Assessment of Maize Food Products in the Kenyan Market for Presence of Genetic Elements and Proteins from Genetically Modified Organisms

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A thesis submitted in partial fulfilment for the degree of Master of Science in Biochemistry in the Jomo Kenyatta University of Agriculture and Technology.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this thesis output to my mum Kathigha Mutoni, dad Mutoni Ngoloi, my husband Dr. Dave Juma Wanzama and to our beloved son Daniel Juma Musikoma. Thanks to you all for the support that you gave me. You laid in me a great foundation that has successfully seen me through to this level of education.

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LIST OF ABBREVIATIONS

AATF	African Agricultural Technology Foundation
ABC	Applied Biotechnology Center at CIMMYT
AREX	Agricultural Research and Extension
B	Billion
BGHC L2	Biosafety Level II Green House Complex
Bio AWARE	National Biotechnology Awareness Strategy
BL2	Biosafety Level 2 laboratory
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower Mosaic Virus
CFT(s)	Confined Field Trial (Sites)
CGE	Capillary Gel Electrophoresis
CIMMYT	International Maize and Wheat Improvement Center
CRMs	Certified Reference Materials
CRY	Crystal
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribo-Nucleic Acid
EDTA	Ethylane-Diamine Tetraacetic Acid
ELISA	Enyme linked immunosorbent assay
EPSPS	Enolpyruval-shikimate-3-phosphate synthase
Et al.	And Others
EU	European Union

FAO	Food Agricultural Organisation
GE	Genetic Engineering/ Genetically Engineered
GEOs	Genetically Engineered organisms
GM	Genetically Modified
GMO	Genetically Modified Organism
Ha	Hectares/ hectarage
Ha^{-1}	Per Hectare
HSRC	Human Science Research Council
IBC	Institutional Biosafety Committee
IRMA	Insect Resistant Maize for Africa
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agriculture Research Institute
KEPHIS	Kenya Plant Health Inspectorate Services
KSTCIE	Kenya Standing Technical Committee on Imports and Exports
M	Million
MAS	Marker Assisted Selection
MBE	Beta Mercapto Ethanol
MBRI	Maize Biotechnology Research Institute
NARL	National Agricultural Research Laboratories
NBA	National Biosafety Authority
NBC	National Biosafety Committee
NCST	National Council of Science and Technology
NIR	Near Infrared

NPRC	National Potato Research Centre
OQS	Open Quarantine Site
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PI	Pollen Intensity
PIFB	Pew Initiative on Food and Biotechnology
PIP	Plant Import Permit
Q_PCR	Qualitative PCR
QC	Quality Control
QTL	Quantitative Trait Loci
RCF	Relative Centrifugal Force
R&D	Research and Development
RNA	Ribonucleic Acid
SFSA	Syngenta Foundation for Sustainable Agriculture
SOPs	Standard Operating Procedures
SSA	Sub-Sahara Africa
STP	Soluble Total Proteins
TBE	Tris-borate-EDTA
UN	United Nations
U.S.A	United States of America
USD	United States Dollars
USDA	United States Department of Agriculture
WEMA	Water Efficient Maize for Africa

ABSTRACT

Transgenic crops were grown in at least 29 countries and by over 15.4M farmers, covering 148M ha of land worldwide by 2010. In 2001, Kenya introduced *cry1Ab* and *cry1Ba* genes for research purposes to control lepidopteran maize stem-borer pests and not for commercial. In 2009 the Kenya Biosafety Bill was assented into law. Nevertheless, Kenya imports foods from countries that grow GM crops. This study assessed maize food products in the Kenyan markets for inadvertent presence of genes and proteins from selected GMOs and the possibility of 'gene-flow' to the surrounding maize farms due to growing of *Bt* maize in confined field trial (CFT) sites at KARI-Kiboko in 2005–2006 period. The target sample (120) was obtained using multistage sampling technique. Conventional PCR and lateral-strip methods were used to analyse the target sample sourced variously as seed, flour and flour products. About 6.7% of the 120 samples tested positive for *cry1Ab* gene and Cry1Ab protein, but were negative for *cry1Ba* gene. Besides, no gene-flow was detected within the vicinity of the Kiboko CFT site (confirming that the regulatory measures employed during the CFTs at Kiboko were effective). These findings indicate that there are GMOs in the maize grain imports into Kenya. Thus NBA and KEPHIS should put foolproof measures including labelling requirements in place to eliminate illegal imports of GM maize food products. Further research should be undertaken on the quantification of GMOs in the maize food products in Kenya.

Key words: *Cry1Ab*, *Cry1Ba*, Biosafety, Genetically Modified (GM), Genetically Modified Organisms (GMOs), Labelling, *Bt* maize.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

1.1.1 Biotechnology Defined

Biotechnology is any technology based on biology, especially when used in agriculture, food science, and medicine. Biotechnology generally refers to the use of micro-organisms to produce certain chemical compounds. Long before the term "biotechnology" was coined for the process of using living organisms to produce improved commodities, people were utilizing living micro-organisms to produce valuable products (Halford and Halford, 2003). The UN Convention on Biological Diversity came up with one of many definitions of biotechnology: "Biotechnology means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use". Biotechnology has, therefore, found a wide use in agriculture, thus the term “agricultural biotechnology”. Most of the biotechnology activities have however, focused on enhancing agricultural productivity (Clive, 2004a).

Genetically Modified Organisms (GMOs) can be defined as organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating or natural recombination. The modification can be done for instance by being genetic engineering (GE) or by recombinant DNA technology (Anklam *et al*., 2002). GM food as defined by the European Community (EC) Novel Food

Regulation is a food that is made from a GMO and contains genetic material of protein resulting from the modification. Some common examples of GMO include herbicide tolerant soybean, insect pest resistant maize, virus resistant papaya and salt resistant tomato (EC, 2003).

1.1.2 Agricultural Biotechnology

Agricultural biotechnology is a term which represents a continuum of different techniques, ranging from non-controversial tissue culture to controversial genetic engineering (GE). GE is a set of techniques from molecular biology (such as recombinant Deoxyribonucleic acid (rDNA)) by which the genetic material of plants, animals, micro-organisms, cells and other biological units are altered in ways or with results that could not be obtained by methods of natural mating and reproduction or natural recombination (Sharma *et al.* 2002). Techniques of genetic modification include, but are not limited to: recombinant DNA technology, cell fusion, micro and macro injection, encapsulation, gene deletion and doubling. Genetically engineered organisms (GEOs) do not include organisms resulting from techniques such as conjugation, transduction and natural hybridization (Njau *et al.,* 2001). Thus simply put, genetic modification describes a series of techniques used to transfer genes from one organism to another or to alter the expression of an organism's genes (Kitch *et al.*, 2002).

In modern agricultural biotechnology, various scientific techniques mostly genetic engineering have been used to modify plants, animals and micro organisms by introducing into their genetic makeup genes with desired traits. A wide variety of

food crops have been genetically engineered in the laboratory to contain beneficial traits such as increased resistance to herbicides, diseases, insects or improved nutritional content. Such genetically engineered crops are referred to as genetically engineered (GE)/genetically modified (GM) crops. They are also commonly referred to as biotech crops (Clives, 2009).

1.1.3 Benefits of Biotechnology

Crop biotechnology can potentially increase agricultural yields; reduce yield losses from insects, diseases and drought, and enhance the nutritive value of crops crucial to poor people's health (Tabashnik *et. al.*, 2003). With the development of modern biotechnology, numerous GMOs have been approved for commercialization. However, GMOs' emergence has caused public debate on the consumers' freedom of choice to purchase GMO-derived products or not. (Dong *et al.*, 2008) However, there is genuine concern expressed by many people about long-term negative health and environmental effects, such as allergies (Domingo, 2000).

1.1.4 Categories of GM Crops and Status of Biotechnology in Africa

GM crops can be categorized into three generations (Smyth *et al.,* 2002). The first generation of GM crops currently available contain input-traits with agronomic benefits to farmers but no direct benefit for consumers. On the other hand, second generation GM crops involve health and nutritional properties that will benefit consumers (e.g. higher content of nutrients), while third generation crops are

aimed at the production of “nutraceuticals" and pharmaceuticals. The use of GM technology to produce medicines rose steeply. Paarlberg (2008), noted that about 25% of new drugs going into the global market are produced using GM technology, while in agriculture, 80% of all cultivated hybrid maize in the US is GM (Cox *et al.,* 2008).

On the whole, African countries are at different stages in the development of biotechnology. Some have moved up the technology ladder and are applying more sophisticated techniques such as molecular markers, while others are still in the tissue culture level of application. For example, Egypt and South Africa have moved rapidly into such areas as gene sequencing, characterization of pathogens and gene promoters, while Tanzania and others are still at rudimentary levels of biotechnology development and application (Clive, 2008).

There are generally three categories of countries in biotechnology: (a) those that are generating and commercializing biotechnology products and services using third generation techniques of genetic engineering; (b) those that are engaged in third generation biotechnology Research and Development (R&D) but have not developed products and/or processes yet; and (c) those that are engaged in second-generation biotechnology (mainly tissue culture). In Africa, the first category includes Egypt, Burkina Faso and South Africa, while Kenya, Uganda and Ghana fall in the second category. Tanzania, Malawi and Zambia are in the third category (Clive. 2009).

South Africa and Egypt are biotechnology leaders in Africa. They have considerable scientific infrastructure and clear programmes on biotechnology. The two countries have focused on cutting-edge biotechnology areas and have commercialized some of their products. South Africa's biotechnology R&D focus is on GE of cereals (maize, wheat, barley, sorghum and millet) soybean, lupins, sunflowers, sugarcane, vegetables and ornamentals, as well as on molecular marker applications. These include diagnostics for pathogen detection; cultivar identification for irish potatoes, sweet potatoes, ornamentals, cereals, cassava; purity testing of cereals seed-lots; marker assisted selection (MAS) in maize and tomato; and markers for disease resistance in wheat. Egypt has invested considerably in GE of potatoes, maize and tomatoes (Clive, 2009).

In South Africa, biotechnology R&D is largely undertaken by departments at universities and national agricultural research bodies. Some of the universities have established units or programmes that are now dedicated to biotechnology R&D. The University of Cape Town, South Africa, has a number of internationally cutting-edge research activities in biotechnology conducted within its Department of Biochemistry, which qualifies as a Centre of Excellence in Biotechnology R&D (Clive, 2008).

In Kenya, most of the agricultural biotechnology R&D activities focus on improving the yield potential of cereals and some export crops such as coffee and pyrethrum. However, field tests and commercialization of genetically modified potatoes are under way. Institutions engaged in agricultural biotechnology R&D

in Kenya include the Kenya Agricultural Research Institute (KARI), the Department of Biochemistry of the University of Nairobi and Kenyatta University, the KARI-National Potato Research Centre (NPRC), and the Jomo Kenyatta University of Agriculture and Technology (JKUAT). KARI focuses on GMOs such as capripox virus and rinderpest recombinant vaccine production, and production of transgenic sweet-potato, cotton which are at the moment in biosafety lab testing (Mugo *et al.*, 2008).

1.1.5 Maize and its Importance

Maize is an impor tant commodity with a global market. Eighty percent (%) of the starch produced in the world is derived from maize. In Eastern, Central and Southern Africa, maize is the major staple food. It is produced and consumed by most households. However, land is limited and yield increases have leveled off. Agricultural productivity has not been able to cope with population growth, leading to annual imports and food insecurity. There is continuing need to increase food production in Kenya through adoption of technologies that will increase productivity and minimize losses from stem borers (Mugo *et al.*, 2008).

It is increasingly becoming clear that the expected maize green revolution in Africa did not take off (De Groote and Mugo, 2005). The reasons are diverse and complex, one being the high costs of inputs compared to decreasing cereal prices. Stem borers are a major constraint to maize production and food security for the majority of maize farmers in Kenya, and are estimated to cause yield losses of

between 13-50% here in Kenya (De Groote, 2002). According to Lutz *et al.* (2006), maize is not only for food, but also it is widely used to produce forage.

The global world rising food prices are a reflection of food scarcity that could affect Kenya and other net food importers. Food security awareness and concerns have become household issues globally, due to the sharply rising food prices estimated at over 40% (Trostle, 2008; FAO, 2008). This exceptional hike in food prices resulted in severe economic, social and political consequences to African agriculture. Unfavourable government policies and low investments have contributed to Africa becoming the worst hit by food insecurity issues (FAO, 2008). Other factors that have contributed to food insecurity include drought, high costs of farm inputs such as fertilizers and improved seeds, pests and diseases, and insufficient quantities of relief food and yield losses (Mugo *et al*., 2008). To minimise these losses and meet the food security requirement there is need for transgenic maize.

1.1.6 Coverage of Transgenic Bt Crops

The development of transgenic maize and the introduction of this product into the global market have made a tremendous impact on maize production, transport and marketing procedures (Scott and Pollak, 2005). According to Clive (2005), the global area of approved biotech crops in 2005 was 90 million (M) ha compared to 81 M ha in 2004 with an increase of 9.0 M ha, equivalent to an annual growth rate of 11% in 2005. This is equivalent to 53-fold increase from 1996 to 2005. In 2006, the first year of the second decade of commercialization of biotech crops, the

global area of biotech crops continued to increase for the tenth consecutive year at a sustained double-digit growth rate of 13%, or 12 M ha, reaching 102 M ha (Clive, 2006). Similarly, the global area of GM crops increased 67-fold, from 1.7 M ha in 1996 to 114.3 M ha in 2007, with an increasing proportion grown by developing countries (Clive, 2007).

Most current GM crops have been modified to resist certain pests or to tolerate a particular herbicide. During the first decade, 1996 to 2005, herbicide tolerance has consistently been the dominant trait followed by insect resistance and stacked genes for the two traits (Clive, 2005). Accordingly, the latter was the fastest growing trait group between 2004 and 2005 at 49% growth, compared with 9% for herbicide tolerance and 4% for insect resistance. The next generation of GM crops will include traits with improved nutritional characteristics. The two most cultivated GM crops are maize and soybean, which represent the staple constituents of many foods (Abdullah et al. 2006). Biotech soybean continued to be the principal biotech crop in 2005, occupying 54.4 M ha (60% of global biotech crop area), followed by maize (21.2 M ha at 24%), cotton (9.8 M ha at 11%) and canola (4.6 M ha at 5% of global biotech crop area) (Clive, 2005).

The world’s leading producers of GM crops are the United States, Argentina, Brazil, Canada, India and China (Clive, 2007). In 2007, the global biotech crop area was 43% up from 40% in 2006 (Clive, 2007). An equivalent to 49.4 M ha was grown in developing countries where growth between 2006 and 2007. This

was substantially higher (8.5 M ha or 21% growth) than industrial countries (3.8 M ha or 6% growth) (Clive, 2007).

1.1.7 Consumer Acceptance of GM Crops

Despite a lot of studies done to analyze consumer acceptance of GM foods in developed countries of the America and Europe as well as some countries in Asia, little research has been done in sub-Sahara Africa (SSA), even though this region could gain substantially from this technology (De Groote *et al.*, 2004). Despite GMOs being grown commercially in South Africa since 1997, there is very little consumer awareness – even with government and non-government organizations (NGOs) making information available on GMOs. A Human Science Research Council (HSRC) client survey in 2004, found that 7 out of 10 respondents from a sample of 5639 who completed a questionnaire, had never heard of a definition for biotechnology (Viljoen *et al.,* 2006). GM technologies continue to be developed for this region, and success of any biotechnology program will depend on consumer acceptance of its products (Baker and Burnham, 2002).

1.1.8 Introduction of Bt Maize in Kenya

In 1999, CIMMYT and KARI, with funding from the Novartis Foundation for Sustainable Development (currently the Syngenta Foundation for Sustainable Agriculture, SFSA), launched the Insect Resistant Maize for Africa (IRMA) project. The goal of IRMA was to increase maize production and improve food security, through the development and deployment of insect resistant maize to

reduce losses due to the stem borers (Mugo *et al.*, 2005). Thus Bt Maize with *cry1Ab* and *cry1Ba* genes was introduced in Kenya for research purposes. They were grown in Kenya during the 2005-2006 period under both confined field trial sites (CFTs) or open quarantine sites (OQS) at KARI-Kiboko and a biosafety level II greenhouse complex (BGHC L2) at KARI-National Agricultural Research Laboratories (NARL), Kabete, Nairobi (Mugo *et al.*, 2008). The details about the IRMA project, the biosafety physical facilities built and measures that were undertaken are explained in Murenga *et al*. (2008).

The IRMA project contributed to moving the biotechnology agenda in Kenya in many ways. A direct contribution was the training of regulators from Kenya Plant Health Inspectorate Services (KEPHIS) and National Biosafety Committee (NBC) through formal courses and visits to research facilities in Kenya, Mexico and the USA. IRMA also provided Bt maize as a product through which the ground work was laid down for the first time in Kenya for a Biosafety regulatory system. However, there is no study on the detection of GM maize food that has been done and published in Kenya to date following those trials and liberalization of maize imports into Kenya. This study, therefore attempted to fill this gap by assessing the maize food products in the Kenyan market for presence of these genetic elements from GMOs.

1.2 Statement of the Problem

There are concerns about the effects of GMOs or GM crops in developing countries that have sparked off debates and controversies (Kuiper *et al.*, 2001).

Kenya like many other developing countries had for many years lacked biosafety legislation 13 years after GM crops were first commercialized. In 2001, Kenya through KARI and its partners introduced *cry1Ab* and *cry1Ba* genes for research purposes aimed at the control of lepidopteran maize stem-borer pests. It was also to investigate the effectiveness of the biosafety measures to prevent gene flow from the sites where the Bt maize was grown. There were, however, no approved introductions of GM crops for commercial production. This not withstanding, it is possible that GM crops might have been introduced into the country without being regulated and detected. This is because it was just in 2009 that the Kenya Biosafety Bill was assented into law.

Kenya has trading relations with countries that are growing GM crops. A lot of maize and its products including the relief maize were imported into the country from some of those countries, especially, USA and South Africa. This was especially more so at the time when maize imports into the country were liberalised. There was a possibility that GM maize products could have found their way into the country without being labelled as 'GM.' Besides, maize seed imports are common due to liberalized maize and maize products trade, hence Kenyan farmers could be growing GM maize crops without knowing. There was, therefore, need to investigate whether the maize food products consumed in Kenya had any *cry1Ab* and *cry1Ba* genes and Cry1Ab and CP4 (Round upvready) proteins. Further, whether there was any gene flow from the experimental sites to the surrounding farmers' maize farms.

1.3 Justification of the Study

There is worldwide debate concerning the safety and desirability of planting, labelling and consuming GM foods. Although there is no evidence of health consequences because of the consumption of GM foods derived from Bt maize and other crops transformed with the same trait (Pryme and Lembecke, 2003), it was of interest to determine the presence and quantity of *cry1Ab* and *cry1Ba* genes and Round up ready proteins that would possibly be present in foodstuffs made with maize (Partridge and Murphy, 2004). This would create awareness on the current status on the GM foods in Kenya. It is documented that Bt maize with *cry1Ab* and *cry1Ba* genes was introduced in Kenya in 2001 and CFTs were carried out from 2004 to 2006 (Mugo *et al.,* 2008).

1.4 Research Questions and Hypotheses

1.4.1 Research Questions:

1. Could the available and consumed maize food products have had inadvertent presence of *cry1Ab* and *cry1Ba* genes?
2. Could the Bt maize grown in the CFTs at KARI-Kiboko have led to gene flow to the neighbouring maize farms in the area?
3. Did food products sold in the supermarkets contain proteins from GMOs?

1.4.2 Null Hypotheses:

1. The available and consumed maize food products in Kenya are not contaminated with genes from GMOs.
2. There was no gene flow from the Bt maize CFTS site at KARI-Kiboko to the neighbouring maize farmers' fields.
3. The maize based food products in the supermarkets in Kenya do not contain proteins from GMOs.

1.5 Objectives of the Study

1.5.1 General Objective

To detect the presence of insect resistance and herbicide tolerance genes and protein in different maize grains, seeds and food products in Kenyan markets and to assess the possibility of any gene flow from the Bt maize CFT at Kiboko to the surrounding farms.

