Kenya, Mozambique, South Africa, Tanzania, and Uganda). The 2009 and 2010 Social Audit Reports of the WEMA Project, along with the WEMA management responses to the reports, are available on one of the managing partners' - the African Agricultural Technology Foundation (AATF) - website: http://www.aatf-africa.org/wema/audit_reports/2009_social_audit_report/en/. This is the first time a Social Audit Model has been applied to a project of this type and we believe it has great utility for other large-scale, agro-biotechnology and global development initiatives led by PPPs. We believe it can help to align the goals and interests of project partners and the public, and help to build trust to mitigate risk in the project[2].

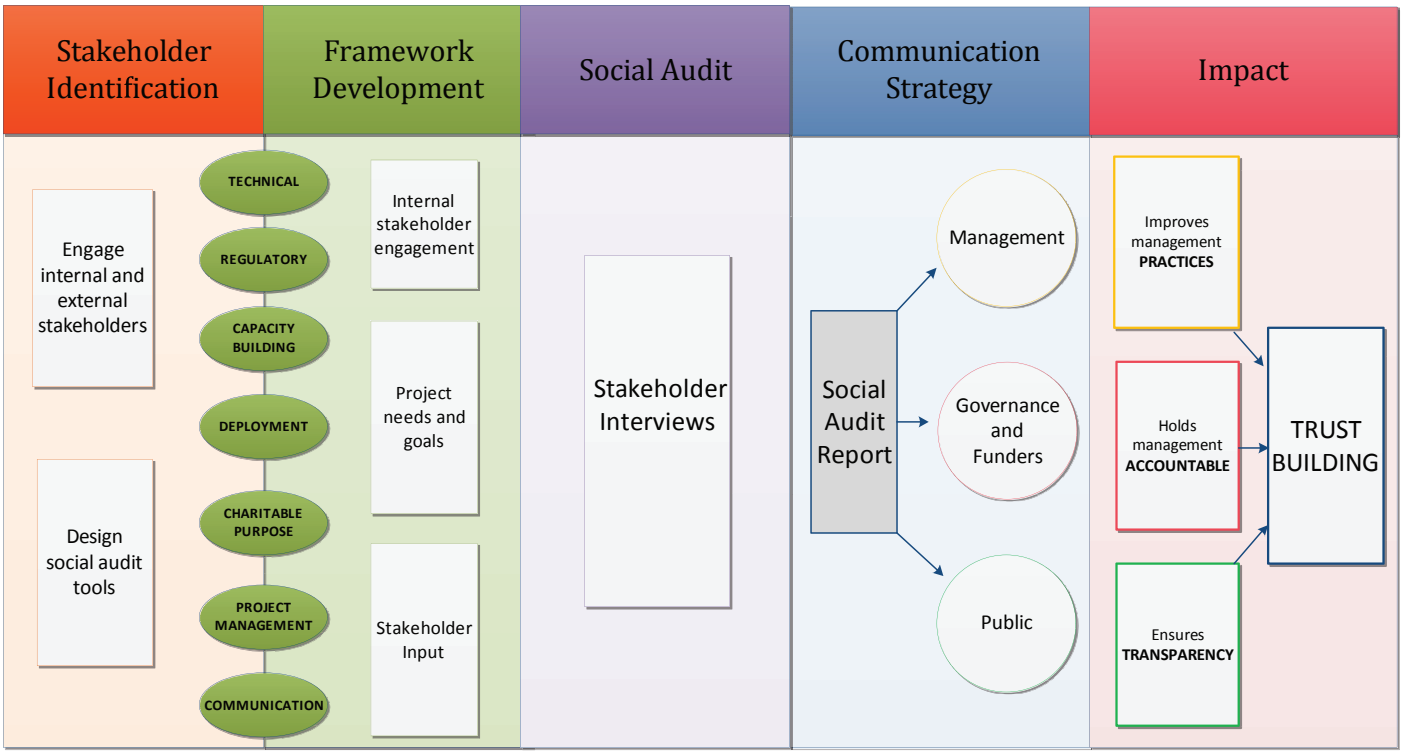

Figure 1: Social Audit Model. The model involves five stages which include stakeholder identification, framework development, social audit, communications strategy, and impact. The first stage is stakeholder identification which involves engagement of internal project stakeholders as well as external stakeholders and the development of social audit tools, including a qualitative semi-structured interview schedule and quantitative questionnaire. Development of a framework for assessing the ESC[2] issues in the project is the second stage of the model. This framework is developed in line with project needs and goals, which involves further engagement and input from project stakeholders. The framework shown here consists of seven domains critical to most agro-biotechnology PPPs and through which we can examine ESC[2] issues illuminated through application of the model. Stage three is the social audit of the project during which stakeholders are interviewed using the ESC[2] interview schedule and questionnaire. The findings from these questionnaires and interviews are analyzed and reported on in the fourth stage of the model – communication strategy. The audit report is presented to and discussed with project management, governance, and funders, and shared publicly with the project stakeholders. In the fifth stage of the model, we see the impact of communicating the Social Audit information. Improved management practices, holding project management accountable to project funders, and ensuring transparency of the project to all stakeholders are the three main envisaged outcomes to help build trust among project partners and between the project and the public.

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[1] Social auditing can be likened to financial auditing. The difference between these processes is that financial auditing deals with financial accounts while social auditing or ESC2 is focused on social accounts. It can be defined as a *"process whereby an audit team collects, analyses, and interprets descriptive, quantitative and qualitative information from stakeholders to produce an account of a project's ethical, social, cultural and commercialization performance and impact"* (Ezezika, *et al.,* 2009).

[2] For information on the Social Audit Model, please refer to: http://www.mrcglobal.org/social_audit_model

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Capacity Development for Agricultural Biotechnology and Biosafety Decision Making: Facilitating Implementation of Confined Field Trials in Uganda

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ABSTRACT

This paper draws upon experiences gained in Uganda, where a hands-on, integrated program for capacity development in agricultural biotechnology and biosafety regulations was implemented, spearheaded by national research and policy-making organizations and financially supported by the Government of Uganda, multilateral and bilateral donor agencies. Uganda is now regarded as a regional hub for agricultural biotechnology innovations, and connected with a range of international projects and programs aimed at developing relevant agricultural innovations. The paper analyzes key factors contributing to progress in biotechnology decision making in Uganda in the last 10 years, which are: (i) Building regulatory capacity and confidence; (ii) developing a biosafety regulatory framework: (iii) scientific and infrastructure capacity; (iv) working on a priority commodity and trait; (v) developing and implementing a communication plan; and, (vi) financial resources and partnerships for engagements in agricultural biotechnology and biosafety.

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Introduction

A growing body of literature confirms the farm-level and societal economic benefits of growing genetically modified (GM) crops, as recently summarized and analyzed by Brookes and Barfoot (2009) and Smale et al. (2009). As the emerging "bioeconomy" expands to include second generation biofuels and biomaterial production, biotechnology and GM crops may play an even greater economic role. It is however, widely accepted that GM crops must be assessed for food, feed and environmental safety before they can be released for commercialisation (McLean et al., 2003). These principles are reflected in an internationally binding agreement, the Cartagena Protocol on Biosafety, which by now (March 2011) has been ratified by 160 countries (SCBD, 2000). Each State Party is obliged to domesticate the Protocol through enactment of relevant policies and regulatory frameworks. Farmers and economies, therefore, will only be able to take full advantage of the bioeconomy under a functional biosafety policy environment.

Despite significant effort and resources devoted to biosafety capacity development, and notwithstanding some good progress, many countries still do not have adequate capacity to design and implement biosafety regulations (SCBD, 2010). This remains a significant barrier to the testing and adoption of new transgenic crop varieties that would offer farmers a means to grow more food, enhance incomes and reduce environmental impacts of agriculture. Furthermore, an uncertain regulatory environment discourages private and public sector investment into the development of the crops and traits that poor farmers need the most.

Numerous programs have attempted to build global capacity for the regulation of biotech crops, with mixed success. Generally, countries with existing capacity for biotechnology research and development (R&D) and high-level political support to biotechnology and biosafety capacity building have made significant advances so far.

This paper draws upon experiences gained in Uganda, where a hands-on, integrated program for capacity development in agricultural biotechnology and biosafety regulations

was implemented, spearheaded by national research and policy-making organizations and financially supported by the Government of Uganda, multilateral and bilateral donor agencies. Uganda is now regarded as a regional hub for agricultural biotechnology innovations, and connected with a range of international projects and programs aimed at developing relevant agricultural innovations.

The lessons learned in Uganda can be applied to ongoing and future biotechnology and biosafety capacity building efforts to ensure that individuals and institutions involved are enabled to make decisions on biotechnology and biosafety in a timely, transparent and science-based manner, and to ensure that all countries can participate in the emerging bioeconomy.

The following sections analyze the key factors contributing to progress in biotech decision making in Uganda in the last 10 years, which are: (1) Building regulatory capacity and confidence; (2) Developing a biosafety regulatory framework; (3) Scientific and infrastructure capacity; (4) Working on a priority commodity and trait; (5) Developing and implementing a communication plan; (6) Financial resources and networking.

Building regulatory capacity and confidence

During the period 1998-1999, the UNCST undertook a scooping study with support from the UNEP-GEF to establish the status and potential for biotechnology applications in Uganda. The profile of biotechnology applications and services revealed that there would be need to develop a National Biosafety Framework for Uganda. The scoping study defined the parameters within which the various institutions involved in biotechnology and biosafety may operate in order to facilitate decision-making across institutions with varied but related mandates for biotechnology.

Uganda ratified the Cartagena Protocol on Biosafety (CPB) in November 2001. Under the CPB, member countries are expected to establish a national biosafety framework (NBF) that will ensure that biotechnology applications, particularly the transboundary movement of "living modified organisms" (LMOs), are regulated in a manner that will minimise any negative effects to human health and the environment.

As a follow up to the scoping study and ratification of the CPB, the UNEP-GEF in 2002 provided support through the biosafety global projects to Uganda to development a National Biosafety Framework. National Biosafety Frameworks may have different components but in the case of Uganda, the following components were pursued during the implementation of the UNEP-GEF project under the auspices of the National Council for Science and Technology (UNCST):

1. A policy on biosafety, which is often part of a broader national policy on biotechnology;
2. A regulatory regime for biosafety, which usually consists of a law or act in combination with implementing regulations;
3. A system to handle notifications or requests for authorisations for certain activities, such as field test releases of GMOs in the environment. The system typically provides for public participation and risk assessment and public participation;
4. A system for monitoring and enforcement; and
5. A system for public information, i.e. a system to inform stakeholders about the development and implementation of the national biosafety framework.

The above components were viewed to constitute a a minimum package for developing a framework that would enable Uganda to fully oblige to the provisions of the CPB and other related international conventions to which Uganda is signatory. Thus, the Government of Uganda has been a consistent and strong supporter to the judicious introduction and application of agricultural biotechnology research in the country.

A key component of the NBF, the proposed national policy on biotechnology and biosafety, aiming to build and strengthen national capacity in biotechnology through research and development; promote the utilization of biotechnology products and processes as tools for national development; and, provide a regulatory and institutional framework for safe and sustainable biotechnology development and application was finalized and presented by the UNCST for enactment in 2002. Following stakeholder consultations and multi-sectoral reviews, the Cabinet of Uganda approved the policy in April 2008. The policy reconfirmed government's balanced position on biotechnology and genetically modified organisms (GMOs), and that the best way to evaluate potential benefits and risks is to have the necessary research and risk/safety assessment capacity in place. This overall position is reflected in the country's regulatory framework.

Biosafety Focal Point and Biosafety Desk established

The Ministry of Water, Lands and Environment serves as the National Focal Point for Uganda and has represented the Country at various Conference of the Party (CoP) engagements. The UNCST is the designated Competent Authority for biosafety in Uganda and has a mandate to coordinate, regulate and make decisions regarding applications and use of biotech in the country.

Right from the time of signing the CPB, the functions of the Competent Authority were executed by UNCST using a project

[1] *UNEP = United Nations Environment Programme; GEF = Global Environment Facility. UNEP manages a range of GEF-funded biosafety capacity building projects worldwide.*

mode approach. During a consultancy exercise conducted in 2002 (Quemada and Traynor, 2002) under USAID support, there was general agreement among UNCST, the research community and government bodies that rising interest and activity in biotechnology and biosafety had created a need for administrative structures for coordination, management and information exchange. This recommendation was implemented in 2004 when a Biosafety Officer was appointed at UNCST to be in charge of the Biosafety Desk. This biosafety desk serves as a central facility to manage, support and effect environmental and health safety in the use of modern biotechnology, and to serve as a national information center. It provides the primary contact point at the Competent Authority Secretariat for national, regional and international activities related to biosafety. Functional linkages were established with other country biosafety coordinating bodies and international support programs in the area of biotechnology and biosafety.

The biosafety desk also serves as Secretariat to the National Biosafety Committee (NBC). It receives and processes applications for the experimental introduction of GM plants in the environment and forwards them to NBC members. It schedules and keeps records of meetings, acquires and delivers relevant publications and provides administrative support to the NBC. The biosafety desk at UNCST is also expected to maintain a roster of local and regional experts and recruit ad hoc expertise for technical assistance as needed. Acting as a bridge between the NBC, government officials and applicants, it facilitates biosafety reviews and decision making, regulatory inspections and monitoring, reporting and record keeping. The managers of this office have been supported by international biosafety initiatives such as the Program for Biosafety Systems (PBS) for training in the management of biosafety as well as in risk assessment, management and risk communication. Having designated biosafety officers at the UNCST to support NBC operations, has been instrumental in enabling review, approval and implementation (including monitoring for compliance) of confined field trials (CFTs), through their support to the application processes, participation in developing the necessary regulatory documents and in the training of regulators, trial managers and inspectors. Clearly, the Government of Uganda assigned high priority to putting a framework in place for developing and regulating modern biotechnology and this has been a key factor in building the necessary institutional and human capacity in biosafety.

Training NBC, IBC and scientists on evaluating applications

Before authorizing GM materials to enter the country, Ugandan scientists had to be trained in preparation of applications while at the same time regulators had to learn how to evaluate such applications. The National Biosafety Committee was established by UNCST in March 1996 when there was an urgent need to make a decision on a livestock product — bovine somatotropine (BST) hormone produced by genetically engineered bacteria. The hormone was intended to be tested for growth and boosting milk production in indigenous cattle. Currently, the main function of the NBC is to provide technical advice to UNCST on science and technology matters related to the safe development and application of biotechnology in Uganda. The NBC comprises members coming from different sectors of government as well as the private sector and general public and these members are selected on personal merit.

Given the rapidly changing and dynamic landscspe of biotechnology applications and services, many members of the NBC took it as their responsibility to learn more about biotechnology and attain competence to advise government from a well informed position. The NBC members attended several training programs to build their capacity for evaluation of applications. Research scientists and other relevant regulators also benefitted from these trainings. Some of the trainees later formed the Institutional Biosafety Committee (IBC) at NARO, when it was established in 2004 through PBS support.

The UNEP-GEF initiated training of biotechnology regulators during their two projects implemented from 1998 to 2005. Several workshops were conducted for various purposes including: improving general understanding of biotech applications and implications for Uganda; risk assessment and risk management principles; monitoring and enforcement mechanisms. Training courses were also conducted to improve knowledge on biosafety legislation, other countries' biosafety practices as well as legal and administrative aspects. Another capacity building initiative, the BIO-EARN[2] project was implemented in Uganda as one of the partners in the region and this project also contributed to capacity building for biosafety through workshops and short courses as well as formal university training leading to the award of postgraduate degrees. Several Ugandans were trained in aspects of risk assessment, monitoring and risk mitigation and management.

When the PBS started its work in Uganda it built on what the UNEP-GEF and the BIO-EARN projects had achieved and continued with capacity building for regulators but initially targeted enabling CFTs of GM crops likely to be planted in Uganda. Capacity for risk assessment and risk management of genetically modified crops was strengthened for biosafety regulators, including members of the National Biosafety Committee (NBC) and the NARO Institutional Biosafety Committee, who were trained through workshops and study visits. The trainings focused on authorization and safe conduct of CFTs, providing information on the characteristics and purpose of these field trials, and providing hands-on

[2] *BIO-EARN = Eastern Africa Regional Programme and Research Network for Biotechnology, Biosafety and Biotechnology Policy Development. BIO-EARN ran from 1999 – 2009 supported by the Swedish International Development Agency, SIDA.*

experience in evaluating applications for introducing GM plants for CFTs.

Training and supporting scientists to develop applications

The first successful application to establish a CFT came from NARO in collaboration with the Catholic University of Leuven, Belgium, where a Uganda scientist had participated in transforming "Gros Michel" banana plants for resistance to black Sigatoka disease (Mycosphaerella fijiensis). The PBS worked together with the Agricultural Biotechnology Support Project (ABSP-II) and NARO to put together a team to produce the field trial application dossier. The team comprised of two Uganda scientists who travelled to Leuven to gain first-hand experience as the banana application was worked on. These Ugandan scientists presented the application to the NBC and continued to participate in addressing queries that came from the NBC before approval. A similar process was followed when field trial applications for testing of insect-resistant (Bt) and herbicide-tolerant cotton were prepared. Again Ugandan scientists traveled to South Africa to participate in completing the application. In the case of GM cassava with resistance to mosaic virus, experts from the Donald Danforth Plant Science Center (DDPSC) collaborated with Ugandan scientists to prepare the CFT application. Currently the capacity to prepare application for CFTs of GM plants is well developed. For example the NARO banana team prepared an application for field trials of GM bananas with improved vitamin-A and iron content and one for bacterial wilt resistance. The NARO IBC is increasingly taking on the role of reviewing applications and helping scientists to complete their dossier before submission to the NBC.

Capacity for environment risk assessment and risk management strengthened

Members of the NBC, IBC, crop inspectors as well as biotech scientists have been targeted specifically for evaluation of applications intending release of GM plants for confined trials and to a limited extent for advanced trials that may have to apply less stringent confinement measures due to their larger size and use of multiple locations. The regulators need to build competence to enable them assess risks that may be associated with products of modern biotechnology and how such risk may be managed. Both NBC and IBC members need to be aware of environmental and socioeconomic implications of adopting or rejecting particular products of modern biotechnology. These committee members and other decision makers need to be conversant with the overall advances in modern biotechnology and its implications to agricultural research advancement and the overall agricultural and industry development. The regulators need technical backstopping from scientists in their decision-making. The regulators and the scientists may access and use existing information for risk assessment but there are cases where locally generated information is critical due to ecological, social economic and other local factors. Hence knowledge and information on risk assessment and risk management is important for both regulators and scientists.

The PBS trained both regulators and scientists for risk assessment and risk management with a focus on plants or products that are likely to be introduced or developed in the country in the foreseeable future. In the same regard physical infrastructure that may be required for containing specific GM plants as they are studied for safety and other desirable traits was designed and later constructed by ABSP-II. Some risk assessment studies of local interest were pursued as part of graduate training programs.

Capacity for regulatory compliance and inspection

Hands-on, on-site trainings were conducted prior to implementing the CFTs to ensure that regulators and scientists clearly understood their role in conducting the trial according to established guidelines and detailed standard operating procedures (SOPs). These trainings were organized by PBS and partners with emphasis on: equipping participants with all SOP requirements in the management of specific crop CFTs; training biosafety inspectors on the procedures in CFT monitoring and evaluation; and, to educate CFT personnel on best practices for communication about the trials. These courses were designed to help participants understand what CFTs are and why there are needed, as well as the critical aspects of compliance and the biology of the test plant as it may relate to the confinement measures adopted. The trainings also covered the CFT reporting requirements in accordance to the SOPs as well as preparedness for incidents and contingency planning. The crop inspectors were particularly assisted to understand the inspection forms that would be used to assess for compliance during the GM crop growth and the post-harvest period. All trainings for compliance had a field visit component to help participants understand the expected layout of the trial and to assess compliance at that stage of implementing the trial.

Learning tours for key regulators and policy makers

Another form of capacity building for policy makers and regulators was achieved through study tours. Ugandan government officials have in different forums pronounced commitment and support to biotechnology application for over a decade. These consistent efforts have yielded desired results and in recent years, Uganda has made very encouraging progress in agricultural biotechnology research, human and infrastructural capacity building, development of regulatory frameworks and technology transfer activities. However, there was need to learn from countries that are intensively using biotechnology applications with regard to overall biotechnology policy design and implementation within the framework of national economic development strategies. In response, PBS in partnership with others players organized a 7-day study tour in 2007 to the Republic of South Africa for key agricultural biotechnology policy makers, regulators and capacity builders in Uganda. The objective was to acquire knowledge and share experience

about biotechnology capacity building, regulation and application from an African country that has made progress with both small and large-scale farmers. There were fifteen (15) participants for this tour including the Minister of State for Industry and Technology; Members of Parliament; senior policy makers from the Ministry of Agriculture; Office of the President; representatives from the Uganda National Council of Science and Technology; the Environment Authority and the National Farmers' Federation and consumer protection representatives. The team visited government research and development biotech programs, University and other training institutions, private sector supporting biotech applications as well as farmers growing GM crops. A similar visit to India was conducted in 2008 for another set of participants nominated from the same institution but with significant participation from Parliament of Uganda. These study visits helped participants to understand how GM crops are evaluated, grown and their benefit to the farmers.

In addition to international trips, policy makers and regulators have visited CFTs planted in the country including those of banana, cotton and cassava and they have been constantly impressed by the CFTs. As Charles Ngabirano, the Former Member of Parliament and Chairperson of the science and technology committee observed "Uganda has capable scientists who are doing a commendable job. The trial is showing positive results. We need to support them by ensuring that there are laws to enable them conduct research which in my view has great potential to address development concerns."

Developing the Biosafety Regulatory Framework

Having a biosafety regulatory framework is critical to enable proper implementation of CFTs. At the time of ratifying the Cartagena Protocol, Uganda was already in process of establishing the national biosafety framework. In 2000, the UNEP-GEF project working in close collaboration with the NBC and other stakeholders assisted the UNCST in developing a proposed National Biosafety Framework, referred to as NBF. Though not fully complete the Uganda NBF has advanced through the support of Uganda Government and other development partners. The NBF derives its authority from the UNCST Statute 1 (1990) which designates the Council as the competent authority in developing strategies for integrating S&T in the national development process. It is under this statute and specifically under the general guideline for biosafety that guidelines and manuals were developed by UNCST and partners through a participatory process to enable handling and implementation of CFTs. Specifically, guidance documents were developed, including the following[3]:

a. ***The Confined Field Trial Guidelines for Uganda:***
   These guidelines provide for a clear and concise summary of the regulatory requirements governing confined field trials of GM plants in Uganda, in accordance with the "Guidelines on Biosafety in Biotechnology for Uganda", which are administered by UNCST. The guidelines are meant for use by applicants and respective regulatory agencies.

b. ***Trial Manager's Handbook:***
   This provides detailed instructions for all aspects of biosafety for confined field trials in Uganda in form of Standard Operating Procedures (SOPs). The SOPs give procedures for shipping and storage, establishment, maintenance and confinement of CFTs; termination and post-harvest management of the trial site; and reporting of results to NBC. The procedures provided are for the use of all Trial Managers, Technical personnel, agents of the Authorized Party, and government officials engaged in planning, conducting or overseeing confined field trials of GM plants in Uganda.

   Procedures for the conduct of CFTs are intended to accomplish three important goals: 1) preventing the spread of novel genes in pollen, seed or other plant parts from the trial site; 2) preventing GM plant material from CFTs being consumed by humans and/or animals before a full food and feed safety assessment is conducted; and 3) preventing GM plants from escaping from confinement and establishing and persisting in the environment. With the achievement of these three goals, novel genes and their products may be confined to the field trial site, and their release into the general environment prevented. In addition to this manual crop specific manuals have been develop to guide research on those specific crops like bananas, cotton and cassava.

c. ***National Guidelines for Containment:***
   These guidelines seek to assist establishment and maintenance and use of containment facilities in order to ensure safety in biotechnology research and development as well as the development of national capacities to identify, assess and manage potential risks as well as establish codes of practice for containment of GM research. These guidelines have been useful in guiding laboratory and screenhouse research involving GM plants at the various NARO institutes.

d. ***Biosafety Inspection Manual:***
   These guidelines provide detailed procedures for inspecting CFTs of regulated genetically modified crops during the crop growth and post-harvest period.

e. ***Resource Book for Regulators:***
   This manual provides procedures and models for regulation of field trials for genetically engineered plants. The manual was also developed to assist NBC in the conduct of their work as they evaluate applications, authorise and oversee implementation of CFTs. Besides its use in field experiments of genetically modified plants, the booklet also provide a useful platform, or expanding and sustaining collective scientific efforts of promoting the safe application of genetic engineering techniques in agricultural production systems in Uganda.

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[3] *Approved guidelines are available online at URL: http://www.biovisioneastafrica.com/regulatory.html*

Scientific and infrastructure capacity

The Uganda agricultural research system embraced biotechnology towards the end of the 1990s when the then Director General of the National Agricultural Research Organization decided to join the international agricultural research consortium under the Consultative Group on International Agricultural Research (CGIAR). This decision was tied to a request for support to biotechnology capacity development, including plant transformation technology for Uganda. A multi-million dollar project on "Novel approaches to the improvement of banana production in Eastern Africa - the application of biotechnological methodologies" was developed and implemented between the banana network of Bioversity International, National Agricultural Research Organization – Kawanda Agricultural Research Institute (NARO-KARI), Uganda; Catholic University of Leuven (KU Leuven), Belgium; Forestry and Agricultural Biotechnology Institute, University of Pretoria (FABI), South Africa; and, the University of Leeds, UK. This project focused on highland bananas, which is an important staple food for the country. Several PhD scientists were trained in various universities with a focus on molecular biology and banana transformation technology.

Phase II of this project focused on developing transgenic East African highland bananas with resistance to banana weevil and nematodes in Uganda. In addition to efforts to develop human resource under this project, a well equipped biotechnology laboratory at NARO-KARI (now the National Agriculture Research Laboratories, NARL), was constructed, so that when the trained officers returned they had good facilities to continue conducting molecular biology research. This facility was officially launched and inducted to conduct research on biotechnology application by H.E. the President of the Republic of Uganda (Y.K. Museveni) August in 2003. Among the products from PhD training and research efforts were transformed plants, notably bananas transformed to resist black Sigatoka. These plants were developed at KU Leuven by a Ugandan PhD student and their being transferred to Uganda for evaluation needed both the scientific and regulatory and infrastructure capacity in place.

The trained molecular biologists could easily understand the language involved in completing a CFT application and so participated in the preparation of the banana application from a well informed position. The regulators including both the IBC and the NBC had also been trained on evaluating CFT applications hence they could consider the application with confidence.

At the time of establishing the molecular biology laboratory, NARO-KARI already had a modern tissue culture facility. On that foundation, a biosafety containment facility level II was constructed with financial support from USAID, and technical guidance by PBS and ABSP-II. This biosafety facility was a great incentive for regulatory agencies to have confidence in the scientific capacity available at NARO to handle GM plant materials. In addition to the biosafety facility, a biotech centre was supported to establish a confinement facility to enable field testing of GM plants under appropriate confinement conditions.

Working on a priority commodity and trait

Highland bananas provided excellent entry point to start CFTs in Uganda not only because a Ugandan scientist was involved in developing the product but also because banana is a key staple crop in Uganda. More than 12 million people depend on banana for food and income. The crop is grown on about 1.5 million hectares of land, which represents about 38% of total arable land in the country. Farmers in various parts of the country rank banana as their most important staple for various reasons including availability of harvest through the year and the relatively low production costs (Kalyebara *et al*, 2003).

