The expression of δ-endotoxins in generations of crosses of tropical inbred lines transformed with Bacillus thuringiensis genes

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A dissertation submitted to the Faculty of Agriculture, University of Nairobi in partial fulfillment of the requirements for the award of a degree in Master of Science in Plant Breeding.

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2008
DECLARATION

The research reported in this thesis is my original work and has not been presented for a degree in any other University.

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Date

This thesis has been submitted for examination with our approval as University Supervisors.

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Very special thanks to my wife Anne A. Mwimali and our sons; Mutola Victor Mwimali, Wesonga Stanley Mwimali, and our daughters Osundwa Jessica Mwimali and Mwenje Agnes Mwimali! You are my greatest source of positive hope and inspiration! May you live long and study beyond what your Dad has done! God bless you all!
ACKNOWLEDGEMENTS

Few people in life honestly reflect on who they are, how they got where they are, and what they think they might do as well. They soon learn that it is a debt to others that spans written history. The work of some unknown persons makes our lives easier everyday. I believe it is appropriate to acknowledge these unknowns persons; but is also necessary to acknowledge those we know have directly shaped our lives and our work.

Adversity is often one of our best teachers. Even the people that somehow gripped our meanest imagination, violated us in some way, even those people are their honor for what they have taught us. I wish to acknowledge all for their role in my life. However, this page is specifically designed to note my appreciation of those persons who stand out most notably in my mind as contributing to the content of what you will find in this Master of Science dissertation.

I would wish to kindly appreciate the steadfast support I have personally received from my supervisors Drs. Githiri S.M., Mugo S.M., Danson J. W. and Olubayo F.M. who urged me on by way of their untiring technical support, guidance, comments and encouragement during my MSc (Genetics and Plant breeding) training. Thank you Wanjala W.B. and Mwasame E.N. for the ‘hands-on’ applications of the plant molecular biology techniques and Gikonyo P., Atieno B. and Apale E. for crop management at the biosafety greenhouse complex without whom there would be no data to report about, and Ngeny J. and Chavangi A. for their assistance with experimental design and some statistical analysis Thanks again! Mr. Matata J.B. you made it easy for me! Thanks a lot!
I would wish to thank and acknowledge The Director, Kenya Agricultural Research Institute (KARI) and the Director General, International Maize and Wheat Improvement Center (CIMMYT) for supporting my research project through the Syngenta Foundation for Sustainable Agriculture (SFSA) supported Insect Resistant Maize for Africa (IRMA) project.

Most of all I would wish to thank my parents Joel Murenga Mutola and Kanaiza Elizabeth, and all my sisters (Mwenje Carolyne. P., Sangula Lilian., Ochutsi Jacklyne., Leya Christine., and Adisa Rose), and brothers (Ong’ato Phanuel. and Wandalo Eric.). You are great to me and God bless you! I thank God for the all the energy, strength, peace, guidance and grace He gave to me to undertake this project to completion.
ABSTRACT
Maize (Zea mays L.) is the third most important cereal grain globally, after wheat and rice, and a significant contributor of food, feed and industrial uses. Infestation and damage by the spotted stem borer, Chilo partellus (Swinhoe) and other stem borer species in Kenya is a major constraint in maize production. The use of genetic engineering tools for control of to C. partellus that utilizes genes derived from Bacillus thuringiensis (Bt) (Berliner), that has been used successfully in other countries has been proposed for Kenya. This study was carried out to determine the differences in the levels of Cry1Ab protein (Bt δ-endotoxins) expression among the parental maize lines transformed using Bt genes carrying events 216 and 223 and the parental non-transformed maize inbred lines CML144 and CML159 and their F1 and F2:3 generations. The crosses involved tropical Bt and non-Bt maize inbred lines. The responses to the control of C. partellus were done using insect bioassays, while the levels of expression of Bt δ-endotoxins among the generations were done using enzyme linked immunosorbent assays (ELISA) and other molecular methods. Bt maize lines carrying events 216 and 223 and non-Bt maize inbred lines CML144 and CML159 were used to form the respective generations. The parental lines, F1 and F2:3 generations were evaluated using; (a) whole plant-insect bioassays to determine the foliar damage ratings, plant height, ear height, number of exit holes, cumulative stem tunnel length, number of larvae recovered, and number of pupae recovered after infestation with C. partellus larvae, (b) differences in the levels of Bt δ-endotoxins expression using dot blot analysis to confirm their presence or absence, and c) enzyme linked immunosorbent assays (ELISA) to quantify the Bt δ-endotoxins in leaf tissue. The treatments consisted parents, their F1 and F2:3 crosses, and four checks. These were replicated 4 times in a 15 x 5 alpha lattice design with each plot containing 4 plants.
The analysis of variance was carried out using PROC GLM of SAS (SAS, 2003) program and the LSD was used for the separation of means.