1.5.2 Specific Objectives

1. To assess the presence of *cry1Ab, cry1Ba* and *CP4-EPSPS* (round up Ready) in maize grains consumed and grown in Kenya.
2. To assess the presence of *cry1Ab, cry1Ba* Bt maize genes representing gene flow from the KARI-Kiboko CFT site to the surrounding farms.

3. To determine whether selected processed maize food products in supermarkets were labelled as GM or non-GM and whether they had proteins from GMOs.

CHAPTER TWO

2.0 LITERATURE REVIEW

This chapter presents a review of literature on GM foods; transgenic Bt maize; impact of GMOs on the environment; gene flow; public debate on GM Crops; commercialization of transgenic crops; public awareness on GM products and their labelling; detection of GMOs in maize food products using DNA based approaches and protein based methods and empirical studies on the detection of GMOs in maize food products.

2.1 Genetically Modified (GM) Foods

The development of GM foods and other agricultural biotechnology products has generated significant public debate. GM foods have continued to make a big splash in the media. According to the chief executive of Pew Initiative on Food and Biotechnology (PIFB), GM foods have the potential to solve many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. GM crops are now extensively cultivated and their adoption and area under cultivation is increasing rapidly. Although growth is expected to plateau in industrialized nations, it is increasing in developing countries (PIFB, 2007).

In Africa, benefits from GM technologies have already been demonstrated. In South Africa, under rain-fed conditions, Bt maize increased yield by 11% that

translated into USD 35 ha^{-1} more revenue (Clive, 2008). In Burkina Faso, field trials on Bt cotton resulted in a two-thirds reduction in insecticide usage and a 15% higher yield, (Vitale *et al.,* 2008), thus promoting farmers' and environmental health while promoting prosperity. In the recent, the African Agricultural Technology Foundation (AATF) initiated several public-private partnerships to enhance agricultural productivity in Africa, including the development of Bt cowpea for protection against the Maruca-pod borer with potential to increase yield from 0.3 to 2.5 kg ha^{-1}. Water Efficient Maize for Africa that is expected to provide about 30% more yield under moderate drought. (Mugo *et al.*, 2008).

Since Africa has diverse cultures, economies, ecologies and politics, conclusions about the risks and benefits of biotechnology likely will differ from country to country. African views may differ from views expressed in developed countries, especially as people in each country weigh their own needs and values against perceived costs and risks. As African people debate whether agricultural biotechnology is appropriate for them, all stakeholders- including smallholder African farmers and consumers- should be included. Ultimately, the final decision on whether to use the technology must be made, and the responsibility borne by Africans themselves (Clive, 2006).

Nonetheless, there is a notion that African farmers ought to continue using seed they inherited from their ancestors, and not improved seed from conventional breeding or biotechnology. There appears to be safety concerns around the

adoption of GM seeds. However, despite over 13 years of increasing adoption of GM crops worldwide, there have been no adverse effects to humans and the environment (EFSA, 2006; Clive, 2008). Yet, the unimproved seed is the same seed that succumbs to a range of biotic and abiotic challenges, resulting in low productivity and even crop failure. It is the same seed that has ensured that African farmers remain trapped in poverty and reliant on food relief. Although this unimproved seed is a gem, it needs not be grown on African farmers' fields in that form, but ought to be improved and/or be archived in gene-banks for conservation of biodiversity (Clive, 2008).

South Africa developed into the 8th largest producer of biotech crops in the world and remains the pioneer and a role model for the adoption of biotechnology by the rest of Africa. South Africa is unique in terms of growing commercial GMOs on the African continent. The GMOs available in South Africa include insect resistant and herbicide tolerant maize, insect resistant and herbicide tolerant cotton and herbicide tolerant soybean (Viljoen *et al*. 2006). It is estimated that biotech crops account for 24% of yellow maize, 10% of white maize, 50% of soybean and 85% of cotton production in South Africa (Clive, 2004b). The commercial hectares totals for all the commercially available transgenic crops in South Africa grew to an estimated 1 813 000 ha in 2008 (Clive, 2009). South Africa is estimated to have enhanced farm income from biotech maize, soybean and cotton between 1998 and 2007 by USD 383M with benefits for 2007 alone estimated at USD 227M (Clive, 2009).

In Zimbabwe many institutions such as universities, government research institutions, parastatal (state-funded), and private organizations were involved in biotechnology research (Sithole-Niang, 2001). As a result, numerous technologies were already being transferred to end-users, thus contributing to economic and social development. Maize Biotechnology Research Institute (MBRI) and Agricultural Research and Extension (AREX) research organisations use MAS for the development off drought tolerant and insect resistant maize (Sithole-Niang *et al*., 2004).

As an initial step, quantitative trait loci (QTL) underlying drought tolerance and insect resistance were identified in the local population that was used. Superior quality inbreeds that are drought tolerant and are resistant to stalk borer attack were then developed using MAS. Hybrids resulting from crossing these parent lines are undergoing multi-locational trials in the country (Sithole-Niang, 2004). As an initial step, quantitative trait loci (QTL) underlying drought tolerance and insect resistance were identified in the local population that was used. Superior quality inbreeds that are drought tolerant and are resistant to stalk borer attack were then developed using MAS. Hybrids resulting from crossing these parent lines are undergoing multi-locational trials in the country (Sithole-Niang, 2004). This small scale field trial of Bt maize was approved and done during the 2001/2002 cropping season. This effort (and two others, one on Bt maize and another on Bt cotton) was not however continued (Sithole-Niang *et al*., 2004).

In Kenya the development of Bt transgenic crops (maize and cotton) has been on-going since 1999, while planting in the CFTs site was carried out in 2005 to 2006. This went on under the national biosafety regulations and guidelines of the National Council for Science and Technology (NCST) (NCST, 1998), until 2009 when the Biosafety Bill was accented into law.

2.2 Transgenic Bt Maize

Transgenic refers to plant cells in which the DNA of interest (the transgene) is integrated into the host genome. Bt is a bacterium of great importance that produces an arsenal of crystal (‘Cry’) proteins during sporulation (Tabashnik *et al.*, 2003) whose toxins kill a large variety of host insects and even nematodes (De Maagd *et al.*, 2001).

Perhaps the most widely utilized modification that confers herbicide resistance is that which confers resistance to glyphosate [N- (phosphonomethyl) glycine]. Glyphosate is a competitive inhibitor of enolpyruval-shikimate-3-phosphate synthase (EPSPS) is isolated from the common soil bacterium, Agrobacterium tumafaciens strain CP4 (CP4 EPSPS). It is required for the production of aromatic amino acids by plants. Expression of the *CP4-EPSPS* (i.e. enzyme CP4-5-Enolpyruvylshikimate-3 Phosphate Synthase) in such modified plants confers resistance to glyphosate. Hence it allows the application of glyphosate to fields of the growing GMO crop, e.g., Roundup Ready soybeans, cotton and maize (Lipton *et al.*, 2000).

Bt hybrids with the Event MON810 were genetically engineered by introducing the *cry1Ab* gene from common soil bacterium Bt, in order to produce its own insecticide, δ-endotoxin, for European corn borer (*Ostrinia nubilalis*), (Sa'zelova *et al*., 2009). The Bt- 176 maize was developed to be resistant to attack by European corn borer (*O. nubilalis*), which is a major insect pest of maize in many countries and proven difficult to handle by conventional approach. Currently, the Bt-maize Event 176 is subjected to European Commission's decision on withdrawal from the market (Sa'zelova *et al*., 2009). The global area of genetically modified crops has grown to 125 million ha in year 2008, up from 114.3 million ha in 2007 (Clive, 2008). In 2009, maize was the second GM crop grown in the world, occupying 41.7M ha that represent about 30% of global biotech crop area (Clive, 2009). Most GM maize hybrids cultivated globally exhibits two output traits, herbicide tolerance and insect resistance, that is double stacked traits. From the genesis of commercialization in 1996 to 2009, herbicide tolerance has consistently been the dominant trait. The insect resistance trait products were the fastest growing trait group (Clive, 2009).

Stacked traits are an increasingly important feature of biotech crops. Eleven (11) countries planted biotech crops with stacked traits in 2009, out of these, 8 were developing countries (Clive, 2009). Stacked products are a very important feature and future trend that meets the multiple needs of farmers and consumers. A total of 28.7M ha of stacked biotech crops were planted in 2009. In Europe, cultivation of Bt maize has increased constantly in recent years and reached 108 000 ha in 2009, amounting to around 1% of the total area cropped with maize. Romania was

the third European country, after Spain and the Czech Republic with a Bt area of about 3 000 ha in 2009 (Clive, 2009).

Bioinsecticides are viable alternatives for insect control in agriculture, and among them, Bt is the most widely used. Bt is compatible with sustainable and environmentally friendly agricultural practices (Monnerat *et al.,* 2006). This bacterium produces insecticidal proteins (Cry protoxins) during sporulation as parasporal crystals that are highly specific to their target insects; safe for humans, other vertebrates and plants, and biodegradable (Roh *et al.*, 2007). By using GE, modified novel genes from the soil dwelling bacteria, Bt were introduced into maize, to control lepidopteran stem borers. The product is Bt maize which has inbuilt resistance to stem borer due to the presence of crystal proteins produced by these genes. Two proteins were found effective against the local lepidopteran stem borers, *cry1Ab::Ubiquitin* and *cry1Ba::Ubiquitin* (Tende *et al.*, 2005). Bt maize is planted in seven countries; USA, Canada, Argentina, South Africa, Spain, Honduras, and Germany (Clive, 2004a). Use of Bt maize may prove to be part of the solution in addressing hunger and food security along side poverty eradication.

Thus Bt maize plants with clean events have been developed with final product carrying only the gene of interest and without the selectable marker gene. These form source lines for the Bt gene which can be used for evaluation and for conversion of other germplasm to develop insect resistant maize cultivars adapted to varying maize agro-ecologies (Monnerat *et al.,* 2006; Mugo *et al.,* 2005).

2.3 Development of Bt Maize in Kenya

The introduction of Bt maize was necessitated by the loss in yield to the farmers occasioned by six major stem borer species *Chilo partellus, Chilo orichalcociliellus, Eldana saccharina, Sesamia calamistis* and the *Busseola fusca* (Mugo *et al.,* 2005). The development of transgenic maize was done by KARI and CIMMYT through the IRMA project from 1999 till the end of 2008 (Mugo *et al.,* 2008). It was intended to develop locally adapted maize with Cry1Ab delta-endotoxins of Bt. Under this project, the Bt maize under development was genetically engineered for resistance to the stem borers using two genes: *cry1Ab* and *cry1Ba* (Mugo *et al.,* 2005). These genes code for a crystal toxin that proteolyses the digestive tract of insect larvae, leading to gut lyses and starvation hence death. The former (c*ry1Ab*) is active against Lepidoptera and stem borer pests while c*ry1Ba*, is active against both coleopteran and lepidopteran larvae (Mugo *et al.,* 2005).

The success of the IRMA project depended on a policy on biotechnology and biosafety, a biosafety law, a system for handling requests or notifications, a system for monitoring and enforcement, and a mechanism for public awareness, education, and participation. Key KARI and CIMMYT scientists were instrumental in the development of the biotechnology and biosafety policy, bills, and biosafety law in Kenya. The Kenyan Biosafety Framework includes the Policy on Biotechnology adopted by the cabinet in 2006, the Biosafety Law (2009), a system for monitoring and enforcement developed by NCST (NCST,

1998). The National Biotechnology Awareness Strategy (Bio AWARE) developed in 2008 by the Ministry of Agriculture (MoA), is a mechanism for Public Awareness, Education and Participation (ROK, 2008). The enactment of the Biosafety Law in 2009 (GOK, 2009) enabled the establishment of the National Biosafety Authority (NBA) in 2010. The IRMA project's regulatory compliance and implementation aided in a noticeable reduction of time between application approvals and regulatory decisions (Mugo *et al.*, 2008).

There are many steps in the regulatory process for the Bt maize GM trials in Kenya. Research proposals are developed by a multidisciplinary team of scientists and forwarded to the KARI Institutional Biosafety Committee (IBC). Generally, the IBC reviews the applications and requests KEPHIS to inspect the target experimental facility through the Kenya Standing Technical Committee on Imports and Exports (KSTCIE). The National Biosafety Committee (NBC) reviews the applications with or without appearance by the applicants. The NBC approves or disapproves the application and forwards it to the NBA. The NBA may also review with or without the applicant, and may request KEPHIS to inspect the facilities. Once the NBC approves the applicant, KEPHIS indicates the conditions included in a compliance document to KARI. Upon satisfaction with the compliance document, KEPHIS issues a letter of authorization and an import permit, if necessary. The applicant then signs a letter of commitment to KEPHIS. Research commences under the supervision of KEPHIS and using standard operating procedures (SOPs) issued by KEPHIS. After research is concluded, a report is written to the NBA (Mugo *et al.*, 2008).

The IRMA project presented four applications all of which were approved (Mugo *et al.*, 2008). These were: (1) Application to introduce Bt maize leaves from first generation CIMMYT events to screening Bt -endotoxins using leaf bioassays for activity against Kenyan maize stem borers; (2) Application for an import permit to introduce Bt maize leaves from cross combinations of CIMMYT first generation Bt maize events to screen for cry proteins Bt-endotoxins for activity against Kenyan maize stem borers using leaf bioassays; (3) Application to introduce maize seeds containing nine second generation Bt maize events from genes c*ry1Ab* and *cry1Ba* for evaluation, seed increase and crossing into other maize lines under biosafety greenhouse containment, and (4) Application to introduce maize Hybrid DKC8073YG and DKC8053YG (with Bt Event MON810 containing *cry1Ab* gene) to carry out greenhouse containment trials to evaluate the efficacy of the Bt-endotoxins against major maize stem borers in Kenya.

The fourth application was made to KARI IBC in November 2006, then to NBC in April 2007, to introduce the two maize Hybrids (DKC8073YG and DKC8053YG). The application was revised from an earlier one approved for research at KARI-Kibos. The revised application was based on a bilateral agreement between KARI and Monsanto to jointly test Bt maize Event MON810 in Kenya. It reflected the new testing location (KARI-Kiboko), and research objectives that would include comparison of efficacy with the IRMA Bt maize public events (Mugo *et al.*, 2008).

The maize was grown under containment in the Biosafety Level II Green House Complex (BGHC L2) at KARI-NARL and under confinement in CFT at an open quarantine site (OQS) at KARI-Kiboko. This had been on-going guided by the regulations and guidelines for Biosafety in biotechnology for Kenya (NCST, 1998) in the absence of biosafety law till 2009 when the biosafety bill became law (GOK, 2009). The transgenic seeds of nine events were sown in pots at the BGHC L2. This was to evaluate the efficacy and effects of these Bt genes against the target stem borer species; they were also increased for future experiments and for conversion of locally adapted germplasm to Bt maize. These initial experiments in the green house involved growing plants from Bt maize seed to confirm results obtained from cut leaf bioassays carried out in the biosafety laboratory (Mugo *et al.*, 2008). In a subsequent trial, the Bt *cry1Ab* gene Event MON810 Bt-endotoxin was tested for efficacy in controlling Kenyan maize stem borers in greenhouse containment trials through PIP KEPHIS/6099/2007 (Mugo *et al.,* 2008). In addition, recently, there was an importation of transgenic drought tolerant maize seed by the Water Efficient Maize for Africa (WEMA) project through Plant Import Permit (PIP) number KEPHIS/9799/2010 (Obonyo *et al.,* 2008).

Nine second generation public Bt maize events were received in 2004 through PIP number PH/408/2004. This was to allow the importation of transgenic maize seed from CIMMYT-Mexico into Kenya for testing in the BGHC L2 and was inspected by KEPHIS on arrival into the country (Mugo *et al.*, 2005). The confined field testing at KARI-Kiboko was carried out using the nine second generation Bt maize events that carried only the trait of interest and no selectable markers. This

was accomplished through co-transformation of the Bt gene and selectable markers that allowed segregating out the selectable marker genes. These were the Bt maize events used in the CFT during 2005B (March planting) and 2006A (October planting) seasons (Mugo *et al.*, 2008). Four Bt maize CFTs were carried out at the OQS. These were first to test the efficacy of the nine Bt maize events against Kenya stem borer in field conditions during 2005A and 2006A (October) seasons and second to backcross Bt maize events to convert eight maize types to Bt during the two October seasons (Mugo *et al.*, 2008).

Fifteen mock trials have been conducted at the CFT since its approval in January 2003 (Mugo *et al.*, 2005). The objectives of the mock trials were to calibrate various activities, train staff and to establish an isolation distance that would avoid unplanned crossing of Bt maize to other cultivars. The experimental trial was under a 0, 1 alpha lattice design with, ten entries, three replications and two five metre long rows for each plot. The row spacing was 75 cm while the plant spacing was 25 cm giving a plant density of 53,000 plants ha^{-1}. Planting was carried out under the supervision of KEPHIS personnel. Fertilizers were applied at the rate of 60 kgN and 60kgP2O5 ha^{-1} as recommended for the maize growing ecology. Nitrogen was applied in two splits of 30 kgN basal application at planting and 30 kgN top dressing at the 5-leaf stage. The trial was rainfed but supplemental irrigation was applied when needed. The fields were kept free of weeds by hand weeding (Mugo *et al.*, 2008).

The training of scientists on biotechnology and biosafety was met through short term visiting scientist model for a maximum of six months. The objectives of the training were to: familiarize the scientists with the protocols used for rearing of stem borers and field infestation of the same at CIMMYT; conduct insect bioassays in the laboratory; learn about biosafety standards maintained at the CIMMYT Applied Biotechnology Center (ABC); learn about the development of Bt maize via biolistic transformation, molecular analysis of putative transgenic plants, and to learn methodology, activities, and biosafety protocols practiced in the ABC biosafety greenhouses (Mugo *et al*., 2008).

Leaf bioassays were carried out to determine responses to other major stem borer species in Kenya, including *B. fusca*, *E. saccharina* and *S. calamistis*. One leaf was harvested from each of the representative plants from the non-infested row at the time of infestation. The leaves were wrapped in wet hand paper towels, packaged and transported to BGHC L2 escorted by KEPHIS personnel. The leaves were cut into 3-cm square sections across the leaf blade. Each section was placed in a five centimetre diameter petri dish containing moistened filter paper with the abaxial side facing up (Mugo *et al.*, 2004b).

Ten neonates were placed on the leaf tissue using a camel hair brush. This was repeated for each of the respective stem borer species: *C. partellus, S. calamistis, E. saccharina* and *B. fusca*. The petri-dishes were sealed with parafilm. Ten replicates were set up for each of the nine events. A control was set up using the susceptible non-transgenic tropical inbred line (CML216). The petri-dishes were

kept at room temperature (25°C) and total darkness in the biosafety laboratory. After five days, the mortality of the larvae was assessed and recorded by counting the number of live and dead larvae. Leaf damage was assessed and recorded by measuring the leaf area consumed using a millimetre grid (Mugo *et al.*, 2004b).

To address the biosafety issues involved, several physical facilities and measures were put in place. These included the setting up of the biosafety level 2 laboratory (BL2) for insect bioassays; BGHC L2 and the CFT site (Mugo *et al.*, 2005; Mugo *et al.*, 2008).

2.4 Impact of GMOs on Environment

The large scale growth of GM plants may have both positive and negative effects on the environment. According to Conner *et al.* (2003) and Wolfenbarger and Phifer (2000) there may be either direct effects, on organisms that feed on or interact with the crops, or wider effects on food chains produced by increases or decreases in the numbers of other organisms. As an example of benefits, insect-resistant Bt-expressing crops will reduce the number of pest insects feeding on these plants, but as there are fewer pests, farmers do not have to apply as much insecticide. This in turn tends to increase the number of non-pest insects in these fields (Roh *et al.,* 2007). Other possible effects might come from the spread of genes from modified plants to unmodified relatives that might produce species of weeds resistant to herbicides (Conner *et al*., 2003). In some areas of the USA "super weeds" have evolved naturally, these weeds are resistant to herbicides and have forced farmers to return to traditional crop management practices

(http://www.france24.com/en/ 20090418). Although insect pests have not evolved resistance to Bt crops, studies with Diamondback moth (*Plutella xylost-ella*) show evolvement of resistance to Bt sprays in the field (Ferré and Van-Rie, 2002).