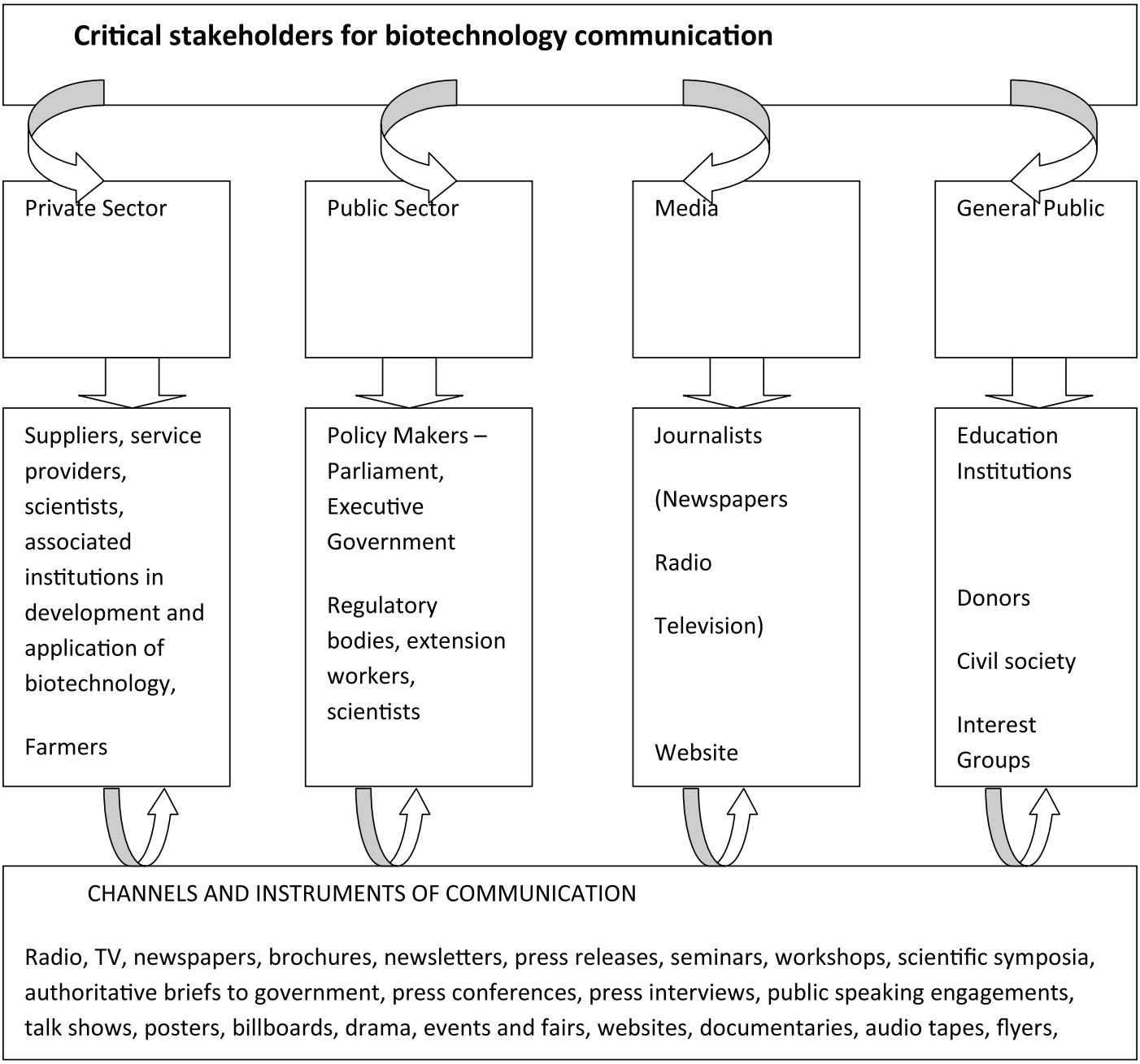
Despite its importance, banana productivity has been in continuous decline in the last 30 years (Insert reference). In the past, banana was a highly sustainable crop in Uganda, with long plantation life and stable yields. Indeed in some areas, one would find women of over 80 years saying that "I found this plantation here when I got married" (insert reference). More recently plantations are short lived requiring replanting every 3-5 years particularly in central Uganda. The most devastating production constraints that have become increasingly serious over the years are black Sigatoka, weevils and nematodes and more recently also the bacterial wilt. These four biotic constraints are very difficult to overcome through conventional approaches hence transformation technology is a welcome option to explore in fighting them. Under these circumstances the CFT application for GM banana was embraced by regulators and scientists considering that it could be a solution to a serious farmers' problem. Having been developed in Belgium, it was also appreciated that the materials needed to be evaluated under field conditions in Uganda where the product was expected to be grown. Government of Uganda has in the past 5 years earmarked and provided funds for research in banana biotechnology.

Communication plan for education, information and creating an enabling environment

While building regulatory capacity for CFT review was in process, program partners were mindful of the needs of policy makers and the public in general to get information on biotech and biosafety, and supporting an enabling environment for its research and development processes. A biotechnology communication strategy (UNCST 2009) was developed to guide the procedure and process for the transfer of relevant knowledge and information to diverse audiences, and to promote public awareness and participation in discussions around the CFT and the development of the biosafety framework in general. The critical audiences within the Ugandan context were defined as: (i) the media (print, broadcast, electronic, multi-media); (ii) policy makers (legislators and regulatory bodies); (iii) scientists; (iv) agricultural extension workers; (v) the private sector (seed companies, processors, exporters); and, (vi) general public (consumers, farmers).

Each of these audiences requires specific communication approaches and activities tailored to suit their information needs with regard to achieving the CFT implementation and overall biotech advancement in Uganda. The communication strategy identified gaps in the transfer of biotechnology information and knowledge to these audiences and on this basis, facilitated audience segmentation and setting objectives for communicating to the different groups (Fig. 1).

Figure 1: Identified audiences for communication strategy



The unique role of the media was recognized. The media, generally, are both a beneficiary and an effective channel for delivering messages to key audiences including policymakers and other decisionmakers (e.g., farmers) Therefore, the communication plan identified the needs of the media and how they could be addressed in order to transform the Uganda media into a communication partner for advancement of the biotechnology. Apart from the media, policy makers, scientists and extension workers were identified as critical audiences in the communication process for biotechnology development and adoption. It was recognized that each of these audiences play a critical role with regard to biotechnology development and use in the Ugandan context, and consequently formed the focus of the communication activities in the strategy. The core objectives for communicating with each of the identified audiences were defined (Table 1).

Table 1 Core objectives for communication across stakeholders

The Media	Policy makers	Scientists	Extension workers	General public	Private sector
Create awareness and promote understanding of Biotechnology	Educate about status of global and regional legislation in biotechnology	Provide opportunities for sharing research and feedback	Educate and inform on global and national biotech advancements and relevant case studies	Enhance awareness and promote understanding	Increase understanding of biotechnology policy and regulatory system
Enhance the image of biotechnology	Stimulate discussion and debate of biotechnology	Stimulate discussion and debate of biotechnology	Demonstrate benefits of bio-tech	Provide avenues for information acquisition	Provide knowledge on investment opportunities in biotechnology
Educate and inform on biotechnology advancements	Change attitudes about biotechnology	Change attitudes about biotechnology	Create dependable information links with farmers	Participate in regular dialogues	Provide avenues for information acquisition
Increase interest in, and coverage of bio-tech issues	Increase/Expand attendance of dialogue sessions	Develop pressure group pro-biotech		Provide information on benefits	
Provide consumers with access to relevant and accurate information	Galvanize strategic action like speedy decision making on biotechnology issues such as the policy	Develop skills and resources to provide accurate information to other audiences			

Source: Modified from UNCST (2009)

Various channels and instruments were used to communicate about the CFT, advances in the regulatory system and biotech in general. Radio was often used to report about specific events, research activities, to host panel discussion and talk shows with an intention of keeping listeners abreast of progress with the technology. The talk shows were particularly useful to capture feedback from the public and provide a two-way communication channel. Besides the Government radio station, Uganda has over 80 private FM radio channels in operation and these have been used to report on biotech and biosafety, particularly by reporters who have attended biotech communication training courses. Television stations have been used with a similar approach but also to air documentaries on the status and advances in biotechnology research and the regulatory system in the country. Other tools of communication that have been used include newspaper articles, brochures and newsletters, policy briefs and posters. A PBS-supported newsletter, BioVision, is published quarterly. This newsletter particularly targets members of parliament and other policy makers to keep them abreast of developments in biotechnology in Uganda and beyond.

The communication process was guided by developing messages that would facilitate acceptance and informed discussion about biotech in the country particularly regarding the safe use of GM crops. The messages were developed according to the audience based on six thematic areas: 1) Definition of biotechnology; 2) Use and footprint of biotechnology; 3) Regulation of biotechnology / biosafety issues; 4) Benefits of biotechnology; 5) Impact of biotechnology on human health and environment; and 6) Product development process (e.g., why CFTs are needed).

The communication strategy was implemented by partners each taking a lead role where they had comparative advantage or core responsibility. The NARO commodity programs took a lead role in communicating about their CFTs while PBS played key role in capacity building for biotech and biosafety communication and for communication to facilitate progress on the legislation process. Two participating civil society organizations, SCIFODE and Consent were the main players for communication to create public awareness. UNCST was responsible for monitoring implementation of the overall communication strategy.

Financial resources and partnerships

Obviously, as noted in the sections above, Uganda has benefited from strong external support in developing its national capacity for agricultural biotechnology and biosafety. However it would be mistaken to regard the process as externally driven, as the leadership and coordination roles are clearly performed by national agencies and organizations, ensuring that international support contributes to a national agenda and does not lead to duplication of efforts.

Building biotechnology and biosafety capacity requires significant levels of funding. By providing an enabling environment for modern agricultural research, Uganda is very well connected in a range of agricultural biotechnology programs (e.g., BIO-EARN, ABSP-II, AATF/WEMA) which have all contributed to establishing R&D infrastructure for safe research and experimentation with GM crops. All international programs active in Uganda have organized hands-on training programs and, in some cases, longer term degree training abroad in relevant disciplines.

In addition, and connected with the above, several international programs support biosafety regulatory capacity development in Uganda. Notable examples include the UNEP-GEF biosafety implementation projects and USAID-supported programs such as PBS and ABSP-II. Other projects supporting biosafety development and training include BIO-EARN and the Danish-supported BioSafeTrain. These programs have operated at the regional level and have contributed significantly to capacity building efforts in the different countries of Eastern Africa.

It should be stressed that those programs would have had limited impact without the strong support from, and active involvement by Ugandan individuals and institutions – UNCST, NARO, Universities (especially Makerere University) and regulatory agencies involved in the overall NBF that have worked in a coordinated fashion to ensure that international investments in agricultural biotechnology and biosafety have paid off over the last 10 – 15 years.

Concluding remarks

The preceding sections exemplified Uganda's progress in developing and implementing biosafety capacity, tailored to the overall needs and development objectives of the country. It has taken a long-term and product-orientated approach, in order to ensure that regulatory development and training were put in practice.

In addition to the activities and developments described above, primarily governing confined field trials, the biosafety regulatory framework's authority and scope would be strengthened when a comprehensive biosafety law is in place. Drafting a biosafety law started in 2002 under the UNEP-GEF project and has advanced since then, through various rounds of review and stakeholder consultations. The Draft Biosafety Bill is currently being finalized for submission and adoption by Parliament as the National Biosafety Act. The Act (biosafety law) will be an essential next step in bolstering Uganda's capacity in the judicious use of agricultural biotechnology.

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Biosafety risk communication and a multidisciplinary approach: The key to adoption of agro-biotechnology applications in Sub-Saharan Africa

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ABSTRACT

The number of people lacking adequate food in the world is increasing, especially among the poor communities in Sub-Saharan Africa (SSA). Constraints to crop production in SSA are many including pests, diseases, weeds, environmental degradation and inadequate food processing facilities. Agro-biotechnology applications hold potential in solving some of the constraints. Two related challenges to the application of agro-biotechnology in SSA are inadequate capacity and public concerns. There are five main public concerns that have hampered adoption of agro-biotechnology in SSA: biosafety issues related to human health, concerns about detrimental environmental impacts, regulatory concerns, economic concerns and ethical concerns. This review will discuss the basis of these fears and concerns. We also identify risk communication as a major factor influencing public concerns and perceptions towards GM agriculture in SSA. We further suggest that scientists have a major role in clarifying issues about the adoption and use of genetically modified (GM) crops in agriculture.

Keywords: *Genetically Modified Crops, public concerns, safety issues*

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Introduction

The number of people lacking adequate food in the world is increasing, especially among the poor communities in Sub-Saharan Africa (SSA) (Dalgado, 1997). Constraints to crop production in SSA are many including pests, diseases, weeds, environmental degradation, soil nutrient depletion and inadequate food processing facilities (Scherr, 1999). Biotechnology tools like tissue culture, genetic transformation and molecular markers can improve efficiency in crop improvement efforts and overcome some of the challenges faced by conventional plant breeding strategies for improvement of food production (Machuka, 2001). The UN Human Development Report (HDR) "Making New Technologies Work for Development" (UN, 2001) identified biotechnology as a key avenue for the socio-economic advancement of the developing countries.

As a tool of biotechnology, genetic transformation is faster, and able to deliver genetic changes that would never occur through conventional methods (Kung and Wu, 1992). Genetic transformation is already being applied in the improvement of crops that are important in SSA. For instance, programs using genetic transformation to enhance nutritional content, to solve environmental constraints such as chilling, freezing, soil salinity, heat stress and biotic constraints such as weevils and viral diseases in sweetpotato have yielded promising results (Yamaguchi *et al.*, 2004; Kasukabe *et al.*, 2006).

Despite the documented potential of agro-biotechnology applications in transforming agricultural economies, its adoption has remained low in SSA (James, 2003). This contrasts with the increase in global area of agro-biotechnology. The global biotechnology crop area reached 250 million hectares in 2006, with more than 10 million farmers in 22 countries planting 102 million hectares of biotechnology crops, up from 90 million hectares planted by 8.5 million farmers in 21 countries in 2005 (James, 2006). The increase of 30 million acres between 2005 and 2006 was the second highest in five years and equivalent to an annual growth rate of 13% in 2006. Of the countries that grew biotechnology crops in 2006, South Africa was ranked eighth in terms of the hectarage devoted to growing biotechnology crops in the world. It is important to note that South Africa

is the leading country in terms of GM commercialization in Africa (James, 2006).

Apart from inadequate capacity in terms of human resource and infrastructure, the dominating public concerns and fears towards agro-biotechnology applications threaten sustainable adoption of the technology in SSA. Other authors have shown that some perceptions have arisen due to a big failure to address concerns that currently fall outside scientific risk assessment; for example fears that agricultural economies will be changed fundamentally by the use of GM crops, leading to undesirable social or political change (Johnson *et al.*, 2006). In this review, we identify risk communication as a key to addressing public concerns and therefore speeding up the adoption of agro-biotechnology in SSA. We briefly review the concerns that have affected adoption of agro-biotechnology in SSA. With the aim of improving effectiveness in biosafety risk communication, we also highlight the historical basis of current perceptions towards genetically modified organisms (GMOs). We finally suggest how scientists can communicate risks and benefits of agro-biotechnology applications to influence opinions and perceptions by the public towards understanding GM agriculture. We also propose how scientists can handle sentiments from commentators who have opted to be denialists regarding GM agriculture.

The major concerns towards GM agriculture

There are five main public concerns that have hampered adoption of agro-biotechnology in SSA: safety issues related to human health, concerns about detrimental environmental impacts, regulatory concerns, economic concerns and ethical concerns. Some of these concerns are legitimate while others clearly result from lack of information and/or misinformation about the technology, whereas others are deliberately not genuine.

Commentators who argue that GM interferes with nature need to understand that GM is a development in a long line of plant breeding techniques. Older techniques shuffled the plant's genes, leading to lots of unintended changes, whereas GM is more precise. The comments that it is "unnatural" are just as true of plants generated through conventional plant breeding programs. It is also surprising that some commentators still talk about "releasing" or "freezing" GM as though it is a one-off decision yet to be taken (James, 2007). GM research and plant trials are on-going worldwide.

Loss of wildlife diversity on farm land is also not a problem specific to GM but of agriculture in general; the losses of habitat, use of fertiliser and pesticides, and changes in crop rotations have all reduced the number of plants, insects and birds (ABE 2002). Research into how GM maize crops influence non-target insects in the environment found that whether the maize is GM or not has much less of an impact than how much insecticide is used (USDA-ARS, 2002).

There are also food safety issues regarding GM agriculture. This still creates fear although in the US, foods containing GM ingredients have been eaten for over a decade. It is estimated that more than 80% of processed foods on US supermarket shelves contain GM traces and over a trillion meals containing GM ingredients have been consumed without revealing any adverse health effects (FSA, 2010)).

Some commentators, including scientists, go into an unfair comparison of the expected impact of GM on world food production in reference to the 'green revolution'. It is important to note that GM is simply a plant breeding technique while 'green revolution' resulted from many factors combined including use of new crop varieties, agrochemicals and improved irrigation. In addition it is necessary to note that the world population has grown from 3 billion in 1960 to 6.72 billion in 2008.

Commentators on the strictness of GM regulation will note that the original regulations on growing GM crops were instigated by scientists doing molecular biology research. The first published GM experiment was a paper in 1972 describing the insertion of bacteriophage genes into an animal virus DNA. It led scientists to raise questions about potential risk to human health and to organize the Asilomar Conference 1975, attended by scientists, lawyers and government officials to discuss the technology. They concluded that experiments could proceed under strict guidelines drawn up by the US National Institute of Health. Unfortunately this has led to regulations that cause most observers to get the impression that GM is dangerous, but it's rather the product of how the regulations developed to allay concerns. Therefore regulation for GM research and application operate proactively, anticipating possible risks. GM regulation is not developed after harm has already been experienced as it is with most existing regulations (Morris, 2007). This is demonstrated best in the regulation against the use of "terminator' technology also referred to as Genetic Use Restriction Technology (GURT).

GURT was previously proposed as a method for restricting the use of GM hybrid seeds by causing second generation seeds to be sterile. Companies thought of using this technology to protect their commercial interests and intellectual property rights in GM crops. This was seen as a violation of the rights of farmers to grow crops from saved seeds. A global moratorium on the testing and commercialization of the technology was established under the United Nations Convention on Biodiversity. It was reviewed in 2006 and still stands. This demonstrates that biosafety regulations are proactive and also shows that scientists are fully aware that multinationals' interests are financial rather than humanitarian. However to entirely refuse GM technology on that basis is throwing the baby out with the bathwater (Nuffield Council on Bioethics, 2003). There are many publicly funded GM schemes that have humanitarian, not financial aims. More productive public research on GM will soon be possible as regulation gets less restrictive.

A brief history of current perceptions towards GM agriculture

Opposition to transgenic crops has diverse origins. Some opposition may be our human tendency to want immediate solutions for perennially difficult problems. Therefore an understanding of the nature of objecting views and opinions is critical for clarifying what issue is actually part of the debate. For instance, one of the reasons for increased negative perception against agro-biotechnology among African smallholders and world consumers is that the first-generation transgenics involved a high cost to create (Stewart *et al* 2000). These were also produced with emphasis on the production traits (e.g. resistance to diseases and tolerance to herbicides) of the most economically important crops (e.g. wheat, cotton, rapeseed and soybean). These crops are grown in Africa but they are certainly not the most important crops in SSA (Bonny, 2003).

In addition, the early studies on risk assessment were scanty, sometimes statistically unsound, but, in hindsight, luckily enough showing the trends which were later asserted by better studies. At the same time firms involved in genetic engineering often initially underestimated the public's questions concerning GMOs. The industry even treated the questions with disdain, apparently considering them to be the result of irrational fears that would disappear gradually with better information and education (Marris *et al.*, 2001). Overall, the biotechnology industry did not seem to take the drawbacks of GMOs (whether real or perceived) seriously enough (Eichenwald *et al*., 2001).

More public suspicion about GMOs arose as the public authorities in Europe and Africa appeared hesitant to comment and often made inconsistent statements on the subject (Tripp, 2001). Scientists are still perceived as being divided on the issue, and the contradicting opinions given about GMOs worry many consumers. In addition, genetic engineering has had few allies or backers considered as credible by the public; whereas on the other side, it has had influential opponents supporting and strengthening the anti-GMO movement. More importantly, the contradictory information received on GMOs from experts compared with the clear message of environmental groups reinforces many people's worries on the subject (Bonny, 2003).

Another reason for the negative perceptions is that genetic engineering became a widespread and frequently reported topic shortly after a period during which various issues of public health, food safety, and pollution, among others, had arisen. Confidence in institutions, in industry, and in certain technological advances had been seriously undermined following the problems of contaminated blood, asbestos, and bovine spongiform encephalopathy (BSE) (Bonny, 2003).

Another factor that has played an important role in creating a negative perception of GMOs is that opposition to GMOs appears to be inherently attractive. Opponents put forward arguments calling upon values that a *priori* generate support, such as the need for caution and wisdom in the use of technology, care and concern for the environment, for public health, for the future of the planet, without forgetting citizen participation and involvement in technological choices (Bonny, 2003). Here, the GMO theme has proved to be an excellent platform for certain associations because it has credited them with a responsible attitude, thus conferring legitimacy upon them.

Various concerns with regard to the trends in techno-economic and socio-economic development nowadays may be added to these determinants of rejection. GMOs sometimes come to be considered as a symbol of changes that many people perceive as negative such as growing concentration in the agro-foods industry and increasing economic globalization. These linkages are bolstered by the fact that opposing organizations and activist groups use GMOs as a springboard for expressing their opinions on much broader related points (Bonny, 2003). Thus GMOs are accused of having negative characteristics which, in fact, are not specific to them e.g. the concentration of firms and difficulties of procurement by poor populations (Bové, 1998).

Risk communication: the key to addressing skepticism towards GMOs

The debate concerning genetically modified crops illustrates confusion between the role of scientists and that of wider society in regulatory decision making (Johnson *et al*., 2006). We identify risk communication as a helpful tool in promoting agro-biotechnology. We also assert that a multidisciplinary approach will speed up the adoption of agro-biotechnology since the challenge has shifted away from public ignorance to mistrust (AEBC, 2001). In this review we are concerned with the adequacy of information flow about GM crops. What type of information do farmers and consumers require in order to recognize and make decisions about GM crops? What type of information is required to assure the farmer that the GM seed for sale is of the traits they seek (Tripp, 2001)? What type of industry is required to deliver agro-biotechnology seed and the necessary information?

Factors that might possibly facilitate a positive change in perception include: companies providing credible answers to a range of GM issues; reliable actors highlighting the advantages of GMOs; changes in the general context such that people come to see GMOs in a different light; or the advantages of genetic engineering being highlighted in precise cases where other techniques were unsuccessful (Bonny, 2003). The way scientists have communicated risks associated with GM crops is blamed as one of the causes of rising fear towards agro-biotechnology (Stewart 2000). It seems scientists needed to be mindful that humans are risk averse by nature. It is possible that this problem comes from the way risk assessment studies are done (Johnson *et al.*, 2006). Scientific studies are frequently undertaken without a view as to how they will help to quantify risk: either there is no conceptual or theoretical model to link the data and the assessment endpoints or, worse, no assessment endpoints are defined (Poppy and Wilkinson, 2005). Scientists either set out to unearth potential intrinsic hazards associated with the stressor, or they focus on the potential for exposure to

some component of the GM plant without demonstrating whether the component is hazardous (Johnson *et al.*, 2006). For risk to be properly estimated and communicated, both components, i.e. hazard and exposure, need to be quantified, if greater focus is on one then the risk assessment loses quantitative power (Poppy, 2004).

Scientists need to always remember that scientific risk assessment, as pure science, should test hypotheses and make predictions from the results of those tests (Johnson *et al.*, 2006). During interpretation of results, scientists need to consider that scientific risk assessment is only a part of wider risk analysis (Johnson *et al*., 2006). The public and opponents of GMO application need to feel that scientists are listening to them. Academic and public scientists have tended to make two mistakes with regard to the current controversy. The first is their reticence to speak directly to the public and the media for fear of being misunderstood. The second is their assumption that the public and the media will take their scientific data at face value (Stewart *et al.* 2000). Placing risk assessments in the wider context of risk analysis enables the wider 'non-scientific' questions to be considered in regulatory decision making (Johnson *et al.*, 2006). Such integration and understanding is urgently required for SSA because the challenges to regulation will escalate as scientific progress advances. This is also our justification for promoting a multidisciplinary approach in this review.

A multidisciplinary approach will improve public trust

It is important to bring together academia, government, and industry to work together towards a public policy (Arthur, 2001). It is also imperative to make the public aware of the importance of science in decision making by moving from "educating the public" to engaging with the public, through discussions with stakeholders such as the farmer, consumer and regulatory organizations (Rose, 2003). For a regulatory system to be successful it must inspire public confidence (Johnson *et al.*, 2006). Much regulation involves the evaluation of technical information by specialists, therefore, for non-specialists there needs to be a strong element of trust, not only in the regulations, but also in the people in charge. Opponents of the current regulations have proposed solutions, including broadening of the regulation to encompass a more holistic approach in assessing and addressing risks, so that not only human and environmental safety are evaluated, but also ethical and economic concerns. There are also calls for the widening of expert panels to include non-expert members to the committees who evaluate applications and advice on decisions for commercial release (Johnson *et al.*, 2006). A PABE report further recommends involving the public as far upstream in the innovation process as at the risk-assessment level through consensus conference, for example (Louët 2011).

Another way of gaining public trust in SSA is ensuring that trained scientists, technical experts, and policymakers from the region are actively involved in communicating agro-biotechnology issues (Bonny, 2003). If policy options regarding the safety of individual organisms and products are made by African scientists in trusted institutions there will be improved public acceptance of agro-biotechnology in SSA. In green revolution era both fundamental research and its application were mainly done by the public sector. Although GMO research is still carried out by public sector, its application largely takes place in the private sector where much of the intellectual property is controlled (Kuyek, 2002). Agricultural biotechnology research and development needs to be done wherever the products of biotechnology are intended to be used, whether in industrial or developing countries.

Suspicions towards GMOs are not a strange phenomenon

It is not our intention in this review to suggest that agro-biotechnology will be accepted in some prescribed period. Scientists communicating benefits of GMOs in SSA need to exercise patience. They need to understand the factors that influence public perception including culture, history, socio-economic conditions, religion, government policies and scientific uncertainty. Genetic engineering is a fairly recent technique, and numerous improvements can be envisaged to reduce risks or to develop innovations with more advantages than those of the very first transgenic plants. Numerous examples of this can be seen in the history of any technology (Bonny, 2003). Many innovations were initially extremely rough and dangerous but gained considerably in safety, usefulness, controllability and ease of use after years and decades of improvement and adaptation to conditions of use.

Conclusion

Biotechnology is a heavily discussed issue in almost every country, where opinions of the different parties vary considerably and sometimes are quite different. Consequently an understanding of the nature of objecting views and opinions is critical for clarifying what issue is actually part of the debate. Some of the negative perceptions towards GM are legitimate while others clearly result from lack of information and/or misinformation about the technology, whereas others are deliberately not genuine. Progress can be made if scientists discuss GM processes and products in comparison with their non-GM counterparts. Additionally such discussions should emphasize a risk-benefit analysis on a case by case basis. Scientists should note that genetic engineering and other areas of biotechnology form a new wave of innovation based on living material. These technologies promise better knowledge of the functioning of organisms and more accurate, finer and closer operation and intervention mechanisms than the empirical techniques used for decades, centuries or even millennia. However, GMOs have to do with food and nature, which have a special place in human culture. Strong societal questioning concerning this change seems logical and pertinent, because of the

risks linked to great power used without sufficient wisdom. In addition, the frequent attitude of suspicion and rejection shown towards GMOs appears fairly logical with regard to the risk/benefit balance drawn up by many people.

Additionally, scientists have a duty not to communicate data in a way that implies that risk has been demonstrated, when all that has been done is that hazard or exposure, or some component of these parameters, has been studied. Importantly, all parties in the GM crop debate should be made aware that scientific risk assessment is one part of risk analysis; it is risk analysis that is used to make decisions and that reflects rational non-scientific concerns. Of course, decisions based on scientific and non-scientific arguments are not easy. One possible answer is to reformulate concerns that appear non-scientific into forms that are amenable to scientific analysis (Johnson *et al.*, 2006). Therefore a multi-disciplinary approach is recommended in this review.

In discussing GM agriculture in public, scientists should always note that the normal academic response of engaging in an opposing argument is not always applicable. It only works when both parties agree to look at evidence as a whole and to reject deliberate distortions and to accept the principles of logic (McKee, 2009). In this review, we argue that it is necessary to shift the debate from the subject under consideration, instead exposing to public scrutiny the tactics employed by anti-GM activists and identifying those tactics publicly for what they are. Denialsts like anti-GM activists use an approach that has the ultimate goal of rejecting a proposition on which scientific consensus exists. The five tactics deployed by denialists are identification of conspiracies or inversionism, use of fake experts, selectivity, creation of impossible expectations and the use of misrepresentation and logical fallacies (McKee, 2009). The controversy surrounding the application of GMOs in agriculture is complex biosafety risk communication and a multidisciplinary approach are alone are not enough to clear the issues discussed. As scientists, we must remain proactive in delivering accurate agro-biotechnology information using the most appropriate tools available (Stewart, 2000).