There were significant differences (p<0.05) among resistant inbred lines (maize lines transformed using Bt genes carrying events 216 and 223) and susceptible inbred lines (CML144, CML159, CML216 and MBR), and the susceptible hybrid lines (CKIR6009 and H513) for foliar damage, number of exit holes, cumulative stem tunnel length, number exit holes, ratio of tunnel length to stem length, and number of larvae recovered and number of pupae recovered. No significant differences (p<0.15) were observed among parents, F₁, and F₃ generations of the crosses of maize lines transformed using Bt genes carrying events 216 and 223. Similarly, no significant differences (p<0.35) were noted among the parents, F₁ and F₃ generations of the non-Bt cross of CML144 x CML159 for all the damage parameters measured. There were significant differences (p<0.05) observed among parents, F₁, and F₃ generations of susceptible x resistant crosses of CML144 x Event 216, CML144 x Event 223, CML159 x Event 216 and CML144 x Event 223. The F₂:₃'s were separated into F₂:₃ resistant and F₂:₃ susceptible based on foliar damage.

The dot blot reactions of Cry1Ab protein extracts from Bt and non-Bt maize and their crosses were used to test for the presence or absence of the protein. Significant differences (p<0.05) were observed among the genotypes used in this study when enzyme linked immunosorbent assays were carried out to determine mean concentrations of Bt δ-endotoxins.

The results from this research experiment reveal that the expression of Cry1Ab proteins (Bt δ-endotoxins) appeared to be stably expressed in the three successive generations of
breeding indicating probably a maintained efficacy and sustainability of the Bt gene and its value in breeding.
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CHAPTER ONE

Introduction

Importance of maize

Maize is the third most important cereal grain in the world, after wheat and rice. Maize contributes substantially to the total cereal grain production in the world economy and trade as food, feed and an industrial grain crop (FAO, 1992; CIMMYT, 1994, 1999). In sub-Saharan Africa, maize is mainly used for human consumption, being a staple food for 50% of the human population (Mugo, 2006). Yields in sub-Saharan Africa (SSA) average 1.4 t/ha, a figure that is 1.0 t/ha below the average for all developing countries (CIMMYT, 1994, 1999). Maize yields in East Africa average 1.5 t/ha while the average farmer in most parts of Kenya gets about 1.1-1.3 t/ha (Mugo, 2006).

The low yields could be attributed to both abiotic (mainly drought) and biotic (diseases, insect pests and weeds) stresses (Waaijenberg, 1994; Wünn et al., 1996; Ghareyzaie et al., 1997; Cheng et al., 1998; Mendelssohn, et al., 2003; Vojtech et al., 2005). The major insect pests of maize include stem borers, the larger grain borer Prostephanus truncatus Horn (Coleoptera: Bostrichidae) and maize weevils Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) (Ampofo and Saxena, 1987; Khan et al., 1997; Mulaa et al., 1999; De Groote, 2002). Field surveys conducted in Kenya between 1995 and 2002 identified five major species of stem borers of economic importance namely; the spotted stem borer (Chilo partellus Swinhoe (Lepidoptera:Crambidae), the African stem borer, Busseola fusca Fuller (Lepidoptera: Noctuidae), the pink stem borer, Sesamia calamistis Hampson (Lepidoptera: Noctuidae), the coastal stem borer, Chilo orichalcociliellus,
Strand (Lepidoptera: Carabidae) and the sugarcane borer, *Eldana saccharina* Walker (Lepidoptera:Pyralidae) (Ajala and Saxena, 1994; Songa *et al.*, 2002). The most widely distributed stem borer species in Kenya are *C. partellus* and *B. fusca* (Figure 1).

Yield losses due to stem borers in maize vary widely in different regions and range from 20-80% depending on the pest population density and phenological stage of the crops at infestation (Grisley, 1997; Songa *et al.*, 2002; Mulaa *et al.*, 2005). An annual loss of 400,000 metric tons of maize estimated at $72 million is incurred due to stem borers in Kenya (De Groote, 2002). This sum represents an average of 14% the farmers' total annual harvest of maize (KARI and CIMMYT, 2003; De Groote *et al.*, 2003, 2004) (Figure 1). The changes in the cropping systems through expansion and intensification of crop monocultures at the expense of natural vegetation worsens the stem borer problems in most farmers fields in sub Saharan Africa (Shelton *et al.*, 2000, 2002; Tang *et al.*, 2000; Songa *et al.*, 2002).
Crop loss from stem borers in Kenya

Figure 1: Maize yield loss (US$ million) from stem borers by agro-ecological zone and species in Kenya. (Source: De Groote et al., 2004)

1.1 Justification
Maize is an important food crop in Kenya. The farmers incur heavy losses from infestation by stem borers. Various methods for the management of stem borers have been suggested namely cultural control, chemical control, biological control and host plant resistance (Kfir et al., 2002). None of these methods has been effective (Kfir et al., 2002; Khan et al., 1997; Songa et al., 2002). Munkvold et al., (1999) showed the efficacy of maize lines transformed using Bt genes in managing both natural and artificial European corn borer (ECB) field infestations by a reduced infestation of over 99 per cent (Alstad et al., 1997).
However, the level of control against late-season maize stem borer infestations varied among events as each event may have produced δ-endotoxins in different parts of the plant (Alstad et al., 1995). There is a potential for rapid development of resistance by target pests (Tabashnik et al., 1993; Ballester et al., 1994; Alstad et al., 1995; Gould, 1998b; Ruud, 1999; Zhaom et al., 2001).