However, in another study in comparing the Bt and non-Bt hybrid pairs the results were different. According to Dien *et al.* (2002), production of fuel ethanol for environmental and economic reasons increased and most was produced from corn grain. Bt and non-Bt hybrid pairs were compared to determine the fate of Bt protein after wet milling and dry grinding for ethanol production. After wet milling the Bt hybrids, *Bt* protein was found in the germ, gluten, and fibre fractions, but not found after liquefaction in the dry grind process. No differences were found between Bt and non-Bt hybrid pairs for yield of ethanol in the dry grind process.

2.5 Gene Flow

In population genetics, gene flow or gene migration is the transfer of alleles of genes from one population to another. Migration into or out of a population may be responsible for a marked change in allele frequencies (the proportion of members carrying a particular variant of a gene). For example, if a field of Bt maize is grown alongside a field of non-Bt maize, pollen from the former is likely to fertilize the latter (FAO, 2002).

The adventitious presence of GMOs as a result of cross-pollination is one of the factors that need to be evaluated in different cropping areas, as local climatic

conditions may influence the extent of pollen mediated gene flow. The maize plant is monoecious and diclinous, with male and female flowers borne separately on the same plant (Messeguer *et al.,* 2006). Maize is protandrous, with pollen being shed before the silks are receptive, but, as there is some overlap, up to 5% self-pollination can occur (Purseglove, 1972).

Numerous trials have been conducted on maize pollen dispersal, because of its economic importance (Brookes *et al*., 2004). These studies clearly show that, although maize pollen is relatively large and heavy, it can travel long distances on the airflow (Jarosz *et al*., 2003). Thus when suitable meteorological conditions occur, cross-pollination will take place to some extent, because it has a high settling speed and rapid deposition (Aylor *et al*., 2003). The rate of cross-pollination between fields depends on pollen viability, synchronization of flowering and the relative concentrations of pollen in the donor and receptor plots. At distances greater than 30–50 m, the level of pollen dispersion is very low (Pleasants *et al*., 2001; Jarosz *et al*., 2005).

In traditional plant breeding programmes, the recommended separation distances within fields of 2 ha or more are 200 m to maintain 99% grain purity and 300 m to maintain 99.5% grain purity (Ingram, 2000). The potential impact of cross-pollination increases notably with the size and number of fields planted (Brookes *et al*., 2004). A farm-scale evaluation of gene flow from GM herbicide resistant maize to non-GM maize has been performed by Henry *et al*. (2003) in the UK, where 55 fields were tested over a 3-year period. Analysis of kernel samples using

real-time PCR showed that there was a rapid decrease in the rate of cross-pollination within the first 20 m from the donor crop, and beyond this distance the rate of decrease was much slower. There was significant variation in the levels of GMO–non-GMO cross-pollination between sites in each year ($P < 0.01$), although the variation between years across all sites was not significant ($P > 0.05$). The authors concluded that an isolation distance of 24.4 m was required to meet the 0.9% threshold recommended by the European Union (EU) for food and feed in the UK.

Another study is that by Ma *et al*. (2004) who conducted field experiments at three sites in Ottawa, Canada, over a 3-year period, using yellow kernel Bt maize and white kernel maize to detect cross-fertilization. In this study, it was concluded that it was possible to produce non-GM maize grains by removing the outer rows of non-GM maize plants (about 30 m) neighbouring the GM maize field concerned, if the acceptance threshold was set at 1% or less, but that the generally recommended 200m distance between two genotypes appeared to be appropriate for Bt and other GM maize.

The results obtained in a field trial carried out in Spain (Brookes *et al*., 2004; Melé *et al*., 2005) were used to estimate the likely levels of adventitious presence of GMO in non-GM maize fields of different sizes and different distances downwind from a GM emitter crop. The level of adventitious presence of GMO likely to be found in non-GM maize crops (1 ha) planted adjacent to a GM plot (0.25 ha) was, on average, 0.83% (measured for the total harvest in the 1 ha plot).

The level of adventitious presence of GMO likely to be found in non-GM maize crops in a same-sized plot as the transgenic nucleus (0.25 ha) planted adjacent to a GM plot was, on average, 1.77%, but this decreased to 0.77% when a 6-m buffer zone was maintained between the GM and non-GM crops.

Other studies performed to evaluate gene flow in maize in Europe (Bannert and Stamp, 2005; Melé *et al*., 2005; Ma *et al*., 2004; Henry *et al*., 2003) confirmed the high interception of pollen by the first few maize rows, and suggested that, by removing 10–20 m from the borders, the 0.9% GMO threshhold allowed would almost never be exceeded in the harvested grain. In the study by Messeguer *et al.* (2006), also carried out in Spain, in which the size of the fields was small (0.5–4 ha), most fields had an accumulation of GMO content in the borders, decreasing towards the centre of the field. Consequently, they pointed out that a large decrease in the GMO content should be obtained by removing 10 m from the borders.

The only laboratory experiments that found maize pollen to be toxic to monarch butterflies at environmentally realistic pollen densities were performed with Event 176 hybrids that used a pollen specific promoter. Hellmich *et al*. (2001) reported deleterious effects on monarch larvae from c*ry1Ab* Event 176, because it had much higher concentrations of endotoxins expressed in pollen than other Bt Events. Event 176 was engineered with a pollen specific promoter and expresses more than 78 times more Bt toxin in its pollen than do other hybrids. It was constructed with the cauliflower mosaic virus 35S promoter or the ubiquitin

promoter. These results illustrate the heterogeneity of commercial Bt maize and their potential toxicity on non-target organisms (Stanley-Horn *et al.*, 2001). Thus the impact of Bt-pollen on monarch populations is restricted to Event 176 that represents less than 2% of the total maize planted in the US; the impact of Bt-maize on monarch butterflies should remain very low.

Studies of the effect of pollen from Bt corn on another non-target insect of public interest, the black swallowtail (*Papilio polyxenes*), led to a similar conclusion but generated less controversy. The initial report with this insect (Wraight *et al.*, 2000) found no impact of Bt pollen on larvae in laboratory and field studies. However, a subsequent report (Zangerl *et al.,* 2001) found deleterious effects on swallowtail larvae for transgenic corn pollen expressing Cry1Ab endotoxin. There have been few published measurements of Bt toxin or pollen intensity (PI) expression levels in pollen or nectar (Malone *et al.*, 2001). Bt toxin (*cry1A(b))* was undetectable in pollen from maize containing a *cry1A(b)* gene under the control of cauliflower mosaic virus (CaMV) 35S promoter, but was present (as 260–418 ng mg^{-1} soluble protein) in pollen/anther preparations from maize containing the same gene on a pollen-specific promoter (Zangerl *et al.,* 2001).

All in all, gene flow from Bt maize to non-Bt maize could accelerate pest resistance in two ways. First, if Bt toxin in refuge plants kills susceptible larvae, fewer susceptible adults will be produced, and the ability of refuges to delay resistance will be diminished. Second, if intermediate toxin levels kill susceptible

larvae but allow survival of heterozygotes, the functional dominance of resistance will increase and resistance will evolve faster (Tabashnik *et. al.*, 2003).

Although as mentioned in section 1.1.8, the Bt maize research at KARI-Kiboko was guided by the regulations and guidelines for Biosafety, aimed at reducing the risk of gene flow to the surrounding maize farms, this has not been proven and documented. There are concerns among Kenya's populace about the impact of GM crops on the environment such as vertical or horizontal gene flow, related ecological impacts especially on non-target insects, effects on biodiversity and the impact of presence of GM material in other maize products (Murenga *et al*., 2008). Hence the detection of the gene either in the maize food products or in neighbouring maize fields and refuges is important. Though all precautions for isolation were in place (Murenga *et al*., 2008), it was postulated that there was a possibility that 'gene-flow' from these experiments could have occurred. It was also espoused that farmers in Kenya could be growing GM crops from other sources without being aware of it. Thus, the emanating information from our study may shed light on the biosafety or agricultural co-existence, in which GM and non-GM cropping systems work side by side (Hino, 2002).

2.6 Public Debate on GM Crops

The development of GM foods and other agricultural biotechnology products has generated significant public debate. Debates over the transformation technology have been, and still are, in many parts of the world very controversial. These debates address ethical, human and animal health related concerns, food safety

and the possible impact on the environment. To reap the many potential benefits from transgenic crops, those crops must be safe to humans and the environment (Jaffe, 2004). The present atmosphere surrounding GM crops has led to a situation where food safety assessment is not just about science, but also about perceptions, concerns and standards about how to ensure “safety”. The reason for the public scepticism towards GM crops is an uncertainty about the longer-term risks and consequences of growing GM crops (Borch and Rasmussen, 2000).

However, despite this scepticism, the chief executive of Pew Initiative on Food and Biotechnology (PIFB) and other proponents assert that GM foods have the potential to solve many of the world’s hunger and malnutrition problems. They help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides (PIFB, 2007). Nevertheless, the critics (environmental activists, religious organizations, public interest groups, professional associations and other scientists, and government officials) have all raised concerns about GM foods. They believe GM foods will lead to an unintended harm to other organisms, increased allergenicity and other unknown effects on human health, reduced effectiveness of pesticides, and gene transfer to non-target species (Chilcutt and Tabashnik, 2004).

To date, there is no evidence of specific harmful environmental effects from the millions of acres of transgenic crops that have been planted worldwide. Besides, there is not any evidence of harm from the many foods that humans have consumed that contain transgenic crop ingredients (Jaffe, 2004). However, Quist

and Chapela (2002) give evidence that transgenic maize had become introgressed by cross pollination into landraces of maize in Mexico. These authors claimed that the transgenes were moving in the genome and thus, were unstable, and that such introgression may threaten the biodiversity of Mexican landraces.

2.7 Commercialization of Transgenic Crops

GMOs were introduced in the world market in the 1996 (Clive, 2004a). The US Department of Agriculture (USDA) approved *cry1Ab* GM maize seeds and *CP4-EPSPS* GM soybean seeds for use as food as well as in animal feeds. Other genetic modifications are restricted to animal feed only, for example *cry9* in corn (Clive, 2004a). Though no allergic reactions to *cry1Ab* or CP4-EPSPS proteins have been reported, concerns have arisen regarding regulatory measures applied to genetically modified foods and has become the focus of novel investigations (PIFB, 2007).

According to PIFB (2007), in May 2004, the EU approved its first GM food under the new regulations and the first since 1998. The Commission approved the import of Syngenta's GM canned sweet corn, under the labelling and traceability provisions of the new regulations. A few months later, in July 2004, the Commission also approved a Monsanto GM Roundup Ready maize variety (NK603) for human and animal consumption, but not for planting. In August 2005, the Commission approved the import of Monsanto GM maize MON863 for animal feed, but not for cultivation or food use (PIFB, 2007).

2.8 Public Awareness on GM Products and their Labelling

2.8.1 Public Awareness on GM Foods

Although GM foods are being widely grown, the public awareness on these foods is low. European public opinion surveys showed strong opposition to GM crops and foods (Carter and Gruère, 2003). An EU-wide study in 2002 found that while attitudes varied among nations, majorities in most EU countries rejected GM foods, which were seen as "risky" and "not useful" for society (Carter and Gruère, 2003). In the different EU countries, between 30% and 65% rejected all the reasons for buying GM foods (Eurobarometer 58.0, 2002). In 2003, a survey in US found that 58% of the consumers polled, believed that they had never eaten GM food (PIFB, 2003) and 30% of respondents wished to avoid GMOs (Baker and Burnham, 2002).

In South Africa, despite GMOs being grown commercially since 1997, there is very little consumer awareness, even with government and non-government organizations (NGOs) making information on GMOs available (Viljoen *et al.,* 2006). A Human Science Research Council (HSRC) client survey in 2004, found that 7 out of 10 respondents from a sample of 5,639 who completed a questionnaire, had never heard of a definition for biotechnology (Rule and Langa, 2005).

In Kenya, Kimenju *et al.*, (2005) show that although GMOs had been introduced for research purposes, most of the people were not aware of the benefits and hazards of GMOs.

2.8.2 Labelling of Products Containing GMOs

Labelling of GM products is very important to help the consumer decide on whether or not to use the GM food. Accurate and reliable detection of GMOs in foods is becoming increasingly important as the demand for labelling of GMO-containing foods increases. According to a new Europan regulation (90/220/CEE), any foodstuff containing more than 1.0% of genetically modified maize or soybean must be labelled as transgenic (Europa, 2004).

In January 2000, an international trade agreement for labelling GM foods was established. More than 130 countries, including the US, the world's largest producer of GM foods, signed the agreement (Helmilth, 2000). Due to consumer pressure many countries have introduced labelling regulations for GI foods. Although labelling does not have any bearing on the safety aspect of GMOs, it is used to give consumers a choice, between GM and non-GM, allowing them to balance concerns of morality and perceived risk (Global Knowledge Center, 2005). All GM food labelling uses predetermined thresholds (Table 2.1), as it is not possible to ensure zero GM in a product once GMOs are present in the production system (Bullock and Desquilbet, 2002).

Different countries set the GMO labeling threshold based on their own criteria. To date, European Union (EU) is still the one and only authority that sets the GMO threshold level of 0.9%, which is most stringent legislation globally (Heide *et al.*, 2008). Australia, New Zealand, Brazil and Saudi Arabia have set GM food labeling threshold of 1.0%, while for South Korea it is 3.0% (Viljoen *et al.*, 2006).

Japan and Taiwan set a 5.0% GM food labeling threshold level. In Malaysia, the Biosafety Bill was approved by Parliament in July 2007, allowing regulations pertaining to labeling of GMOs in food and feed to be introduced and enforced in the near future (Jasbeer *et al*., 2008). A 3.0% GMO labeling threshold has been approved but is yet to be implemented.

New South African labelling legislation came into effect on the 16th of January 2004 (Viljoen *et al.,* 2006). This was to guide the labelling of a foodstuff significantly different in respect of the composition, nutritional value, and mode of storage, preparation or cooking, allergenicity or containing genes of human or animal origin. Although no provision is made for labelling that allows consumers the choice of preference between GM and non-GM foods, many products can be found in retail and health outlets with "non-GM", "GMO free", "organic" and even "may be GM" labels (Viljoen *et al.,* 2006). Presumably the type of label being used is aimed at perceived consumer perception and preference, especially products marketed for vegetarians (Viljoen *et al.,* 2006).

To make sure the successful enforcement of the GMO labelling, protein and nucleic acid-based detection techniques have been developed, or are in the process of development, such as Enzyme-Linked Immunosorbent Assay (ELISA), lateral flow strip, Polymerase Chain Reaction (PCR), and micro-array (Yang *et al.*, 2008; Bulcke *et al.* 2007; Miraglia *et al.*, 2004; Holst-Jensen *et al.*, 2003).

Table 2.1: GM Food Labelling Regulations and Thresholds for Different Countries

Country	Labelling	% Threshold	Scheme
Australia & New Zealand[1,2]	Mandatory	1.0%	GM
Brazil[2,3]	Mandatory	1.0%	GM
Canada[2,3,4,9]	Voluntary	5.0%	Non-GE or GE
China[2,5]	Mandatory	1.0%	GM
European Union[6]	Mandatory	0.9%	GM
Indonesia[2,5]	Mandatory	5.0%	GM
Israel[7]	Mandatory	0.9%	GM
Japan[2,3,7]	Mandatory	5.0%	GM
Philippines[2,5]	Voluntary	N/A	N/A
Russia[2,5]	Mandatory	0.9%	GM
Saudi Arabia[2,5,7]	Mandatory	1.0%	GM
South Korea[2,5,7]	Mandatory	3.0%	GM
Switzerland[5]	Mandatory	0.9%	GM
Taiwan[2,5,7]	Mandatory	5.0%	GM
Thailand[2,7]	Mandatory	5.0%	GM
USA[7,8]	Voluntary	5.0%	Organic

Source: Viljoen *et al.* (2006)
[1]Food Standards Australia and New Zealand (2005), [2]Foster *et al.* (2003), [3]Agrifood Awareness Australia (2004), [4]Health Canada (2003), [5]The Center for Food Safety (2005), [6]Europa (2004), [7]Global Knowledge Center on Crop Biotechnology (2005), [8]United States Department of Agriculture (2002) and [9]Canadian General Standards Board (2004).

2.9 Detection of GMOs in Maize Food Products

Screening techniques are of great importance in routine GM food detection. They rapidly provide information about the presence or absence of a GM food. Thus,

detection and quantification methods of GM food (Hernandez *et al.*, 2005) were developed to label the food correctly. To comply with the legislation, reliable and accurate methods for the identification of GM food in either raw materials or processed food products are required. Japan for example, has developed and validated many detection methods for newly approved GM events. Moreover, time- and cost-effective detection methods are currently under development (Kitta *et al.*, 2009).

Methods for detection and quantification of GMO in grains and foodstuffs include analyses of DNA (end-point and real-time PCR, dot and Southern blots) and proteins (lateral flow strip, ELISA, Western blot) (Ahmed, 2002). Being able to detect a GMO is an important part of food safety, as without detection methods the traceability of GMOs would rely solely on documentation (Ahmed, 2002).

Among these methods, PCR is the most popular technique for GMOs analysis because of its versatility (from screening to identification), specificity, high throughput, and efficiency, therefore the PCR detection methods have been developed for many GMOs. The introduced DNA fragments are usually integrated into the genome of GMOs by random transformation events: based on the different transformation methods of the inserted DNA sequences (Holst-Jensen *et al.*, 2003).

2.9.1 DNA Based Approaches

2.9.1.1 Polymerase Chain Reaction

The polymerase chain reaction (PCR) technique is widely used for detecting GMOs and can be used for both screening and quantification (Holst-Jensen, 2003; Cankar *et al.*, 2005). PCR detection methods of GMOs are grouped into four types, such as the screen-, gene-, construct-, and event-specific PCR methods (Miraglia *et al.*, 2004).

PCR method is based on detection of the control sequences flanking the newly introduced gene, namely the 35S promoter from CaMV and the NOS terminator from the *Agrobacterium tumefaciens* Ti plasmid. It may also have the kanamycin-resistance (*npt*II) marker gene (Tozzini *et al*., 2000; Querci, 2004). PCR is a highly reproducible and sensitive technique that can be successfully used in detecting transgenes for screening GM maize (Rhandawa and Firke, 2006).

However, a study done by Tengel *et al*. (2001) showed that DNA extraction for PCR from highly processed foods such as pastries, some breakfast cereals, ready meals, or food additives can be difficult because of the DNA-degrading action of some manufacturing processes. For example, the detection of GMO material in cocoa that contain high levels of plant secondary metabolites can lead to an irreversible inhibition of the PCR process. Such substances must be completely removed before PCR analysis can take place. To overcome the difficulties in obtaining the certified reference materials (CRMs) of GMOs, standard reference

molecules have been developed and are being used in PCR detection (Lee *et al.*, 2006; Yang *et al.*, 2005)

2.9.1.2 Quantitative End-point PCR

The quantitative competitive PCR method relies on the competition between the template DNA originating from the sample and a synthetic DNA fragment competing for the same primers (EC, 2000). In this method, a shorter fragment (compared to the target DNA sequence to be amplified in GM plants), the internal standard, is synthesized, which has the same sequence to which the primers may anneal (Pöpping, 2001). It requires that the two DNA targets are amplified with equal efficiency and by setting two competitive PCRs, one for the GMO and the other for the species of interest. This includes competitors in both the quantity of GMO relative to the species so that it can be estimated by extrapolation from the degree of dilution and concentration of the competitors (Holst-Jensen, 2003). The methodology is less expensive than the real time technology, but the necessary dilution series is considerably more time consuming (Pöpping, 2001).