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Exploiting the use of biotechnology in sweetpotato for improved nutrition and food security: Progress and future outlook

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ABSTRACT

Sweetpotato (*Ipomoea batatas*) production is expanding faster than any other major food crop in sub-Saharan Africa (SSA), with about 13.4 million tons of roots from 3.2 million hectares in 2005. However, major constraints, including sweetpotato weevils (SPWs) and sweetpotato virus disease (SPVD) cause almost total crop loss on susceptible cultivars. This paper reviews examples where biotechnology in particular biofortification and genetic transformation could be used to improve sweetpotato for nutritional quality and food security. Expression of Cylas-active *Bacillus thuringiensis* (Bt) Cry proteins in sweetpotato could result in varieties potentially with field resistance against SPWs. Post transcriptional gene silencing (PTGS) mode of resistance to SPVD is promising. Quantitative trait loci (QTL) for dry-matter, starch, ß-carotene content, and yield have been identified in a hexaploid sweetpotato mapping population of 'Tanzania' (female, cream-colored flesh) x 'Beauregard' (female, orange-fleshed storage roots). Biotechnology approaches offer an attractive option of integrating some desired traits into farmer preferred sweetpotato cultivars in a more effective manner than conventional breeding.

Keywords: *Ipomoea batatas, Biofortification, Cry toxins, Quantitative trait loci*

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Importance of Sweetpotato in Sub-Saharan Africa (SSA)

Sweetpotato, *Ipomoea batatas* L. (Lam.) is an important food crop in more than 100 countries, and is ranked according to FAO data as the seventh most important food crop globally, grown on 9 million hectares, yielding about 124 million tons, with an average of about 13.7 ton/ha (FAOSTAT, 2007). In the developing world, sweetpotato is especially important because it is a highly adaptable crop that generates large amounts of food per unit area and unit time during relatively short rainy periods, giving it an advantage over major staples. Sweetpotato also has flexible planting and harvesting times, tolerates high temperatures and low fertility soils, is drought tolerant, is easy to propagate and maintain, and yields well in adverse conditions. Furthermore, compared to other crops, sweetpotato requires fewer inputs and labour, making it particularly suitable for households threatened by migration or diseases such as HIV/AIDS (Jayne *et al*., 2004).

These characteristics make sweetpotato ideal as a crop for poverty alleviation. They also make it more suitable for mitigating food shortages and famines that occur under the most severe socio-political circumstances such as in war-zones that are common in Africa. Although China accounts for about 85% of the world production, sweetpotatoes are also an important food staple in Asia, Africa, and the Caribbean, and South America, where they are an important source of carbohydrates, vitamins A and C, fibre, iron, potassium, and protein (Table 1) (Woolfe, 1992).

Table 1. Energy and Vitamin A yields of sweetpotato and other major starchy staples

Crop[a]	Average tropical yield (tons/ha)	Edible energy value (MJ/kg)	Edible energy per ha (10^3 MJ)	Available vitamin A value RAE[c] µg/100 gms	Vitamin A per hectare (RAE/ha)	Mean growth period (Days)	Edible energy (MJ/ha /day)
Sweeetpotato[d]	7	4.8	27.2	0-2500	0 to 175 million	140	194
Cassava[d]	9	6.3	45.6	0-75	0 to 6.5 million	330	138
Yam[d]	7	4.4	26.2	0-7	0 to 490,000	280	94
Banana	13	5.4	41.4	3	0 to 390,000	365	113
Rice[b]	2	14.8	20.8	0	0	140	149
Maize	1	15.2	18.8	0-30	0 to 300,000	130	145
Sorghum	<1	14.9	11.2	0	0	110	101
Millet	<1	15	8.2	0	0	100	82

Source: Low et al. (2009)

[a] Cereals, air-dry; roots/tubers/bananas fresh. [b] Paddy Rice. [c] RAE = Retinol Activity Equivalent

[d] Andrade et al. (2009); cassava varieties above 5 µg/100 gms still under development.

Sweetpotato is one of the most widely grown root crops in SSA, covering about 3.2 million hectares, with estimated annual production of 13.4 million tons of roots in 2005 (FAOSTAT, 2007). It is predominantly grown in small plots by poorer farmers, and is often referred to as the "poor man's food" (Woolfe, 1992). Its ability to produce relatively good yields under marginal conditions, its flexible planting and harvesting times provide roots and leaves during hunger seasons, and its good yield response to better management are factors driving its expansion in SSA, especially in land constrained countries with high population densities, justifying its reputation as the "classic" food security crop (Woolfe, 1992). FAOSTAT (2007) show that the area planted to maize in SSA is 9 times greater than to sweetpotato, but the latter is expanding faster than any other major food crop in SSA. All African countries recorded significant growth (3.1% annual growth) in sweetpotato area during 1991-2006. Currently 34.5% of global sweetpotato area is in Africa representing a significant increase from 4.6% crop area in 1961 (Srinivas, 2009).

Major production and nutrition constraints

Although the area under sweetpotato is expanding rapidly in SSA farmers have to struggle to overcome several challenges. The major constraints to increased sweetpotato productivity include, declining soil fertility, drought, low yielding varieties, sweetpotato diseases (mainly sweetpotato virus disease (SPVD), and *Alternaria* blight), and insect pests, (mainly, sweetpotato weevils). Shortage of high quality planting materials, and limited range of processing and utilization options, leading to high post harvest losses, estimated between 30-35%, are also important production constraints. In addition, low nutritive value (low ß–carotene) of non-orange-fleshed sweetpotato and marketing problems limit availability of food with health attributes and processed products to consumers. Research to address these production and market constraints requires a sustained effort in the medium (2-5 years) to long term to produce adapted technologies such as pest resistant varieties. Biotechnology approaches offer an attractive option of integrating desired traits into farmer and consumer preferred sweetpotato cultivars. Examples where biotechnology could be used to improve sweetpotato significantly for nutrition and food security are highlighted below.

Target traits and strategies using biotechnology tools

Biotechnology tools are used to complement conventional approaches particularly for traits where conventional breeding efforts and integrated crop management efforts have till now not produced durable resistances particularly for pests and diseases. In sweetpotato, the target traits are mainly sweetpotato weevil and virus disease resistance.

Sweetpotato genetic transformation for weevil resistance. One of the most important causes of sweetpotato production losses worldwide is the sweetpotato weevil, *Cylas* spp. (Sutherland, 1986). Sweetpotato weevils are the most important biological threat to productivity, marketability, and sustainability in areas with significant dry periods.

The most ubiquitous *Cylas* species worldwide is *C. formicarius elegantulus* (Summers). However, in SSA the primary species are C. *puncticollis* (Boheman) and *C. brunneus* (Fabricius), which may cause losses of up to 60-100% depending on severity of attack (Smit, 1997; Stathers *et al.*, 2003). The primary damages caused by *Cylas* spp. larvae are partial consumption of the root, unacceptable microbial contamination, and production of toxicants by the sweetpotato root making it unfit for consumption (Uritani *et al.*, 1975; Sato and Uritani, 1981; Woolfe, 1992). The eggs are laid right underneath the skin and when hatched larvae tunnel into the sweetpotato root. This makes weevils extremely difficult to control because strategies such as use of insecticides, and integrated pest management (IPM) have primarily targeted adults. Pheromone mass-trapping of *Cylas* males has been successful in Cuba for the control of *C. formicarius* (Lagnaoui *et al.*, 2000). However, no differences in storage root infestation levels and female mating was observed when mass-trapping experiments were conducted

in Uganda using pheromones of the African species *C. puncticollis* and *C. brunneus* (Smit *et al*., 2001). The losses due to weevils limit the crop's potential contribution to reducing poverty and under-nutrition.

A recent survey on the socio-economic impact of weevils in Uganda, reports an average yield loss of over 28% between wet and dry seasons (Kiiza *et al.*, 2009). According to Qaim (2001), weevil resistance sweetpotato cultivars generated from biotechnology applications if deployed in Kenya would create welfare gains of US $ 9.9 million and an approximate internal rate of return on research investment of between 33 to 77%.

Breeders and biotechnologists seek to develop plants that are resistant to the weevils. Conventional breeding of sweetpotato is problematic due to factors such as hexaploid genomics, high heterozygosity, low seed set and self- and combining- incompatibilities. Conventional breeding alone is unlikely to provide all the solutions to sweetpotato improvement.

Weevils have the capacity to adapt and develop resistance to active proteins and compounds found or introduced into new varieties. Hence, long term control requires use of multiple strategies, to increase barriers to the rapidly evolving pests that pose a threat to the durability of available resistance.

Progress in breeding with resistance to weevils has been slow mainly due to scarcity of varieties with significant levels of resistance (Stevenson *et al.*, 2009). Nonetheless, weevil resistance was among the first traits for which genetic transformation was applied in the crop. Early work focused on transformation with proteins that decrease the digestibility of sweetpotato for insects. Sweetpotato was transformed with a cowpea (*Vigna unguiculata*) trypsin inhibitor (CTI) and the mannose binding snowdrop lectin (Newell *et al*., 1995); a soybean (*Glycine max*) Kunitz-type trypsin inhibitor (SKTI) and a rice (*Oryza sativa*) cystein proteinase inhibitor (OCI) (Cipriani *et al*., 1999, 2001).

Initial results in no-choice feeding tests showed moderate increase of weevil resistance in two transgenic events produced by Newell *et al*. (1995) with the West Indian sweetpotato weevil (*Euscepes postfasciatus*) in a screenhouse bioassay (Zhang *et al*., 2000). However, the strategy of using proteinase inhibitors was later abandoned due to the relatively small increase of resistance observed and because there were concerns regarding nutritional impact of such proteins on the human diet. More recently, toxins from *Bacillus thuringiensis* (Bt) were tested against the two African weevil species, *C. puncticollis* and *C. brunneus*, and against the American and Asian species, *C. formicarius*.

A diet incorporation methodology provided reliable toxicity measures of seven Cry proteins from Bt strains, which were chosen based on prior evidence of toxicity (Maingi *et al*., 2002) or known anti-Coleopteran activity. All Cry proteins evaluated were toxic to both species at concentrations less than 1 µg/gram diet, and Cry7Aa1, ET33/34, and Cry3Ca1 had LC_{50} values below 1 µg/gram diet against both species (Ekobu *et al.*, 2010). These tests demonstrated the feasibility of transformation of sweetpotato varieties potentially conferring field resistance against these pests. Four of these Cry toxins were selected for plant expression because of their toxicity and low sequence identity, which is important for the potential of cross-resistance. Studies to help establish the potential for cross-resistance are currently ongoing. Several gene constructs have been developed using chemically synthesized sequences for the coding and polyA regions and optimized for sweetpotato, and sweetpotato promoters to express high level in the storage root. For weevil and virus resistance, transformation with *Agrobacterium* is underway for African varieties that are amenable to transformation and regeneration both in Kenya and Uganda (Gichuki *et al*. 2007; Kreuze *et al.*, 2009).

Sweetpotato genetic transformation for virus disease (SPVD) resistance. SPVD is a synergistic viral disease caused by co-infection of a crinivirus, *Sweet potato chlorotic stunt virus* (SPCSV) with a potyvirus, *Sweet potato Feathery Mottle Virus* (SPFMV). Whereas most sweetpotato cultivars are highly resistant to most viruses including SPFMV, infection with SPCSV renders them highly susceptible and the severe disease, SPVD, follows. Therefore SPCSV can be considered a major target for which resistance is required, although increasing resistance to SPFMV may also reduce damage caused by SPVD. The causal viruses of SPVD are transmitteed by whiteflies (*Bemisia tabaci;* SPCSV) and aphids (*Myzus persicae;* SPFMV), and during vegetative propagation of sweetpotato by humans. The disease causes stunting, mottling and deformation of sweetpotato leaves and up to 99% loss in yield (Njeru *et al.*, 2004; Mukasa *et al*., 2006).

Sources of good resistance, useful in breeding programs, are yet to be identified for SPCSV and SPVD. However, local varieties and improved varieties such as New Kawogo and NASPOT 1, respectively, exhibit reduced incidence of the SPVD in the field compared to other cultivars, a type of resistance for which the mechanism has not yet been elucidated (Mwanga *et al*., 2002; Kreuze *et al.*, 2009).

Viruses that cause SPVD belong to the family *Closteroviridae* and *Potyvidiridae* which are known to be highly variable and have a high rate of evolution leading to emergence and re-emergence of epidemics. Therefore, long term control requires use of multiple strategies, to increase barriers to the rapidly evolving pathogens that pose a threat to the durability of available resistance (Kreuze *et al*., 2009).

A transgenic approach is a good option for integrating SPVD resistance into farmer and consumer preferred cultivars. Indeed, for other crops, transgenic plants genetically transformed with similar genes have been produced, tested in greenhouse and field conditions, and in several cases developed for commercial release. Field trials of crops engineered for resistance to viral diseases have been approved in Canada, the United States, Mexico, China, Kenya and other countries (Fuchs and Gonsalves, 2007). Sweet potato transformed with the coat protein (CP) encoding sequence of SPFMV was resistant to SPFMV following experimental inoculation by grafting (Okada *et al.*, 2002). This type of pathogen-derived resistance (PDR) has been used against some viruses in many crop species (Latham and Wilson, 2008).

However, resistance that works under controlled experimental conditions may not necessarily work under field conditions. This was the case for the first transgenic sweetpotato lines engineered for resistance to SPFMV using PDR. Their resistance broke down in East Africa (New Scientist, 7 February 2004, p. 7) possibly because the transgene was not from a locally prevalent SPFMV strain or because the plants became infected with SPCSV (Kreuze *et al.*, 2009). Resistance to potyviruses mediated by a rice cystein proteinase inhibitor (OCI) has been reported in tobacco (*Nicotiana tabacum*) (Gutierrez-Campos *et al.*, 1999). The OCI mediated resistance to potyviruses is thought to act through inhibiting the viral cystein proteinase NIa that processes the potyviral polyprotein. Closteroviruses also encode cystein proteinases to modify some of their proteins. Therefore, it was considered that expression of cystatins in transgenic plants might confer resistance to both viruses involved in SPVD. Cipriani *et al.*, (2001) reported increased resistance to SPFMV in sweetpotato plants of cv. 'Jonathan' transformed with the *OCI*. However, mixed infection with SPCSV still caused SPVD.

Post transcriptional gene silencing (PTGS) or RNA silencing, is a particular form of PDR. The PTGS strategy is based on the action of short interfering RNA (siRNA) molecules, which are formed in the plant in response to double stranded RNA (dsRNA) (Lindbo and Dougherty, 2005; MacDiarmid, 2005). The dsRNA molecules expressed in the transgenic sweetpotato events trigger naturally occurring defence mechanisms in the plants and serve as guides for enzymatic cleavage of complementary RNAs produced by SPFMV and SPCSV, thus destroying the corresponding viruses.

Recently, Kreuze *at al.* (2008) genetically engineered a Peruvian landrace sweetpotato variety 'Huachano' that is extremely resistant to SPFMV for resistance to SPCSV. In the case of Jonathan and Huachano as in many others (Karyeija *et al.*, 2000) the high levels of resistance to SPFMV breaks down following infection with SPCSV and the plants succumb to the severe SPVD. This shows the importance of the resistance to SPCSV in protecting sweetpotatoes against SPVD and other severe synergistic diseases induced by SPCSV with other viruses. The transgene was designed to express an SPCSV-homologous transcript that forms a double-stranded structure to efficiently prime virus-specific resistance through PTGS. Several transgenic lines accumulated only low concentrations of SPCSV after infection and no or only mild symptoms developed. These results showed that sweetpotato could be protected against the disease caused by SPCSV using PTGS. However, the low concentrations of SPCSV in the transgenic plants were still enough to break down the natural high levels of resistance to SPFMV. Hence, immunity to SPCSV seems to be required for prevention of the severe virus diseases in sweetpotato (Kreuze *et al.*, 2008).

PTGS may be lost following infection of the plants with a virus that is not targeted by PTGS (Latham and Wilson, 2008). This is explained by suppression of RNA silencing by the untargeted virus. RNA silencing is a fundamental antiviral defence system in plants and other cellular organisms. It becomes activated or primed by transgene-driven over-expression of viral RNA in cells of transgenic plants and by double-stranded structures of the viral RNA during infection of non-transgenic plants (Haasnoot *et al.*, 2007). Hence, PTGS actually activates the natural antiviral resistance to be ready for action when the target virus initially infects cells. However, PTGS is sequence-specific and therefore not able to target viruses that show less than ca. 87–90% sequence identity with the transgene sequence (Jones *et al.*, 1998). Consequently, the virus that circumvents PTGS will accumulate and produce proteins that suppress RNA silencing (Voinnet, 2005), which will convert the plant susceptible also to the virus that was the target of PTGS. For these reasons, the commonly encountered mixed virus infections in the field and the genetic variability of sweetpotato viruses pose an important challenge that needs to be addressed before sustainable virus resistance can be obtained (Tairo *et al.*, 2005).

Interference with silencing suppressor of SPCSV for controlling SPVD is another strategy of sweetpotato genetic transformation for virus disease resistance. SPCSV encodes an RNase3 protein which is a silencing suppressor (Kreuze *et al.*, 2005) and this protein by itself is able to provoke SPVD-like disease in RNase3 transgenic plants infected with SPFMV (Cuellar *et al.*, 2009). The RNase3 protein functions as a dimer and a mutation in the catalytic RNase3 signature motif (RNase3-Ala37,44) renders it dysfunctional, hence unable to suppress silencing (Kreuze *et al.*, 2005; Cuellar *et al.*, 2009). It is hypothesized that over- expression of the RNase3-Ala37,44 protein will interfere with the function of the wild-type protein expressed by SPCSV by binding to it, resulting in a dysfunctional RNase3-RNase3-Ala37,44 dimer, unable to suppress RNA silencing. Such transgenic plants might become resistant to SPVD.

Other Target Traits for Genetic Modification. There are other traits that could be targeted for genetic modification, for example, drought tolerance (particularly survival of shoots during drought, and sprouting of storage roots at the beginning of the rainy season), starch (modification for quality and quantity), baking quality, protein content, and nematode resistance; some of these have been described by Kreuze *et al.* (2009).

Sweetpotato genetic mapping and genomics

Vitamin A deficiency (VAD) remains a serious threat to children under five years of age in SSA. In 2005, an estimated 43 million children in SSA under 5 years old were still at risk of VAD (Aguayo and Baker, 2005). The causal link between VAD and associated increased child mortality is well-established. Extreme mortality rates (60%) are linked with severe VAD, and even sub-clinical deficiency is associated with a 23% increase in pre-schooler mortality (Sommer and West, 1996). Development of orange-fleshed sweetpotatoes (OFSP) is essential for the improvement of the food supply and nutritional status of millions of people in developing countries, particularly in SSA. However, sweetpotato breeding is challenging due to its genetic complexity and marker-assisted breeding tools are needed to facilitate crop improvement.

Recently, the North Carolina State University, U.S.A. and the National Agricultural Research Organisation research team of Uganda identified quantitative trait loci (QTL) for nematode resistance, dry-matter, starch, ß-carotene content, and yield in a hexaploid sweetpotato mapping population derived from a cross between 'Tanzania', a cream-fleshed, high dry matter African landrace, and 'Beauregard', an orange-fleshed, low dry matter sweetpotato cultivar popular in U.S.A. Two parental maps were constructed using a population of 240 clones (Cervantes *et al.*, 2008a, b). In both parental maps, QTL analysis revealed the presence of 13 QTL for storage root dry-matter content, 12 QTL for starch content, 8 QTL for ß-carotene content, and 18 QTL for yield. Multiple QTL regression models developed for segregation of alleles in each parent explained 15-24% of the variation in dry matter content, 17-30% of the starch content, 17-35% of ß-carotene content, and 12-30% of the variation in yield (Cervantes, 2006; 2010). Molecular markers are an important genetic diversity analysis tool for increasing sweetpotato breeding efficiency (Yada *et al.*, 2010; Elameen *et al*., 2008). This work is a first step toward the long-term goal of developing marker-assisted breeding tools to aid sweetpotato breeding efforts. It also improves our understanding of the inheritance of these important traits in sweetpotato.

CIP and collaborating partners have also developed some genomic resources for sweetpotato breeding, supported by the Generation Challenge Program. They have 454-sequenced two normalized cDNA libraries and have established a gene index consisting of about 30,000 contigs and another 29,000 singletons. The gene index and database are available at the CIP website *(http://www.cipotato.org/sweetpotato_gene_index)*. CIP has produced a population from two heterozygous (2x) *Ipomoea trifida* accessions. This population serves for establishing a diploid reference map for sweetpotato. Currently, CIP has about 680 Diversity Arrays Technology (DArT) markers and 50 simple sequence repeat (SSR) markers to genotype this population; and the map based on *I. trifida* is expected to be ready by 2013. For rapid progress there is a strong need to make available more and better genomic resources for sweetpotato by increasing available sequence information. This could be done by increasing transcriptome sequencing of different tissues and clones for functional genomics. CIP, in collaboration with the University of Ghent is sequencing the genome of sweetpotato, cv Huachano (SOLiD4 complete genome shotgun sequencing with paired ends at 90x coverage of the haploid genome) which is expected to provide a wealth of new data for genomic studies and development of new molecular markers. To study QTL for some traits, it would be easier to do in the 2x population than in hexaploid sweetpotato, however, it might not be suitable to assess most traits of interest, although the parents do produce storage roots. The available DArT resource could be used to increase the number of markers for genotyping the mapping populations.

Conclusion

Africa faces major sweetpotato production constraints, including SPWs and SPVD which cause almost total crop loss on susceptible cultivars. Biotechnology could be used to improve sweetpotato significantly for nutrition and food security. Expression of *Cylas*-active *Bacillus thuringiensis* (Bt) Cry proteins in sweetpotato could result in varieties potentially with field resistance against SPWs. Post transcriptional gene silencing (PTGS) mode of resistance to SPVD is promising. Quantitative trait loci (QTL) for dry-matter, starch, ß-carotene content, and yield have been identified in a hexaploid sweetpotato mapping population of 'Tanzania' (female, cream-colored flesh) x 'Beauregard' (female, orange-fleshed storage roots). Biotechnology approaches offer an attractive option of integrating desired traits into farmer and consumer preferred sweetpotato cultivars to improve sweetpotato significantly for nutrition and food security.

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Bioconversion potential of common agricultural lignocellulosic wastes

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ABSTRACT

With increasing interest in organic farming in the developing world, the potential use of common agricultural wastes in wild *Pleurotus* species cultivation in Mid-Western Uganda was investigated. Growth rate, yield performance and nutrient contents of the mushroom were investigated on different agricultural wastes with and without supplementation of rice bran or molasses. Sugarcane bagasse (24.7days) gave minimum time throughout the growth period, then rice hulls (34.3 days) and coffee husks (35 days). Maximum mushroom growth period was observed on rice straws (44.7 days) and saw dusts (47 days). Organic supplementation improved growth rate, yield performance and nutrient composition of the mushroom. Molasses was the best supplement for growth and rice bran in addition, enhanced yield performance. Organic supplementation of soybean straws and saw dusts exhibited drastic results on growth rate. The cultivated *Pleurotus* species had significant nutrient profile compared to the wild species. Crude protein was significantly high ($p<0.05$) for cultivated mushrooms with supplementation, relatively high mineral contents (K, P and Mg) and no significant change on crude lipid and fatty acids. Bioconversion of lignocellulosic biomass by mushroom cultivation may increase productivity of high quality food, a solution to malnutrition and food security in the country.

Keywords: *Growth rate and yields, mushroom, Pleurotus species, nutrition, supplementation.*

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Introduction

Unlike green plants, mushrooms lack chlorophyll and are unable to produce their own food but derive all their energy and growth materials from their growth medium, through biochemical (decomposition) processes. Mushrooms serve as the most efficient and economically living organisms that can convert lignocellulosic materials into high quality food and feed rich in protein (Vijay *et al*,. 2007). They can grow well on all crop residues such as cereals and legumes, corncobs, tree leaves, sawdust, coffee wastes, banana leaves, bagasse, cotton wastes, brewer's wastes, papyrus reeds and elephant grass (Quimio, 1986). The spent wastes can be reused in agriculture and agroforestry for soil conditioning, as fertilisers or as animal fodder as well as reducing environmental pollution (Zadražil, 1993).

Uganda being an agricultural country has a big potential to produce mushrooms due to availability of large quantities of agro residues. Unfortunately, these agro wastes are generally low in nutrients required for mushroom production. Supplementation of these wastes with the required nutrients such as nitrogen or a combination of one or more substrates is a crucial factor for growth and yield of mushrooms (Royse, 2002). This being the case, however, it remains unclear why the world mushroom thrive without supplementation and hence the need for robust data to guide decisions on supplementation in the production of edible mushrooms. In this study, therefore, growth performance, yield and nutrient composition of wild *Pleurotus ostreatus* on supplemented and main substrates compared to the edible wild *Pleurotus* species were established.

Uganda has a rich diversity of edible and medicinal mushrooms like *Armillaria mellea*, *Lentinus prolifer*, *Termitomyces aurantiacus*, *Termitomyces eurrhizus*, *Pleurotus* species and *Termitomyces microcarpus* which grow in most parts of the country (Katende *et al.*, 1999, Opige *et al.*, 2006, Engola *et al.*, 2006). These mushrooms constitute a traditionally very important nutritious food besides having broad ritual and medicinal acceptance (Kamatenesi *et al.*, 2006, Opige *et al.*, 2006, Nakalembe *et al.*, 2009). Mushrooms contribute to the welfare of some households when they are sold to earn an additional income (Nakalembe *et al.*, 2009). *Pleurotus*

species are grown on small-scale basis, specifically to improve on the socio-economic status of farmers, and they can easily be cultivated on various agricultural wastes (Igbal *et al.*, 2005, Vijay *et al.*, 2007, Pushpa and Manonmani, 2008) with minimal input to give high yield of valued food protein for direct human consumption. In this study, six common main substrates namely: rice straws, rice hulls, saw dusts, coffee husks, sugarcane bagasse and soybean straws were selected to examine their bioconversion potential through determination of the growth rate and yield performance of Ugandan edible wild Pleurotus species as well as the nutrient composition of the fruit bodies.

Materials and Methods

Wild Pleurotus species was obtained from Mid-Western Uganda, in the Albetine region. Identification of this species of mushroom was done locally by the indigenous people and then identified up to genus level by Dr. Ipulet Perpetua of the department of Botany, Makerere University. A fresh and healthy mushroom provided the required cultures through tissue culturing. The cultures were maintained on acidified potato dextrose agar (PDA). A small sterile tissue (3x3mm) was obtained from inside of the stalk near the veil of the mature, fresh and health mushroom with a sterile blade and inserted onto the sterile agar under the laminar flow hood.

The cultures were incubated at room temperature. Radial growth of mycelium of different portions was observed everyday until the Petri dishes were approximately 90% filled with mycelia. Subculturing was done three times on PDA until a pure culture was obtained. Spawn preparation was carried out on millet grain in a small-necked glass flask. Dried and clean grains (200 g) of millet were boiled for 10-15 min. The excess water was removed leaving the grains with approximately 60% of moisture content. Then the grains were spread on newspapers for cooling and mixed with 2% of lime (to improve and maintain pH) and gypsum (to prevent stickiness and absorb excess moisture) before sterilization in flasks at 15 psi at 121 ºC for 45 minutes. The sterilized grains were inoculated aseptically with small squares of 4x4mm mycelial culture from the full-grown pure agar culture and incubated at room temperature until mycelium fully covered the grains.

The dried substrates were chopped into small pieces of 2-3cm, with exception of sugarcane bagasse, weighed and soaked in water overnight before boiling them for one hour. The substrates were spread over a clean, inclined surface for cooling and draining off the excess water. Half a kilogram of each substrate (about 65-70% of moisture content) was packed in polythene bags before sterilization in an autoclave at 15 psi for 45 min. Some bags of each main substrate were supplemented with 10% of sterile molasses by weight (with exception of sugar baggasse) or 38% of rice bran alone by weight in dry form. Three replicates of each substrate were also prepared. The bags were inoculated with the pure grain spawn of Pleurotus species at the rate of 20g/kg of substrate dry weight. The inoculated bags were placed in a dark room with 65-70% humidity, temperature of (27±2)°C, and good ventilation for spawning run. Time of spawning run i.e. the time mycelia is seen in the substrate to full growth and that of pin-heads starting to appear were recorded daily for all treatments. The bags were mouth opened and slits made on their sides to facilitate development of fruit bodies. Watering of the mushrooms was done three times by hand spraying and discontinued a day prior harvest of the fruiting bodies. The time taken for the maturity of fruiting bodies was recorded while the mushrooms harvested in three flushes on each substrate with or without supplementation were weighed in g/kg substrate and the maximum average yield estimated from each substrate.

Chemical compositions of all fruit bodies with or without supplementation were determined. Proximate compositions were performed in accordance with the official Methods of Analysis of the Association of Official Analytical Chemists, AOAC (2002). Minerals were determined using an atomic absorption spectrophotometer and a flame photometer (AOAC, 2002) while fatty acids were determined by AOAC Official Method Ce-2-66 (Modified) Gas-liquid chromatography). The content of total carbohydrates was calculated using the following formula: Total carbohydrates (g/100g fresh weight) = 100 - moisture (g/100g fresh weight) - protein content (g/100g fresh weight) - crude fat (g/100g fresh weight) - ash (g/100g fresh weight). Data were subjected to a one-way ANOVA test and differences between means were detected using the t-test and least significant differences (LSD) at the 95% confidence level. All analyses were computed using SPSS 12.0 Windows program (SPSS Inc., 2003, Chicago, IL, USA).

Results and discussion

The growth rate of the cultivated Pleurotus species was determined using the spawn running time, pin-head-formation time and maturation time. The spawn running ranged from 16-40 days on the main substrates (Tables 1-3). The spawn running completed earlier on sugarcane bagasse at 16th day followed by rice hulls (24 days) and coffee husks (26 days). Maximum time was observed on saw dusts and rice straws at 40.3 days and 35 days, respectively. This could be attributed to the fact that the substrate needed to undergo fermentation due to tough lignin mainly in the saw dusts. Shah *et al.*, (2004) reported spawn running time of 17.33 days for Pleurotus species on fermented saw dusts while Iqbal *et al.*, (2005) reported spawn running time of 14 days on sugarcane bagasse.

Substrate supplementation significantly reduced the spawn running time in all treatments. The best supplementation was observed with molasses (15-29 days) compared to 17-32 days of rice bran supplementation. Supplementation of soybean straws and saw dusts tremendously decreased the days of spawn running with the minor decrease on sugarcane bagasse (Table 1).

Table 1 Time (days) taken for completion of spawn running on different agricultural wastes

Agricultural wastes	Supplementation		
	Main substrate	Rice bran	Molasses
2Rice grass	35.3	30.3	24
2Rice hulls	24.7	18.7	16.3
1Saw dusts	40.3	32.3	29
2Coffee husks	26	20.3	17.7
Sugarcane baggasse	16	14	ND
1Soybean straws	32.3	17	15

ND, not done, [1]tremendous decrease in time (days) on supplementation;

[2]moderate decrease in time (days) on supplementation

The same trend as in spawn running was observed in the case of pin-head formation for all treatments. Sugarcane bagasse was the best main substrate whereby 20 days were required for the first appearance of pin-heads after spawning of the substrate. This was followed by rice hulls (28.3 days). Longer time was taken for the appearance of pin-heads on rice grass and saw dusts with 39 and 43 days, respectively. Several studies report different timings of pin-head formation of *Pleurotus* species on various substrates. For instance, Iqbal *et al.*, (2005) reported time for pin-head formation ranging from 43-47 days and 16-23 days on wheat straw and sugarcane bagasse, respectively; while Kirbag and Akyuz, 2008 reported 26.2 days on wheat straw, 2-4days on composted sisal decortications residue (Mshandete and cuff, 2008) and 11.3days on paddy straws (Jansi, 2010).

The best main substrate to give drastic results for pin-head formation after supplementation was soybean straws (Table 2). *Pleurotus* species mushrooms attained maturity at an early time on sugarcane bagasse (24.7 days) and maximum on rice straws (44.7 days) and saw dusts (47 days). A minimum time of maturation of fruiting bodies (20.3, 22 and 37 days) for *Pleurotus* species was reported by Iqbal *et al.*, (2005) on sugarcane bagasse while the first fructification on coffee husks started on the 20 days. Supplementation of main substrates significantly reduced the maturation time in all substrates. Soybean straws supplemented with molasses exhibited drastic results on all stages of mushroom growth (Table 3). This shows that supplements modified the substrates for better conversion and utilization of the substrates. This observation concurs with Zadražil (1993) report that supplements usually change the decomposition rate and the sequence of decomposition of substrate components during mushroom growth. Zadražil, (1980) also observed that organic supplements such as soybean meal, alfalfa meal, and cotton seed powder increase not only yields but also proteins of mushrooms. Okeke *et al*., (1994) also observed that high levels of soluble protein provide greater biomass in mushroom cultivation. Therefore, the stimulating effects of rice bran may be due to the presence of high levels of carbohydrates, amino acids and minerals in the supplement (Fasidi and Kadiri, 1993) whereas the presence of the additional sugars in molasses facilitated degradation of lignocelluloses in the main substrates, and hence their utilization by the growing mushrooms. Molasses, in addition, provides relative levels of nitrogen, minerals, protein and non-nitrogenous acids (Paterrson-Beedle *et al.*, 2002) needed for the growth of the fungus.

Table 2 Time (days) taken for completion of pin-head formation on different agricultural wastes

Agricultural wastes	Supplementation		
	Main substrate	Rice bran	Molasses
2Rice grass	39	34	27
2Rice hulls	28.3	22	20
2Saw dusts	43	36	33
2Coffee husks	30.7	24	22
Sugarcane baggasse	20	19	ND
1Soybean straws	35.7	21.7	17

ND, not done, [1]tremendous decrease in time (days) on supplementation;

[2]moderate decrease in time (days) on supplementation

Table 3 Time (days) taken for the fruit bodies to attain maturity on different agricultural wastes

Agricultural wastes	Supplementation		
	Main substrate	Rice bran	Molasses
Rice grass	44.7	38.7	31
Rice hulls	34.3	27	23.7
Saw dusts	47	40.7	37.7
Coffee husks	35	27.7	25
1Sugarcane baggasse	24.7	22	ND
Soybean straws	39	27	26.7

ND, Not done, 1Presented early maturity of fruit bodies and late for rice grass and saw dusts

Table 4 Yield of Pleurotus species on different agricultural wastes with or without supplementation

Agricultural waste	Mean fresh weight (g/kg substrate)		
	Main substrate	[1]Rice bran	[2]Molasse
Rice grass	321.05±1.34[a]	489.55±6.85[b]	432.00±5.11[bc]
Rice hulls	406.56±4.51[ab]	**590.45±1.76[d]	434.74±1.92[bc]
Saw dusts	177±5.06[ac]	**392.92±2.13[ed]	240.55±7.44[e]
Coffee husks	[a]476.2±2.99[b]	601.85±8.21[d]	484.86±3.35[b]
Sugarcane bagasse	[a]430.6±3.06[c]	522.32±1.25[ad]	ND
Soybean straws	[a]500.62±1.50[c]	525.22±6.42[ad]	518.62±7.71[ad]

*Figures having different letters are significant different at 5% level of probability,

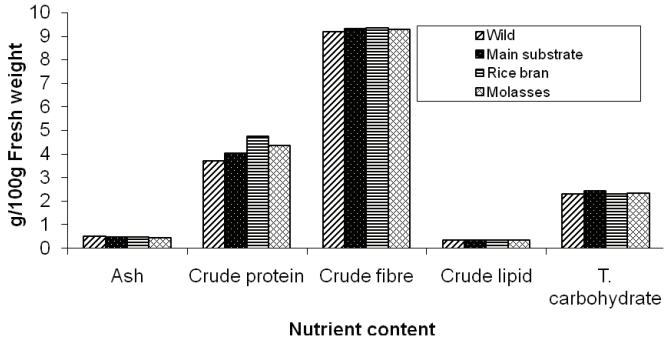
ND= not done [1]Moderate increase in fresh weight, [2]Minimal increase in fresh weight, [a]High mean fresh weight on main substrate

**Drastic increase in mean fresh weight on Rice bran supplementation.

There was a significant difference between the mean fresh weights of fruit bodies produced on all the substrates. However, molasses supplementation had minimal increase in mean fresh weights of fruit bodies compared to rice bran. Considering the main substrates, mushrooms cultivated on sugarcane bagasse, soybean straws and coffee husks weighed higher than those of rice straws, rice hulls and saw dusts (Table 4). Supplementation of the main substrates produced more mean fresh weight of fruit bodies. This could be attributed to the improved nutrient content of the main substrates. Saw dusts and sugarcane bagasse showed a drastic increase in mean fresh weight of fruit bodies in three flushes on rice bran supplementation with the lowest being observed on soybean straws.

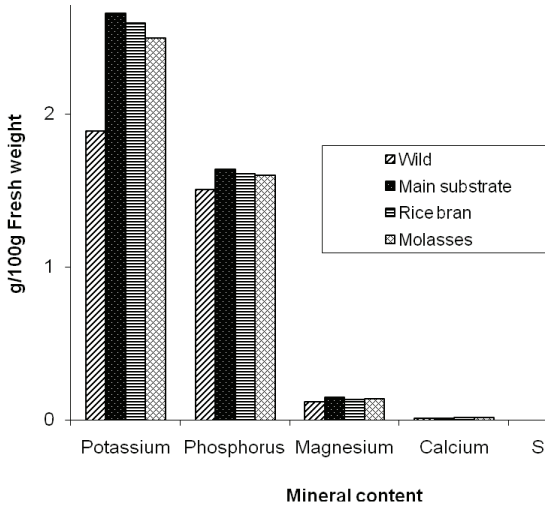
There were also significant differences of some nutrient composition among the fruit bodies of wild Pleurotus species and the cultivated ones (Figs. 1-3). Crude protein was significantly ($p<0.05$) high for cultivated mushrooms as compared to the wild Pleurotus species, ranging from 4.42-5.91±(0.02-0.05) g/100g fresh weight. This was attributed to the presence of high protein and the availability of the degraded components in the substrates. There were no significant differences in crude fat and their fatty acids among the respective treatments (Fig. 3). Potassium (K), phosphorus (P) and magnesium (Mg) were relatively higher in cultivated mushrooms. This shows that the mushrooms could be able to assimilate additional minerals from the media tested.

Figure 1 Mean proximate chemical composition of a wild and cultivated mushrooms (Mean±SD, n=2).



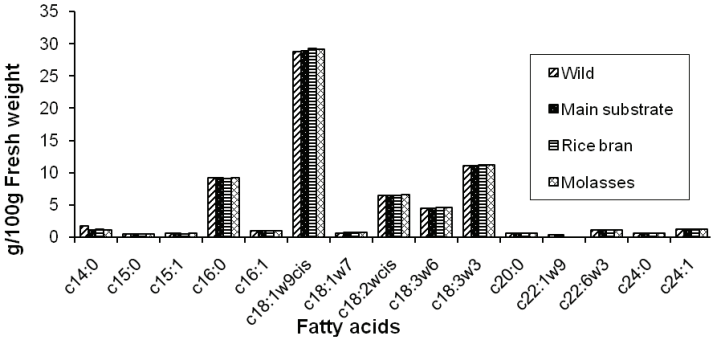
*An arrow indicates significant difference between crude proteins at 5% level of probability

Figure 2 Mineral contents of wild and cultivated mushrooms (Mean± SD, n=2).



*Relatively high Potassium, Phosphorus and Magnesium contents, low sodium content

Figure 3 Mean fatty acid contents of wild and cultivated mushrooms. (Mean±SD, n=2)



Legends: c14:0 (Myristic acid), c15:0 (Pentadecanoic acid), c15:1 (cis-10-pentadecanoic acid), c16:0, (Palmitic acid), c16:1 (Palmitoleic acid), c18:1w9cis (Oleic acid), c18:1w7 (Vaccenic acid), c18:2:cis (Linoleic acid), c18:3:w6, gamma-linolenic acid, c18:3:w3, Alpha-linolenic acid, c20:0 (Arachidic acid), c22:1w9 (Erucic acid), c24:0 (Lignoceric acid), c22:6w3 (Docosahexaenoic acid (DHA), c24:1 (Nervonic acid)

Conclusion

Wild Pleurotus species has a potential for domestication using Uganda's available agro wastes namely: sugar bagasse, rice hull, saw dusts, rice straws, soybean straws and coffee husks. The best main substrates were sugar bagasse, rice hulls and coffee husks. Supplementation of the main substrates enhanced the growth rate, the yield and the nutritive value of the cultivated mushrooms. Molasses and rice bran gave promising results as supplements in cultivation of wild Pleurotus species. Molasses being the best supplement during mushroom growth but it does not significantly affect the yields. Rice bran would be another alternative supplement as it increases growth rate of mushrooms and their yields. Therefore, more sensitization about use of these available agro wastes in the domestication of wild Pleurotus species should be done. This will help to reduce on the environmental pollution, and enhance better utilization of the agro-wastes in agriculture.

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Industrial Biotechnology Opportunities and Progress in Uganda

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ABSTRACT

Industrial biotechnology has potential to enliven and promote growth of the manufacturing industry of Uganda through creating intricate value chains with biomass as raw material for high value products. Uganda is endowed with biomass and many communities are engaged in manufacturing industry with prime focus in fermentative production of bio-chemicals including alcohols and fermented foods. Nonetheless, the scale and scope as well as the quality of products generated remain a challenge which effectively curtails growth of the industry. Consequently the contribution in terms of foreign exchange earnings from industrial biotechnology is extremely low. Key constraints which should be addressed with a view to enhance industrial biotechnology applications and services in production of biochemicals in Uganda include the following, among others: lack of systematic scientific promotion and failure to extend the technology frontier; lack of a long term strategy or policy; unplanned craft and job production design; focus on social welfare and survival instead of economic market behavior; lack of public awareness and acceptance of the potential of the technology; lack of necessary facilities to demonstrate commercial feasibility; and insufficient connectivity between the key players. Government should apply market pragmatism by systematic scientific promotion. Policy direction should complement markets in order to achieve a better long term outcome for the country's economy and society and provide necessary facilities to demonstrate commercial feasibility.

Keywords: *Biochemicals, economic markets, technology frontiers, value chains, Uganda*

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Introduction

This article evaluates current and future contributions of industrial biotechnology and potential for progress in Uganda. Industrial biotechnology has a role in ensuring the steady growth of Uganda's manufacturing industry development. There are enormous opportunities, not just for the economy, but also to our environment and society. Industrial biotechnology has been practiced for a long time in a craft and job manufacturing system even to produce high quality products of world class. The technology identifies with the situation in developing countries where innovation is born out of need and there is no incremental innovation once something becomes useful (Dr. Kazungu D. Personal Communication, 2010 [1]). Moreover Ugandan manufacturers employ basic techniques where harnessing the environment is key to wealth creation. Industrial biotechnology is the use of biotechnology in industrial processes. Application of nature's tool set to the production of bio-based chemicals, materials and fuels.

Industrial biotechnology

Industrial biotechnology also known as "white biotechnology" is the use and application of biotechnology for the sustainable production of bio-chemicals, biomaterials and bio-fuels from renewable resources, using living cells and or their enzymes (Tang and Zhao, 2009). In the past, biological processes have been applied in industrial production such as making cheese, wine, beer, and with the advancement in

[1] Dr. David Kazungu commented in relation to indigenous innovations in a survey under IDRC funded project *"Traditional Science Technology and Innovations Systems in the Context of a Modern Incubator Research and Development Agency"* results are not yet published

scientific developments the list of products has expanded to include vaccine and hormones and biogum, among others. Enzymes which are derived from these products, organisms, are applied to catalyze a conversion in order to generate the desired products (Fig. 1).

Opportunities for industrial biotechnology

Whenever there is a market potential, there is an industrial opportunity. Uganda has a manufacturing industry with the prime focus in the second part of the value chain (Fig. 1), for production of biochemicals, biofuels and biomaterials. Typically the industry involves fermentative production of bio-chemicals including alcohols, and fermented foods. Value can be created in the social, environmental and economic sectors of industrial biotechnology which is yet to be uncovered.

Examples of emerging efforts to harness industrial biotechnology in Uganda include ongoing studies at the Uganda Industrial Research Institute (UIRI) to use Neurospora sitophila fungi to produce cassava detoxifying enzymes which precondition the cyanogenic glycosides for Xanthomonas campestris bacteria in the derivation of biogum a high value product. Social utility is created by providing safe cassava products of consistent quality on time, thus ensuring food security. Production of bitter cassava has both social and environment impacts since women prefer to grow them to allay household thieves (Wendiro and Otim-Anyoni, 2004[2]). This variety stays long in the soil hence is the only available source of food in periods of food shortage. Moreover bitter cassava varieties are resistant to pests and diseases and do well on marginal land (Aerni, P., 2004; NAARI, 2009).

In another study at UIRI, laccase enzymes (Novozymes 18043) derived from white rot fungi are being used in the removal of lignin from bark of Ficus species (Unpublished, ongoing study at UIRI). The use of enzymes has been shown to improve the fibre characteristics for textile manufacture. The bark is locally used to make bark cloth http://www.worldagroforestrycentre.org/sea/Products/AFDbases/AF/asp/SpeciesInfo.asp?SpID=866#Uses. Ficus species have for long been used as shade in banana and coffee plantations, its leaves are feed to small ruminants, it has medicinal value, and as wind breakers, and currently as timber for furniture since more traditional tree species are scarce (Zziwa, *et. al*., 2006; Brink, 2010). Innovative value addition to the fibre, which has so far been used for traditional purpose, would thus have resounding social, economic and environment impact, since harvesting the bark is usually non-destructive.

From the products generated, it is clear that industrial biotechnology has the potential for more sustainable livelihoods and competitive enterprises in terms of: low carbon revolution offering businesses the capability to develop and use less carbon intensive products and processes; reducing costs of production; and opening new, emerging and established markets. Market opportunities have been identified in many sectors; fibre based materials, bio-plastics and other biopolymers, surfactants, bio-solvents, bio-lubricants used in cosmetics, household and industrial detergents, paints and adhesives, ink and paper making, ethanol and other chemicals, pharmaceutical products, vaccines, enzymes, and cosmetics (Yang *et al*., 2002; Hsu and Lo, 2003; Rosalam and England, 2005; Salah *et al.*, 2010) .

Biomass energy is derived from trees and agricultural residues from the surrounding land cover such as farm lands, bush lands, woodlands, forests and grasslands. There is a rapid gain in transition towards renewable bio-based raw-materials. This market change will lead to further opportunities. The Lead Market Task Force on bio-based products predicted that the global market for bio-based products will grow to $ 250 billion by 2020 for a range of bio-based products including: fibre based materials (i.e. for construction sector or car industry); bio-plastics and other bio-polymers; surfactants; bio-solvents; bio-lubricants; ethanol and other chemicals and chemical building blocks; pharmaceutical products including vaccines, enzymes, and cosmetics (http://ec.europa.eu/enterprise/policies/innovation/files/lead-market-initiative/bio_based_products_taksforce_report_en.pdf).

Recently Government of Uganda is placing focus on science, technology and innovation (STI) and high value manufacturing. Industrial biotechnology resonates strongly in these areas considering that the prime focus of our traditional STI in the value chain is the fermentative production of bio-chemicals. Biomass is measured as the mass of organically bound carbon (C) that is present. The total live biomass on earth is about 560 billion tons of carbon. Forests contain about 80% of global terrestrial above-ground carbon stocks (biomass). Africa has the second largest block of rainforest in the world and is diverse in terms of the wide range of ecosystems. Uganda has a total area of about 241,551 km^2, out of which, farmland is the most extensive, followed by grasslands, woodlands, water bodies, bush land, tropical high forest (normally stocked), tropical high forest (degraded) and others in that order. The land area excluding water is about 20.5 million ha, out of which 4.9 million ha (about 24%) is covered by forests (plantations both hard and softwoods) tropical high forests (normal and degraded), and woodlands (Drichi, 2002).

In years to come Uganda's success will increasingly be defined by her competitive edge in industrial biotechnology and other knowledge-intensive industries. Industrial biotechnology has multiple impacts: it increases process efficiency and enables the use of renewable feedstock. It is a tool in the development of sustainable production processes. At global level, an environmental indicator that is relevant for all case studies on global scale is green house

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[2] *Comment by a woman respondent in focus group discussion in Osukulu Subcounty Tororo District of Uganda, 2004 in a research on "Alternative technology approach to food security: A case of using enzymes in the detoxification of cassava." The use of enzymes was being tested for consumer acceptability. The woman commented in relation to cassava saying the husbands steal sweet cassava from house hold gardens and sell it to drink alcohol. With bitter cassava the situation is different because it requires processing, which the man cannot do stealthily.*

emissions. Biotechnology is among the key technologies that can help to address global warming, one of the most pressing environmental challenges; cleaner industrial biotechnology processes could thus enable countries to meet the international objectives in terms of carbon dioxide emissions, and pollution through anaerobic digestion, bioremediation or environment sequestration (Kumar, *et. al.*, 2011).

Industrial biotechnology makes industry more sustainable, it is expected that the benefits will be seen across a range of critical society-based arenas: job retention and creation, development of new technology platforms, and the reduction of society's dependence on valuable fossil resources, thereby conserving them for future generations. High-level education and research will be stimulated by providing high qualification employment and by developing R&D initiatives.

In Uganda, the niche for industrial biotechnology applications and services is apparent in aspects and sectors of food security and health intervention efforts for securing improvements in livelihoods. Examples of candidate projects for which industrial biotechnology can create value addition include the following:

- Local alcoholic beverages commonly called *tonto, malwa, mwenge bigere, muramba* etc contributes a percent to the "informal sector" economy. Some Ugandans are educated by proceeds from that industry, and some jobs are created in this way such as maize growing, and sieving (Mwesigye and Okurut, 1995; Namugumya and Muyanja, 2009)
- Processing and use of fermented soybean popularly known as tempeh to fight persistent diarrhea would have economic, health and food security benefits. It has been reported that 50% of diarrhea related deaths in developing countries are associated with persistent diarrhea disease. Isolation of enteropathogenic *Escherichia coli* in stool samples was associated with 28.4% of conversion to persistent diarrhea (de Andrade, *et. al.*, 2011), Invitro extracts of tempeh were found to inhibit adhesion of enterotoxigenic *Escherichia Coli* to intestinal brush boarder membrane of piglets (Kiers, *et. al.*, 2007).
- The alcohol powered bicycle launched by Honorable Jimmy Akena of Lira Municipality in Lira Town (Kiwuuwa, 2008) could contribute to improved health access; and poverty reduction by opening new markets for an already existing product – waragi, which is currently sold cheaply to local consumers whose health is also at risk due to consuming it
- Low carbon revolution offering businesses the capability to develop and use less carbon intensive products and processes (van der Zwaan, *et. al*., 2002, Chen, *et. al.*, 2011)
- Specialty cosmetic products that are over 60% organic and high value using organic stabilizers, and herbal extracts targeting lucrative niche markets of upscale consumers who prefer organic products
- Unprocessed essential oils extracted from natural herbs are being exported and or sold locally including thyme, white eucalyptus, citronella, ginger, coriander, ocimum, and tagette, to mention but a few (Wren, 2003), which have great opportunity in high value market sectors such as pharmaceuticals (Chen, *et. al*., 2011) and cosmetics (Prabuseenivasan, *et. al.*, 2006)

Based on the value chain approach, industrial biotechnology has a lot to offer (Figs. 1 - 3) in terms of value addition to raw materials, job creation, innovation and saving resources for future use. The pace of technology development is one of the most important factors that determine the rate of market growth. There is limited systematic scientific promotion and extension of technology frontier, which has resulted into slow growth of the manufacturing industry.

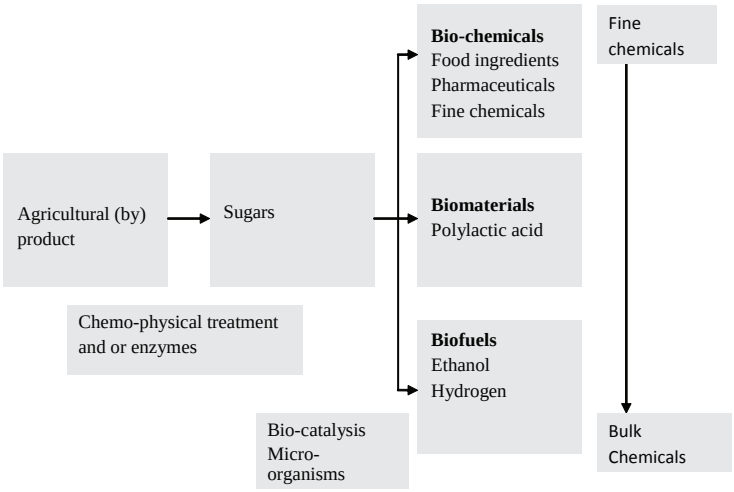

Figure 1: Industrial Biotechnology Value Chain

Figure 2: Impact of industrial biotechnology on society

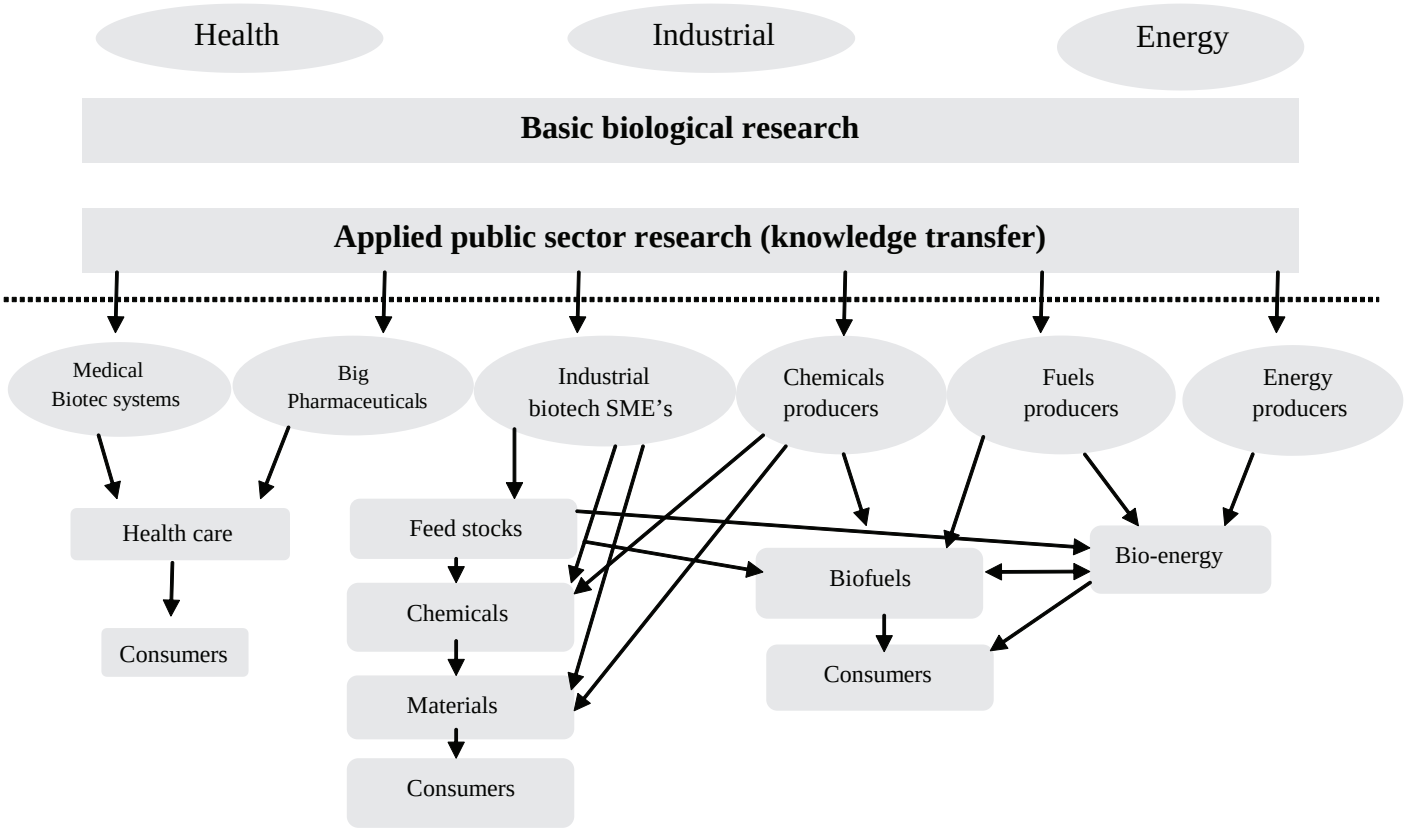

Figure 3: Impact of industrial biotechnology on society

Key issues which should be addressed to catalyze the deployment of industrial biotechnology include the following, among others: establishment of systematic scientific promotion; adoption of a long term strategy or policies; crafting and adoption of job design; focus on social welfare and survival instead of economic market behavior; enhancement of public awareness and acceptance of the potential of industrial biotechnology applications and services; investments to establish facilities to demonstrate commercial feasibility; and enhanced coordination among the key players in industry.

Government should apply market pragmatism to policy direction to complement markets and achieve a better long term outcome for the economy and society. The criteria for selection should include indigenous STIs that could generate viable products for niche markets; opportunities provided by trade and availability of natural resources; prospects for deepening value chains (opening way for other uses); environment impact, social cultural factors such as gender issues and geographical proximity to the central marketing district; available technological capability; and strategies to exploit new opportunities, for high value products like biogum processing, biofuels and textiles from bark of Ficus species. More attention should be paid to factors controlling science, technology and innovation (STI), diffusion and commerce.