2.9.1.3 Real-time PCR

Real-time PCR is the most commonly used technology. It requires a special thermal cycle and an addition of fluorescence probe. Real-time PCR with TaqMan chemistry has been used in various kinds of qualitative and quantitative detection (Kuribara *et al.*, 2002; Holst-Jensen *et al.*, 2003) for GMOs as the risk of carry over contamination in routine analysis is reduced (Miraglia *et al.*, 2004; Lyon and

Wittwer, 2009). Real-time PCR measures the amount of molecules produced during each stage of the PCR rather than just at the end (Pöpping, 2001). In its first applications, the real-time PCR was a system that was based on the fluorometric measurement of an internal probe (EC, 2000). TaqMan PCR provides higher specificity than conventional PCR due to the chemistry with TaqMan probes. These characteristics are advantageous for a universal detection platform. Several types of hybridization probes are available that emit fluorescent light corresponding to the amount of synthesized DNA (Holst-Jensen, 2003). The amount of products synthesized during PCR is measured by detection of fluorescence signal produced as a result of specific amplification

According to Holst-Jensen (2003), with the use of fluorescence, it becomes possible to measure exactly the number of cycles that are needed to produce certain amount of PCR product, which corresponds to the amount producing a fluorescence signal. The number of cycles is called a Ct-value. Since the PCR is a linear function at certain cycles, the number of starting molecules can be extrapolated (Pöpping, 2001).

The standard curves are generated both for the crop-specific reference and a specific event by plotting the Ct-values measured for the calibration points against the logarithm of the DNA copy numbers, and by fitting a linear regression line into these data. The standard curves are used to estimate the copy numbers in the unknown sample DNA by interpolation from the standard curves (EC-CRL, 2005). This means, by comparison of Ct-values for the GMO target sequence and

the reference gene it becomes possible to estimate the ratio of the GMO target sequence to the reference sequence in terms of difference in number of cycles needed to produce the same quantity of product (Holst-Jensen, 2003). Although a real-time system is relatively expensive, it can analyze, like the ELISA plate, a maximum of 96 assays at a time (Pöpping, 2001).

2.9.1.4 Qualitative PCR (Q_PCR)

Q-PCR is what is used to detect whether a GMO is present in a food substance or not. However multiplex PCR can also be employed in this detection. Multiplex PCR uses multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences, i.e. different transgenes. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the amount of reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair (bp) length, should be different enough to form distinct bands when visualized by gel electrophoresis (**Southern Blot**

Southern blot involves fixing isolated sample DNA onto nitrocellulose or nylon membranes, probing with double stranded (ds)-labelled nucleic acid probes specific to the GMO, and detecting hybridization radio graphically, fluorometrically or by chemiluminescence (Ahmed, 2002). This method relies on the complementary specificity of the two strands that form the double helix of double stranded DNA; that is, the two complementary DNA strands anneal or

hybridize in a sequence specific manner and this specificity is exploited in the detection process (Magin *et al*. 2000). It is the technique most commonly used to analyze transgene insertion events for both research and regulatory purposes (Kohli *et al.* 2003).

2.9.2 Protein Based Detection Methods

The protein based methods include western blot, ELISA and lateral flow strip.

2.9.2.1 Western Blot

In western blotting, the extracted proteins (resulting from the translation of the inserted genes) are subjected to polyacrylamide gel electrophoresis (PAGE) and are then transferred onto nitrocellulose membrane to which they bind. Nitrocellulose membrane is then used for probing with a specific labelled antibody. The antibody may be labelled as 125I and the signal is detected with autoradiography (Ahmed, 2002).

2.9.2.2 ELISA.

ELISA involves testing for the presence of specific proteins by exploiting the specificity of binding between expressed antigen and target antibody (Querci, 2004). It tests for the presence of the specific protein that the GM DNA produces in the plant. It uses antibodies that react with specific proteins produced by the GMO. In ELISA, which is a plate test, the intensity of colour indicates the amount of the protein present. It employs more than one format: a micro well plate (or strip) format, and a coated tube format (Ahmed, 2002).

2.9.2.3 Lateral Flow Strip Test (Serological Method)

Strip test is considered as a version of ELISA. According to Ahmed (2002), a variation on ELISA using strips rather than micro titer wells, led to the development of lateral flow strip technology. Protein strip tests are simple, fast, cheap and reliable, making them a complementary tool to the PCR-based GMO detection methods (Van Duijn *et al.*, 2002). All protein strip test analyses are performed following the manufacturer's procedures (*incasu* Envirologix, Neogene or Strategic Diagnostics) (Van den Bulcke *et al.,* 2005).

The lateral flow test (dipstick format) uses a membrane- based detection system. The membrane contains two capture zones, one captures the bound transgenic protein, the second captures colour reagent. Paper strips or plastic paddles are used as support for the capture antibody that is immobilized onto a test strip on specific zone. The lateral flow strip is dipped into the prepared sample in extraction solution and the sample migrates up the strip by capillary action. As the sample flows through the detection antibody strip and the capture antibody strip, the protein of interest will accumulate and thus give a high intensity band, but the volume is not as well controlled. These tests generally provide qualitative or semi-quantitative results using antibodies and colour reagents incorporated into a flow strip (Ahmed, 2002).

This method has been used in various studies to detect presence of Cry1Ab protein. For example, a study done in Argentina by Margarit *et al.* (2006), the CryIAb protein was undetectable or almost undetectable by ELISA in a group of

samples previously identified by PCR as containing transgenic maize and derived some of them from highly processed foodstuff like pellets. The CryIAb protein was also undetectable in other group of samples (puffs and Cornflakes) where the DNA isolated was not satisfactory for amplification. These results suggest that the CryIAb protein is highly degraded under the harsh conditions during food elaboration as suggested by Terry *et al.* (2002). It was also not possible to determine if foodstuffs like maize oil and maize syrup were derived from transgenic grains, due to the fact that neither DNA nor proteins were detected in the samples.

As a result of the frequent addition of newly approved GM lines, the ideal system for the detection of GM is easily updatable and customizable as the situation demands. In that regard, a universal detection platform was developed where many analyses can be performed in a single system for the detection of genetically modified crops (Mano *et al.*, 2008).

2.9.2.4 Near Infrared (NIR) Spectroscopy

Near-infrared (NIR) spectroscopy is a proven workhorse in the area of food quality control. It is one of the other GMO detection methods. Certain genetic modifications may alter the fibre structure in plants, whereas no significant differences could be observed in the content of protein and oil (e.g. RR soybeans). These could be detected by NIR spectroscopy (Hurburgh *et al.* (2000). However, the capacity of NIR to resolve small quantities of GMO varieties in non-GMO products is assumed to be low, as is true for the chromatographic methods.

NIR transmittance spectroscopy has been used by grain handlers in elevators in most of the world for non-destructive analysis of whole grains for the prediction of moisture, protein, oil, fibre and starch. In combination with chemometrics techniques, several vis/NIR-based methods are being developed as powerful GMO detection tools (Michelini *et al.*, 2008). In a related application area, investigators with Pioneer Hi-Bred in Iowa (US) have developed a novel NIR method to enable single-kernel determination of oil content in maize, with an eye towards the continued improvement of food, feed and fuel traits in new maize varieties (Janni *et al.,* 2008).

The key merits of NIR spectroscopy as an analytical tool for GMO detection are (1) its relatively high speed of analysis, (2) the lack of a need to carry out complex sample preparation or processing, (3) its low cost, (4) its suitability for on-line process monitoring and quality control (QC), (5) it is fast (less than 1 min), (6) sample preparation is not necessary because it uses whole kernels (about 300 g), which are dropped into measurement cells or flow through the system, and (7) it is therefore cheap (Cozzolino *et al.*, 2008; Hurburgh *et al.*, 2000).

However, NIR's major demerit is that it does not identify compounds, thereby necessitating a large set of samples to generate spectra for subsequent multivariate analysis. This calibration dataset is then used to predict the GMO event. Thus, this method cannot be more accurate than the reference method used to build the model. Moreover, a calibration needs to be developed for each GMO to be predicted. Furthermore, although NIR is sensitive to major organic compounds

(e.g. vibration overtones of C–H, O–H and N–H), its accuracy is limited. For example, with respect to GMOs, it does not detect a change in DNA or a single protein, but much larger unknown structural changes, such as those linked to the parietal portion of the seed (e.g. lignin or cellulose) that are introduced by the presence of the new DNA (Janni *et al.,* 2008).

2.10 Empirical Studies on the Detection of GMOs in Maize Food Products

Following the introduction of GM crops in various countries a lot of studies on detection of GMOs in food products using PCR, especially in maize products have been done. This is especially in those countries that are growing GM crops. Such studies have provided information to the consumers to enable them make informed decisions on what to buy and consume. Kimio *et al.* (2004) using PCR and real-time PCR techniques, explored genetically modified organisms (GMOs) in food samples including 70 processed corn foods. These were obtained from November 2000 to March 2003 in the Tokyo area, Japan. Qualitative and quantitative analyses of GM corn were performed on corn and semi-processed corn products such as corn meal, corn flour and corn grits. GM corns were detected in 8 of 26 samples. The amount of GM corn in GM corn-positive samples was in the range of 0.1-2.0%.

Another study done in Argentina by Margarit *et al.* (2006) carried out PCR analyses with primers that amplified a 204 bp (Permingeat *et al.*, 2002) fragment from all *cryIAb* transgenes present in commercial maize food products. This study detected 2 precooked and 1 non-cooked polentas, 2 cracked maize branches and 3

foodstuffs for monogastric animals (1 for chicken and 2 for pigs) contained transgenic maize. To identify the transgenic events present in the foodstuff other primers were used that showed the amplification products of 180 and 420 bp corresponding to the *CryIAb* fragment from Events Mon810 and 176, respectively. The experiments revealed that most of the samples contained maize derived from the 2 transgenic events and at equal or higher levels than 0.1% (Permingeat *et al.*, 2002). However, the authors did not analyse the presence of event Bt 11 because it was not grown in Argentina at the time when the feedstuffs were obtained. In addition they detected Cry1Ab protein by use of ELISA Kit from low processed maize food products. Out of all processed food precooked polenta had the highest amount of CryIAb protein. On the other hand, it was not possible to obtain good quality DNA for PCR from highly processed food, such as corn flakes.

A similar study was done in South Africa by Viljoen *et al.* (2006). In this study, out of 58 off-the-shelf food products sampled randomly from different retail and health outlets, 76% tested positive for GM. This was irrespective of the fact that the sampling used did not take batch effects into account. For maize, GM was detected in 63% of local products. These results indicate that the GM production in South Africa may be higher than the estimated 24% for yellow maize, 10% for white maize and 50% for soybean (Clive, 2004b). However, the South African Grain Laboratory determined that for 2003/2004, only 3% white maize and 2% yellow maize was found to contain GM (South African Grain Laboratory, 2005).

This suggests that either there is a delay of GM entering the food chain possibly due to the existence of reserves or that a diffusion of GM is occurring in non-GM product in the food chain during processing. Of the products tested, 7 maize products carried a GM related label. GM was detected in 57% of the labelled maize. Two out of the three maize products with a “may be GM” label were found to contain GM. GM was also detected in 71% of all products with either a “non-GM”, “GMO free” and/or “organic” label. Of the products with a negative GM label, GM was present in 50% maize.

There is no study found where *cry1Ba* gene was detected in maize food products since it has not yet been commercialized. The USDA approved *cry1Ab* GM maize seeds and *CP4-EPSPS* GM soybean seeds for use as food as well as in animal feed (Clive, 2004a).

From the foregoing elucidation on the GM crops, *Bt* maize, impact of GMOs on the environment, gene flow, public debate on GM crops, commercialization of transgenic crops, possible detection methods and empirical studies, it is clear that there are still unresolved issues especially on the public debate about the pros and cons of GM crops.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The maize seeds and maize food products were obtained from different open markets, supermarkets and fields in Kenya. The laboratory work was carried out at the KARI Biotechnology Center at National Research Laboratories Biotechnology, Molecular laboratory.

3.2 Sampling

Multi-stage sampling technique was adopted in this study (Castillo, 2009). In the *first stage,* provinces were selected. North Eastern province, which is neither a major maize producer nor consumer, was purposively eliminated from the sampling frame. Out of the remaining 7 provinces, 4 provinces: Nairobi, Coast, Eastern and Western were randomly selected. In the *second stage,* 10 towns were selected from urban centres in the selected provinces: Nairobi, Mombasa, Kitui, Machakos, Mwingi, Makueni, Kakamega, Mumias, Busia, and Bungoma. Kiboko town where the CFT site is situated was later purposively added resulting in a total of 11 towns. The *third stage* was the categorization of the chosen towns relative to the importance in the maize industry. All the selected towns were confirmed important based on the following criteria:

i. Mombasa: is the main port which receives most of the imported food products, maize included into the country.

ii. Nairobi: is the main city which receives and processes imported maize grains, a major consumer and the distribution centre.

iii. Kitui, Machakos, Mwingi, and Makueni: they are the main towns in areas frequently hit by drought and which receive and consume a lot of the relief food grains.

iv. Kakamega, Mumias and Bungoma: Are major maize seed growers and important grain maize producers.

v. Busia: a border town so a lot of trade located on a major transit route to Uganda and a major maize producer too.

In the *final stage*, a total of 120 samples were randomly selected from 21 open air markets and Kenya, Monsanto, Seedco and Pannar Seed Companies shown in Table 3.1, and Nakumatt and Uchumi supermarkets in Table 3.2. .Maize grains were also collected from farms within a radius of about a kilometre from the CFT site where the Bt. maize had been grown between 2005 up to 2007. The samples were analysed to determine the presence of the *cry1Ab* and *cry1Ba* genes by Polymerase Chain Reaction (PCR). However, we did not test for CP4-EPSPS gene because positive control for it is not available in the country. Cry1Ab and CP4-EPSPS proteins were tested by Lateral flow strips (Dipsticks) methods. Cry1Ba protein was not tested because the transgene has not yet been commercialised hence strips are not available for the test.

Table 3.1: Sources of Maize Samples and Description

Province	Sample source (markets)	Description	Number of samples
Eastern	Makueni	Maize grains	5
	Mwingi	Relief food (maize)	1
		Relief yellow maize	1
		Maize grains	5
	Kitui	Maize grains	8
	Machakos	Maize grains	5
	KARI Kiboko	CML202	1
	KARI Kiboko	CML204	1
	Kiboko farmers	Maize grains	10
Nairobi	Kangemi	Maize grains	5
	Wakulima	Maize grains	5
	Ngong	Maize grains	5
	Kitengela	Maize grains	5
	Karen	Maize grains	5
	Toi	Maize grains	5
	Mulolongo	Maize grains	4
	Seed Companies:		
	Monsanto Seed Company	DK 8031 maize seed	1
	Kenya Seed Company	H513 maize seed	1
	Kenya Seed Company	DH04 maize seed	1
	Seedco Limited	D41(Duma) maize seed	1
	Pannar Seed Company	PAN 67 maize seed	1
Coast	Kongowea	Maize grains	5
	Kilindini	Maize grains	2
	Marikiti	Maize grains	5
Western	Busia	Maize grains	5
	Bungoma	Maize grains	5
	Kakamega	Maize grains	5
	Mumias	Maize grains	5

Source: Researcher Personal Data Collection and Processing, July-December 2009.

Table 3.2: Products from Supermarkets, Description and Manufacturer

Origin	Product Name	Description	Producer
Nakumatt and Uchumi Supermarkets	Corn Flakes1	Cereal	Bokomo Foods
	Corn Flakes2	Cereal	Kellogg Company
	Corn Flakes3	Cereal	Magic Time Int'l LL (MTI)
	Corn Flakes4	Cereal	Nestle Products
	Corn Flakes5	Cereal	Mass food
	Popcorn1	Popcorn	Natural popcorn
	Popcorn2	Popcorn	American popcorn
	Popcorn3	Popcorn	Merican popcorn
	Unga1	Maize flour	Capwell industries
	Unga2	Maize flour	Capwell industries
	Unga3	Maize flour	Unga mills
	Baby Porridge	Maize flour	Proctor & Allan

Source: Researcher Personal Data Collection and Processing, July-December 2009.

3.3 Determination of Sample Size

The sample size (120) was determined according to the formula in Fisher *et al.* (1991) and given as:

$$n = \frac{Z^2\ pq}{d^2}$$

Where:

n = the desired sample size;

z = 1.96 for a confidence limit of 95%;

p = the proportion of the target population estimated to have the genes and gene flow being tested (in this study was estimated at 8.5%);

q = 1-p;

d = level of statistical significance set (in this study was 0.05 i.e. 5%).

3.4 DNA Extraction from Maize Seeds

DNA extraction was carried out from maize grains and seeds collected from the following 21 markets in Kenya: Makueni, Mwingi, Kitui, Machakos, Kiboko, Kangemi, Wakulima, Karen, Ngong, Kitengela, Toi, Mulolongo, Kongowea, Kilindini, Marikiti, Busia, Bungoma, Kakamega, Mumias, Seed Companies (for maize seeds) and Supermarkets (for processed maize food products).

3.4.1 Sample Preparation

Thirty (30) seeds of each of maize grains and seeds were ground into fine flour with mortar and pestle. Care was taken to prevent contamination of flour following the method described by Mace *et al.* (2003). Flour (0.02 g (gram) was transferred into eppendorf tube. Extraction buffer (0.45 g CTAB, 0.6 millilitres (ml), EDTA 20 mM, 4.2 ml NaCl 1.4 mM, 1.5 Tris 10 mM and 0.0255 ml BME) (450 µl) was added followed by 10 minutes (mins) incubation period at 65 ^{0}C in a water bath with occasional shaking.

Hydrophobic components were removed by adding 450 µl chloroform: isoamyl alcohol (24:1) to each sample and inverted twice to mix. Phases were resolved by centrifuging the sample for 10 mins at 9660 x g (relative centrifugal force i.e. rcf). Then 400 µl of the supernatant was transferred to a fresh eppendorf tube and 700 µl of isopropanol (stored at -20^{0}C) was added to each of the sample and inverted once to mix. Using mini-spin table centrifuge, the samples were centrifuged at 9660 x g (rcf) for 15 mins. This was done so as to precipitate the DNA. The pellet was then washed in 70% ethanol where 500 µl of it was added to each sample then short-spanned at 9660 x g (rcf) for 2 mins. After precipitation, the pellet was dissolved in 50 µl of deionised molecular water. Five (5)-µl aliquot was loaded in 1% (w/v) agarose gel stained with ethidium bromide. This was electrophoresed at 40 mA for 1 hour to assess DNA yield and quality.

3.4.2 Determination of Quality and Quantity of DNA

Purified DNA was quantified with UV spectroscopy at 260 nm. Each DNA sample (15 µl) was added to 73 µl of sterilized double distilled water in 1.5 ml eppendorf tube and vortexed to give a dilution of 1:50. Absorbance was measured in a spectrophotometer using distilled water as a blank. A pure solution of double stranded DNA at 50 µg ml^{-1} has an optical density 1.0 at 260 nm (Santella, 2006). The ratio of absorbance at 260 and 280 nm ($A_{260\ nm}/A_{280\ nm}$) was around 1.8 and indicated good-quality DNAs. If the ratios were below 1.8, could indicate protein contamination because proteins have a peak in absorption at 280 nm resulting

from the aromatic amino acids. While higher values indicate RNA contamination (Santella, 2006).

3.4.3 Agarose Gel Electrophoresis

The buffer and agarose concentration were established according to the size of nucleic acids expected. Agarose 0.3 g was added to 30 ml tris borate EDTA (TBE) buffer. Agarose was melted in a microwave oven and allowed to cool to 50 ^{o}C before pouring it into the tray that had been sealed using masking tape. Wells for loading DNA were made by inserting a comb 1.5 – 2 cm at one end of the gel tray and it was allowed to set for 30 mins. After that it was placed in a gel tank which had been filled with TBE buffer and the gel was completely covered by the buffer. DNA samples (5 µl) were loaded containing (3 µl) loading dye. Electrophoresis was carried out at 40 V at room temperature for 1hr. Since DNA is negatively charged, samples loaded in the wells at the cathode migrated towards the anode at the rate dependent on the fragment size and the voltage used. Stained gel was visualized under UV light using the Gel Doc 1000 Image Analysis System (Biorad).

3.5 Biochemical Analysis

The randomly collected maize seeds and maize food products from the 21 different markets in Kenya were screened for *cry1Ab*, *cry1Ba* genes and for Cry1Ab and CP4-EPSPS proteins by molecular and lateral flow strip analyses.