There is need for specific scientific promotion, advancement of technology frontier and application of a business support regimen representing a precise flexible pattern to complement efforts of indigenous STI to reach a threshold level for development; develop S&T capabilities that are critical in creatively exploiting knowledge for economic, social and political aims (like a wide variety of design and engineering activities) (Wamae, 2009). This would aid local firms to acquire state-of-the-art technologies that would enhance their competitiveness in global trade. Centres for indigenous science, technology and innovation systems should be established to identify viable innovations, study, develop and package them for the market as typically African products to fast track industrial development, and courses in innovation should be included in curriculum at all education levels.

The next generation of biotechnological advancements will continue to require interdisciplinary communication among engineers, biologists, and physical scientists. Likewise, in all aspects of bioengineering, biology helps provide the framework for understanding which questions and problems are important, while the engineering is critical to developing effective solutions. Hence the cross-disciplinary exposure gained by engineers and biologists working in interdisciplinary teams, will have an impact beyond the development of immediate applications.

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Sources of resistance to stem rust among selected wheat germplasm

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ABSTRACT

Wheat (*Triticum aestivum*) is an important staple food crop contributing to food security and income generation among resource poor farmers. However, the crop is threatened by stem rust which pose a major constraint to wheat production in East Africa. This is because the *Ug99* (TTKS) a virulent strain of the *Puccinia graminis fsp tritici* Eriks and Henns, has overcome major resistance genes; *Sr31, Sr36* and *Sr24* previously deployed against the stem rust. This has led to significant reduction in the wheat yields or sometimes to total crop failure under heavy epidemics. Thus, host resistance remains vital in combating the *ug99* spread. A study carried out at Kenya Agricultural Research Institute (Njoro) in the field aimed at identifying sources of resistance to stem rust. This study revealed some promising wheat lines; R07F4-21258 and THELIN#2/TUKURU CGSS02Y00118S-099M-099Y-099M-16Y-OB which should constitute appropriate material for breeding programs. These promising lines have already been used in intercrosses and populations are being advanced into further generations for genetic studies and mapping of the resistance genes. The recurrent selection will be used to accumulate these resistance genes into high yielding wheat background in further breeding work to help avert further wheat yield losses in East Africa which is faced with acute malnutrition, famine and drought.

Keywords: *Host resistance, Puccinia graminis, Triticum aestivum, virulent strain, ug99*

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Introduction

Wheat (*Triticum aestivum*) production is constrained by many factors despite its economic importance in terms of calorific input of 16% in the developing countries (Dixon *et al*., 2009). In Kenya, the growing of wheat at different agro ecological zones provides a green bridge for rust inoculum throughout the year (Singh *et al.*, 2008). This has led to unacceptable yield losses of over 70% by the small scale farmers who produce 20% of the wheat consumed in East Africa (Wanyera *et al.*, 2004). In 2007, 100% yield losses were reported among farmers in Kenya (Wanyera, 2008). The stem rust disease caused by *Puccinia graminis f. sp. tritici* (Eriks and E. Henn) is currently the greatest threat to wheat production due to the emergence of the *Ug99*, a virulent strain of the *Puccinia graminis fsp tritici* which was designated TTKS based on North American pathotype nomenclature system (Wanyera *et al.*, 2004). This has led to increased prices of wheat grain and its food products, increased alternative food prices due to increased demand, increased net food imports and loss of the high investment crop.

The fungus, *Puccinia graminis fsp tritici*, exhibits high genetic diversity (Groth *et al.*, 1995) that has resulted in breakdown of major resistance genes namely *sr31, sr24* and *sr36*, which were deployed in stem rust resistant varieties. This has been associated with mutation, sexual and para-sexual genetic recombination and the current climate change.

Over 45 stem rust resistance (Sr) genes have been deployed worldwide against the different races of stem rust (Roelfs, 1988; McIntosh *et al*., 2003 and Steffenson *et al.*, 2007). However, most of these resistance genes show dominant inheritance and are race specific (Spielmeyer *et al*., 1998). Thus, the use of single resistance genes has been considered a threat to wheat production and is highly discouraged.

The erosion of the few resistance genes against the *ug99* race has been attributed to the arms race between the pathogen and the host. The host has to continuously evolve to produce new forms of resistance genes while the pathogen is forced to alter effector genes to avoid its recognition. Hence, this calls for use of durable sources of resistance to stem rust which can remain effective for long duration over generations under environments with disease pressure (Johnson, 1984). This has involved the use of wheat lines containing *Sr2* gene which is non race specific. This would help to stabilize the existing populations of the pathogen preventing further evolution. These resistance genes provide resistance in mature plants close to immune when used in combination. Thus, this study set out to identify new sources of resistance effective against *Ug99* strain of the *Puccinia graminis fsp tritici.*

Materials and Methods

Twenty five wheat lines plus susceptible checks containing *Sr24* gene were planted in the field at Kenya Agricultural Research Institute (KARI) Njoro (0^0 20'S; 35^0 56' E, and 2166 m above sea level) during the off season, 2009 (November 2009 to April, 2010) and main season, 2010 (June to October, 2010). The site is a hotspot for stem rust disease with all the major pathotypes including the *ug99*. It also has the facilities and fits well with the shuttle breeding program adopted by CIMMYT. The shuttle breeding has been involved in the screening of segregating populations developed by wheat breeding programs at CIMMYT-Mexico nurseries and other international wheat breeding programs (Singh *et al.*, 2009).

Each wheat line was grown on two rows each of one metre in length in an alpha lattice design. Disease spreaders involving a mixture of the seven susceptible *Sr24*-gene containing wheat lines were grown perpendicular to all the plots as infector rows and inoculated with the stem rust spores using a syringe (Liu and Kolmer, 1998).

The field assessment for disease resistance was achieved by making a score of the disease severity which includes estimating the proportion of the stem affected by the disease from time of disease appearance until physiological maturity. The stem rust disease severity and reaction was scored based on modified Cobb's scale (0-100%) and their field responses. The field responses involved resistant (R) to moderately resistant (MR) (small-size uredinia); moderately resistant (MR) to moderately susceptible (MS) (medium-size uredinia) while the moderately susceptible (MS) to susceptible (S) showed medium to large-size uredinia (Peterson *et al.*, 1948). Other agronomic data collected were plant height, stem lodging, yellow rust and maturity. All these data was subjected to ProcGLM of the SAS program (SAS institute Inc., 1996).

Results and Discussion

All the traits measured among the wheat genotypes showed variation at $p<0.001$. It was also noted that the disease severity did not show variation across the two seasons (Table 1). There were favourable environmental conditions for rust development in both seasons.

The genotypes R07 F4-21258 and THELIN#2/ TUKURU CGSS02Y00118S-099M-099Y-099M-16Y-OB exhibited the best agronomic traits. These lines had statistically low disease severities, early maturity, low plant height, low yellow rust severity and resistance to stem lodging (Table 2). The early maturing lines exhibited disease resistance whereas the short plants produced thick stems which withstood stem lodging while supporting larger seed heads. From this work, wheat lines with trace responses (TR) were noted. These lines were characterized by chlorotic flecks implying they may contain major genes attributed to hypersensitive responses. The use of wheat lines containing only major genes has been prohibited due to the erosion of the resistance through mutations and sexual recombination (Ayliffe *et al.*, 2008). Further improvement of these lines could be achieved through pedigree selection to select for major gene effects. The involvement of these lines in intercrosses and recurrent selection could enhance the value of these lines.

Table 1 ANOVA for agronomic traits among selected wheat germplasm

Source	df	% stem lodging		a%DS		maturity		plant height		yellow rust	
		mean deviance	chi pr	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Pedigree	24	31.075	<.001	370.5	<.001	50.26	0.101	147.29	<.001	396.51	<.001
season	1			28.4	0.609	4746.33	<.001	12168.85	<.001	4872.04	<.001
Pedigree. Season	24			97.4	0.587	18.39	0.935	40.45	0.41	208.58	<.001
Residual	48										
Total	98										

[a]%DS = percentage disease severity; MS= mean squares

Table 2 Means of the stem rust disease and other agronomic traits among the wheat germplasm

Entry	Pedigree	%DS (0-100)	*a*Field Response	yellow rust (0-100)	[b]PBC	maturity (days)	plant height (cm)	stem lodging (0-100)
1	1168.6	1	TR	16.5	+	84.5	66.31	70
19	SUNCO//TNMU/TUI	1	TR	7.5	-	78.5	74	35
7	SERI.1B*2/3/KAUZ*2/BOW// KAUZ/4/PBW343*2/TUKURU/5/ C80.1/3*BATAVIA//2*WBLL1	1.8	TR	18.75	-	85.5	67.06	65
21	R07 F4-21258	4.5	TR	19	-	82	70.31	0
3	MON'S'/ALD'S'//TOWPE'S'	12.5	RMR	3.75	+	87.75	85.12	0
5	THELIN#2/ TUKURU CGSS02Y00118S-099M-099Y-099M-16Y-OB	12.7	MR	8.75	-	74.25	71.19	0
4	87	15	RMR	10.25	-	83	78.56	0
24	CHEN/AEGILOPS SQUARROSA (TAUS)// BCN/3/VEE#7/BOW/4/PASTOR/5/ VERDIN CMSS02M00361S-030M-16Y-0M-040Y-16ZTB-0Y-03B-0Y	16.2	MR	20	-	79	79.94	0
20	CHEN/AEGILOPS SQUARROSA (TAUS)// BCN/3/VEE#7/BOW/4/PASTOR/5/ VERDIN CMSS02M00361S-030M-15Y-0M-040Y-6ZTB-0Y-03B-0Y	17.5	RMR	23.75	+	81.75	76.56	0
2	CWANA 1st SR RESIS. ON - ETH - OS71	20	RMR	5.25	+	87	86.16	0
9	WHEAR/KUKUNA//WHEAR	21.2	MR	32.5	+	78.5	85.56	0
14	WHEAR/VIVITSI//WHEAR	21.2	RMR	33.75	-	80.25	83.12	0
15	WHEAR/SOKOLL	21.2	RMR	13.75	-	75.5	84.88	0
22	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	21.2	M	12.5	-	80.5	82.97	0
13	SUPER SERI#1	23.7	MR	22.5	+	81.25	86.88	0
25	(yield trial 2007)	23.7	MR	7.75	-	87	85.38	0
11	PBW343*2/KUKUNA//PBW343*2/ KUKUNA/3/PBW343	25	MR	21.25	-	82.5	79	0
12	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	25	M	37.5	-	83	78.44	0
10	WHEAR/JARU//WHEAR	26.2	M	35	-	79	77.62	0
17	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	27.5	RMR	15	-	81.27	84.44	0
16	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	28.7	M	14	+	75	81.84	30
23	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	28.7	MR	33.75	-	83.5	84.94	0
18	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	30	M	17.5	-	81	83.94	0
8	WHEAR/VIVITSI//WHEAR	32.5	M	18.75	-	81.25	81.06	0
6	IGW3207	33.8	MS	6.5	-	79.5	84.56	0
	Grand mean	19.7		18.22		81.29	79.99	
	Standard error of differences (s.e.d)	7.31		4.914		4.041	4.35	

[a] Field responses involved trace response (TR), resistant (R), moderately resistant (MR), resistant to moderately resistant (RMR), moderately resistant to moderately susceptible (M), moderately susceptible (MS), and susceptible (S).

[b] PBC is pseudo black chaff suggesting presence of the Sr2 gene; its presence is indicated by a plus (+) while the lack of it is represented by a minus (-).

%DS implies percentage disease severity

Among the 25 wheat lines screened for resistance to stem rust, *ug99*, 72% of them expressed resistance responses (R to MR). The wheat lines used in this experiment had been selected during the 2008 (main season) international screening nursery grown at KARI, Njoro based on their level of resistance. Some of these lines exhibited the pseudo black chaff phenotype (Table 2). The pseudo black chaff phenotype has been associated with the *Sr2* gene. The *Sr2* gene in combination with other minor genes forms the '*Sr2*' complex forming the basis of durable resistance in wheat against the stem rust. The *Sr2* has been effective against *Puccinia graminis fsp tritici* since 1920 (Ayliffe *et al.*, 2008; Liu and Kolmer, 1998). With durable sources of resistance, the Sr genes remain effective over a vast area and longer period of time (McIntosh and Brown, 1997). This will make it difficult for new races of *Puccinia graminis fsp tritici* to overcome the prevailing resistance. 24% of these lines expressed moderately resistant to moderately susceptible responses. It is crucial to test these lines for partial resistance to stem rust for further screening in combination with the resistant wheat lines. These lines could also be involved in gene pyramiding experiments to make use of any available resistance.

The results of this experiment suggest the presence of good sources of resistance to the stem rust *ug99* among the wheat lines involved.

Conclusion

More work is ongoing and intercrosses have been made. The population is also been advanced through the single descent method to study the genetics of resistance and map the stem rust resistance genes. It is crucial to accumulate the resistance into high yielding wheat background while testing the advanced generations for adaptability in areas that are vulnerable to the *ug99* strain especially in East Africa which is faced with acute malnutrition, drought and famine. The genotypes could also be used to replace the susceptible wheat varieties to ensure food security within the region.

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Does horizontal gene flow occur in transgenic banana/ *Fusarium oxysporum* (V5W2-9 NH3) associations?

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ABSTRACT

Fusarium oxysporum (V5W2-9 NH3) a fungal non-pathogenic *Fusarium* species is useful in the bio-control of nematodes in bananas. With the advent of the usage of biotechnological approaches to mitigate crop production constraints, several biosafety concerns have emerged. Of interest is geneflow to phyllosphere microorganisms. In this study, transgenic banana plantlets having the hygromycin resistance (*hpt*) gene as a selection marker and ß-glucuronidase (*gus*A) gene as reporter marker were used to investigate gene flow to *F. oxysporum* (V5W2-9 NH3). The plantlets were inoculated with *F. oxysporum* (V5W2-9 NH3) and transferred into 10 litre buckets containing sterile soil. After colonisation, the endophytes were re-isolated from the plants on selective medium. DNA was extracted from the re-isolates and amplified using hpt and *gusA* specific primers to confirm putative gene flow. Results indicated that although plant colonisation was significantly affected by media composition (P≤0.0001), there was no interaction between media composition and the banana genomic structure (P=0.31). PCR screens also showed that the microorganisms were not able to take up the transgenes from the plants suggesting therefore that the cultivation of transgenic bananas will not be a threat to the fungal communities with which they are associated.

Keywords: *biosafety, Fusarium species, endophyte, non-pathogenic fungus.*

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Introduction

Endophytes generally refer to fungi inhabiting plant tissues, though there are species of bacteria that have been observed to live endophytically within the plant tissues where they play crucial roles (Stoltzfus *et al.*, 1997). Fungal endophytes play important roles in the growth of plants through mutualistic associations. There are various types of endophytes, some occurring in monocots, while others in dicot plant species. The most important genera that have been isolated from bananas are *Fusarium* and *Acremonium*. Others include; *Penicillium*, *Aspergillus*, *Gongromella* and *Trichorderma*. *Fusarium* is the most dominant genus with *F. oxysporum* being the most widely spread species (Hallman and Sikora, 1994; Niere *et al*., 2002). There are various strains of *Fusarium oxysporum* some of which are pathogenic mainly causing vascular wilts. Some *fusarium* species such as *Fusarium oxysporum* (V5W2-9 NH3) are non-pathogenic and play important roles in improving plant growth through inhibitory effects on nematodes and weevil development (Hallman and Sikora, 1994; Niere *et al*., 1999; Paparu, 2008). With respect to the environment, *Fusaria* unlike other biological control agents reduce the risk of side-effects on non target organisms including crops and humans since they live inside plant tissues (Niere *et al*., 2002).

Due to free lying DNA within plant tissues, endophytes by virtue of their location are predisposed to this DNA and ultimately take it up through horizontal gene flow using mechanisms such as homologous and illegitimate recombination (Magee *et al*., 2003; Zhang *et al*., 2005). Use of biotechnological approaches in the mitigation of biotic constraints in bananas for example the use of chitinases from

rice and papaya in the induction of resistance in bananas to black sigatoka (*Mycosphaerella fijiensis*) (Swennen and Sagi, 1996) and maganins to induce resistance to *Fusarium oxysporum* f.sp. *cubense* and *Mycosphaerella musicola* in rasthali cultivar (AAB) (Atkinson *et al*., 2003), raises biosafety concerns especially gene flow. The occurrence of horizontal gene flow to these microbial communities can have potential negative effects on their major biological and ecological functions (Dröge *et al.*, 1999). Due to the fact that fungi release large amounts of DNA in the environment during their life cycles in addition to the occurrence of conjugatory gene transfers between fungi and bacterial communities (Paul *et al.*, 1987; Dunn-Coleman and Wang, 1998), uptaken DNA will be shared with other microorganisms in the environment thereby leading to the emergence of new populations that may even be pathogenic to the landraces and other plants and disrupt the biological diversity in the environment (Teycheney and Tepfer, 1999; Cooper and Sweet, 2001). This study therefore sought to investigate the occurrence of horizontal gene transfer of a *ß- glucuronidase* and *hpt* gene in transgenic bananas to an associated fungal endophyte, *Fusarium oxyporum* (V5W2-9 NH3).

Materials and methods

The materials used in the study included; transgenic banana plantlets from local cultivars (Mbwazirume and Mpologoma) transformed with *ß-glucuronidase* (gusA) reporter gene under the control of CaMV35S promoter and a hygromycin resistance gene as the selectable marker and terminated by a *nos* sequence. The microorganisms tested were *Fusarium oxysporum* (V5W2-9 NH3).

Experimental design. The experiment was set up following a Complete Randomised Design (CRD). The treatments comprised of transgenic banana plantlets containing *gus*A and *hpt* genes and non transgenic control banana plants. These plantlets were innoculated with *F. oxysporum* (V5W2-9 NH3). The experiment was run for a period of 13-16 weeks during which normal agronomic practices were done to ensure proper growth. Each replicate had 5 transgenic plants and 2 controls. The experiment was repeated three times. Re-isolation of the fungal organisms was done by destructive sampling of the roots. The re-isolations were done three times.

Media preparation. The fungal organisms were cultured on half strength Potato Dextrose Agar (PDA) which was prepared by autoclaving constituted media for 15 minutes at 121°C and cooled prior to the addition of streptomycin sulphate (Duchefa Biochemicals, Netherlands), penicillin (Duchefa Biochemicals, Netherlands) and chlorotetracyclin (Duchefa Biochemicals, Netherlands) antibiotics as well as potassium chlorate (Lab Express Inc New Jersey, USA). The media was subsequently dispensed into petri-plates (90mm). Normal half strength PDA comprised of the antibiotics above and potassium chlorate (Lab Express Inc New Jersey, USA). Selective half strength PDA contained all the above antibiotics, potassium chlorate (Lab Express Inc New Jersey, USA) as well as hygromycin (50 µg/ml) (Duchefa Biochemicals, Netherlands).

Inoculation and re-isolation of experimental organisms

The transgenic plantlets including the controls were established in a nutrient solution for 2 months for adequate root mass development (Fig. 1). At inoculation stage, pure *Fusarium oxysporum* (V5W2-9 NH3) cultures (Paparu, 2008) raised on half strength potato dextrose agar (Duchefa Biochemicals, Netherlands) were used as inoculum. Spore counts were standardised using a haemocytometer. The banana plantlets were exposed to the fungal inoculum by immersion in ritar jar flasks containing 1.5 x 10^6 spores/ml for four hours. Inoculated plantlets were later transferred to 10 L buckets having sterile soil which had been steam sterilised at 100°C using moist heat over a water bath in a metallic cylindrical drum for a period of up to 3-4 days.

After the first eight weeks, two root samples were taken from each of the plants. The root samples were washed and sterilised in 57% sodium hypochlorite (Reckit Bectinson, East Africa Limited Nairobi, Kenya) for 30 seconds, 75% ethanol for one minute and finally rinsed in sterile water for 1 minute. Each root was cut into 12 small cylindrical discs of which 6 root discs were plated on normal half strength PDA (Duchefa Biochemicals, Netherlands). The remaining 6 root discs were plated on selective half strength PDA. This procedure was repeated for each of the 2 roots excised from all the experimental plants. The plates were incubated for 7-10 days at room temperature. A total 1584 banana root cylindrical discs were assessed for *Fusarium oxysporum* (V5W2-9 NH3) (Paparu, 2008) colonisation with major emphasis on its microscopic characteristics.

Molecular analyses to establish evidence of gene flow. Fungal colonies growing on selection media were sub-cultured onto fresh selective half strength PDA plates and incubated for 7-10 days at room temperature for genomic DNA extraction. This was done to confirm the ability of the

Fig. 1: A transgenic plantlet establishing in nutrient solution prior inoculation.

fungal colonies to re-establish on selection media. Where wild fungal isolates were observed, they were subjected to the same procedures as above.

Genomic DNA extraction and PCR screens. Thirty six (36) fungal isolates (including 30 *Fusarium oxysporum* and some wild fungal isolates, and 6 re-isolates from the controls) were used for genomic DNA extraction according to Mahuku (2004). The mycelia of the fungal colonies were crushed in sterile sand using miniature pestles. The DNA extraction buffer (Tris EDTA SDS (TES) contained: 0.2 M Tris-HCl [pH 8], 10 mM EDTA [pH 8], 0.5 M NaCl, 1% SDS. The quality of the extracted DNA was assessed by running 5 µl of the DNA and 2 µl of loading dye in a 1% agarose gel in TAE buffer. The gel was stained in 1% ethidium bromide for 20 minutes and visualized under an ultra violet trans-illuminator. The bands on the gel were documented in a gel documentation system. PCR was performed using primers specific to the gusA and hpt genes. The primers used were; primer 1- 5' TTTAACTATGCCGGGATCCATCGC 3' and primer 2- 5' CCAGTCGAGCATCTCTTCAGCGTA 3'. PCR involved a 2 minute initial denaturation step at 94°C and 30 cycles consisting of 1 minute denaturation at 94°C, 1 minute primer annealing at 60°C and a 1 minute extension at 72°C followed by a 10 min extension step at 72°C. The amplification products were size fractionated by agarose gel, electrophoresed in TAE buffer at 80 volts for one hour (Maniatis *et al*., 1989). The gels were stained with 1% ethidium bromide in an aqueous solution and were examined for amplification of the *gus*A and *hpt* genes.

Statistical analysis of the results. The data were subjected to analysis of variance (ANOVA) using the Statistical Analysis Systems (SAS) (version 9.1) (SAS Institute Inc, North Carolina, USA). Isolation frequencies of *F. oxysporum* (V5W2-9 NH3) among the plant roots were analysed using categorical logistic regression. Likelihood ratio tests were performed to investigate differences within factors (*F. oxysporum* (V5W2-9 NH3) and plant roots). In cases where significant differences ($P \leq 0.05$) were detected, the Dunn-sidak correction factor was used as a posterion testing tool (Sokal and Rolf, 1995) using the SAS system (SAS, 1989).

Results

Gene flow analysis from transgenic plants to *F. oxysporum* (V5W2-9 NH3). Plant colonisation was low with only 40 root segments colonised by the fungus. A total 36 fungi grew on normal media of which 20 where from the transgenic plants and 16 from the controls. Four (4) fungi established on selection media of which 3 were from the controls and only one established from the transgenic plants. Overall, *F. oxysporum* colonisation averaged 27.3% in transgenic plants and 20.5% in the non-transgenic plants. Plant colonisation by *F. oxysporum* (V5W2-9 NH3) was significantly influenced by the banana genotype ($P \leq 0.034$) (Table 1). Although there was no effect of sampling time on colonisation ($P = 0.076$) among plants of the same genotype, plant colonisation was significantly affected by the media composition ($P \leq 0.0001$), with higher percentage colonisation observed for roots that were plated on non-selective media compared to those that were plated on selective media ($P \leq 0.025$) (Table 1). There was no interaction between media composition and banana genomic structure ($P = 0.31$). Some wild fungal isolates were similarly observed to grow especially on selective media as well as even on the non-selective media.

Table 1. Logistic regression analysis for Fusarium oxysporum (V5W2-9 NH3)

Source of variation	Degrees of freedom	Sums of squares	Probability
Media	1	23.16	<0.0001
Treatment	1	4.48	0.034
Weeks	2	5.14	0.076
Interactions			
Media * treatment	1	1.04	0.308
Media * weeks	2	4.61	0.0997
Treatment*weeks	2	0.79	0.675

Molecular analysis and PCR screens for gusA and hpt gene. PCR was performed using *gusA* and *hpt* specific primers. The amplicons were electrophoresed on a 1% agarose gel. An amplified fragment of about 500bp corresponding to the internal fragment of *gusA* gene was observed in the positive control (plasmid DNA (pCAMBIA1201)) (Plate 1C) and an amplified fragment corresponding to the *hpt* gene was also observed in the positive control (Plate 1D). No amplified fragment was observed with DNA extracted from the fungi re-isolated from the phyllosphere of the experimental plants (Plate 1C and 1D).

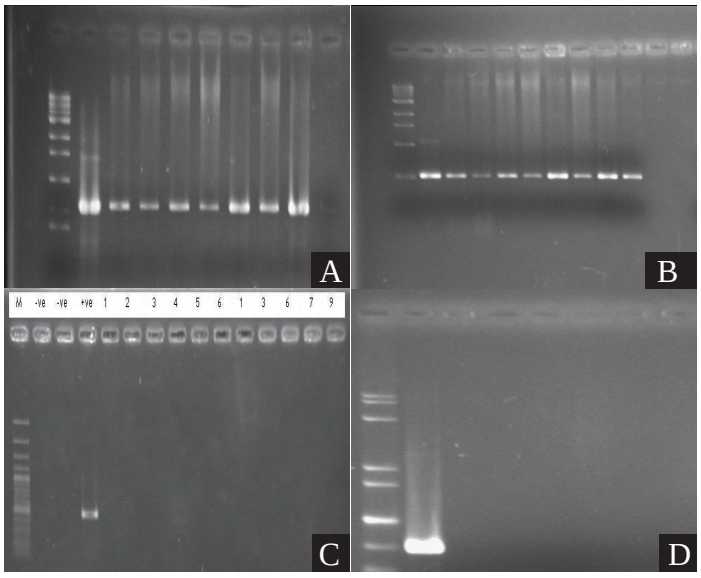

Plate 1. Electropherogram showing the presence of the hygromycin hpt (A) and β–glucuronidase (B) genes in the genetically engineered banana plants; and the absence of β–glucuronidase (C) and the hpt genes (D) in the microbial DNA.

Discussion.

In the study, the fungal re-isolates from transgenic banana phyllospheres were not transformed with the *gus*A and *hpt* genes. The test fungi were able to survive on selective media suggesting endogenous capacity to degrade the antibiotics. However, attempts to amplify the transgene were negative with no amplicons affirming that *F. oxysporum* (V5W2-9 NH3) re-isolates were indeed not transgenic. Among other fungi however, horizontal gene flow has been demonstrated. Whereas gene flow may occur through homologous and illegitimate recombination (Magee *et al*., 2003; Ruiz-Díez, 2002), up taken DNA are rapidly destroyed (Hoffman *et al*., 1994). This phenomenon may explain in part the survival of re-isolates on selective media and failure to detect the *gus*A and *hpt* gene. Nevertheless, the survival of re-isolates even prior to exposure to transgenic banana suggests these fungi (*F. oxysporum* (V5W2-9 NH3)) possess mechanisms to degrade hygromycin. This study thus found no evidence for gene flow. These results agree with earlier studies (Zhang *et al*., 2005) which failed to show any gene flow of the *Streptomyces hygroscopicus* bar gene under the control of a *Cochliobolous heterostrophus* glyceraldehyde-3-phosphate dehydrogenase (GPD) promoter in transgenic poplar to *Amanita muscaria* ectomycorrhizas. However, other studies have detected horizontal gene flow events to *Aspergillus niger* in soil microcosms after co-cultivation with transgenic plants of *Datura innoxia* expressing the hygromycin B gene under the control of the 35S promoter (Hoffman *et al*., 1994). This is interestingly one of the few reports of horizontal gene flow to fungal organisms. Taken together, this study found no empirical evidence of the occurrence of horizontal gene flow from transgenic banana to root endophytic *Fusarium oxysporum* (V5W2-9 NH3) suggesting therefore that cultivation of such transgenic bananas may not be a threat to the beneficial fungal communities with which they are associated. However, the differences in physiology in plants and their interactions with micro-organisms would warrant pursuing these studies on case by case basis.