The two analyses are hereby respectively expounded in the two sections that follow:

3.5.1 Molecular Detection of *cry1Ab* and cry1Ba genes

Conventional PCR for amplification of *cry1Ab* and *cry1Ba* were carried out in a thermocycler using an Applied Biosystems GeneAmp PCR System 9700. This was in a final volume of 25 µL, containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 µM primers, 1.25 U Taq polymerase, 1 X PCR buffer and 40 ng DNA template. All reagents were obtained from Invitrogen except for primers which were synthesized by Biosynthesis Inc. Conditions for amplifications were as follows: denaturation of DNA at 94°C for 2 mins, 35 cycles of 1 min at 94 ^{o}C, 1 min at 68 ^{o}C, 1 min at 72 ^{o}C, and a final extension at 72 ^{o}C for 10 mins. With exception of *cry1Ba* where the annealing temperature was at 54°C. Five µL of the amplified products was electrophoresed in 2% agarose gel for 1 hr at 40 mA, and stained with EtBr for visualisation. Primers used for amplification are described in Table 3.3. *Bt* maize line containing *cry1Ab* gene (Bt maize Event 223::*cry1Ab*) and *cry1Ba* gene (Bt maize Event 6::*cry1Ba* and Bt maize Event 10::*cry1Ba*) were used as positive controls while CML 216 was the negative control for the PCR reactions.

Table 3.3: Primers Used for Amplification

	Primer	Sequence	Product (bp)	Reference
Cry1Ab	Forward Reverse	5'-ACCATCAACAGCCGCTACAACGACC-3' 5'-TGGGGAACAGGCTCACGATGTCCAG-3'	200	Danson *et al.* (2006)
Cry1Ba	Forward Reverse	5'-CCATGGTTACCTCCAACCGT-3' 5'-GGATGATCTCGATCTTGTCGA-3'	70	Danson *et al.* (2006)

Source: Danson *et al.* (2006)

3.5.2 Detection of Cry1Ab and CP4-EPSPS Proteins

All protein strip test analyses were performed following the manufacturer's procedures i.e. *incasu* Envirologix, Neogene or Strategic Diagnostics (EnviroLogix Inc., 2003). The Lateral flow strips method was carried out by adding 2.5 ml of deionized water to each of the eppendorf tubes containing 2 g of the ground maize flour. The mixtures were shaken thoroughly to ensure that all the flour was completely wet. Then 0.5 ml of the supernatant was transferred into a clean sterilize eppendorf tube. One lateral strip was placed into each of the eppendorf tubes and left to stand for 5 mins. The results were read and recorded accordingly. The Bt maize containing Cry1Ab protein (Bt maize Event

223::*cry1Ab*) was used as the positive controls while CML 216 and CML 144 non Bt maize were used as the negative controls for this reaction. The appearance of 1 line at the control line indicated negative results, while the appearance of 2 lines both at the control and test line indicated positive results.

3.6 Data Analysis

To test the hypotheses, the study employed *'order statistics'* or *'nonparametric statistics'* or *'distribution free'* as opposed to *parametric statistics* (Kothari, 2001). This was chosen because in this study we were testing for the presence versus the absence of the GMOs in the maize food products. Hence the one sample *sign test* was used. This is a test based on the direction of the plus (+) for presence and minus (-) for absence, the signs of observations in a sample and not on their numerical magnitudes. It is applicable when we sample a continuous symemetrical population in which case the probability of getting a sample value less than mean (μ_{Ho}) is ½ and the probability of getting a sample value greater than mean is also ½. This is used to test the null hypothesis $\mu = \mu_{Ho}$ (population mean is equal to sample mean) against an appropriate alternative on the basis of a random sample size 'n'. MS-Excel Version 2007 was used to compute the values and the table of binomial probabilities and or a normal approximation to binomial distribution (depending on the size of 'n') were used to compare the calculated values with the z-values (Kothari, 2001).

CHAPTER FOUR

4.0 RESULTS

4.1 Detection of *cry1Ab* genes in Maize from different locations in Kenya

PCR amplification of DNA extracted from 96 maize grains and seeds sampled from various randomly selected retail markets in Kenya were used to detect *cry1Ab* genes using specific primer set (Table 3.3). Amplification products were separated in 2% agarose gel stained with ethidium bromide for visualization. Positive samples had PCR products of 200 bp for *cry1Ab* (Plate 4.1a). A negative score was made where no band was detected by visual inspection. Bands of 200 bp were clearly visible under the UV light. From this result 5 (5.2%) of the 96 samples were found to contain *cry1Ab* genes. The samples include: Mombasa 1, Mombasa 2, Mombasa 3, Mombasa 4 sampled from Mombasa town and Kangemi 1 sampled from Nairobi. The rest of the maize samples collected tested negative for the *cry1Ab* gene. There was no amplification for non *Bt* control maize CML216, lane 7 (Plate 4.1a).

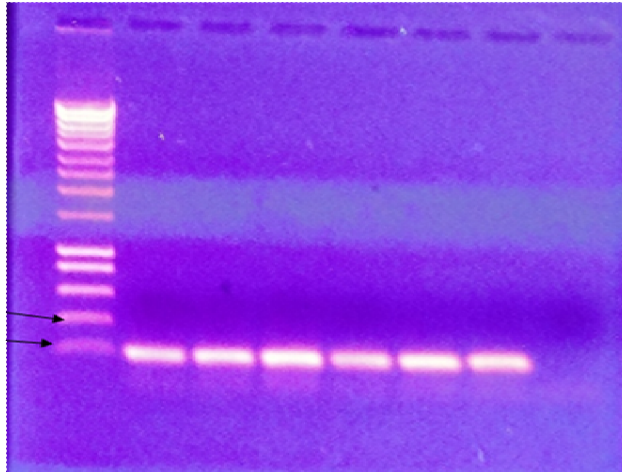

Plate 4.1a: PCR Results for five maize samples, a positive and negative control lanes amplified with *cry1Ab* primer
M: Molecular weight Ladder (Hyper ladder 1 bioline-ranging from 200bp to show the band of interest; Lane 1: Mombasa 1; Lane 2: Mombasa 2; **Lane 3: Bt event::*cry1Ab* (Positive control)**; Lane 4: Mombasa 3; Lane 5: Mombasa 4; Lane 6: Kangemi 1; Lane 7: Negative control (CML 216). The samples were amplified with *cry1Ab* primers.

Bt maize event 6::*cry1Ba* and *Bt* maize event 10::*cry1Ba* samples for *cry1Ba* were amplified with *cry1Ab* primers (Plate 4.1b). The amplification was carried out together with other samples collected from various markets to test the specificity of the Primer.

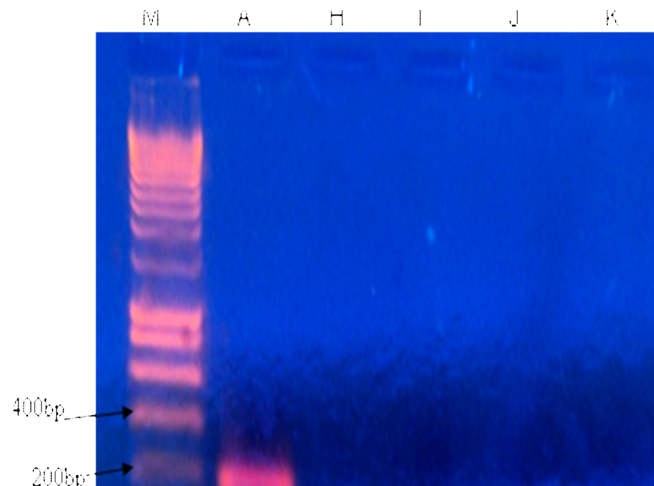

Plate 4.1b: *cry1Ab* specific amplification test on both *cry1Ab* and *cry1Ba* Bt Event maize and some maize samples collected from markets.
M: Molecular weight Ladder (Hyper ladder 1 bioline-ranging from 200 bp-10,000 bp); A: Bt maize Event 223::*cry1Ab*; H: Bt maize Event 6::*cry1Ba*; I: Bt maize Event 10::*cry1Ba*; J: Kangemi 3; K: CML 216.

The Bt maize event samples taken from *cry1Ba* (lanes H to I) did not amplify with *cry1Ab* primers but the Bt Event 223::*cry1Ab*, which was a positive control amplified the expected band of 200 bp (Plate 4.1b). This is consistent with other studies which found primers to be specific to the Bt genes (Danson *et al.,* 2006).

4.2 Detection of Cry1Ab Protein in Maize from different towns in Kenya

The PCR results for detection of *cry1Ab* gene above were compared with detection of Cry1Ab protein by lateral flow strips technique (Plate 4.2).

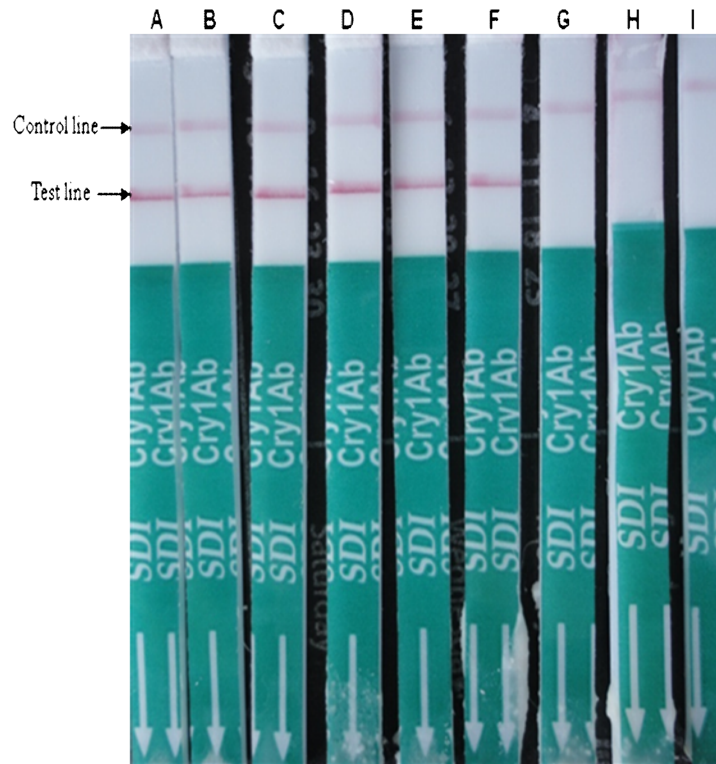

Plate 4.2: Cry1Ab protein results for some of maize samples collected and two *cry1Ba* Bt Event maize.
Strip A: Mombasa 1; strip B: Mombasa 2; strip C: Mombasa 3; strip D: Bt maize Event 223::*cry1Ab*; strip E: Mombasa 4; strip F: Kangemi 1; strip G: CML 216; strip H: Bt maize Event 6::*cry1Ba;* and Strip I: Bt Event 10::*cry1Ba.*

Strips A – F showed the same bands. This confirms that those maize samples that were positive for *cry1Ab* gene by PCR method, had two lines indicating they were also positive for Cry1Ab proteins. This confirmed that the 5 samples contained *cry1Ab* gene. On the other hand, the 2 Bt maize Events for *cry1Ba* positives (lanes H and I) and the CML216 control (lane G) were negative for the Cry1Ab protein. This shows that the strips used were specific for Cry1Ab protein. The results for all maize sampled from different markets in the country showed that out of 96

maize samples randomly collected from retail markets, open air markets and seed companies in Kenya 5 (5.2%) tested positive for Cry1Ab protein (Table 4.1).

4.3 Detection of CP4-EPSPS (Round up ready) Protein in Maize Grains and Seeds from different towns in Kenya

The maize grains and seeds samples randomly collected from different town in Kenya were tested for CP4-EPSPS protein by use of strips specific to this protein. Out of 96 maize samples, the same set of 5 (5.2%) maize grains samples tested positive for the round up ready protein (Plate 4.3). The 5 maize grains samples that tested positive were: Mombasa 1, Mombasa 2, Mombasa 3, Mombasa 4 and Kangemi 1. It was not possible to run PCR on this due to lack of positive control which requires a lot of legal procedures to import.

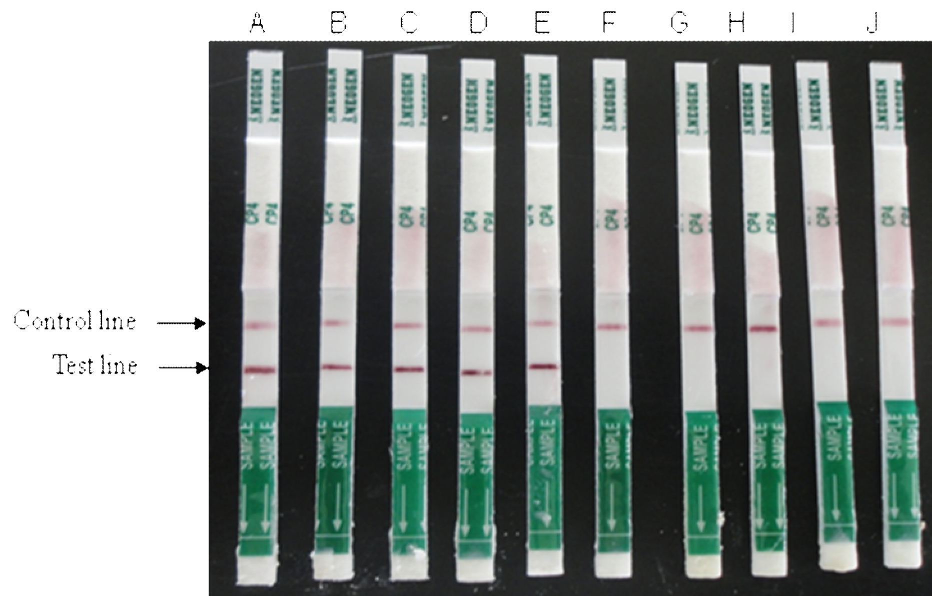

Plate 4.3: CP4-EPSPS Lateral Strip results for Maize Grains and Seeds
A: Mombasa 1; B: Mombasa 2; C: Mombasa 3; D: Mombasa 4; E: Kangemi 1; F: Bt Event 223::*cry1Ab* (positive control for Cry 1Ab); G: Bt Event 6::*cry1Ba*; H:

Bt Event 10::*cry1Ba*; I: CML 216 (negative control) and J: CML202 (negative control).

Table 4.1: The Comparison between lateral strip method and PCR results for Cry1Ab Protein and *cry1Ab* Gene of the Maize Seed

Province	Sample source (Market)	Sample Identity	Cry1Ab Protein	PCR amplification for *cry1Ab*	CP4-EPSPS
Eastern	Makueni	Makueni 1-5	_	_	
,,	Mwingi	Mwingi 1-7	_	_	
,,	Machakos	Machakos1-5	_	_	
,,	Kitui	Kitui 1-8	_	_	
Nairobi	Kangemi	Kangemi 1	+	+	+
,,	Kangemi	Kangemi 2-6	_	_	
,,	Wakulima	Wakulima1-5	_	_	
,,	Ngong	Ngong 1-5	_	_	
,,	Toi	Toi 1-5	_	_	
,,	Karen	Karen 1-5	_	_	
,,	Kitengela	Kitengela 1-5	_	_	
	Mulolongo	Mulolongo1-4			
Seed Companies	Monsanto S. Co.	DK 8031	_	_	
,,	Kenya S. Co.	H513	_	_	
,,	Kenya S. Co.	DH04	_	_	
,,	Seedco Limited	D41 (Duma)	_	_	
,,	Pannar S. Co.	PAN 67	_	_	
Mombasa	Kongowea	Mombasa 1	+	+	+
,,	Kongowea	Mombasa 2	+	+	+
,,	Kongowea	Mombasa 5	_	_	
,,	Kongowea	Mombasa 6	_	_	
,,	Kongowea	Mombasa 7	_	_	
,,	Kilindini	Mombasa 3	+	+	+
,,	Kilindini	Mombasa 4	+	+	+
,,	Marikiti	Mombasa8-12	_	_	
Western	Busia	Busia 1-5	_	_	
,,	Bungoma	Bungoma 1-5	_	_	
,,	Kakamega	Kakamega1-5	_	_	
,,	Mumias	Mumias 1-5	_	_	

Key: - (Negative); + (Positive); S- Seed; Co. Company.

Source: Researcher Personal Data Analysis, July-September, 2010.

The results for the 96 maize samples were analyzed using one sample sign test (an aspect of 'nonparametric' or 'distribuition-free') at 5% level of significance to test the null hypothesis (that there were no GMOs in maize food products in Kenyan markets). The observed proportion of the positive samples was computed and found to be (5/96) 0.052083. The standard error of the proportion was found to be 0.053634. To test the null hypothesis against the alternative, a one-tail test was appropriate. By using the table of area under normal curve, the z- value for 0.45 of was found to be 1.64. This was used to work out the limit (on the lower side as the alternative hypothesis is of the < type) of the acceptance region which was found to be 0.412041. Since the observed proportion of positives is 0.052083 which falls in the rejection region, the null hypothesis was rejected and accepted the alternative hypothesis. The conclusion was that the maize grains sold in the various Kenyan open air markets contains *cry1Ab* gene, the extensively commercialized gene.

4.4 Assessment of Gene flow at KARI Kiboko by PCR

PCR analysis was done for DNA extracted from 10 maize grains collected from farmers' around Kiboko and 2 non Bt maize CML 202 and CML 204 that were sampled from KARI Kiboko field trials located at least 800 m away from where the Bt maize CFT site at an open quarantine station at KARI-Kiboko. The samples were amplified using *cry1Ab* the specific primers (Table 3.3). Bt maize Event 223::*cry1Ab* was used as positive control for *cry1Ab* gene while the non Bt

maize CML 216 was used as the negative control for PCR reactions. The results obtained showed that the samples collected from KARI Kiboko and from Kiboko farmers as well as the negative control did not amplify the expected band of *cry1Ab* gene (Plate 4.4). However, the positive control amplified the expected band of 200 bp. Similar results were also obtained by the lateral strip method. This implies that there was no gene flow that occurred from the confined field trials to other maize in its vicinity.

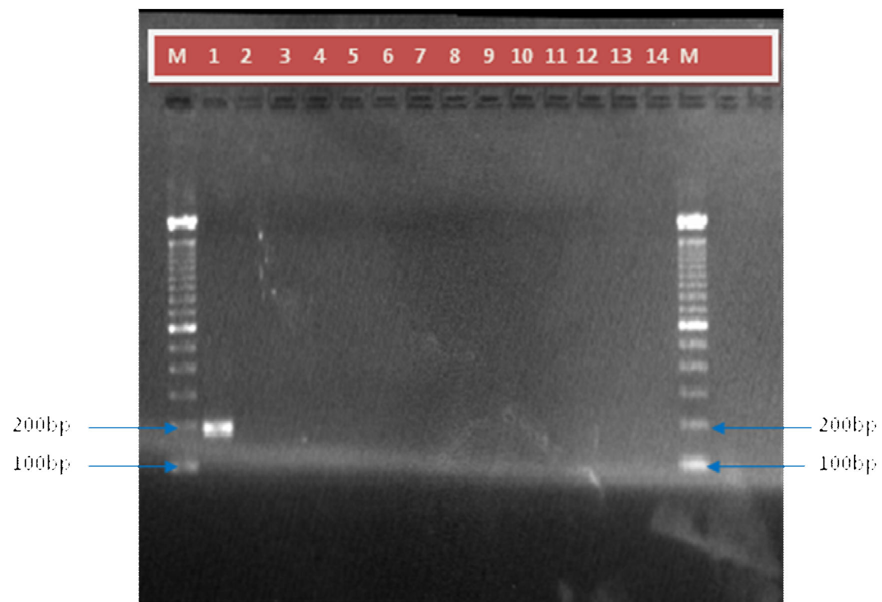

Plate 4.4: Detection of *cry1Ab* Gene for Gene flow to non target Maize Fields

M: Molecular weight ladder 100 bp from Invitrogen; Lane 1: Bt maize Event 223::*cry1Ab*; Lane 2: CML202; Lane 3; CML204; Lane 4: CML216; Lanes 5 to14: maize samples from farmers in Kiboko.

4.5 Detection of *cry1Ba* genes in Maize from different towns/markets in Kenya

PCR of DNA extracted from maize grains and certified hybrid seeds sampled from various randomly selected markets (Table 3.1) in Kenya were used to detect *cry1Ba* genes using specific primer set (Table 3.3). Two Bt maize events (Event 6::*cry1Ba* and Bt maize Event 6::*cry1Ba*) containing the *cry1Ba* genes were used as positive control while the non Bt maize CML 216 was the negative control for PCR reactions. Positive controls amplified PCR products of 70 bp (Plate 4.5). On the other hand, no band was observed for the negative control and for all the samples collected from the various markets in Kenya. This shows that none of the samples collected from the various markets in Kenya contained *cry1Ba* gene.

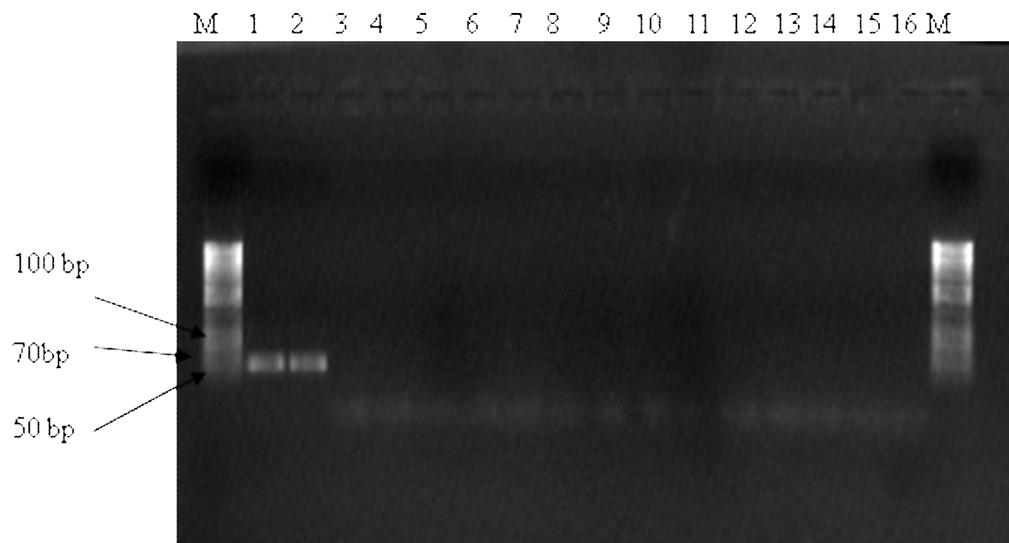

Plate 4.5: Amplification of DNA from Maize grains with Cry1Ba Primer to Detect *cry1Ba* Gene
M: Low molecular weight ladder 50 bp from fermenters; Lanes 1-2: Bt positive control Maize grains (Bt maize Event 6::*cry1Ba* and Bt maize Event 10::*cry1Ba*); Lane 3: Bt maize Event 223::*cry1Ab*; Lanes 4-9: *cry1Ab* samples amplified with

cry1Ba primers; Lane 10-11: non Bt control maize samples; Lanes 12-16: shows negative results for collected maize samples amplified with cry1Ba primers.

Since Cry1Ba gene is not yet a commercialized gene, there are no strips for it. Iit was therefore not possible to test for Cry1Ba protein by lateral strip method.

4.6 Detection of Cry1Ab Proteins in Processed Food Products

During the random selection of the processed maize food products it was noted that none of the samples was labelled whether it contained GM or not. The 12 samples collected from supermarkets were tested for Cry1Ab proteins by use of Cry1Ab protein strips and the following results were obtained (Plate 4.6 and Table 4.2). Out of the 12 samples tested, 3 (25%) were positive for Cry1Ab protein.

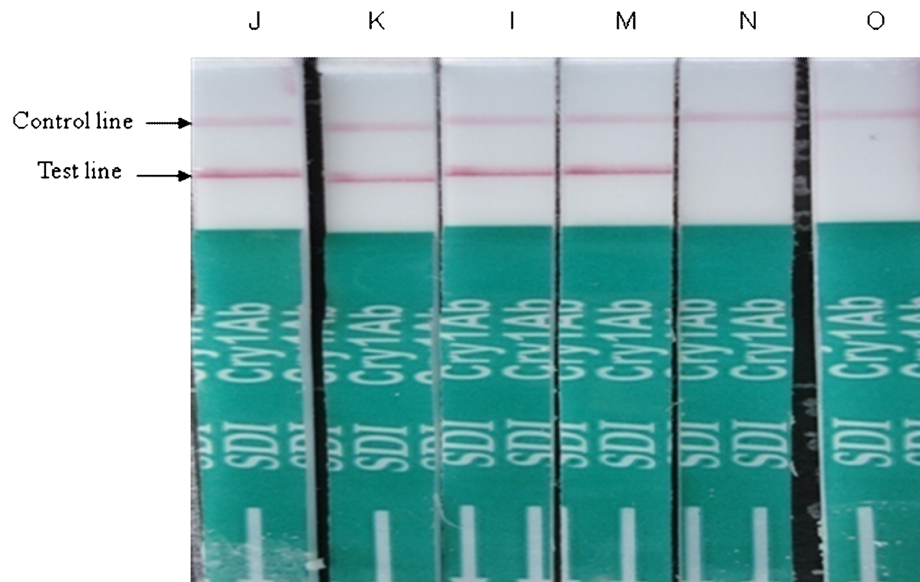

Plate 4.6: Lateral Flow Strip results for cornflakes tested for Cry1Ab Protein.
Strip J; Bt Event 223::*cry1Ab*, strip K Corn[2]; strip I: Corn[3]; strip M: Corn[4]; strip N: CML 216 and Strip O: Corn[1].

Origin of the cornflake: [1]Magic foods; [2]Kellog company; [3]Magic times Int'l LL (MTI); [4]Nestle Products.

From the results some of the processed maize products sold in the supermarkets contain Cry1Ab proteins. However, since there were no face labels on those products, specific guidelines and regulations are needed in place for food labelling in Kenya. This is because GM products can be found in the country's supermarkets and retail outlets without being labelled as GM or GM-free. In that case a system must be put in place to verify both local and imported food products in Kenya so as to ascertain the validity of unlabelled and labelled food products to give the consumers the choice of preference between GM and non GM food products.

Table 4.2: Detection of Cry1Ab Protein in Unlabelled Processed Maize Food products

Origin	Product Name	Description	Producer	CRY 1Ab Protein
Supermarkets	Corn Flakes1	Cereal	Bokomo Foods	-
	Corn Flakes2	Cereal	Kellogg Company	+
	Corn Flakes3	Cereal	Magic Time Int'l LL (MTI)	+
	Corn Flakes4	Cereal	Nestle Products	+
	Corn Flakes5	Cereal	Mass food	-
	Popcorn1	Popcorn	Natural popcorn	-
	Popcorn2	Popcorn	American popcorn	-
	Popcorn3	Popcorn	Mericana popcorn	-
	Soko	Maize flour	Capwell Industries	-
	Pendana	Maize flour	Capwell Industries	-
	Jogoo	Maize flour	Unga mills	-
	Baby Porridge	Maize flour	Proctor & Allan	-

Source: Researcher Personal Data Analysis, July-September, 2010.

The results of the 12 samples of the processed maize food products obtained from supermarkets were analyzed using one sample sign test at 5% level of significance to test the null hypothesis (that the food products sold in the supermarkets in Kenya do not contain proteins from GMOs). The observed proportion of positive samples was computed and found to be (3/12) 0.25. The standard error of the proportion was found to be 0.144336. To test the null hypothesis against the alternative, a one-tail test was appropriate. By using the table of area under normal curve, the z value for 0.45 was found to be 1.64. This was used to work out the limit (on the lower side as the alternative hypothesis was of the $<$ type) of the

acceptance region that was found to be 0.263. Since the observed proportion of positives is 0.25 which, falls in the rejection region, the null hypothesis was rejected and accepted the alternative hypothesis. This confirms that there were Cry1Ab proteins in the processed maize foods products sold in the Kenyan supermarkets.

4.7 Geographical Spread of GM Samples

The distribution of maize containing *cry1Ab* gene came from 2 geographical areas, Coast and Nairobi provinces. The samples from Coast (Mombasa markets) had a higher percentage of 4.2% while that for Nairobi province (Nairobi City, Kangemi market) was 1.0%. It was further noted that out of the 12 processed samples collected from supermarkets in Nairobi, 3 cornflakes contained Cry1Ab protein and are manufactured from those countries which grow GM crops .

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 DNA Extraction

Good quality DNA was recovered for PCR amplifications. These confirmed that the CTAB DNA extraction method used was adequate for the extraction of amplifiable maize DNA from the samples and this is in agreement with Pirondini *et al*. (2010).

However, extraction from the 5 corn flakes (Kellogg's, Nestle, Magic, Bokomo and Temmy) was not successful. This findings concur with (Margarit *et al.*, 2006) who found out that it was not possible to obtain good quality DNA for PCR from highly processed foods, such as corn flakes, corn puffs and corn syrup. These results suggest that high temperatures or other factors involved in the processing of the cornflakes degraded the *cry1Ab* DNA as earlier reported by Terry *et al.* (2002).

In the case of popcorns, the extraction of DNA was not as successful as in the ordinary maize grains. The popcorn samples were not amplified. This could have been due to either the DNA content being insufficient, or there were PCR inhibitors affecting them. It is known that food samples comprise of complex mixture containing PCR inhibitors that may compromise the amplifiability of DNA (Pirondini *et al*., 2010).

5.1.2 Assessment of Presence of cry1Ab Genes by PCR

In this study, PCR results provided evidence for the presence of *cry1Ab* gene in maize grains consumed in Kenya by amplifying a fragment of *cry1Ab* with molecular weight of 200 bp in the following maize grains: Mombasa 1, Mombasa 2, Mombasa 3, Mombasa 4, Kangemi 1 and Bt event 223::*cry1Ab* (Plate 4.1a).

Out of 96 DNA samples analysed of maize seeds and grains collected randomly from various towns, 5 of them showed presence of *cry1Ab* gene and this constitutes 5.2% of the total samples analyzed. Since there is no similar studies carried out in Kenya, these findings are in agreement with other studies done in other countries where *cry1Ab* gene has been detected in maize food product using PCR.

For example a study by Yoke-Kqueen *et al.* (2011) on detection of GMOs targeting *EPSPS* and *cry1Ab* genes in maize products from 60 samples that were randomly collected from local retail markets and supermarkets in Malaysia found out that 42 (70%) out of 60 samples were positive for GM events. Of these positive samples 5 (11.9%) were found to contain *EPSPS* gene whereas 37 (88.1%) contained *cry1Ab* gene.

Another study is that by Margarit *et al.* (2006), done in Argentina where a fragment of 204 bp (Permingeat *et al.*, 2002) was amplified by PCR. This study used commercial maize. They detected that 2 precooked, 1 non-cooked polentas and 2 cracked maize contained cry1Ab genes. Our findings also concur with those

of Kyrova *et al.* (2008) who conducted a study in the Czech Republic from 2002 to 2007 on the detection of GM foods on the market. Out of 1164 samples found that 3 varieties of GM maize were positive. In Canada, out of the 35 representative food products selected based on their commercial importance (corn, soybean and canola), about one-third of the samples were GM positive (Gobeil *et al.*, 2008). A study in Turkey (Ertugrul *et al.*, 2008) also found the presence of GMO content in some of the maize samples. Finally, is a study done in South Africa Viljoen *et. al.* (2006). The authors of this study detected GMOs in maize food products randomly collected in different retail outlets including Pick 'n Pay, Shoprite Checkers, Spar and Woolworths as well as small retail outlets, including health food shops.

It is worth noting here that when samples positive for *cry1Ba* genes were tested with *cry1Ab* primers there was no amplification detected. The results of samples positive for *cry1Ab* genes when tested with *cry1Ba* primers were similar in that no amplification was detected. This showed that the primers were specific for *cry1Ab* Bt gene and this finding concurs with that of Danson *et al.* (2006) who found out that all Bt plants from *cry1Ab* did not amplify with the *cry1Ba* primers. Similar results were obtained for Bt plants from *cry1Ba* amplified with *cry1Ab* primers.

5.1.3 Assessment of Presence of Cry1Ab Protein by Lateral Flow Strip Method

The maize samples were also analyzed for Cry1Ab protein by lateral flow strip method and those samples that were positive for *cry1Ab* gene were also positive

for Cry1Ab proteins giving the same percentage of 5.2%. Thus the 2 methods (PCR and lateral flow strip) are in agreement. Protein strip tests, thus, confirmed that it can be used as a cheaper and more rapid tool than PCR based GMO detection method, which concurs with the findings by Van Duijn *et al*. (2002). Besides, it is also confirmed by Albuquerque *et al.* (2011) who used this method in determining trans fatty acids in content food and found it to be cost effective and faster than PCR.

5.1.4 Assessment of Presence of CP4-EPSPS Protein

Those maize samples that were positive for *cry1Ab* gene and Cry1Ab protein (Mombasa 1, Mombasa 2, Mombasa 3, Mombasa 4 and Kangemi 1) were also positive for CP4-EPSPS protein when protein strips specifically for it were used. This also gave a percentage of 5.2%. The results show that the 5 samples contained stacked traits. The maize samples were likely double stacked with pest resistance (Cry1Ab) and herbicide tolerance traits (CP4-EPSPS) protein. This finding again concurs with that of Clive (2011) on double stacked maize with pest resistance and herbicide tolerance traits' growth in the world up to 2010. Since Kenya has not legalized GM stacked events the availability of GM stacked products could be considered as illegal or non approved introductions (Mugo *et al.*, 2011c). According to the regulatory practices within the EU, stacked events are considered as new GMOs. Hence prior to their marketing they need regulatory approval, including an assessment of their safety, similar to single events (De Schrijver *et. al.,* 2007).

5.1.5 Assessment of Presence of c*ry1B*a Genes by PCR

The *cry1Ba* gene was not detected in the maize samples. This is can be attributed to the fact that *cry1Ba* is not commercialized in Kenya or in any other country and for that reason, there are no strips for protein test yet. However, it was tested in the biosafety level 2 laboratory, biosafety level 2 greenhouse and under CFTs in Kenya 2001-2007 (Mugo *et al.*, 2011b). According to Clive (2004a), the USDA approved only *cry1Ab* GM maize seeds and CP4-EPSPS GM soybean seeds for use as food as well as in animal feeds.

5.1.6 Assessment of Gene flow to non targeted Maize farms in Kiboko due to concluded Bt Maize Experiments in KARI–Kiboko.

The second objective of this study was to assess the presence of gene flow to non targeted maize farms in Kiboko area due to concluded Bt maize experiment, IRMA project at KARI–Kiboko. The samples collected from KARI-Kiboko and Kiboko farmers showed no amplification both by PCR and lateral flow strip methods. Hence there was no gene flow detected from the CFTs to other maize in its vicinity. This shows that the Biosafety regulations and precautions that were in place were adhered to and were effective in ensuring genetic confinement of the Bt maize (Mugo *et al.*, 2011c). Among the measures were isolation by distance, detaseling Bt maize, harvesting before maturity, disposal in pits, restricted access to the site among others. These were followed by postharvest monitoring during which volunteers in the CFT site were destroyed at the seedling stage (Mugo *et al.*, 2011a). Since maize is wind pollinated, with fertilization occurring at up to

200 m (Ma *et al*., 2004; Ingram, 2000), doubling of the distance between the experimental site and the near by maize farm from the recommended distance of 400 m to 800 m (required for breeder seed production) reduced the chances of gene flow from any pollen to occur (Mugo *et al.*, 2011a).

Given that pollen concentrations and thus cross-fertilization levels rapidly decrease with increasing distance from the pollen source, spatially isolating GM maize fields from non-GM maize fields is an effective on-farm strategy to reduce the extent of cross-fertilization. As maize pollen is fairly heavy, the vast majority is deposited within a maximum of 18–20 m distance of the emission source, minimizing the chances of cross-fertilization occurring beyond this distance (Devos *et al*., 2005).

In defining science-based isolation distances between GM and non-GM maize fields cross-fertilization rates have been studied both in experimental and commercial fields. Gene flow was followed based on Bt maize sequence information (Sorina *et al.*, 2010). The results obtained from experimental fields in Romania's 3 refugee areas (Chiciu, Chirnogi and Gătaia), the maximum distance where the GM content was below 0.9% for all of the four geographic directions was 21 m in 2007, and 25 m in 2008.

In the study by Messeguer *et al.* (2006), bearing in mind the real situation of coexistence that exists in Spain they evaluated the rate of cross-fertilization in several non-GM maize fields. This was in 2 different regions in which Bt and conventional maize are commonly used. They showed that the effect of distance

could be confirmed by examining the spatial distribution of the receptor fields in relation to the donor field distances. They suggested that a distance of about 20 m would be sufficient to control the adventitious presence of GMOs as a result of pollen-mediated gene flow and to obtain GMO content below the 0.9% EU threshold. However, they added that larger security distances, such as 30–50 m, are useful to maintain the GMO content at a very low level, and perhaps could contribute to minimize the effect of other factors, such as mixing during sowing, harvesting, storage and the presence of volunteers in the field. They concluded, based on the available data that only those Bt donor fields within 0–150 m can significantly influence the rate of cross-pollination. Any cross-pollination beyond this distance could be attributed to other factors other than distance.

In 2002, Chilcutt and Tabashnik (2004) conducted 2 field experiments in USA at the Texas Agricultural Research Station (Corpus Christi). The minimum distance between Bt and non-Bt maize was 1 m in experiment 1 and 15 m in experiment 2. In both experiments, non-Bt maize was planted north of Bt maize. The predominant wind direction was from the southeast (16 kph mean daily wind speed), which favoured gene flow from Bt maize to non-Bt maize. In both experiments, ELISA tests of non-Bt maize showed that the mean concentration of Bt toxin *cry1Ab* in kernels and the percentage of kernels with *cry1Ab* decreased with distance from Bt maize. The results obtained show low to moderate levels of Bt toxin *cry1Ab* in ears of non-Bt maize refuge plants within 31 m of Bt maize. Maximum concentrations of Bt toxin in non-Bt maize that occurred within 2 m of Bt maize, were 43–45% of the mean concentrations in Bt maize. The mean

concentration of Bt toxin *cry1Ab* in kernels and the percentage of kernels with *cry1Ab* decreased with distance from Bt maize, which implies that pollen-mediated transgene flow from Bt maize caused contamination of non-Bt maize refuge plants.

5.1.7 Detection of Cry1Ab Protein in Unlabelled Processed Maize FoodProducts

This objective (third objective) was achieved through lateral strip method by detecting the presence of Cry1Ab protein. Out of the 12 samples 3 tested positive for cry1Ab protein representing 25% of the total samples. These were cornflake2, cornflake3 and cornflake4 manufactured by Kellogg Company, Magic Time Int'l LL (MTI) and Nestle Products respectively indicating that those cornflakes were made from low processed GM maize and that there were some traces of protein left during processing. In cornflake5 there was no reaction suggesting that there was either no GM, or high temperatures and other factors involved in the processing of the cornflakes had degraded the Cry1Ab protein as suggested by Terry *et al*. (2002). This is also in agreement with Yoke-Kqueen *et al.* (2011) who found that as high as 45.5% of the processed food samples had low protein yield. This finding is common in processed food due to degradation of protein during food processing. This is in accordance with a study by Ahmed (2002) which revealed that heating and other processes associated with food production can degrade proteins in those foods.

Although some of the selected corn flakes obtained from the Nakumatt and Uchumi supermarkets outlets in Kenya tested positive for Cry1Ab protein the labels were silent on those food products. A consumer who purchased such food products was not informed whether those products were GM or not GM. It is important to note that the amount of GM in the samples was not quantified. Thus it is likely that the samples, which tested positive for GMOs, the transgenes present in them could be above or below the accepted thresholds of 1-5% (Viljoen *et. al.,* 2006). However, Botha and Viljoen (2009) who used PCR GM detection to screen and quantify GM content in food products in South Africa found that voluntary labelling was not sufficient to provide consumers with a choice between GM and non-GM food products and that unregulated GM labelling would result in consumers inadvertently being misled.

5.2 CONCLUSIONS

There are GM maize food products in the Kenyan market carrying the maize Bt gene *cry1Ab* and herbicide resistant *CP4-EPSPS* (Round up ready) genes that are GM products. The high presence of GM in the Mombasa samples has showed that the major source is import.