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Advances in insect pest management technologies of agricultural crops: an integrated approach

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ABSTRACT

The paper presents a critique of advances and applications of technologies in the management of insect pests of agricultural crops. The management technologies discussed include; biological control, host resistance, use of genetic modification of crops, traditional pesticide materials, and legislative control. Further, the implications of each management technology to the welfare of the communities and the ecosystems in general are discussed. These are technologies which could form components in the Integrated Pest Management (IPM) approach to combating insect pests of crops. It is revealed from the analysis that when wisely employed, developments in pest management play a great role in increasing food security, environmental conservation, reduction of poverty and ultimately improving the peoples' quality of life.

Keywords: *Integrated pest management, food security*

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Introduction

Insect pest infestations cause greater losses of agricultural products in developing countries thereby hindering agricultural development resulting in food insecurity. For crops in storage, insect infestations cause great losses given their low Economic Injury Levels (EIL) (FAO. 1996). In controlling crop losses the major thrusts reported in the advances of pest management mainly focus on field crops in farms amongst rural communities (Abate *et al.*, 2000; Chapman, 2000; Rugumamu, 2005). At international border posts of many countries, government agencies are also reported to enforce plant quarantine regulations as pest control measures.

In principle pest control measures have to be extended to various ecosystems and integrated into an operational system, be it large or small in scale, if they are to be effectively applied. The major objective of this paper is to present and critically analyse major advances in pest management technologies and their application in order to recommend wise uses in an integrated approach. Development and applications of technologies in the management of insect pests forms a sound basis for a better understanding of their contribution to the Integrated Pest Management (IPM) approach. The current thrust in pest management is on IPM, a domain of extension science referring to a management system that combines all economically, technically and ecologically applicable technologies to keep pest populations below those causing economic injury while minimizing unwanted side effects of the applied measures (Hill, 1987; Benbrook, 1996; Matteson, 2000; Neuenschwander *et al.*, 2003).

Timely application of control measures following fluctuations of pest populations in relation to their general equilibrium position, economic threshold and economic injury levels was illustrated by Hill (1987). Various scientific and technological discoveries and developments over time have been contributing vastly in managing insect pests and vectors of crop diseases particularly in farm fields at varying degrees. In this regard Chapman (2000) outlined landmark events in insect-related basic biology and applied entomology of the twentieth century in agricultural development (Table 1). The technological discoveries were in line with efforts to attain food security.

Table 1: Major advances in insect pest management over time

Time	Development in pest control
1920s – 1930s	Biological control campaign against prickly pear in Australia
1940	DDT first synthetic insecticide used
1950s -200….	Varietal resistance to insect pests broadly classified by Painter (1951) and Russell (1978). Silent spring published. Synthetic pyrethroids based on structure/activity relations. Sterile male technique eliminates screw worm from most of North America. IPM concepts established. Transgenic cotton containing *Bacillus thuringiennsis* toxin commercially available. More GMO crops developed (OFAB 2007-2008)

Food security is conceived to be a situation in which people do not live in hunger or fear of starvation (FAO 2003). Around 852 million men, women and children worldwide are chronically hungry due to extreme poverty, while up to 2 billion people are intermittently food insecure due to varying degrees of poverty (FAO, 2003). According to FAO (1996), food security exists when all people, at all times, have access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. FAO is currently carrying out a Special Programme for Food Security (SPFS) assisting some governments replicate successful food security practices on a national scale. The SPFS also encourages investment in rural infrastructure, off-farm income generation, urban agriculture and safety nets. Noting that a household is the lowest level of community organization in Africa the above conception merits high consideration in this regard.

In Tanzania for instance, the overall policy in the food and agriculture sector is to achieve self-sufficiency in food and food security through increased food production as well as increased commodities for export and hence reduction of poverty (URT, 2005). It places emphasis on, among other things, food production and underscores the need to utilize science and technology in order to develop the agricultural sector and aiming at the maximization of productivity through introduction of improved scientific and technological initiatives in the use of appropriate seed varieties and better methods of food and crop processing, preservation and storage.

Scientific and technological initiatives generally encompass their relationship in pest management to produce desirable approach to solve problems of pest infestations in crops (Hill 1987). This paper reviews the different integrated pest management (IPM) strategies available and proposes a way forward in employing search practices under African agriculture.

Insect Pest Infestations of Crops

The most important step towards controlling insects and minimizing losses is the correct identification and application of proven control measures. Bhatia (1976) advanced that the harvested crop is the net result of all prior production efforts and any subsequent losses incurred are absolute losses with no possibilities for compensatory action. It is imperative therefore, to develop effective management technologies against both field and stored pests especially in systems where rural livelihoods are based on agriculture. Cereals, pulses and root crops are commonly grown in almost all ecosystems in African. Maize, *Zea mays*, for example, is a staple food and cash crop grown and stored in almost all the regions of the tropics (Abate *et al.*, 2000). The recent technological development including the introduction of many new maize varieties to farmers have triggered the crop's propagation into highlands and mid-altitudes. In the early 1980s problems associated with maize and cassava production in some East and West African countries for instance, have been aggravated by the damage caused by the Larger Grain Borer (LGB), *Prostephanus truncatus* (Golob, 1988; Rugumamu, 2003).

Prevailing relative increases in crop yields have been facilitated partly by scientific and technological advances in breeding for genotypes of greater resistance to field pests and diseases and partly by application of modern farm practices and implements. However, Abate *et al.* (2000) noted that greater storage losses to insect pests may also result from some of the improved technologies. Some new crop varieties, however, are mostly susceptible to insects in farms and in storage. Arnason and Gale (1992); Rugumamu (2005) later demonstrated that some grain physical and chemical/nutritional characteristics incorporated during breeding could result into its susceptibility to insects in storage. It may be cautioned that whereas some measures have proved successful some other relief forms perpetuate famine. Following from the above, users should be cautioned given that some technological development efforts knowingly or unknowingly could aggravate socio-economic problems.

Application of Industrial Chemical Pesticides

Historically, when synthetic chemical pesticides mostly the organochlorine group came into widespread use in the 1940s, they promised an era of abundant agricultural yields. However, Carson (1962), and Hill (1987) have their thoughts shared with Edward Groth who in his foreword in the book "Pest Management at the Crossroads" by Benbrook (1996) noted that it didn't take long to recognize that these miracle chemicals had costs and risks as well as benefits. The chemicals were highly toxic to most insects with control levels of 98 – 99% or even higher, broad spectrum and persistent thereby becoming unfriendly to the environment. Currently, control of insect pests is achieved by mainly the application of industrial pesticides (Kilimo/GTZ 1996; Golob, 2002) even though this strategy has several shortcomings, economically, technically and ecologically.

Insect Growth Regulators (IGR) and Juvenile Hormones (JH) are also included in the pesticides groups which are specific and have minimal disruptive effects on the environment. JH if applied to full-grown larva disturbs the process of metamorphosis and the insect dies as a deformed pupa/ adult (Chapman, 2000). Nonetheless IGRs are not as specific as JH but they interfere with cuticle formation at the time of ecdysis and hence killing the moulting larva.

Field evidence shows that, only rarely does industrial chemical application kill all the pests, and that the few which survive during successive generations develop slight genetic differences from the main stock of the insect species which become biotypes, usually giving serious problems as they develop resistance to the chemicals (Hill, 1987; Golob, 2002). Incidentally, Benbrook (1996) reports that genetic resistance to pesticides in pest populations and outbreaks of new pest problems when broad-spectrum insecticides remove natural checks and balances, have led to escalating dependence on pesticide use with no real decline in pest-induced crop losses. It is further urged that if not well monitored, continued additions of chemicals result into general ecological disturbance as well as causing residue in ecosystems. Edge and Schauber (2000); Fischel, (2005) report that toxicological experiments have showed that pesticides could cause cancer and birth defects awnd damage or interfere with nervous, endocrine, reproductive and immune systems in mammals.

Socioeconomic status in Africa has made the use of synthetic pesticides the lowest among all regions of the world (Sangodoyin, 1993). They are very expensive and most governments have reduced subsidy to farm inputs especially to pesticides (Arthur, 1996). Further, misuse of chemicals during application, non-availability when most required and incorrect timing of treatment given the low EIL of most crop pests, aggravate chemical control problems. In this regard, Benbrook (1996) lamented that many chemical pesticides cost comparatively little to use, in large part, because the risks and social costs associated with their use are not included in their price.

To this end, in Tanzania for example, a full-fledged Tropical Pesticides Research Institute (TPRI) was established in the 1970s with a purpose of institutionalizing a system for both research and regulation of pesticides in use in the country. It supervises and regulates the manufacturing, importation, distribution, sale and use of pesticides and to administer the regulations made under the Act establishing it (Kilimo/ GTZ 1996; Nakora, 2005). As argued by Carson (1962); Dendy *et al.*, (1991); Hodges (1994) and Arthur (1996), any rational decision on the use of chemical pesticides in pest management must be based on the cost–benefit analysis and environmental impact considerations. It is against this background that strategies for minimizing expenditure in pesticide use will be a factor to sufficient food supply, reinvesting of finances obtained from other sectors and last to the reduction of health hazards of the pesticides which in some cases are unauthentic.

Biological Control of Insect Pests

Another important component of IPM in agriculture is biological control which, in a broad sense, includes all types of control involving the use of natural organisms which have a long history of evolution (Rees, 1988; Dick, 1990; Scholler *et al.*, 1997; van Emden, 1999; Neuenschwander *et al*., 2003). Biological control is the reduction of pest populations by natural enemies and typically involves an active human role. The enemies kill or debilitate their host and are relatively specific to certain insect groups. Conservation, classical biological control, and augmentation are three basic types of biological control strategies. The main attractions of this control are that it reduces the necessity of using chemical poisons and in its most successful cases gives long-term control from one introduction (van Emden, 1999). In this regard, biointensive IPM is advocated in agricultural systems. Benbrook (1996), however, emphasized that expanded reliance on biontensive IPM could work when far-sighted policies are in place from both government and private sector. Biological control is most effective against pests of exotic crops which often do not have their full complement of natural enemies in the introduced locality. On rare occasions, a local predator or parasite will successfully control an introduced pest.

Terestriosoma nigrescens Lewis, a predator was released and established as a natural enemy for the control of P. truncatus in some African countries including Kenya (Giles *et al.*, 1996; Meikle *et al.*, 2002; Holst and Meikle, 2003). However, initial studies on the impacts of this entomophagous insect to control the target insect have concentrated on observing its spread and the effects on loss reduction in experimental maize stores (Rees, 1988; Borgemeister, 2001). It may not be surprising now, however, if T. nigrescens has already been established in more African countries. Entomopathogenic fungi, Beauveris spp was reported to infect P. truncatus, S. zeamais, Tribolium spp, Carpophilus spp. in Kenya (Oduor *et al.*, 2000). Scholler *et al.*, (1997) report that protection by natural enemies should be taken much early during storage given the low EIL of infested stored crops.

A major limitation to this technology is that most predators are not host-specific and hence not particularly confined to any specific host (1988; Bottrell *et al.*, 1998). Further, the enemy requires longer periods to be effective. It is thus advanced that ecological research on specificity of agents to the pests may allow a wide introduction of more predators; pathogens; parasites and parasitoids of common insect pests as biological control measures. It is hence important to preserve natural enemies whenever possible and to facilitate their identification.

Male Sterilization Technique (MST) and use of pheromones are other biological methods of insect pest control. Male sterilization was first proposed in 1955 (Klassen and Curtis, 2005) and is effective when applied to restricted populations and also in species where females mate only once and unable to distinguish or discriminate against sterilized males. Recently, recombinant genetic technology has been applied to SIT and transgenic (GM) sterile males have been the focus of extensive research efforts (Morrison *et al*.,

2010). Attractant pheromones are used in pest population monitoring so that control measures may then be exercised if necessary with precise timing. It should be appreciated moreover that aggregation pheromones could be employed in insect pest behavioural control where insects are induced to fly to inappropriate hosts.

Genetic Modification of Crops for Pest Resistance: Over the last two decades major advances in the field of agriculture biotechnology and in particular recombinant DNA technology have enabled the production of crops that are insect pest resistant due to expression insecticidal proteins. The most studied and widely applied insecticidal protein in crops is the Bt toxin which is produced by the bacterium *Bacillus thirungiensis* (Cranshaw, 2003). The bacterium forms crystals of proteinaceous insecticidal δ-endotoxins (called crystal proteins or Cry proteins), which are encoded by cry genes. Using the recombinant DNA technology (rDNA) cry genes have been transferred into crops such maize (Bt maize) and cotton (Bt Cotton). It is reported that Cry toxins are effective against some insect species of the orders Lepidoptera, Diptera, Coleoptera, Hymenoptera and also Nematodes. Proponents of the rDNA technology (GM technology) list the following among benefits of using Bt crops; (i) reduced environmental impacts from pesticides, (ii) increased opportunity for beneficial insects (iii), Bt proteins will not kill beneficial insects, (iv) reduced pesticide exposure to farm workers and non-target organisms (Cranshaw, 2003; Federici, 2007 NewAfrican, 2009). Alongside the benefits the following potential risks are reported (i) invasiveness (ii) development of resistance to Bt – (iii) Cross-contamination of genes-whereby genes from GM crops can flow into related native species (Paalberg, 2006; Yarobe and Quicoy, 2004; Hosea *et al*., 2005).

Successful biological control are commonly permanent and also self-propagating and self-perpetuating and hence self-adjusting (Scholler *et al*., 1997; van Emden, 1999). Despite the importance the control, there can arise shortcomings, for example, most predators used to attack pests and vectors are not host-specific hence could attack beneficial organisms (van Emden, 1999). The genetically manipulated parasites or pathogens also when misused may result into undesirable consequences. In this regard, concern is usually expressed over the dual-use nature of biological agents due to the ease with which they could be directed to antagonistic use for biological warfare against crops and animals including humans.

Host plant resistance in pest management

Resistant crop varieties are an aspect of pest control of great importance whereby plant breeding is a very specialized subject in its own rights and hence it is dealt with separately, not just within biological control (Hill, 1987). Varietal resistance to pests was broadly classified by Painter (1951) into three categories which are non-preference, antibiosis and tolerance. Hill (1987) further noted that, non-preference and/or antibiosis types of resistance have adverse effect on the bionomics of the pest by causing its death or decreasing the rate of its development and reproduction. Until recently, it was commonly believed that resistance of a crop variety could only be effective to a growing plant in the field.

It is emphasized by Bosque-Perez and Schulthess (1998) that host-plant resistance as a pest control method is environmentally safe, economically acceptable to farmers and most compatible with other components in IPM initiatives. Further, Bhatia (1976); Rugumamu (2006) reported resistant varieties as one of biological components in the IPM which could significantly reduce losses of agricultural crops. As a contribution to this control initiative, varying levels of resistance of some maize varieties to *P. truncatus* and *S. zeamais* were determined in the laboratory and in the farm stores studies by, among others Derera *et al.*, (2001); Rugumamu (2005), the findings indicate significant differences among the maize varieties tested according to statistical methods by Fowler *et al.* (1999) and Sokal and Rohlf (1998). These results did shed light to the importance of pursuing a search on resistance levels of many more maize varieties developed and grown by farmers in order to identify resistant varieties to the common insect pests.

Under subsistence food production, however, it has been noted that the availability of resistant varieties has, to some extent, failed to achieve a major impact (Mohamed and Teri, 1989; Hillocks, 1995; Abate *et al.*, 2000). This is reported to be a result of, first, local varieties were probably most resistant due to co-evolution and selection by farmers over many years; second, farmers in unstable and variable environments plant mixtures of varieties that are more able to respond to erratic rainfall, fluctuations in soil conditions and to pest and disease problems; and, third, breeding physical characteristics in varieties may have a detrimental effect on either palatability or cooking time or both and therefore unacceptable to farmers. However, given the potency of resistant varieties to insect pest control, it is recommended that deliberate effort by policy markers be directed towards dissemination of the knowledge to stakeholders, the smallholder farmers in order to enhance food security and poverty reduction.

Among the various methodologies currently used for assessing and determining varying resistance of crop varieties to insect pests are presented by Dobie (1977) and Rugumamu, (2006). These innovations are intended to positively contribute to the welfare by reducing food insecurity. Different crop varieties are produced in various breeding programmes and it is now known that some crop physical and chemical/nutritional characteristics could affect their susceptibility to insect attack and damage (Chapman, 2000; Aluja *et al.*, 2001). The methodologies for screening crops for resistance are in line with the need to monitor the possible misuse of breeding technologies which could lead to mass production and distribution of varieties with poor qualities leading to losses of higher magnitude.

Application of Traditional Pesticides

Farmers' ingenuity in rural areas has enabled them, through time, to apply indigenous pesticide materials to protect crops (Rugumamu and Mtumbuka, 1998; Rutatora and Matee 2001). It is advanced by UNESCO (2002) that pest

management practices in traditional African agriculture have a built-in mechanism in the overall crop production systems. It is acknowledged by Elwell and Maas (1996); Rutatora and Mattee (2001) among others, that the majority of smallholder farmers in most African countries employ only Indigenous Knowledge Systems (IKS) in their agricultural production processes.

It is reported that due to increased applications of chemical insecticides by some communities over the last few decades, some traditional methods of protecting stored seeds and food crops are being forgotten to the extent that some farmers are now unaware that traditional low-cost alternatives do exist. It is however cautioned by Golob (1988); Golob and Hanks (1990) that rampant claims of the effectiveness of traditional grain protectants need further research to establish their efficacy and full potential as well as any possible toxicological hazards associated with their use. The current emphasis upon IPM is, in effect, a reassertion of the need to put traditional good husbandry practices in place as a fundamental component of pest control (Haines, 1999; Abate *et al.*, 2000).

Legislative Control

Legislative measures of pest control such as restrictions of movement of produce at entry points at international borders are usually enforced by state agencies as a pest control strategy (Golob, 1988). The programme for example is practiced in Tanzania and is adaptable as a model for other countries in Africa to prevent the exotic insect pest, *P. trucatus* infestations from spreading to neighbouring countries (FAO, 2003). The strategy, however, is reported to work with limited success given free movement of people in this global village.

Way Forward

Based on the above analysis, it is concluded that development and applications of science and technology in insect pest management cannot be overemphasized. It is evident that when wisely used control strategies form components of IPM and the main advantages of various insect pest management technologies have been highlighted for increased agricultural production. It is, however recommended that extensive and in-depth multidisplinary research is essential in order to eliminate possible dual uses.

Although development of components of IPM was recommended over thirty years ago the thrust was on the effective uses of various control measures in farm field environments (Hill, 1987). Given the existing situation regarding insect infestations Adda *et al*., (2002) call for research in the field of IPM that will reduce reliance on only chemical. It is noted, however, that IPM strategies are specific to each pest, climatic conditions and other local factors. It is against this background that IPM requires multidisciplinary research, often years of it to develop successful IPM methods and unlike chemicals, once developed; IPM strategies cannot be packed and sold everywhere.

On another front, it is advanced here that instead of IPM "technology transfer" through training and visit (T&V) system, the "farmer first" paradigm of participatory non-formal education led by IPM extensionists in farmer field schools followed by community IPM activities is highly recommended. This approach emphasizes farmer-training-farmer and research by farmers (Meikle *et al.*, 2002; Scoones and Thompson, 2009). In essence, with well synergized IPM components, it would result in a total well being of crops and the ecosystem in which they grow. When scientific and technological advances are wisely applied in agricultural production systems in accordance with the knowledge on biological conservation, crop pest management and food security will be significantly promoted.

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Potential role of GMOs in adapting Agriculture to Climate change in Sub-Saharan Africa

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ABSTRACT

Sub-Saharan Africa (SSA) is the most vulnerable region to climate change in the world because of its low adaptive capacity. Over 70% of the labour force depends on agriculture, which is extremely vulnerable to climate change. The purpose of this review is to foster policy discourse on challenges of agriculture in SSA due to climate change and explore potential adaptation strategies. Ongoing studies of climate change and agriculture have recommended adoption of stress-resistant crop varieties as a feasible strategy. Genetic engineering provides tools that can be used to develop stress resistant transgenic plants. For SSA to adopt products of genetic engineering known as genetically modified organisms (GMOs) as an adaptation strategy to mitigate effects of climate change in the agricultural sector, a number of concerns must be addressed and strategies implemented. These include investing in research and development, putting in place mechanisms for risk assessment and effective governance, managing intellectual property rights (IPR), and addressing trade issues.

Keywords: *Agriculture, Adaptation, Climate change, GMOs, Sub-Saharan Africa*

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Introduction

Climate change occurs naturally over time however its trends have been altered by human activity leading to what has often been called global warming. The major causes of global warming and associated climate changes such as shift of the precipitation patterns are increased emissions of green house gases (GHG) from fossil fuel use, waste management, industry, forestry, agriculture, buildings, and transport, and reduced sinks and increased emissions from the clearing of natural vegetation such as deforestation (IPCC, 2007a). The impacts of these climatic shifts have already been experienced by many people around the world and they present a future challenge because effects of GHG emissions will continue to be felt for decades or even millennia.

While industrial countries are responsible for majority of the GHG emissions, developing countries are likely to be most gravely affected by climate change (IPCC, 2007a). There is growing evidence that negative impacts of climate change are already being felt by many developing countries (FAO, 2008; UNFCCC, 2007). For example in 2004 severe flooding in Bangladesh caused the death of over 600 people and displaced over 20 million. Increased drought events have contributed in a rise in malnutrition, which is responsible for an estimated 3.5 million deaths each year in developing countries (FAO, 2008). More than 42 million children in Sub-Saharan Africa (SSA) are malnourished, and with continued climate change the number is expected to increase to 52 million by 2050 (Nelson *et al.*, 2009). Sub-Saharan Africa contributes only 5 percent of the total global GHG emissions, but it is considered the most vulnerable region to climate change due to limited adaptive capacity. The causes of SSA's vulnerability include: the fact that a large portion of the region is desert or dry land, high exposure to drought and floods, economies heavily dependent on natural resources, limited infrastructural and technological resources, and high disease prevalence (WDR, 2010). In this region, agriculture is vital; 70 percent of the labour force and over 25 percent of GDP in most countries' economies depend on agriculture (UNECA, 2009). In order to address SSA's vulnerability, there is need to alleviate the negative impacts of climate change on agriculture. The purpose of this paper is foster policy discourse on the problems SSA's agriculture, particularly crop production, is likely to face due to climate change. The paper uses the example of genetically modified organisms (GMOs) to explore the challenges that are likely to be encountered when implementing some of the suggested adaptation mechanisms.

Impacts of Climate Change on Agriculture

Agriculture in many regions of the world is extremely vulnerable to climate change. Changes in temperatures and precipitation patterns are predicted to result in positive and negative effects on agriculture globally (Table 1). Climate change is expected to contribute to loss of habitat for wild relatives of crops, which are a vital source of genetic diversity for crop improvement (FAO, 2008). It is projected that continued climate change will affect wild relatives of three major food security crops: groundnuts, cowpea and potato causing of extinction of 16 to 22 percent of wild species by 2050 (FAO, 2008). Climate change is also likely to cause increased manifestation of human diseases. Increases in temperature and humidity will create ideal conditions for vector-borne diseases such as malaria, sleeping sickness and other infectious diseases that will directly affect the availability of human resources for the agriculture sector. While some parts of the world are expected to have agricultural benefits from climate change at least in the short-term, many African countries are likely to experience more negative impacts (IPCC, 2007b) that will threaten national economies and food security.

Table 1: Climate change impacts on agriculture

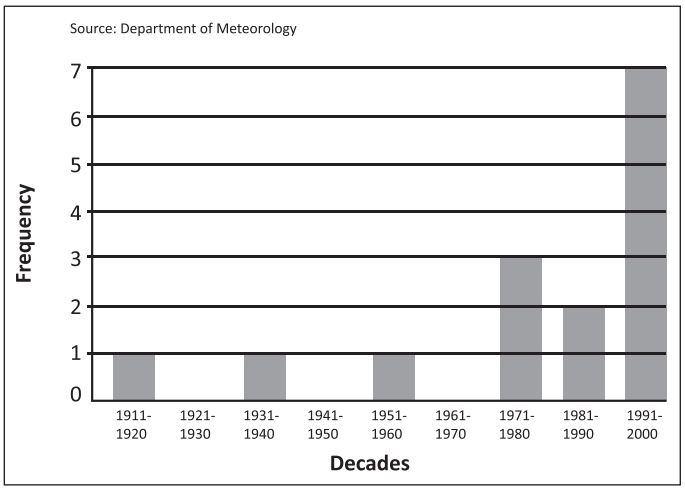
Climate event	Possible impact on agriculture
Warmer and fewer cold days and nights; warmer and more frequent hot days and nights over most land areas (virtually certain)	Increased yields in colder environments; decreased yields in warmer environments; increased insect pest outbreaks
Warm spells and heat waves increasing in frequency over most land areas (very likely)	Reduced yields in warmer regions due to heat stress; increased crop damage due to wildfire
Heavy precipitation events increasing in frequency over most areas (very likely)	Damage to crops; soil erosion; inability to cultivate land due to water-logging of soils
Drought-affected area increases (likely)	Increase in land degradation and soil erosion; lower yields from crop damage and failure; increased risk of wildfire; loss of arable land
Intense tropical cyclone activity increases(likely)	Damage to crops
Extremely high sea levels increase in incidence (excludes tsunamis) (likely)	Salinization of irrigation water; loss of arable land and increase in migration

Source: FAO, 2008

In Africa, 220 million people are affected by drought each year (UNFCCC, 2007) and this number is projected to rise to 350-600 million by 2050 (IPCC, 2007b) as a result of both population growth and increased drought frequency. For example, in Uganda there is already evidence of increased drought events (Figure 1). An estimate 25–42 percent of crop species habitats is expected to be lost across Africa due to drought leading to species range shifts and extinction of some species including wild indigenous foods and plant-based medicines (FAO, 2008). Between 9 and 20 percent of SSA's arable land is expected to become much less suitable for agriculture by 2080 (Fischer *et al.*, 2002; WDR, 2008).

Sub-Saharan Africa's agriculture is extremely vulnerable to climate change because it is largely rain-fed (97 percent) (Alvaro *et al*. 2009), and is therefore greatly affected by precipitation pattern variability. Crop yields from rain-fed agriculture are expected to be halved by 2020 (Boko *et al*. 2007; FAO, 2008). According to a crop model by International Food Policy Research Institute (Nelson *et al.*, 2009), cereal production in sub-Saharan Africa will decline by 22 percent for wheat, 14 percent for rice and 5 percent for maize by 2050 due to climate change. Similar maize yield losses were predicted for East Africa by Thornton *et al.*, (2009). Some vulnerability mapping for the continent has identified hotspots including arid–semiarid systems in the Sahel, arid–semiarid rangeland systems in parts of eastern Africa, the coastal regions of eastern Africa, and dry-lands in southern Africa. These are areas where agricultural populations are already vulnerable, and the situation is expected to get worse in the future (Thornton *et al.*, 2009).

Fig 1: Occurrence of drought in Uganda. Source: Department of Meteorology, Ministry of Water and Environment 2007



Floods are also a major cause of vulnerability in SSA. For example floods in 2007 affected 22 districts in northern and eastern parts of Uganda, affecting more than 200,000 people who lost 90 percent of their crops, which created food insecurity that will last throughout 2008 (FEW NET, 2008). This crop loss resulted in food shortages causing increased food prices with a 20 to 65 percent rise for cereals, and a 60 percent increase for beans during that time period. Floods also cause infrastructure damage, displacement, destruction of livelihood assets and disease epidemics, which increases peoples' vulnerability (McGrath, 2008).

Future predictions indicate that if global GHG emissions continue to remain high then Africa temperatures will rise by more than 2°C above the current temperature (IPCC, 2007b). This temperature rise will have a great negative impact on many crops that are important for Africa for example a 2°C temperature rise is expected to significantly affect Uganda's

coffee production potentially causing a US $ 265.8 million income loss that is 40% of export earnings (Hepworth and Goulden, 2008). With these crop productivity reductions, and expected increase in population (UN, 2009), SSA needs to have in place an adaptation system to assuage the devastating consequences expected due to climate change.

Climate adaptation strategies for SSA's agriculture

Many SSA countries have adopted a national adaptation programme of action (NAPA) in compliance with international policies such as United Nations Framework Convention on Climate Change (UNFCCC, 2007). Some countries have developed a number of national policies related to adaptation and mitigation impacts of climate change (WDR, 2010). In relation to agriculture, such policies advocate for adaptation to climate change through adoption of stress resistant crop varieties, improved water and soil management, land management systems, and market and extension services as well as enhance communication (Nelson *et al.*, 2009). All the above-mentioned strategies are important but this paper will focus on adoption of stress resistant crop varieties.