The study did not find any 'gene-flow' from the quarantine to the farmers' farms in Kiboko implying that the isolation and measures required by KEPHIS were sufficient to confine the transgenes**.** Likewise the Biosafety Green House Containment (BGHC) was effective as none of the products sampled showed

presence of the Bt maize *cry1Ab* or *cry1Ba* that were only instituted for use in the BGHC and in the Kiboko open quarantine station.

The food stuffs in the major supermarkets operating in Kenya are not labelled as GM or non-GM, yet there are GM products being sold. Hence this denies the consumers the opportunity to make informed choices.

5.3 RECOMMENDATIONS

- Labelling of the food stuffs in the countries exporting grain and other products from those countries largely growing GM crops, would give the consumers a chance to choose between GM and non-GM.
- Quantification of the transgenes in those samples that tested positive for *Cry1Ab* would show the degree of the presence of GM and whether they are within the acceptable thresholds.
- The Kenya Biosafety regulations are needed to operationalize the Biosafety Law that would guide the legal importation of GM food and feeds into the country.
- There is also need to screen more samples so as to give a good representation of the country and collect more data for consumer data bank. It would also be desirable to have these tests formalized and carried out regularly by NBA, the regulatory institution for GM products.

REFERENCES

Abdullah, T., Radu, S., Hassan, Z., Hashim, J. K. 2006: Detection of genetically modified soy in processed foods sold commercially in Malaysia by PCR-based method. *Food Chemistry,* **98**: 575–579.

Agrifood Awareness Australia 2004: Biotech Bull-Global Labelling Laws. Available at http://www.afaa.com.au (accessed 6 October 2010).

Ahmed, F. E. 2002: Detection of Genetically modified organisms in foods. *Trends in Biotechnology,* **20**: 215-223.

Albuquerque, I. G., Costa, H. S., Castilho, M. C., Sanches-Silva, A. 2011: Trends in the Analytical methods for the determination of trans-fatty acids in content food. *Trends in Food Science and Technology,* **22:** 543-560.

Anklam, E., Gadani, F., Heinze, P., Pijnenburg, H., Van den Eede, G. 2002: Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products. *European Food Research and Technology,* **214**: 3-26.

Aylor, D., Schultes, N., Shields, E. 2003: An aerobiological framework for assessing cross-pollination in maize. *Agricultural and Forest Meteorology*. **119**: 111–129.

Baker, G. A. and Burnham, T. A. 2002: The market for genetically modified foods: consumer characteristics and policy implications. *International Food and Agribusiness Management Review,* **4**: 351-360.

Bannert, M. and Stamp, P. 2005: Cross-fertilization in maize–results of a Swiss study. In: Conference on Coexistence of GM and Non-GM Crops: Scientific Data, Practical Applications and Perspective for the Next Decade. Zurich, Switzerland: Agroscope FAL Reckenholz. URL: http://www.coexistence.ethz.ch. PDF/Summary/Coexistence/Zurich/June2005.pdf (accessed on18 October 2009).

Borch, K. and Rasmussen, B. 2000: An analytical approach to the implementation of genetically modified crops. *Trends in Biotechnology,* **18:** 483–486.

Botha, G. M. and Viljoen, C. D. 2009: South Africa: A case study for voluntary GM labelling. *Food Chemistry,* **112:** 1060-1064.

Brookes, G., Barfoot, P., Melé, E., Messeguer, J., Bénétrix, F., Bloc, D., Foueillassar, X., Fabié, A. and Poeydomenge, C. 2004: *Genetically Modified Maize: Pollen Movement and Crop Coexistence.* Dorchester, UK: PG Economics Ltd. 20pp.

Bulcke, M., De Schrijver, A., De Bernardi, D., Devos, Y., Mbongo-Mbella, G., Casi, A. L., Moens, W., Sneyers, M. 2007: Detection of genetically modified plant products by protein strip testing: an evaluation of real-life samples. *European Food Resource Technology,* **225**: 49-57.

Bullock, D. S. and Desquilbet, M. 2002: The economics of non-GMO segregation and identity preservation. *Food Policy,* **27**: 81-99.

Canadian General Standards Board, 2004: Voluntary Labelling and Advertising of Foods That Are and Are Not Products of Genetic Engineering (CAN/CGSB-32.315-2004). Available at http://www.pwgsc.gc.ca/cgsb/032_025/ standard-e.html (accessed 18 October 2009).

Cankar, K., Ravnikar, M., Zel, J., Gruden K., Toplak, N. 2005: Real-time polymerase chain reaction detection of Cauliflower mosaic virus to complement the 35S screening assay for genetically modified organisms *Journal of the Association of Official Agricultural Chemists, International,* **88:** 814-822.

Carter, C. A. and Gruère, G. P. 2003: Mandatory labelling of genetically modified foods: Does it really provide consumer choice? *AgBio Forum,* **6**: 68-70.

Castillo, J. J. 2009: Statistical Sampling Techniques. Retrieved from Experiment Resources: http://www.experiment-resources.com/statistical-sampling-techniques.html (accessed on 16 September 2009).

Chilcutt, C. F. and Tabashnik, B. E. 2004: Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. *Proceedings of the National Academy of Sciences,* **101**: 7526-7529.

Clive, J. 2011: Global Status of Commercialized Biotech/GM Crops: 2010. ISAAA Brief No 42-2010. Ithaca, NY.

Clive, J. 2009: Global Status of Commercialized Biotech/GM Crops: 2009. ISAAA International Service for the Acquisition of Agri-Biotech Applications *Briefs* No. 41-2009. Ithaca, NY.

Clive, J. 2008: Global Status of Commercialized Biotech/GM Crops: 2008. ISAAA Brief No. 39 – 2008. ISAAA: Ithaca, NY. ISBN 978-1-892456-44-3.

Clive, J. 2007. Global Status of Commercialized Biotech/GM Crops: 2007. Highlights of International Service for the Acquisition of Agri-Biotech Applications (ISAAA) Briefs No. 37-2007. Ithaca, NY.

Clive, J. 2006. Global status of commercialized biotech/GM crops: 2006. Highlights of International Service for the Acquisition of Agri-Biotech Applications (ISAAA) Briefs No. 36-2006. Ithaca, NY.

Clive, J. 2005. Global status of commercialized biotech/GM crops: 2005. Highlights of International Service for the Acquisition of Agri-Biotech Applications (ISAAA) Briefs No. 34-2005. Ithaca, NY.

Clive, J. 2004b: Global Status of Commercialized Biotech/GMO Crops: 2004. ISAAA Briefs No. 32- 2004: Ithaca, NY.

Clive, J. 2004a: Global Review of Commercialized Transgenic Crops: 2003. Feature: Bt Maize. ISAAA *Briefs* No. 29 – 2003: Ithaca, NY.

Conner, A. J., Glare, T. R., Nap, J. P. 2003: The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment. *Plant Journal,* **33**: 19–46.

Cox, B., Atkins, P., Shields, E., Bergstrom, G. 2009: Field-Scale Studies Comparing the Base Genetics of Corn Hybrids with Double and Triple-Stacked Hybrids. *Newsletter for New York Field Crops and Soils,* **19**: 1-20.

Cozzolino, D., Fassio, A., Restaino, E., Fernandez, E., La Manna, A. 2008: Verification of silage type using near-infrared spectroscopy combined with multivariate analysis. *Journal of Agriculture and Food Chemistry,* **56:** 79-83.

Danson, J. W., Kimani, M., Mbogori, M. 2006: Detection of *Bacillus Thuringiensis* Genes in Transgenic Maize by the PCR Method and FTA Paper Technology. *African Journal of Biotechnology,* **5**: 2345-2349.

De Groote, H. 2002: Maize yield losses from stem borers in Kenya. *Insect Science Applications,* **22**: 89-96.

De Groote, H. and Mugo, S. 2005: Can GM crops alleviate poverty in Africa? Evidence from Bt maize research in Kenya, Paper presented at the 9th International Conference on Agricultural Biotechnology: Ten Years After, organized by the Internatioal Consortium on Agricultural Biotechnology Research (ICABR), Ravello (Italy) July 6 - 10, 2005.

De Groote, H., Mugo, S., Bergvinson, D., Odhiambo, B. 2004: Debunking the myths of GM crops for Africa: The case of Bt maize in Kenya. Paper prepared for presentation at the American Agricultural Economics Association. Annual Meetings, August 1-4 2004, Denver Colorado. Version 3, DRAFT - May 17, 2004.

De Maagd, R. A., Bravo, A., Crickmore, N. 2001: How Bacillus thuringiensis has evolved specific toxins to colonize the insect world. *Trends in Genetics,* **17**: 193–199.

De Schrijver, A., Devos, Y., Van den Bulcke, M., Cadot, P., De Loose, M., Reheul, D., Sneyers, M. 2007: Risk assessment of GM stacked events obtained from crosses between GM events. *Trends in Food Science and Technology*, **18:** 101-109.

Devos, Y., Reuhel, D., De Schrijver, A. 2005: The co-existence between transgenic and nontransgenic maize in the European Union: a focus on pollen flow and cross-fertilization; *Environmental Biosafety Research,* **4:** 71-87.

Dien, B. S., Bothast, R. J., Iten, L. B., Barrios, L., Eckhoff, S. R. 2002: Fate of Bt protein and influence of corn hybrid on ethanol production. *Cereal Chemistry,* **79:** 582–585.

Domingo, J. L. 2000: Health risks of genetically modified foods: Many opinions but few data. *Science,* **288:** 1748-1749.

Dong, W., Yang, L, Shen, K., Kim, B., Kleter, G. A., Marvin, H. J P., Guo, R., Liang, W., Zhang, D. 2008: GMDD: a database of GMO detection methods; BioMed Central Ltd. http://www.biomedcentral.com/1471-2105/9/260 (accessed on 20 April 2010).

EC. (European Commission Regulation) 1829/2003 and1830/2003, 2003: *Official Journal of European Communities,* **L268:** 1-28.

EC. (European Commission) 2000: Validation of analytical methods for the identification and determination of genetically modified organisms (GMOs) in food and food ingredients. Final report of the EC. Food Products and Consumer Goods Unit, DG Joint Research Centre, Institute for Health and Consumer Protection, Ispara, Italy.

EC-CRL. (European Commission-Commission Regulation License) 2005. Event-specific method for the quantification of maize line DAS-59122-7 using real-time PCR. Validation report. Biotechnology & GMOs Unit, DG Joint Research Centre, Institute for Health and Consumer Protection, Ispara, Italy.

EFSA. (European Food Safety Authority) 2006: Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to genetically modified crops (Bt176 maize, MON810 maize, T25 maize, Topas 19/2 oilseed rape and Ms1xRf1 oilseed rape) subject to safeguard clauses invoked according to Article 16 of Directive 90/220/EEC: *European Food Safety Authority Journal*, **338**: 1-15.

Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R., Schwagele, F., Jahreis, G., Flachowsky, G. 2001: The fate of forage DNA in farm animals: A collaborative case-study investigating cattle and chicken fed recombinant plant material. *European Food Research and Technology,* **212**: 129–134.

EnviroLogix Inc. 2003: CryIAb/c Plate Kit EnviroLogix 500. Riverside Industrial Parkway, Portland, Maine 410301418, USA. Available from http://www.envirologix.com/library/ap003insert.pdf (accessed 30 December 2009).

Ertugrul, A., Budak, H., Cetiner, S. 2008: The DNA based qualitative and quantitative analysis of genetically modified products in Turkish market. The 1st Global Conference on GMO Analysis (organized by the Joint Research Centre of the European Commission) in Villa Erba, Como, Italy. 24-27 June 2008. (http://gmoglobalconference.jrc.it). (accessed 20 April 2010).

Eurobarometer 58.0. Europeans and Biotechnology in 2002: http://europa.eu.int/comm/publicopinion/archives/ebs/ebs177en.pdf (accessed October 1, 2010).

Europa, 2004: New regulations concerning the traceability and labelling of geneticallymodified organisms (GMOs). Available at http://europa.eu.int/scadplus/ leg/en/lvb/l21170.htm (accessed 12 January 2009).

FAO. (Food Agricultural Organisation of the United Nations) 2002: Gene flow from GM to non-GM populations in the crop, forestry, animal and fishery sectors, Background document to Conference 7: May 31 - July 6, 2002; Electronic Forum

on Biotechnology in Food and Agriculture, Food and Agriculture Organization of the United Nations (FAO).

FAO (Food Agricultural Organisation of the United Nations) 2008: Soaring food prices put further pressure on African agriculture, FAO Director-General Jacques Diouf address at the 25th FAO Regional Conference for Africa. Nairobi: Food and Agriculture Organization.

Ferré, F., and Van-Rie, J. 2002: Biochemistry and genetics of insect resistance to *Bacillus thuringiensis. Annual Review of Entomology,* **47**: 501-533.

Fischer, A. A., Laing, J.E., Stockel, J. E., Townsend, J. W. 1991: *Handbook for Family Planning Operations Research Design.* Population Council New York, pp. 45.

Food Standards Australia New Zealand, 2005: Labelling genetically modified foods. Available at http://www.foodstandards.gov.au/mediareleasespublications/factsheet/factsheets2000 (accessed 5 September 2010).

Foster, M., Berry, P., Hogan, J. 2003: *Market access issues for GM Products: Implications for Australia.* Australian Bureau of Agricultural and Resource Economics (ABARE), Canberra, Publication 12559; pp. 67.

Global Knowledge Center on Crop Biotechnology 2005: Global status: International approaches to labelling. Available at http://www.isaaa.org/kc (accessed 3 February 2009).

Gobeil, F., Hottin, L., Coenen, K., Brunelle, F., Michaud, D. 2008: Recombinant DNA material in foods: a preliminary assessment of the situation in Quebec, Canada. The 1st Global Conference on GMO Analysis (organized by the Joint Research Centre of the European Commission) in Villa Erba, Como, Italy. 24-27 June 2008. (http://gmoglobalconference.jrc.it) (accessed 20 April 2010).

GOK. (Government of Kenya) 2009: Biosafety Law, Government of Kenya, Government Press, Nairobi, Kenya.

Halford, C. and Halford, N. 2003: *Genetically Modified Crops.* Imperial College Press, pp. 300.

Health Canada, 2003: Frequently Asked Questions: Biotechnology and Genetically Modified Foods Part 3: Labelling of Novel Foods Derived Through Genetic Engineering. Available at http://wwwhc-sc.gc.ca/fnan/gmf-agm/fs-if/fa3e.html#2 (accessed 22 September 2010).

Heide, B. R., Heir, E., Holck, A. 2008: Detection of eight GMO maize events by qualitative, multiplex PCR and fluorescence capillary gel electrophoresis. *European Food Research and Technology*, **227**: 527-535.

Hellmich, R. L., Siegfried, B. D., Sears, M. K., Stanley-Horn, D. E., Daniels, M. J., Mattila, H. R., Spencer, T., Bidne, K. G., Lewis, L. C. 2001: Monarch larvae sensitivity to Bacillus thuringiensis-purified proteins and pollen. *Proceedings of the National Academy of Science of the United State of America,* **98:** 11925–11930.

Helmilth, L. 2000: Biotechnology: Both sides claim victory in Trade Pact. *Science,* **287**: 782-783.

Henry, C., Morgan, D., Weekes, R., Daniels, R., Boffey, C. 2003: Farm Scale Evaluations of GM Crops: Monitoring Gene Flow from GM Crops to Non-GM Equivalent Crops in the Vicinity. Contract Reference EPG 1/5/138. Final Report 2000/2003. URL: http://www.defra.gov.uk/environment/gm/research/pdf/epg_1-5-138.pdf (accessed 10 March 2010).

Hernandez, M., Rodriguez-Lazaro, D., Ferrando, A. 2005: Current methodology for detection, identification and quantification of genetically modified organisms. *Current Analytical Chemistry,* **12:** 203-221.

Hino, A. 2002: Safety assessment and public concerns for genetically modified food products: The Japanese experience. *Toxicopathology,* **30**: 126–128.

Holst-Jensen, A., Rønning, S. B., Løvseth, A., Berdal, K. G. 2003: PCR technology for screening and quantification of genetically modified organisms (GMOs), *Analytical and Bioanalytical Chemistry,* **375:** 985-993.

Hurburgh, C. R., Rippke, G. R., Heithoff, C., Roussel, S. A., Hardy, C. L. 2000: Detection of genetically modified grains by near-infrared spectroscopy. Proceedings PITTCON 2000–Science for the 21st Century, #1431. New Orleans, La. 12–17 March 2000. Available at http://pittcon24857.omnibooksonline.com (accessed 20 March 2010).

Ingram, J. 2000: Report on the Separation Distances Required to Ensure Cross-Pollination is Below Specified Limits in Non-Seed Crops of Sugar Beet, Maize and Oilseed Rape. Report Commissioned by the UK Ministry of Agriculture, Fisheries and Food (MAFF). Project No. RG0123 URL: http://www.agindustries.org.uk/scimac/otherdoc/NIABSepDistReview.pdf (accessed on 20 February 2010).

Jaffe, G. 2004: Regulating transgenic crops: a comparative analysis of different regulatory processes. *Transgenic Research,* **13:** 5–19.

Janni, J., Weinstock, B. A., Hagen, L., Wright, S. 2008: Novel near-infrared sampling apparatus for single kernel analysis of oil content in maize. *Applied Spectroscopy,* **62:** 423-6.

Jarosz, N., Loubet, B., Durand, B., Foueillassa, R. X., Hube, R. L. 2005: Variations in maize pollen emission and deposition in relation to microclimate. *Environmental Science and Technology*, **39**: 4377–4384.

Jarosz, N., Loubet, B., Durand, B., McCartney, A., Foueillassar, X., Huber, L. 2003: Field measurements of airborne concentration and deposition rate of maize pollen. *Agricultural and. Forest Meteorology,* **119**: 37–51.

Jasbeer, K., Ghazali, F. M., Cheah, Y. K., Son, R. 2008: Application of DNA and Immunoassay Analytical Methods for GMO Testing in Agricultural Crops and Plant -Derived Products. *Association South East Asian Nations Food Journal,* **15**: 1-25.

Kimenju, S. C., De Groote, H., Karugia, J., Mbogoh, S., Poland, D. 2005: Consumer awareness and attitudes toward GM foods in Kenya. *African Journal of Biotechnology,* **4:** 1066-1075.

Kimio, M., Rie, A., Hisatsugu, I., Masaki, S., Naomichi, U., Kazue, S., Hideo, K., Takeshi, M., Akihiro, H. 2004: Detection of Genetically Modified Organisms from Food Samples Obtained. *Journal of the Food Hygienic Society of Japan,* **45:** 184-190.

Kitch, L., Koch, M., Sithole-Niang, I. 2002: Crop biotechnology. A working paper for administration and policy makers in sub-Saharan Africa. FAO sub-Regional Office, Harare, Zimbabwe, pp. 55.

Kitta, K., Mano, J., Furui, S., Hino, A. 2009: The development of detection methods for the monitoring of GMO in Japan. INRA/AFFRCS French-Japanese Seminar on Food safety, novel food, and sustainable environment to promote animal and human health, INRA, Versailles-Paris, France, June 8-10, 2009.

Kohli, A., Twyman, R. M., Abranches, R., Wegel, E., Stoger, E. Christou, P. 2003. Transgene integration, organization and interaction in plants. *Plant Molecular Biology,* **52**: 247–258.

Kothari, C. K. 2001: *Research Methodology: Methods and Techniques*. Fourth Edition: 2001. Wiley Eastern Limited, New Delhi, pp. 468 (331-367).

Kuiper, H. A., Kleter, G. A., Noteborn, H. P. J. M., Kok, E. J. 2001: Assessment of the food safety issues related to genetically modified foods. *Plant Journal,* **27**: 503–528.

Kuribara, H., Shindo, Y., Matsuoka, T., Takubo, K., Futo, S., Aoki, N., Hirao, T., Akiyama, H., Goda, Y., Toyoda, M., Hino, A. 2002: Novel reference molecules for quantification of genetically modified maize and soybean; *Journal of the Association of Official Agricultural Chemists, International,* **85**: 1077-1089.

Kyrova, V., Ostry, V., Laichmannova, L., Ruprich, J. 2008: GENOMON - Specialized monitoring of GM foodstuffs in the Czech Republic during 2002-2007. The 1st Global Conference on GMO Analysis (organized by the Joint Research Centre of the European Commission) in Villa Erba, Como, Italy. 24-27 June 2008. (http://gmoglobalconference.jrc.it (accessed 20 April 2010).

Lee, S. H., Min, D. M., Kim, J. K. 2006: Qualitative and quantitative polymerase chain reaction analysis for genetically modified maize MON863. *Journal of Agriculture and Food Chemistry,* **54**: 1124-1129.

Lipton, C. R., Dautlick, J. X., Grothaus, G. D., Hunst, P. L., Magin, K. K. M., Mihaliak, C. A. 2000: Guidelines for the validation and use of immunoassays for determination of introduced proteins in biotechnology enhanced crops and derived food ingredients. *Food and Agricultural Immunology*, **12**: 153–164.

Logan, J., Edwards, K., Saunders, N. (Eds). 2009: *Real-Time PCR: Current Technology and Applications.* Caister Academic Press, pp. 23-46.

Lutz, B., Wiedemann, S., Albrecht, C. 2006: Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. *Journal of Animal Physiology and Animal Nutrition,* **90:** 116–123.

Lyon, E., Wittwer, C. T. 2009: LightCycler Technology in Molecular Diagnostics. *J. Molecular Diagnostics,* **11:** 93-101.

Ma, B. L., Subedi, K. D., Reid, L. M. 2004: Extent of cross-fertilization in maize by pollen from neighbouring transgenic hybrids. *Crop Science*, **44**: 1273–1282.

Mace, S. E., Hutokshi, K. B., Crouch, J. H. 2003: A High-Throughput DNA Extraction Protocol for Tropical Molecular Breeding Programs. *Plant Molecular Biology Reporter,* **21:** 459a–459h.

Magin, K., Mihaliak, C., Somerville, L., Privalle, L., Charlton, S., Porter, L. 2000: Methods for detection of GMO grain in commerce. American Crop Protection Association, http://www.acpa.org (accessed on 10 March 2010).

Malone, L. A., Burgess, E. P. J., Gatehouse, H. S., Voisey, C. R., Tregidga, E. L., Bruce, A. P. 2001: Effects of ingestion of a *Bacillus thuringiensis* toxin and a trypsin inhibitor on honey bee flight activity and longevity. *Sciences Apidologie,* **32:** 57–68.

Mano, J., Shigemitsu, N., Futo, S., Akiyama, H., Teshima, R., Hino, A., Furui, S., Kitta, K. 2008: Real-Time PCR Array as a Universal Platform for the Detection of Genetically Modified Crops and Its Application in Identifying Unapproved

Genetically Modified Crops in Japan. *Journal of Agricultural and Food Chemistry,* **57:** 26-37.

Margarit, E., Reggiardo, M. I., Vallejos, R. H., Permingeat, H. R., 2006: Detection of Bt. transgenic maize in foodstuffs. *Food Research International,* **39:** 250–255.

Melé, E., Peñas, G., Serra, J., Salvia, J., Ballester, J., Bas, M., Palaudelmàs, M., Messeguer, J. 2005: Quantification of pollen gene flow in large maize fields by using a kernel colour trait. In: 2nd International Conference on Coexistence Between GM and Non-GM Based Agricultural Supply Chains, Montpellier, 14–15 November 2005 (Messéan A., Ed.), Montpellier: Agropolis Productions; pp. 289–291.

Messeguer, J., Peñas, G., Ballester, J., Bas, M., Serra, J., Salvia, J., Palaudelmàs, M., Melé, E. 2006: Pollen-mediated gene flow in maize in real situations of coexistence. *Plant Biotechnology Journal,* **4:** 633-645.

Michelini, E., Simoni, P., Cevenini, L., Mezzanotte, L., Roda, A. 2008: New trends in bioanalytical tools for the detection of genetically modified organisms: an update. *Analytical and Bioanalytical Chemistry,* **392:** 355-67.

Miraglia, M., Berdal, K. G., Brera, C., Corbisier, P., Holst-Jensen, A., Kok, E. J., Marvin, H. J., Schimmel, H., Rentsch, J., van Rie, J. P., Zagon, J. 2004: Detection and traceability of genetically modified organisms in the food production chain. *Food Chemistry and Toxicology,* **42**: 1157-1180.

Monnerat, R., Martins, E., Queiroz, P., Ordu´z, S., Jaramillo, G., Benintende, G., Cozzi, J., Real, M. D., Martinez-Ramirez, A., Rausell, C., Cero´n, J., Ibarra, J. E., Del Rincon-Castro, M. C., Espinoza, A. M., Meza-Basso, L., Cabrera, L., Sa´nchez, J., Soberon, M., Bravo, A. 2006: Genetic Variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) Populations from Latin America is Associated with Variations in Susceptibility to *Bacillus thuringiensis* Cry Toxins. *Journal of Applied and Environmental Microbiology,* **72:** 7029–7035.

Mugo, S., Murenga, M., Taracha, C., Songa, J., Gichuki, S., Tende, R., Karaya, H., Bergvinson, D., Pellegrineschi, A., Hoisington, D.. 2011a: Testing public Bt maize event for control of stem borers in the first confined field trials in Kenya. *African Journal of Biotechnology,* **10:** 4713-4718.

Mugo, S., Mwimali, M., Karaya, H., Tende, R., Taracha, C., Gichuki, S., Ininda, J., Kinyua, M., Chavangi, A. 2011b: Control of Busseola fusca and Chilo Partellus stem borer by Bacillus thuringiensis (Bt) – endotoxin from *cry1Ab* gene Event MON810 in greenhouse containment trials. *African Journal of Biotechnology,* **10:** 4719-4723.

Mugo, S., Gichuki, S., Murenga M., Taracha, C., Macharia, H. 2011c: Experiences with the biosafety regulatory system in Kenya during the introduction, testing and development of Bt maize. *African Journal of Biotechnology,* **10**: 4682-4693.

Mugo, S., Gethi, J., Ouma, J. O., Ajanga, S., Beyene, J., Murenga, M., Mulaa, M., Likhayo, P., Gichuki, S., Kega, V., De Groote, H., Chavangi, A. (Eds). 2008: Book of abstract of the "Consolidating Experiences from IRMA I and II: Achievements, Lessons and Prospects", IRMA Project End-of-Phase II Conference, 28-30 Oct 2008, Nairobi, Kenya: IRMA Document No. 35: KARI and CIMMYT.

Mugo, S., De Groote, H., Bergvinson, D., Mulaa, M., Songa, J., Gichuki, S. 2005: Developing Bt. maize for resource-poor farmers – Recent advances in the IRMA project. *African Journal of Biotechnology,* **4:** 1490-1504.

Mugo, S., De Groote, H., Songa, J., Mulaa, M., Odhiambo, B., Taracha, C., Bergvinson, D., Hoisington, D., Gethi, M. 2004a: Advances in Developing Insect Resistant Maize Varieties for Kenya within the Insect Resistant Maize For Africa (IRMA) Project. *In* Palmer F. E. (Ed.): Integrated Approaches to Higher Maize Productivity in the New Millennium. Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference, Nairobi, Kenya, 11 - 15 February 2002. Mexico, D. F.: CIMMYT, pp. 31-37.

Mugo, S., Taracha, C., Bergvinson, D., Odhiambo, B., Songa, J., Hoisington, D., McLean, S., Ngatia, I., Gethi, M. 2004b: Screening cry proteins produced by Bt maize leaves for activity against Kenyan maize stem borers. *In* Friesen D. K., Palmer A. F. E. (Eds.): Integrated Approaches to Higher Maize Productivity in the New Millennium. Proceedings of the 7th Eastern and Southern Africa Regional

Maize Conference, Nairobi, Kenya, 11 - 15 February 2002. Mexico, D. F.: CIMMYT, pp. 102-105.

Mugo, S., Bergvinson, D., Hoisington, D. 2001: Options in Developing Stem borer-Resistant Maize: CIMMYT Approaches and Experiences. *Insect Science Applications* **21**: 409-415.

Murenga, M., Mugo, S., Gichuki, S., Taracha, C. 2008: Manual for Biosafety Level II Greenhouse for Research on Transgenic Plants at KARI Biotechnology Centre. Insect Resistant Maize for Africa (IRMA) Project. IRMA Project Document No. 14. Nairobi, Kenya: KARI and CIMMYT.

NCST. (National Council for Science and Technology) 1998: Regulations and guidelines for Biosafety in biotechnology for Kenya. National Council for Science and Technology, Nairobi, Kenya.

Njau, P. N., Kinyua, M. G., Pathak, R. S., Kimurto, P. 2001: Biotechnology in quick response to bread wheat breeding in Kenya. Proceedings of the first Egerton- KARI Annual Symposium. 2001.

Paarlberg, R. L. 2008: *Starved for Science: how biotechnology is being kept out of Africa.* Harvard University Press, Cambridge, pp. 235 (111-112).

Partridge, M. and Murphy, D. J. 2004: Detection of genetically modified soya in a range of organic and health food products. *British Food Journal,* **106**: 166-180.

Permingeat, H., Reggiardo, M. I., Vallejos, R. H. 2002: Detection and quantification of transgenes in grains by multiplex and real-time PCR. *Journal of Agricultural and Food Chemistry*, **50:** 4431–4436.

PIFB. (Pew Initiative on Food and Biotechnology) 2003: Public sentiment about genetically modified food. Awareness of genetically modified foods remains low. Available at http://www.pewagbiotech.org/polls (accessed 20 February 2009).

PIFB. (Pew Initiative on Food and Biotechnology) 2007: Applications of Biotechnology for Functional Foods, Pew Initiative on Food and Biotechnology, Brussels.

Pirondini, A., Bonas, U., Maestri, E., Visioli, G., Marmiroli, M., Marmiroli, N. 2010: Yield an amplifiability of different DNA extraction procedures for traceability in the dairy food chain. *Food Control,* **21:** 663-668.

Pleasants, J. M., Hellmich, R. L., Dively, G. P., Sears, M. K., Stanley-Horn, D. E., Mattila, H. R., Foster, J. E., Clark, P., Jones, G. D. 2001: Corn pollen deposition on milkweeds in and near cornfields, *Proceedings of the National Academy of Science of the United State of America,* **98:** 11919–11924.

Pöpping, B. 2001: Methods for the detection of genetically modified organisms: Precision, pitfalls and proficiency Available at http://www. American clinicallaboratory.com/articles/al/a0102pop.pdf (accessed 20 April 2010).

Pryme, I. F. and Lembecke, R. 2003: In vivo studies on possible health consequences of genetically modified food and feed with particular regard to ingredients consisting of genetically modified plant materials. *Nutrition and Health,* **17**: 1–8.

Purseglove, J. W. 1972: *Tropical Crops. Monocotyledons.* Vol I. London: Longman Group, pp. 334.

Querci, M., Jemini, M., Van Den Eede, G. 2004, *The Analysis of Food Samples for the Presence of Genetically Modified Organisms* – Institute for Health and Consumer Protection of the Joint Research Centre of the European Commission.

Quist, D. and Chapela, I. H. 2002: Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico, *Nature,* **414:** 541–543.

Randhawa, G. J. and Firke, P. K. 2006: Detection of transgenes in genetically modified soybean and maize using polymerase chain reaction. *Indian Journal of Biotechnology,* **5:** 510-513.

Roh J. Y., Choi J. Y., Li M. S., Jin B. R., Je Y. H., 2007: "Bacillus thuringiensis as a specific, safe, and effective tool for insect pest control". *Journal of Microbiology and Biotechnology,* **17**: 547–559.

ROK (Republic of Kenya), 2008: National Biotechnology Awareness Strategy 2008 - 2013. Government Printers, Nairobi, Kenya.

Rule, S., and Langa, Z. 2005: Public understanding of Biotechnology in South Africa. HSRC client survey 2004. Report to Public Understanding of Biotechnology. Available at http://www.pub.ac.za/resources/research.html (accessed 4 October 2010).

Sa´zelova, P., Kasˇicˇka, V., Iba´n˜ez, E., Cifuentes, A. 2009: Extraction and separation of water-soluble proteins from Bacillus thuringiensis transgenic and non-transgenic maize species by CZE. *Journal of Separation Science.* **32**: 3801–3808.

Santella, R. M. 2006: Approaches to DNA/RNA Extraction and Whole Genome Amplification. *Cancer Epidemiology Biomarkers Preview,* **15:** 1585-7.

Scott, M. P. and Pollak, L. M. 2005: Transgenic Maize. WILEY-VCH Verlag GmbH & Co. KGaA, *Starch/Starke,* **57:** 187-195.

Sharma, H. C., Crouch, J. H., Sharma, K. K., Seetharama, N., Hash, C. T. 2002: Applications of crop biotechnology for crop improvement: Prospects and constraints. *Plant Science,* **163:** 381-395.

Sithole-Niang, I. 2001: The Future of Plant Science in Zimbabwe. *Trends in Plant Science,* **6**: 493-494.

Sithole-Niang, I., Mujaji, C., Chikosi, E. 2004: Inventory on plant breeding and agro biotechnology activities and capacities in Zimbabwe. A study Commissioned by the International Institute for Tropical Agriculture, pp. 29.

Smyth, S., Khachatourians, G. G., Phillips, P. W. B. 2002: Liabilities and economics of transgenic crops. *Nature Biotechnology,* **20**: 537-541.

Sorina, P., Leprince, F., Ioja-Boldura, O., Măruțescu, A., Sabău, I., Marcela, B. E 2010. Quantification of pollen mediated gene flow in maize. *Romanian Biotechnological Letters*, **15**: 5351-5360.

South African Grain Laboratory, 2005: SA averages – maize analysis. Available at http://www.sagl.co.za/maize_sa_averages.aspx (accessed 3 October 2010).

Stanley-Horn, D. E., Dively, G. P., Hellmich, R. L., Mattila, H. R., Sears, M. K., Rose, R., Jesse, L. C. H., Losey, J. E., Obrycki, J. J., Lewis, L. 2001: Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies, *Proceedings of the National Academy of Science of the United State of America,* **98**: 11931–11936.

Tabashnik, B. E, Carrière, Y., Dennehy, T. J., Morin, S., Sisterson, M. S., Roush, R. T., Shelton, A. M., Zhao, J. Z. 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. *Journal of Economic Entomology,* **96**: 1031-1038.

Tende, R. M., Nderitu, J. H., Mugo, S., Songa, J. M., Olubayo, F., Bergvinson, D. 2005: Screening for development of resistance by the spotted stem borer, *Chilo Partellus* Swinhoe (Lepidoptera: Pyralidae) to Bt-maize delta-endotoxins. African Crop Science Conference Proceedings, *African Crop Science Society,* **7:** 1241-1244.

Tengel. C., Schler, P., Setzke, E., Balles, J., Sprenger-Hauels, M. 2001: PCR-based detection of genetically modified soybean and maize in raw and highly processed foodstuffs. *BioTechniques,* **31**: 426-429.

Terry, C. F., Harris, N., Parkes, H. C. 2002: Detection of genetically modified crops and their derivatives: critical steps in sample preparation and extraction. *Journal of the Association of Official Agricultural Chemists, International,* **85**:768–774.

The Center for Food Safety 2005: Genetically engineered crops and foods: Worldwide regulation and prohibition. Available at http://www.centerforfood safety.org (accessed 12 September 2010).

Tozzini, A. C., Martínez, C. M., Lucca, M. F., Rovere, C. V., Distéfano, A. J, del Vas M, Hopp, H. E. 2000: Semi-quantitative detection of genetically modified grains based on CaMV 35S promoter amplification*.* *Electronic Journal of Biotechnology,* **3**: 2.

Trostle, R. 2008: Global Agricultural Supply and Demand: Factors Contributing to the Recent Increase in Food Commodity Prices. A Report from the Economic Research Service. *United States Department of Agriculture,* **WRS-0801:** 1-30.

USDA. (United States Department of Agriculture) 2002: The National Organic Program. Program standards. Available at http://www.ams.usda.gov/nop/ NOP/standards.html (accessed at 21 September 2010).

Van den Bulcke, M., Leunda, A. C., De Bernardi, D, De Schrijver, A., Mbongo-Mbella, G., Sneyers, M. 2005: *Detection of genetically modified crops in real-time practice: a state of the art*. Ravello, Italy: Internatioal Consortium on Agricultural Biotechnology Research (ICBAR).

Van Duijn, G., Van Biert, R, Bleeker-Marcelis, H., Van Boeijen, I., Adan, A. J., Jhakie, S., Hessing, M. 2002: Detection of genetically modified organisms in foods by PCR- and DNA-based techniques: bridging the methods. *Journal of the Association of Official Agricultural Chemists, International,* **85:** 768–774.

Viljoen, C. D., Dajee, B. K., Botha, G. M. 2006: Detection of GMO in food products in South Africa: Implications of GMO labelling. *African Journal of Biotechnology,* **5**: 073-082.

Vitale, J., Harvey, G, Greenplate, J., Abdennadher, M., Traoré, O. 2008: Second-Generation Bt Cotton Field Trials in Burkina Faso: Analyzing the Potential benefits to West African Farmers. *Crop Science,* **48:** 1958-1966.

Wolfenbarger, L.L. and Phifer, P. R. 2000: "The ecological risks and benefits of genetically engineered plants". *Science,* **290**: 2088–2093.

Wraight, C. L., Zangeri, A. R., Carroll, M. J., Berenbaum, M. R. 2000: Absence of toxicity of Bacillus thuringiensis pollen to black swallowtails under field conditions. *Proceedings of the National Academy of Science of the United State of America*, **97:** 7700–7703.

Yang, L., Guo, J., Zhang, H., Liu, J., Zhang, D. 2008: Qualitative and quantitative event-specific PCR detection methods for oxy-235 canola based on the 3' integration flanking sequence. *Journal of Agriculture and Food Chemistry,* **56**: 1804-1809.

Yang, L., Pan, A., Zhang, K., Yin, C., Qian, B., Chen, J., Huang, C., Zhang, D. 2005: Qualitative and quantitative PCR methods for event-specific detection of genetically modified cotton Mon1445 and Mon531. *Transgenic Resources,* **14**: 817-831.

Yoke-Kqueen, C; Yee-Tyan, C.; Siew-Ping, K., Son, R. 2011: Development of multiplex- PCR for Genetically Modified Organism (GMO) detection targeting EPSPS and Cry1Ab genes in soy and maize samples. *International Food Research Journal,* **18**: 512-519.

Zangerl, A. R., McKenna, D., Wraight, C. L. Carroll, M., Ficarello, P., Warner, R., Berenbaum, M. R. 2001: Effects of exposure to event 176 Bacillus thuringiensis corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proceedings of the National Academy of Science of the United State of America,* **98:** 11908–11912.

APPENDIX

APPENDIX 1: Extraction Solutions

1 CTAB Extraction Buffer

- % CTAB extraction buffer (1 lt) was prepared from;
- 100 mM Tris–HCl (100 ml of 1 M Tris-HCL, PH8.0)
- 20 mM EDTA (40 ml of 0.5 M EDTA, PH 8.0)
- M NaCl (280 ml of 5 M NaCl).
- % (w/v) CTAB (30 g) using this formula: $c_1v_1 = c_2v_2$
- It was made up to 950 ml with de-ionized water and adjusted to PH 8.0 using HCL and adjusted the total volume to 1 lt with de-ionized water. On day of extraction 0.17% β-mercaptoethanol was added. (i.e. for 15 ml = 25.5 µl). Preheated the buffer at 65^0C

2 Chloroform: Isoamyl Alcohol (24:1) (100 ml)

- Chloroform 48 ml
- Isoamyl Alcohol 2 ml
- Isopropanol (stored at -20^0C
- Low salt TE Buffer PH8.0 (1 lt)
- 10 mM Tris (10 ml of 1 M Tris-HCL, Ph 8.0)
- mm EDTA (500 µl of 0.5 M EDTA, PH 8.0)

- Made up to 950 ml with deionised water and adjusted to PH 8.0 using HCL and total volume adjusted to 1 lt. 70% Ethanol (stored at -20^0C) (Ethanol absolute 70 ml, adjusted to 100 ml with double distilled water).

3 Components of the Gel

- Agarose
- Ethidium Bromide.
- Electrolyte (Buffer- TAE = tris acetate EDTA or TBE tris borate EDTA)

4 Equipment of Electrophoresis

- Gel tank connected to the power supply (220V).
- Casting tray.
- Combs (depending on the number and size of wells to be used).