GMOs and Agriculture

Ongoing studies of climate change and agriculture have recommended adoption of stress-resistant crop varieties as a feasible strategy (FAO, 2009a). These crop varieties will have to be resistant to pest and diseases, and tolerant to drought and increased soil salinity. These varieties can be developed by crossing, irradiation or chemical treatment, marker assisted breeding or genetic engineering. Genetic engineering (GE) provides breeders the tool to transfer characteristics within species, and between species that cannot naturally cross. For example through GE plant breeders have transferred a disease-resistant characteristic from rice to banana (Arinaitwe, 2008). This tool broadens potential sources of characteristics for crop improvement for both productivity and nutrient enhancement especially in situations where conventional breeding may not be possible. Products developed by GE are known as genetically modified organisms (GMOs). Genetically modified (GM) crops have been grown in various parts of the world for the last fifteen years (James, 2010). Currently, a few crops species have commercially grown GE varieties including soybean, maize, and cotton, and the common traits are herbicide tolerance, insect resistance, and a combination of the two traits. "Since their introduction in 1996, the cultivation of GM crops has grown rapidly and accounted for over 80 percent of soybean, maize, and cotton acreage in the United States in 2009" (NAS, 2010). Globally, there has been an increasing adoption rate of GM crops starting with 1.7 million hectares of biotech crops in 1996 in USA to 148 million hectares in 2010 in 29 countries (James, 2010). GM crops can affect all the three pillars of sustainable agriculture: environmental protection, economic and social development. However, this adoption rate is much lower than expected, especially in developing countries (Paarlberg, 2008).

Benefits and Concerns with GMOs as an adaptation strategy for climate change

In relation to crop agriculture, GMOs can be considered a climate change adaptation strategy because it can contribute to sustainable agriculture by reducing cost of production incurred by farmers or increasing crop productivity thus increasing the net income per land unit. Genetic engineering technology can facilitate speeding up of breeding and introduction of new desired trait in crops, for example resistance to high mineral content, drought tolerance or pest and disease resistance that reduce the requirement for expensive and hazardous chemicals, increase productivity per unit area on the current 1.5 billion hectares of arable land, and enable expansion of agriculture to accessible marginal land, which could probably reduce the driving forces for deforestation thus enabling preservation of the forest biodiversity (Ferry and Gatehouse, 2008; James, 2010). Additionally, the technology may provide crop varieties tolerant to abiotic stresses such as drought and salinity, which would expand agricultural production to less arable land such as saline soils and areas with extended dry seasons.

However there are potential risks associated with GMOs including invasion and persistence of GMOs or their genes which could cause loss of plant biodiversity (Pimentel, 2005). For example if a herbicide resistant GM crop such as rice was introduced in an environment with its weedy relatives then the off-springs could persist in the environment and compete with other plant diversity for the limited arable land. Some GM crops require increased application of chemicals, which increases the cost of production for the resource poor farmers. This may increase rather than reduce the vulnerability of resource-poor farmers to climate change. All GMOs, currently on the market, have been tested to alleviate these concerns (Paoletti *et al.*, 2008). Another concern associated with GMOs is the fact that most of the products currently on the market are owned by multinational companies, thus farmers will have to depend on these companies as the source of seed.

Trade issues are another concern because the European Union, which is the biggest customer of global agricultural commodities, has adopted a precautionary approach to the use of GM crops (Paarlberg, 2008). This caused a mechanism known as "trading up" (Vogel, 1995) where producers interested in selling their commodities to a particular market have to produce what the customer wants, at the quality level and using production process demanded by these customers thus following the quality and regulatory standards in the import countries. Even the United States of America is strongly affected by European standards, as Mitchner (2003) stated, "Americans may not realize it, but rules governing the food they eat, the software they use and the cars they drive increasingly are being set in Brussels". This has contributed to low adoption of GMOs and implementation of very stringent regulatory procedures in countries interested in selling their agricultural commodities to Europe.

Solutions to some of the concerns related to GMOs

To address the issue of the environmental and human health concerns associated with GMOs, there is a need for thorough risk assessment on a case-by-case basis for each GM crop before it is introduced into the environment. All countries that have adopted GMOs set up or modified existing formal risk assessment systems to screen all new GMOs before they are introduced in the environment (Paarlberg, 2008).

Concern about multinationals can be addressed by developing GM crop varieties from locally adapted plant materials (Cohen, 2005) addressing native production constraints. Such products may not be of interest to the multinational companies, therefore public research and development is most likely to take into consideration the local farmers' need and local conditions. As Kofi Annan, then UN Secretary General, said in a 2004 speech "there is need to generate a uniquely African green revolution focusing around ensuring food security and agricultural development because this will go a long way to help foster dignity and peace" (Paarlberg, 2008).

An example of a successful story of adopting new crop varieties is the New Rice for Africa (NERICA) rice scheme (Africa Rice Center, 2008). The rice was developed using conventional methods by crossing high yielding Asian rice with poor yielding but better adapted African rice. The new varieties have high yields, have shorter growing seasons and are tolerant to the local conditions. The NERICA is not restricted to working on rice growing in paddies; thus it may enable farmers to grow rice in environments not previously thought possible. Uganda as an example launched an Upland Rice Project in 2004 and NERICA is a major component. In 2007, 35,000 farmers were involved in rice production compared to 4,000 in 2005 (Karembu *et al.*, 2009). Over the same period, the country reduced its rice imports from 60,000 to 35,000 tonnes, which saved approximately US$ 30 million. Plans are under way to enhance the nutrient content of this rice using biotechnology tools so as to alleviate malnutrition (Karembu *et al*., 2009).

An example of a GMO currently undergoing regulatory assessment that will be important for enhancing SSA's agricultural resilience to climate change, if successful, is drought tolerant maize commonly known as water efficient maize for Africa– WEMA (AATF, 2009). Drought tolerant crops are going to became increasingly essential because projected climate change effects include 10-20 percent reduction in rain in eastern Africa by 2050 (Kigotho, 2005), increased soil moisture loss and reduction in arable land (WDR, 2008). Certain characteristics may be necessary to facilitate GMOs adoption by a social system including: the ability to try them on a limited basis without a commitment, superiority to the traditional varieties, ease of use, compatibility with existing practices, the ability to observe others utilizing them and affordability (Rogers, 1995).

Trade issues can be mitigated by encouraging more internal and regional marketing of the agricultural products. While Europe is still an important importer of SSA's agricultural commodities, the trade trends have changed. Currently there are many regional markets in SSA for example Kenya, Sudan, Rwanda and Democratic Republic of Congo are major importers of Uganda's agricultural commodities with a share of 39 percent, which is higher than the European share of 25 percent (CIA, 2008). According to a study carried out by the Regional Approach to Biotechnology and Biosafety Policy in Eastern and Southern Africa (RABESA) in 2006, although Africa's total agricultural export value to Europe was US $ 6.1 billion, only 6.64 percent of that value represented crops that have commercially available GM varieties grown in different parts of the world including South Africa (Minde and Kizito, 2007). Maize and cotton are the only export commodities with GM varieties currently on the market, and most of the crops necessary for food security in SSA for example beans, cowpea, cassava and sweet potato are not exported to Europe. Regional and internal markets need to be encouraged so that the country has flexibility to adopt technologies to improve productivity and quality of crops required for local and regional utilization, while respecting the requirement for products imported to Europe. A similar approach was adopted by the United States when they decided not to produce GM varieties for crops like rice and wheat to avoid losing their export markets in Europe, but they continue to grow GM crops that are utilized locally or exported to parts of the world that do not restrict GMO imports.

Challenges to adoption of GMOs in SSA's agriculture

Adoption of GMOs presents a number of challenges including issues related to ownership of the technology (intellectual property rights), trade related issues, the need for investment in research and development, and capacity for regulation and effective governance of the technology. The major challenge facing SSA will be the cost of building human and infrastructural capacity required for adoption of this technology.

Most GMO technologies and seeds are protected by utility patents and not plant variety protection therefore the intellectual property rights (IPR) issues are much more complex. Public institutions must take care when developing GM crops so that they do not use patented genes or germplasm without the authorization of the patent owners. This is essential because before releasing a product, the developer will need to gain the freedom to operate for most of the patented technologies (Byerlee and Fischer, 2002). Currently, the African Agricultural Technology Foundation (AATF) an Africa not-for-profit organization assists public research institutions to develop partnerships with other private/public institutions so that they can access proprietary technologies that maybe relevant for small-scale farmers (AATF, 2009). Nevertheless, countries and the institutions involved in biotechnology research need to develop their own capacity in IPR so that they can actively participate in negotiations for access and benefit sharing of genetic resources. Effective IPR systems may be necessary both at national and institutional level to attract external technologies and generation of local innovations that address national priorities. Critics of IPRs

indicate that control over seed and other farm inputs are in the hands of a few multinationals (IAASTD, 2008a), however, not all IPRs are in these companies. Plant variety protection is necessary to promote plant breeding in both private and public research institutions, and it encourages importation of foreign varieties, plus it also provides breeders with bargaining power for genetic resource access and benefits sharing, thus should be implemented.

A trade-related challenge is the vulnerability of farmers to fluctuating international market prices, where their products sometimes receive prices below the cost of production (IAASTD, 2008a). If GMOs are adopted as an adaptation strategy for climate change, there is likely to be an increase in productivity and thus enhance need to reach new markets. There is need to improve competitiveness of the SSA agricultural commodities on international markets. This can be achieved by encouraging local value-addition through providing credit and markets to small-scale processors, and reducing tariffs on these commodities. Farmer collective action should be facilitated to encourage more value-addition and bulking, improve negotiation power, and to identify alternative markets. At the same time the Governments needs to negotiate removal of trade barriers on SSA products that have a comparative advantage at international markets. All these efforts will contribute to reducing the vulnerability of SSA's agriculture to fluctuating international market prices which are expected to increase due to climate change (IAASTD, 2008b).

Sub-Saharan African countries experienced a reduction in foreign investment in agriculture in the 1980s and this caused a decline in funding of research and development of technologies relevant to local priorities (IAASTD, 2008a). International donors especially from US and Japan have been reducing support to agricultural research particularly for the CGIAR system, leaving European nations as the major donors (Paarlberg, 2008). This may hamper research on GMOs, since many European nations oppose the application of this technology.

Many donors are currently advocating for organic farming. Organic agriculture contributes to crop production with minimum inputs, decreases environmental and human health hazards due to chemical application, and provides farmers with comparative advantage over conventional agriculture in European markets (Vaast *et al*, 2009), however it is not certain whether or not organic farming will produce enough food to meet current and future needs in response to continued population growth (Scialabba, 2007; UNCTAD, 2008). FAO Director-General Jacques Diouf indicated that "while organic agriculture contributes to poverty reduction and should be promoted, it cannot feed 6.8 billion today and 9.1 billion in 2050" (FAO, 2009b).

For example, in 1960s and 1970s Asian countries including China, India and Philippines underwent a "Green Revolution" by adopting new improved crop varieties especially hybrids of wheat and rice, and increasing application of irrigation, fertilizer and pesticide (Paarlberg, 2008). The Green Revolution is widely criticized for causing environmental damage, however, the positive impacts were that this move helped Asia to feed its rapidly growing population, reduce its permanent dependence on foreign food aid, and increase farmers' income and the country GDP making them more affluent (IFPRI, 2002). Currently these countries are also utilizing GM technology to further improve their crop varieties so as to reduce their dependence on inputs like pesticides, and to increase resilience of their crops to climate change. Asian countries have ensured substantial continued investment in research and development and human and infrastructural capacity building even after the initial donors such as the Rockefeller and Ford Foundation ceased their funding. Given the Europeans dominance of funding and their reluctance regarding GMOs, use of GMOs as a strategy to adapt to climate change will require finding alternative source of funding. This can be achieved by providing an enabling environment through legislation, and having clear national priorities. Governments should also put in place mechanisms to ensure continued sustainable investment in agriculture even after donors withdraw.

To provide an enabling environment for investment in research and development, and application of GMOs, SSA countries need to have in place updated laws related to GMOs. Many SSA countries are signatory to international treaty responsible for transboundary movement of GMOs known as the Cartagena Protocol on Biosafety, which advocates for a "precautionary approach" to GMO adoption (CBD and UNEP, 2003). While there are benefits to the precautionary approach, the stringent regulatory system adopted from the EU by many African countries may hinder utilization of the technology. This may especially disadvantage technologies that could benefit of the small-scale farmers because only wealthy seed companies interested in export crops will be able to afford the regulatory approval requirements. No comparable source of funding exists to support regulatory approval for technologies developed for poor producers. Also such a system may create a difficult environment for external investment and partnerships in GM technology. Therefore, SSA countries need to develop national legislation that allow for flexibility in governance so that technologies that are likely to address the food security situation and enhance the livelihood of resource-poor farmers are given greater consideration. The legislation needs to clearly state the national priorities for utilisation of GM technology for development but should also allow for flexibility taking into consideration changes in knowledge and local needs.

As SSA countries prepare to adopt GMOs, regulatory authorities need to institute a system for a thorough case-by-case risk assessment of GM crops to enable the country to utilize the benefits of the technology while controlling for any potential risks. Countries should have in place national biosafety committees responsible for reviewing all applications related to GMOs including assessing potential risks for introducing them to the local environment. These committees need to comprise members from various relevant sectors such as scientists, health workers, policy makers, consumers and farmers' representatives. Thus risk assessment should be a multidisciplinary approach with stakeholder engagement.

The multidisciplinary approach for risk assessment is necessary for effective governance of GMOs as the decision takes into consideration a diverse scope of the local concerns and values. However, this approach may result in a delayed or erroneous decision. This outcome can be prevented by conforming to the requirements for effective governance (Dietz *et al*. 2003; Ortwin, 2008) described in the subsequent paragraphs.

First, timely distribution of accurate information to stakeholders is necessary to develop trust and enhance confidence in the processes. Information provided should be comprehensive and relevant so that the decision-making process is focused and considers all pertinent concerns. Oversimplifying the science or skewing the results may cause inaccurate risk management decision, while providing too much detail that does not address key concerns can undermine the audience's trust in the system (Stern and Fineberg, 1996). The information provided should include uncertainties and tradeoffs to be considered in the decision (Ortwin, 2008). To achieve this, clear policies are needed on what is nationally considered valuable and risky, as well as standards for information sharing.

Second, involvement of a wide range stakeholders' in the decision-making process is likely to result in conflict due to the diverse interests and concerns of the different sectors. Addressing such conflicts is another requirement for effective governance (Dietz *et al.*, 2003; Ortwin, 2008). Various approaches can be used to achieve conflict resolution including voting by ballot, intense deliberations, or legal redress. Deliberations allow for a negotiated and comprehensive decision-making process, however sometimes this approach can also be costly and cause prolonged.

Third, effective governance requires that standards must be developed and complied with by all stakeholders to ensure safe application of the technology. The countries and institutions need to establish mechanisms to ensure homogeneous compliance of these standards. This can be achieved through "command and control" where violation results in sanctions such as fines or jail terms. The severity of the sanction should increase with number of violations (Ostrom *et al.*, 1994). This approach requires funding to monitor compliance and ensure enforcement of these standards. An alternative approach is to encourage "voluntary compliance" through incentives (Dietz *et al.*, 2003). Incentives may include research grants, tax reductions and compliance recognition through awards.

Fourth, effective governance also requires human and infrastructural capacity to regulate introduction of technologies (Dietz *et al.*, 2003; Ortwin, 2008). As discussed earlier, SSA countries need to build a substantial and competent human resource as well as well-equipped testing facilities for environmental and food safety assessment of GMOs. Other infrastructures such as communication and transportation systems are also necessary for effective governance. Finally, effective governance requires flexibility so that the regulatory system evolves with changes in knowledge, social values, and local and international situations.

Conclusion

With growing evidence that the threats of climate change are real and already happening, SSA countries need to start implementing a plan for adaptation, including ensuring food security and increased income for majority of the citizens who depend on agriculture. Increasing resilience to climate change is going to require utilizing "all the innovations and ingenuity that the human race is capable of" (WDR, 2010).

If safely used, GMOs can contribute to overcoming the challenge from climate change by speeding up breeding, and developing stress-resistant crop varieties when conventional breeding is not effective. However, for effective utilization of GMOs as an adaptation strategy, the country will need to: develop capacity in IPR management at national and institutional level; have in place appropriate legislation that clearly states the national objectives and concerns; and increase investments in agricultural research and regulation to facilitate introduction of GMOs that address local needs. It may also be necessary to encourage regional and internal markets, while respecting the quality requirements for the international markets so as to reduce farmers' vulnerability to fluctuating international prices. Setting up these systems requires substantial funding and most SSA countries will require external assistance so that it safely and effectively integrates GMOs with other improved technologies to meet the growing demand for food, and increase the adaptation capacity to climate change for SSA's agriculture.

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Prevalence of Columnaris, ecto-parasite and fungal conditions in selected fish farms

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ABSTRACT

The study investigated prevalence of columnare disease, fungal infections, ecto-parasites and underlying factors in ten (two intensive and eight low production) fish farms for six months. A questionnaire was used capture data on farm infrastructure, feeding practices, health and disease control measures, sensitization and level of awareness in aspects of fish health. Fish were examined for ecto-parasites using a hand lens and light microscope followed by collection of swabs on lesions for culture. The mean water temperature; dissolved carbon dioxide and oxygen were: 23.6^{0}C 0.132; 42 ppm 2.91 and 3.4 ppm 0.145, respectively. *Flavobacterium. columnare* and fungal infections was prevalent in 35% and 25% of the samples cultured from lesions, respectively. The ecto-parasites seen were *Gryodactylus spp, Dactylogrus spp*, Trichodina spp and leeches. Diseases were more on intensive farms as the water sources had relative low or no oxygen and high stocking densities. It was recommended that farms using ground water aerate the water in the tank and avail prophylactic measures to control columnaris disease and ecto parasites on the farm.

Keywords: *Fish diseases, hatchery, stocking densities, Uganda*

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Introduction

Aquaculture is a fast growing food-producing sector and it has immensely contributed to economic development and food security worldwide (F.A.O, 2008). Africa's output remained stagnant over the last ten years, with declining availability per inhabitant and the consumption of fishery and aquaculture products from 8.8 kg per capita in 1990 to about 7.8 kg in 2001(F.A.O, 2008). In Uganda only 0.1% of the 25,000 metric tons exported world wide in 2003 was obtained from aquaculture (Nyombi, 2009) and this fetched US $ 70 million. Parasitic conditions and nutritional deficiencies in fish have largely been reported in the last three years and the common parasites reported include: *Dactylogarus, Gyrodactylus,* leeches and anchor worms ((Isyagi, 2006); (Akoll P; Konecny, 2006).

Fungal infections are common in fish and these include water moulds from the class Oomycetes

Although there are several water moulds that can affect fish, the most common and significant are the saprophytic water moulds *(Saprolegnia and Achyla), Saprolegna* are aquatic decomposers which grow as cottony masses on dead algae or fish, mainly in freshwater habitats (Brown, 1995 & Stevenson, 2002).

The majority of fish bacterial pathogens are short gram negative rods belonging to the families Enterobacteriaceae, Pseudomonadaceae and Vibrionaceae and these cause septicaemic and ulcerative disease conditions (Robert, 1989). Columnare is world wide in distribution and most species of fresh water and anadromous fish are susceptible. Under intensification practices on fish farms; the morbidity and mortality have reached 100% and 70% respectively (Post, 1984).

There is currently limited empirical data on fish health status in Uganda largely because the impact of fish production under aquaculture was insignificant as compared to capture fisheries. However, with the increase in levels of

intensification especially in hatcheries, economic impact of fish disease has become significant in aquaculture (Isyagi, 2006).

The overall objective of the study was to establish the prevalence of columnaris disease, ecto- parasite infestations and fungal conditions and associated risk factors in selected fish farms in eastern and central Uganda

Materials and methods

All the 10 fish farms covered by the FISH (Fish Investment for Sustainable Harvest) project located in the districts of Wakiso, Mpigi, Iganga, Jinja, Mukono were included in this study from March to August 2007. Retrospective and longitudinal studies were undertaken. The farms were disaggregated (according to production level as intensive (n=3) and those with low production (n= 7); and according to water supply system as those with a flow through (n= 9) and those with a re-circulated water system (n=1).

Ten semi structured questionnaires were administered to managers or the owners in all the farms. Questions were read to the manager or farm owner and his/her answers filled in by the researcher. The questionnaires captured data on background information on the farm, farm infrastructure, feeding practices, health and disease control measures, sensitization and level of awareness in aspects of fish health. The data from the questionnaires were coded, cleaned and descriptive statistics computed using SPSS version 17. In addition, to administration of the questionnaires, an 8-hour observation was done to record a daily activity schedule of each study farm.

Preliminary visits were made to the study fish farms (n=4) in order to establish the number and composition of the tanks and ponds. This was done in March and the researcher was tasked with documenting the stocking rate, ages, fish species, feeding regime, stocking density and management practices for each pond/ tank..

In each of the selected ponds, about 30 fish were targeted for physical examination for presence of ectoparasites and lesions basing on random sampling. the selected fish in each pond fish were visually examined by use of a hand lens for presence or absence of any skin lesions and ecto-parasites. Wet mounts from both lesions and scrapings (gill and skin) were put on microscopic slides and examined for presence of the bacteria, ectoparasites or hyphae. Where lesions occurred, swabs were taken from four to six fish (Brown, 1993) for culture *Flavobacterium columnare* and fungi. The swabs were kept in Stuart's transport media, in an ice box before being transported to the laboratory for culturing. Any ecto-parasites found were preserved in 10% formalin for further identification.

Flavobacterium columnare isolation was carried out according to (Roberts, 1989; Valerie, 1993.). Fungal isolation was achieved by inoculation on to Sabourauds agar (Oxoid, Basington, England).The medium was acidified to pH 3.5 by adding 1.0 ml of lactic acid 10% to each 100 mls of sterilized medium (Roberts ,1989). Skin and gill scrapings from the sampled fish were mounted on a slide in saline, and were examined under a microscope at ×10 for presence of ecto-parasites (Robert, 1989; (Brown, 1993)

In each of the study ponds and tanks, the following physico-chemical properties of water were measured for a total of six months: pH, dissolved oxygen, temperature, ammonia, nitrite, carbondioxide, and water hardness. A corning 313 pH/T^0C meter (YSI, USA) was used to measure the pH and the temperature of the water in the tanks and ponds; while the dissolved oxygen levels were measured using an oxygen meter. All the other parameters were measured using a viscolor water kit (Lamotte, USA) and this involved use of ingredients which were compared against standards all present in the water kit according to the manufacturer's instructions.

Results

Farm characteristics

Farm characteristics were as shown in Table 1 below.

Disease occurrence on study fish farms

Of the four farms chosen for the cross sectional study, two were classified as intensive production farms based on the stocking densities of fish (1kg of fish stocked per cubic litre of water) and the amount of fingerlings produced per month (above 100,000 fingerlings); and the other two had less stocking densities and production capacities than the above. The farms were also classified depending on the system of water flow (either as a flow through system or recirculated system). Disease conditions were found in both intensive and non-intensive systems.

Intensive production with aeration and a flow through water system

Columnare disease

Out of the 20 samples that were cultured in the laboratory, *F. columnare* was isolated in seven samples, giving a prevalence of 35% as shown in Table 2. All the seven samples that were positive for *F. columnare spp* were taken from fish in the hatcheries.

Fungal infections

Out of a total of 20 samples that were cultured, fungi were isolated in only five samples giving a prevalence of 25% Table 2.

Diseases detected and control measures in farms with different production systems

At the two farms with high intensive production with aeration and re-circulation system, two diseases were observed, which included gas bubble disease and columnaris disease as shown in Table 3.

Table 1: Farm characteristics

Parameters	Attribute	Frequency	%
Types of feeds used	floating feeds sinking feeds	2 8	18.2 72.7
Water sources on the farm	Ground/ spring water Stream/ river water Pipped water Lake water	4 3 1 1	44.4 33.3 11.1 11.1
Onset of farm (2000)	Before After	2 5	26.8 74.2
Nature of farm	H & G only H, G & B Cages G & B	1 5 2 1	11.1 55.6 22.2 11.1
Size (acres)	1 - 3 4 - 7 >8	4 1 3	50 12.5 37.5
No. of hatchery tanks	1 - 5 6 - 10 >10	1 2 3	16.7 33.3 50
No of grow out tanks	1 - 5 6 - 10 >10	3 4 1	37.5 50 12.5
No. of brood stock ponds	1 - 5 6 - 10	7 1	87.5 12.5

Table 2: Samples Taken For Lab Analysis on Farm

Date Of Collection	Section	Findings
18/6/2007	GT7	Columnare
22/6/2007	GT9	Fungal
24/6/2007	GT10	Columnare
25/6/2007	GT7	Columnare
7/7/2007	GT9	Columnare
8/7/2007	GT 1	Fungal
8/7/2007	GT8	Columnare
15/7/2007	GT8	Columnare
23/7/2007	GT 7	Fungal
25/7/2007	GT9	Fungal
10/8/2007	GT10	Fungal
11/6/2007	GT7	Columnare

Table 3: Fish disease conditions detected and control measures in farms with different production systems

Type of production system	Pond identification	Fish population	Disease condition	Preventive measures	Weekly Mortality rates
Intensive (recirculation)	A	15,000	Gas bubble	None	None
	E	20,000	None	None	None
	Green House	25,000	Columnaris disease	Salt & Oxytet in feeds	None
Low production (urban based)	N/A	N/A	Accumulated feed in tanks	None	0.67%
	N/A	N/A	Predators	None	0.01%
	N/A	N/A	Columnaris disease	None	0.53%
	N/A	N/A	Dactylogyrus spp	None	0.53%
	Holding tank	N/A	Trichodina	None	None
	Brood stock	N/A	Trichodina Gryodactylus spp Leeches	None	None
	Grow out		Trichodina	None	None
Low production (rural based)	Hatchery tanks	N/A	Anoxia (0-1.5ppm)	None	1.0%
	Hatchery tanks	N/A	Dropsy	None	None

Discussion

A narrow majority (55.6 %) of the farms studied had hatcheries, grow out and brood stock sections while the rest had one of the three. This impacted on the ability to produce fingerlings and stockers in large numbers. Due to absence of brood stock on 33.3% of the farms studied, the latter had to depend on other farms to supply disease free stock for their farms. This could lead to disease outbreaks on the recipient farms since F .columnare and Saprolegna are able to survive long spells in the environment of high water hardness and organic matter, ((Valerie, 1993.);(Brown, 1993); (Stevenson, 2002);)

Only 44.5 % of the farms studied used ground/ spring water as the primary water source and this was attributed to the fact that underground water had its own limitations such as little or no oxygen and a lot of carbon dioxide hence mitigation measures have to be put in place to combat the shortfalls in water quality. This is in agreement with earlier studies which observed that ground and spring waters are not saturated with oxygen but supersaturated with nitrogen and if acidic have high levels of carbon dioxide (Roberts, 1989). Intensive aeration may be necessary to add oxygen and remove nitrogen and carbon dioxide.

Most of the diseases were observed on intensive farms as compared to the low production farms and this was attributed to the fact that the water sources on the farms had relatively low or no oxygen hence the need for aeration and degassing; the high stocking densities on the farm easily lead to stress of the fry and fingerlings which in turn is the major cause of disease on the fish farms. Previous research showed that physiological stress and physical injury are the primary contributing factors of fish disease and mortality in aquaculture (Rottmann 1992). Many potential fish disease pathogens are continually present in the water, soil, or fish. In nature, fish are often resistant to these pathogens, and they are able to seek the best living conditions available and this explains why fish when stressed will begin showing clinical signs after three days (Rottmann 1992). Regardsless of the system of management, columnaris disease was observed on all the four farms studied due to stress arising from increased fish density and poor water quality (low dissolved oxygen, undesirable temperature or pH, increased levels of carbon dioxide, ammonia, nitrite, hydrogen sulfide, organic matter in the water) (). Other factors include: injury during handling during capture, sorting, transporting; inadequate nutrition; and poor sanitation. The most common stressors observed on the intensive farms studied were water shortage and high stocking densities, while on low production farms the major stressor was poor water quality (low levels of dissolved oxygen and anoxic conditions).

The common ectoparasites observed on the low production farms were Gryodactylus, Dactylogrus, Trichodina, leeches and none was found on the intensive farms. In the former, the parasites were found in the brood stock and grow out sections of the farm especially where happas are used and were only introduced into the hatcheries if the brood stocks were not dipped in a salt or potassium permanganate bath. In happas due to the high stocking density and the poor water quality, Trichodina and other ecto parasites will always be present and if there numbers are so vast then treatment will be sought and this was consistent with earlier studies which showed that heavily infested fish may have an increased production of cuticular material, frayed fins, skin ulcers and damaged gills ((Akoll P; Konecny, 2006); Roberts, 1989). The study revealed that the major predilection sites for the Trichodina were the gill and skin (Roberts, 1989) and this was the only parasite which was transported to the holding tank during conditioning of the fish before spawning.

Fungal diseases were only found in the intensive farm with flow through system and none in the low production or recirculation farms. This occurred concurrently with columnaris disease and this was mainly attributed to water shortage which usually stresses the fish. Work done by(doctor, 2007.), also found out that fungi are opportunistic parasites, able to take advantage of damaged or stressed fish. In addition, (Maria Laura 2007) and (Stevenson, 2002) established that Saprolegniasis is mainly a secondary infection seen after damage to the fish integument (skin and gills) caused by parasites, viruses, bacterial infections and other skin damage.

Conclusions

Of the fish farms studied, 45% used ground water as the source of water on the farms. The prevalence of Columnaris disease and fungal infection in the high intensive production farms with a flow-through system were 35% and 25% respectively. Ecto parasites were observed only on low production farm and the former were mainly found in the broodstock and grow-out sections of the farms.

Recommendations

All farms using ground and spring water as main water sources must have an efficient degassing mechanism and use aeration to improve on the water quality in the hatchery tanks. Future studies should be set up to categorise Flavobacterium columnare strains found in the hatchery. Prophylactic measures should be instituted to control columnaris disease and ecto parasites in the broodstock and growout sections of the farms.

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Assessment of the potential for horizontal gene flow from transgenic bananas to rhizosphere inhabiting microorganisms

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ABSTRACT

Recent advances in plant biotechnology, especially genetic engineering have heightened promises for mitigating biotic and abiotic constraints. The use of biotechnology to mitigate biotic constraints has raised several biosafety concerns mainly gene flow. Of interest in crop cultivation is gene flow to rhizosphere microorganisms. In this study, transgenic banana plantlets having the hygromycin resistance (*hpt*) gene as selection marker and beta-glucuronidase (*gus*A) gene as a reporter marker were used to investigate gene flow to *Agrobacterium tumefaciens, Escherichia coli* (DH5α) and natural soil bacteria. The plants were potted in soil in which the microorganisms were inoculated and later isolated from the rhizosphere on selective medium supplemented with hygromycin. DNA was extracted from bacterial organisms that established on selective medium and subsequently amplified using *hpt* and *gus*A specific primers to confirm putative gene flow. Highly significant differences (P=0.001) were observed among the means of the CFUs of *E. coli* and natural soil bacteria that were re-isolated on normal and selective media however, plant genotype effects on the re-isolation of the test organisms were not observed (P≥0.05). PCR screens for the marker genes revealed no uptake of the transgenes by the microorganisms suggesting therefore a very low probability of gene flow from banana. Given the differences in physiology and root exudation patterns among different crops, and their ability to induce competence among microbial organisms, its worthwhile pursuing these studies on a case by case basis with respect to different crops and antibiotic markers.

Keywords: *Biosafety, biotechnology, biotic constraints, Musa* sp*, Uganda*

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Introduction

Rhizosphere microorganisms are those that live within a volume of soil that is in direct contact with plant roots and thereby influenced. They comprise fungal, bacterial and protozoans amongst other microbial groups and in crops like bananas, often reach upto very high densities per gram of soil due to the highly diverse habitat provided (Siciliano *et al*., 1998). Bacteria constitute an important component of these microorganisms and they have developed associations with plants which range from parasitic, symbiotic, epiphytic to endophytic associations. The diversity and development of these microorganisms is to a large extent stimulated by root exudates and other factors such as root exudation patterns, root structure, duration of the season/ growth, soil type, crop stage, cropping practices and other environmental factors (Smalla *et al*., 2001). Furthermore, species diversity of plants affects the amounts of root exudates and rhizo-deposition in the different root zones. This significantly affects the structural and functional diversity of the rhizosphere microorganisms (Dunfield and Germida, 2004). Therefore, the influence of plants on soil microbes is greatest in the rhizosphere and the magnitude of this influence is determined by the extent of microbial interactions and the plant (Dunfield and Germida, 2004; Kowalchuk *et al.*, 2003).

Changes in plants due to genetic modifications such as the use of biotechnological approaches to develop transgenic plants could have alterations on these effects through horizontal geneflow, a process which involves the uptake and eventual expression of plant DNA by competent microorganisms (Cooper and Sweet, 2001; Nielsen, 2003). The use of biotechnology approaches to develop transgenic plants has followed the promise of the technology in the control of emergent pest and disease problems over other strategies

such as cultural, chemical, biological and conventional breeding that have various associated constraints which make them inefficient at providing the requisite control (Okori, 2004; Tripathi *et al*., 2004; Vuylsteke *et al*., 1995).

In bananas, which are a major food source in East Africa and especially in Uganda where they are an important source of food security and household incomes for the local farming communities, biotechnological approaches have been employed in the mitigation of some of these constraints. For example the use of anti-microbial proteins and chitinases in AAB plantains and grande naine cultivars to induce resistance to black sigatoka (*Mycosphaerella fijiensis*) and; the use of maganins in rasthali cultivar (AAB) to induce resistance to *Fusarium oxysporum* f.sp. *cubense* and *Mycosphaerella musicola* (Atkinson *et al*., 2003). In Uganda, research has been undertaken on the use of chitinases from rice and papaya in the induction of resistance in bananas to black sigatoka (*Mycosphaerella fijiensis*) (Swennen and Sagi, 1996); while there are ongoing efforts on the use of *hrap* genes in bananas to control banana bacterial wilt. These research efforts should consider potential gene flow concerns.

The occurrence of horizontal gene flow to rhizosphere microbial communities can have potential negative effects on their major biological and ecological functions (Dröge *et al*., 1999). Moreover, if the genes taken up have the ability to improve the fitness of the recipient microorganisms, directional selection may ultimately lead to the emergence of new populations that may even be pathogenic to the landraces and other plants and disrupt the biological diversity in the environment (Cooper and Sweet, 2001). The objective of this study therefore was to investigate horizontal gene flow from transgenic bananas to rhizobacteria.

Materials and methods

Study materials. The materials used in the study included; transgenic banana plantlets from local cultivars (Mbwazirume and Mpologoma) transformed with *ß-glucuronidase* (*gus*A) reporter gene under the control of *CaMV35S* promoter and terminated by a nos sequence with a hygromycin resistance gene used as the selectable marker. The microorganisms tested included: *Agrobacterium tumefaciens* (EHA 105) resistant to the gentamycin sulphate antibiotic, *Escherichia coli* (DH5α) and naturally occurring soil bacteria obtained from the rhizosphere of banana plants.

Experimental design. The experiment was set up with treatments that comprised of transgenic banana plantlets containing the *gus*A and *hpt* genes and non transgenic banana plants that were used as the control. The plantlets' rhizospheres were innoculated with either *A. tumefaciens* or *E. coli*. The experiments were run for a period of 13-16 weeks during which normal agronomic practices were done to ensure proper growth. Each experiment had 5 transgenic plants and 2 controls. The experiments were repeated three times. Re-isolation of the bacterial organisms from the rhizosphere region was done three times.

Media preparation. For culture of *E. coli* and natural soil bacterial isolates, Luria Bertani (LB) media (Cools *et al*., 2001) was used which was prepared by autoclaving constituted media for 15 minutes at 121 ^{o}C and cooled prior to addition of a fungicide, cyclohexamide (150 µg/ml) (VWR International, England). The media was subsequently dispensed into petri-plates (90 mm). Selective LB media was prepared by adding an antibiotic, hygromycin (50 µg/ml) (Duchefa Biochemicals, Netherlands) and a fungicide, cyclohexamide (150 µg/ml) (VWR International, England) to autoclaved and cooled media. The selective media was dispensed into petri-plates (90mm). Normal and selective media for *A. tumefaciens* re-isolation was prepared as for *E. coli* except that gentamycin sulphate (50 µg/ml) (Duchefa Biochemicals, Netherlands) was added as a selective antibiotic.

Re-isolation of inoculated experimental organisms

Inoculum dosage optimisation experiments

The bacterial innocula used comprised of *A. tumefaciens* and *E.coli*. Dosage optimisation was initially done to establish the right amounts of bacteria to apply in the soil that would be easily re-isolated. The optimisation process involved the use of tissue culture banana plantlets grown in plastic pots (400 ml volume) in sterile soil for a period of 2 months. Some of the plants were inoculated with an overnight culture of *E. coli* using either 1 ml, 2 ml, 3 ml or 4 ml. The other plants were inoculated with a 48 hour old culture of *A. tumefaciens* using the same volumes that were used with *E. coli*. The inoculation process was done by pouring the bacterial culture solution concentrically around the stem base region of the plants. The experiment was left to stand for 10 days prior to the re-isolation of the bacteria. The 3ml volume gave optimal results for *A. tumefaciens* and was therefore adopted for the study while in *E. coli*; the 2ml inoculum volume was adopted.

Inoculum re-isolation optimization

Plantlets inoculated with the 2 ml and 3 ml bacterial innoculum dosages were initially used during the re-isolation of *A. tumefaciens* and *E. coli*, respectively. One gram soil samples were derived from each of the pots from the stem base region of the plant and vortexed for 2 minutes in 2 ml of sterile water in sterile 50 ml centrifuge tubes. The soil sample was subsequently serially diluted to 10^{-2}, 10^{-4} and 10^{-6}. This procedure was followed for the rhizosphere soil samples that were respectively inoculated with *E. coli* and *A. tumefaciens*. Diluted solutions (100 µl) were spread on LB media (Cools *et al*., 2001). The plates spread with *E. coli* were incubated overnight at 37^{o}C while the *A. tumefaciens* culture plates were incubated at 28^{o}C for two days. Colony forming units (CFUs) were compared across the different plates for each of *E. coli* and *A. tumefaciens* to establish the optimum dilution for usage in the experiments. In *A. tumefaciens* and *E.coli* respectively, the 10^{-2} and 10^{-4} dilutions gave the most distinct and countable re-isolated colonies and were therefore adopted for use in the experiments.

Gene flow analysis to the experimental organisms

Agrobacterium tumefaciens and *E. coli* were inoculated into the rhizospheres of the respective experimental plants using optimised innoculum volumes. This was followed with the re-isolation of the bacteria from the rhizospheres of transgenic and non-transgenic banana plantlets for the study of geneflow. Two 1 g soil samples collected three times at intervals of 10 days from the rhizosphere of each plant were used for re-isolation purposes. The intervals used were adopted to enable acclimatisation of the microorganisms to the banana rhizosphere and also allow for adequate exposure to root exudates. The soil samples derived from the rhizosphere of plantlets inoculated with *A. tumefaciens* were suspended in sterile water and diluted to 10^{-2} CFU. Of this solution, 100 µl was inoculated into LB plates containing selective media (2 LB plates containing 50 µg/ml hygromycin) and a control (without the antibiotic). A similar procedure was used for soil samples derived from the rhizospheres of transgenic plantlets inoculated with *E. coli*. The exception was the initial dilution of the bacterial suspension to 10^{-4} CFU. Both *A. tumefaciens* and other bacterial cultures were incubated at 28^{0}C for 48 hours while the *E. coli* cultures were incubated at 37^{0}C for 24 hours. Colony counts were taken and used to compute the CFUs to enable working with the actual amounts of bacterial organisms that were in the sample of soil taken from the rhizosphere region of the experimental plants.

Agrobacterium tumefaciens colonies that grew on selective media were subjected to a second round of selection on plates containing hygromycin (50 µg/ml) (Duchefa Biochemicals, Netherlands) antibiotics to confirm their survival. A similar procedure was used for *E. coli* and the natural soil bacterial isolates that grew on selective media. The selection plates used for the re-isolation of *E. coli* and the natural soil bacteria isolates had hygromycin (50 µg/ml) (Duchefa Biochemicals, Netherlands) as the selection antibiotic. In order to culture sufficient quantities of bacteria for DNA isolation, re-isolated colonies of *A. tumefaciens*, *E. coli* and natural soil bacteria were cultured in LB broth media. A 25 mls solution of LB broth in 100 ml erlenmeyer flasks containing only hygromycin (for *E. coli* and natural soil bacteria) or gentamycin sulphate and hygromycin (for *A. tumefaciens*) was used to culture the test isolates. The flasks containing *E. coli* were incubated overnight at 37^{0}C while those that had *A. tumefaciens* and natural soil bacterial isolates were incubated at 28^{0}C for 48 hours. Optical densities were taken at the end of the incubation period for all the bacterial cultures. The bacterial cells in solution were harvested by centrifugation (MIKRRO 250, Berlin Germany) at 6000 rpm for 15 minutes at 4^{0}C in sterile 50 ml centrifugation tubes.

Genomic DNA extraction and PCR screens. Genomic DNA was extracted from the harvested bacterial cells that grew on selection media using a protocol by Mahuku (2004). The bacterial cells were crushed using sterile sand and miniature pestles. The DNA extraction buffer (Tris EDTA SDS (TES) contained: 0.2 M Tris-HCl [pH 8], 10 mM EDTA [pH 8], 0.5 M NaCl, 1% SDS. The quality of the extracted DNA was assessed by running 5 µl of the DNA and 2 µl of loading dye in a 1% agarose gel in TAE buffer. The gel was stained in 1% ethidium bromide for 20 minutes and visualized under an ultra violet trans-illuminator. The bands on the gel were documented using a gel documentation system. The PCR was performed using primers specific to the gusA and hpt genes. The gusA primers used were; primer 1- 5' TTTAACTATGCCGGGATCCATCGC 3' and primer 2- 5' CCAGTCGAGCATCTCTTCAGCGTA 3'. The PCR involved a 2 min initial denaturation step at 94^{o}C and 30 cycles consisting of 1 min denaturation at 94^{o}C, 1 min primer annealing at 60^{o}C and a 1 min extension at 72^{o}C followed by a 10 min extension step at 72^{o}C. The amplification products were size fractionated by agarose gel, electrophoresed in TAE buffer (Maniatis *et al.*, 1989) at 80 volts for one hour. The gels were stained with 1% ethidium bromide in an aqueous solution and were examined for amplification of the *gus*A and *hpt* genes.

Statistical analysis of the results. The bacterial colonies that grew on media were expressed as CFUs using the formula below;

$$CFUs = \frac{\text{No. of colonies}}{\text{Dilution factor}} \; X \; \text{volume of sterile water used to dilute the soil sample}$$

The data collected were subjected to t-tests for comparison of means. The analysis for geneflow to rhizo-bacteria data were subjected to ANOVA using the GenStat Discovery Edition 3 (Lawes Experimental Trust Rothamstead Experimental Station UK). Where significant differences were found between treatment means, these were compared using Fishers Protected Least Significant Difference Test (Steel *et al*., 1997).

Results

Re-isolation of rhizosphere inoculated bacteria

Inoculum dosage optimisation. A comparison of best dilution volumes revealed significant differences at 5% level (P<2.35, t = 1.19) in *A. tumefaciens*. Distinct colonies were obtained with the 3 ml volume (13 x 10^4 CFUs as compared to the 8.60 x 10^4 CFUs, 16 x 10^4 CFUs and 26 x 10^4 CFUs obtained with the 1ml, 2 ml and 4 ml volumes, respectively. In *E. coli*, the inoculum dosage used influenced the bacterial colony counts at re-isolation at the 5% level (P < 2.35, t= 1.24) with the highest colony count observed where the 2 ml dosage volume was used and the least in the 3 ml inoculum volume.

Inoculum re-isolation optimisation. In *A. tumefaciens,* significantly more colonies established with the 10^{-2} dilution compared to other dilution factors (P< 6.31, t=4.30). About 83 colonies were obtained with the 10^{-2} dilution whereas 1 colony and 0 colonies where observed for the 10^{-4} and 10^{-6} dilution factors, respectively. Results from the re-isolations in *E. coli* revealed that all the dilutions gave similar results and none were significantly different (P ≥ 0.05). The 10^{-4} dilution plate however, had more distinct colonies as compared to the 10^{-2} and 10^{-6} dilutions.

Analysis of geneflow to the experimental organisms. On plates, a total 1089 colonies (853 on normal media and 236

on selection media) were obtained from *E. coli* while in *A. tumefaciens* and natural soil bacterial isolates, 12503 colonies (12457 on normal media and 46 on selection media) and 615 colonies (564 on normal media and 51 on selection media) respectively were observed to grow. Generally more isolates from *E. coli* grew on selection media than from *A. tumefaciens* and natural soil bacteria. The lowest count of isolates that grew on selection was observed in *A. tumefaciens*. The total CFUs that grew on selective media were 8.0×10^6 for natural soil bacterial isolates, 4.26×10^7 for *E. coli* and 5.80×10^4 for *A. tumefaciens*. Highly significant differences (P=0.001) were observed amongst the means of the CFUs of *E. coli* that were re-isolated on both normal and selective media (Table 1). Marked differences (P=0.001) were similarly observed amongst the means of the CFUs derived from the different plants. However significant plant genotype effects on the re-isolation of *E. coli* were not observed (P≥0.05) (Table 1). In general more bacteria were re-isolated from non- selective media (Table 2). Interestingly, plants that supported high numbers of bacteria on non selective media similarly generated high numbers on selective media.

Table 1: Analysis of variance for the means of the CFUs of the experimental microorganisms that established on normal and selective media

Source of variation	Degrees of freedom	Mean square	F. pr
E.coli			
Replicate	2	2480289	
Plant	5	1465884	<.001
Treatment	1	3206770	<.001
Plant.Treatment	5	3206770	0.911
Error	22	146660	
Total	35		
Natural Soil bacterial isolates			
Replicate	2	207515	
Plant	8	195282	0.748
Treatment	1	30380866	<.001
Plant.Treatment	8	252260	0.597
Error	34	310619	
Total	53		
A.tumefaciens			
Replicate	2	914930	
Plant	4	194385	0.583
Treatment	1	1840816	0.017
Plant.Treatment	4	192720	0.587
Error	18	266403	
Total	29		

Data were transformed using the square root transformation to normalize the variances (Sokal and Rolf, 1995)

Table 2. Mean colony forming units of experimental organisms that established on both normal and selective media

Plant	Treatments	
	Normal media	Selective media
Escherichia coli		
Transgenic plants		
EP2	1753	1240
EP3_1	1491	1054
EP5	2507	1772
EP6	1644	1162
Non-transgenic (controls)		
EPN2	1854	1311
LSD		264.7
CV%		26.1
Natural soil bacterial isolates		
Transgenic plants		
EP1	1886	404
EP2	1594	364
EP3_1	1965	316
EP3_2	1846	574
EP4	1512	428
EP6	2676	499
EP7	2095	236
Non transgenic controls		
EPN1	1703	779
EPN2	2141	316
LSD		308.3
CV (%)		47
Agrobacterium tumefaciens		
Transgenic plants		
EP1	982	45.0
EP3_2	199	79.0
EP4	469	15.0
EP7	827	47.0
Control		
EPN1	202	15
LSD		396
CV (%)		105

Data were transformed using the square root transformation to normalize the variances (Sokal and Rolf, 1995)

In the naturally occurring re-isolated rhizobacteria, highly significant (P=0.001) differences were observed amongst the treatments though the re-isolates from the plants were not markedly different (P≥0.05). Plant genotype did not influence (P≥ 0.05) bacterial re-isolation (Table 1). The means among bacteria re-isolated from the rhizosphere of the transgenic plants were not significantly different although there were

some differences between reactions of the isolates (Table 2). With *A. tumefaciens*, no significant differences (P≥0.05) were observed among the means of the CFUs of the isolates derived from the plants used in the experiments though differences (P≤0.05) in treatments used were noticeable. The re-isolation of *A. tumefaciens* was not influenced (P≥0.05) by the genotype of the plants (Table 1). Among the means of the CFUs of *A. tumefaciens* from the rhizospheres of transgenic plants and the non transgenic plants (controls), no significant differences (P ≥ 0.05) were similarly observed though some re-isolates reacted differently (Table 2).

Molecular analysis and PCR screens for *gus*A and *hpt* genes. A total 36 bacterial re-isolates (8 from *E. coli*, 9 from *A. tumefaciens* and 22 isolates from natural soil bacteria) were used for DNA extraction. The PCR was performed using *gus*A and *hpt* specific primers and, the amplicons were electrophoresed on a 1% agarose gel. Initial amplification of the DNA extracted from the experimental plants with *gus*A and *hpt* specific primers revealed amplicons corresponding to *hpt* and *gus*A genes respectively in plates 1A and 1B. An amplified fragment of about 500bp corresponding to the internal fragment of *gus*A gene was observed in the positive control (plasmid DNA (pCAMBIA1201)) (Plate 1C) and an amplified fragment corresponding to the *hpt* gene was also observed in the positive control (Plate 1D). No amplified fragment was observed with DNA extracted from the bacteria re-isolated from the rhizosphere of the experimental plants (Plate 1C and 1D).

Discussion. The results from this study suggested that no horizontal gene flow occurred to the bacteria tested. The non influence of the genotype of the plants used in the study on the re-isolation of the bacterial organisms suggests all re-isolated bacteria from the rhizosphere of transgenic plants reacted in a similar manner to the bactericide and were thus non-transformed. Interestingly for all bacteria re-isolated, some survived on selective media suggesting uptake of the transgene. However, the absence of PCR amplicons of the *gus*A and *hpt* genes in all the re-isolated bacteria surviving on selective media provides support for absence of gene flow and for the theory that the bacteria used in this study possess endogenous capacity to degrade antibiotics. Indeed, other studies on *E. coli* have revealed the presence of a plasmid encoded antibiotic resistance gene to hygromycin (Rao *et al.*, 1983). Elsewhere studies have demonstrated the occurrence of antibiotic resistance genes on plasmids (Riesenfeld *et al.*, 2004). However, it should be noted that in respect of horizontal gene flow, natural events have been detected in *E. coli*. Studies by Doolittle *et al*. (1990) revealed that *E. coli* integrated a second glyceraldehdye-3-phosphate dehydrogenase gene from a eukaryotic host. In case of *A. tumefaciens*, a similar response was found on both selective and non-selective media suggesting absence of geneflow from bananas. Similar reports have been made in other studies involving *A. tumefaciens* (Broer *et al.*, 1996).

The absence of gene flow could be attributed to various reasons. Firstly, transformation of bacteria requires that cells be competent (able to take up exogenous DNA). The ability to naturally develop competent cells has been reported among bacteria (Palmen and Hellingwerf, 1997). However, this capacity may be compounded by the physiological state of the cell and the influence of environmental factors (Lorenz and Wackernagel, 1992). In other bacteria, competence "state" development is influenced by absence or presence of certain amino acids and glucose availability which modulate DNA-binding and uptake machinery (Palmen and Hellingwerf, 1997). Availability of amino acids and glucose are inadvertently influenced by rhizosphere conditions especially enzyme activities that may enhance bio- degradation of both DNA and these ingredients (Bertolla and Simonet, 1999; Nielsen *et al*., 1998). The bio-degradation process may also degrade DNA reducing dosages for adsorption and ultimately uptake (Bertolla and Simonet, 1999). In this study, bacteria

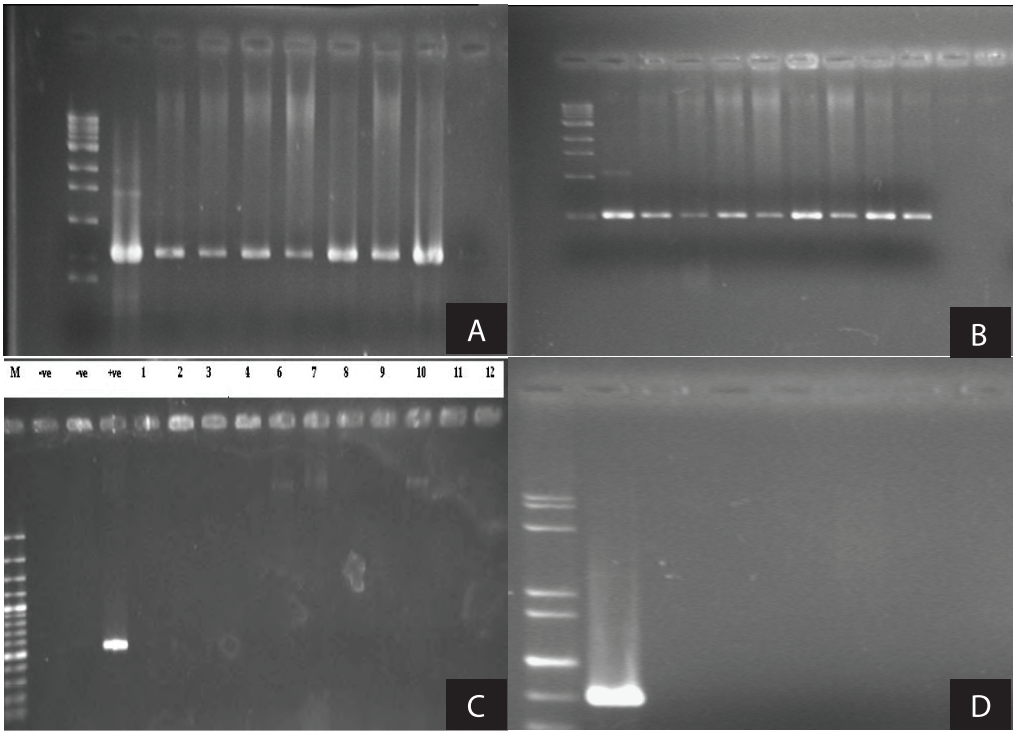

Plate 1. Electropherogram showing the presence of the hygromycin hpt (A) and β–glucuronidase (B) genes in the genetically engineered banana plants; and the absence of *β–glucuronidase* (C) and the hpt genes (D) in the microbial DNA

were inoculated into the rhizosphere and grown in soil for 13 weeks. The failure to uptake DNA from plants (banana) could thus be attributed to inavailability of transgenic DNA or more importantly, the lack of competent state limited gene transfer. Furthermore, eukaryotic DNA molecules are usually associated with proteins such as histones which condense DNA and could therefore drastically interfere with the uptake and recombination mechanisms of bacteria (Bertolla and Simonet, 1999). These studies on Agrobacterium and other soil inhabiting bacteria suggest a very low probability of gene flow from banana.

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NOTE: ALWAYS PRESS
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COMMUNIQUÉ

AGBIOSAFESEED2010: Kampala, Uganda 08 - 11 March 2010 - International Conference on Agro-biotechnology, Biosafety and Seed Systems in Developing countries.

This international conference has brought together over 120 stakeholders that included policymakers, scientists, government regulators, media and communication experts. The theme of this conference was; "Tapping Agro-Biotech Potential for Improved Seed Production and Utilization".

Noted that:

1. High population growth rates particularly in SSA are placing a huge demand on available global natural resources. There is thus a need to quadruple our efforts in food production in order to cope with the current unfolding food crisis;
2. Various emerging challenges, including the increased need for sustainable energy and climate change and variability are further exacerbating the dilema;
3. Biotechnology is a tool that offers immense potential for stimulating agricultural development in SSA;
4. There are a number of biotechnology research for developments efforts in Africa at various levels of development, and similarly a number of countries are at different stages of development their regulatory systems
5. There is need to improve communication of GM technologies through various strategies including improved packaging of information
6. Need for involvement of all stakeholders in research and dissemination of biotechnology products and related technologies
7. Most of the crops of economic importance to Africa are considered orphan crops by the developed world and African stakeholders need to ensure initiatives to harness their potential are pursued
8. Need to regularly update development partners / policymakers and other stakeholders on progress of research and development, particularly those that make use of biotechnology, to increase their involvement for policy making and implementation
9. Need for social and environmental audit of agricultural development projects
10. There is a need for substantial investment in capacity development for biotech and other related fields but there is continuous brain drain due to poor conditions of service

Agreed and recommended that:

1. African governments should operationalize the Maputo Declaration on investing 10% of national budget into agriculture
2. Biotechnology is one of the key tools in agricultural research for development that can positively contribute to agricultural growth to cope with the increasing population growth and the diversity of biotech and abiotic constraints
3. Need to build a critical mass of human resource and other competencies as well as infrastructure for the needs of cutting edge sciences
4. There is need for increased resources for undertaking basic research on biotechnology particularly GM technology in addition to support for technology evaluation
5. Careful and well thought dissemination of information packages on GM can significantly contribute to the acceptance and adoption
6. There is good political will for biotechnology advancement in many African countries. However, countries need fully functional biosafety frameworks backed up by appropriate legislations. For example, in Uganda, we request that the biosafety policy be brought to the Parliament for debate as soon as possible.
7. Need to improve on the remuneration and other incentives systems for scientists to minimize brain drain
8. Need to strengthen regional policy and technical collaboration to optimize resource use.

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