Genetic transformation of plants and other organisms occurs naturally through organisms such as *Agrobacterium tumefaciens* and *Agrobacterium rhizogene* which cause crown gall disease and hairy root syndrome respectively (Gelvin 1994; Chawla, 2002). In the laboratory, the DNA of interest carrying valuable agronomic traits replaces the disease genes (Gelvin 1994; Chawla, 2002). Bt genes are used to confer insect resistance to stem borers in maize. Other methods used for transformation are particle bombardment, which uses tungsten microprojectiles coated with DNA of interest. The particles are accelerated into plant cells and are incorporated at random. Electroporation, microinjection and others are methods other methods used (Gelvin 1994; Chawla, 2002).

Stable integration and expression of transgenes is of great concern in hosts with large genomes (McGauchey and Whalon, 1992; Estruch et al., 1997). Recent research revealed that once foreign genes are integrated into host cells, they could be faithfully transmitted through sexual generations and retain(s) high meiotic stability and expression stability (Duan et al., 1996; Fearing et al., 1997; Scott et al., 1998; Adamczyk et al., 2001). However, transgenes may be lost through meiosis, rearrangements or gene inactivation or gene silencing in the progenies of transgenic plants (Assaad et al., 1993; Finnegan and McElroy, 1994; Matzke and Matzke, 1995; Srivastava et al., 1996; Iyer et al., 2000; Zhang et al., 1996, 2001).
The stability of transgene expression is imperative for transgenic crop/plants to become an integral part of agricultural systems (Koziel et al., 1993; Dutton et al., 2005). The effectiveness and sustainability of Bt-transgenic technology in the control of major target insect pests will depend on the levels of expression of Bt δ-endotoxins in adequate quantities in appropriate plant parts at the requisite time in successive generations (Estruch et al., 1997; Wu et al., 2000; Tabashnik et al., 2000, 2002; Mugo et al., 2004; Kranthi et al., 2005; Olsen et al., 2005; Wei et al., 2005).

This study was to determine the stability of expression of levels of Bt δ-endotoxins in successive generations of crosses between maize lines transformed using Bt genes carrying events 216 and 223 and the non-transformed maize inbred lines CML144 and CML159. The information will help to understand the effects of successive generations of breeding on the levels of Bt δ-endotoxins as it relates to control of target insect pests (Yong-Biao et al., 2001; Bourguet et al., 2003; Wei et al., 2005).

1.2.1 Hypotheses

1. There are no differences in the resistance to C. partellus due to in expression of Bt δ-endotoxins among the crosses between maize lines transformed using Bt genes carrying events 216 and 223 and the non-transformed maize inbred lines CML144 and CML159, their F1 and F2:3:s.

2. Bt δ-endotoxins (CrylAb protein) are present in leaf tissues of the crosses above.

3. There are no differences in the quantities of Bt δ-endotoxins expressed in the crosses between maize lines transformed using Bt genes carrying events 216.
and 223 and the non-transformed maize inbred lines CML144 and CML159, their F₁ and F₂:₃:₃s.

1.2.2 Overall objective

The overall objective of this study to determine the effectiveness of the resistance conferred by Bt genes in tropical maize lines and their successive generations of crosses

Specific objectives

1. To determine the differences in the resistance to *C. partellus* due to expression of Bt δ-endotoxins among the crosses between maize lines transformed using Bt genes carrying events 216 and 223 and the non-transformed maize inbred lines CML144 and CML159, their F₁ and F₂:₃:₃:₃s.

2. To determine the presence of the Bt δ*-endotoxins* (*CrylAb* protein) in leaf tissues of crosses

3. To determine differences in the quantities of Bt δ-endotoxins expressed in the crosses between maize lines transformed using Bt genes carrying events 216 and 223 and non-transformed maize inbred lines CML144 and CML159 and their F₁ and F₂:₃:₃:₃s
2.0 CHAPTER TWO

Literature Review

2.1 Origin of Maize

Maize (Zea mays L.) is thought to have originated in Mexico and Central America (southern Mexico) or in the Andean highlands (Peru, Ecuador and Bolivia). Despite increasing acceptance in the current taxonomy that teosinte (Zea ssp.) is the immediate ancestor of maize (Z. mays ssp. mays); there is no consensus in understanding the derivation of modern maize. Two leading hypotheses emerge, namely; the Tripsacum-Zea diploperennis and the teosinte hypotheses (Beadle, 1939, 1977; 1980; Eubanks, 1997, 2001).

The tripsacum-zea diploperennis hypothesis proposes that maize arose from the progeny of a cross between Z. diploperennis and Tripsacum dactyloides (Eubanks, 1995, 1997, 2001). It suggests that two putative hybrids, dubbed ‘Tripsacorn’ and ‘Sundance’ originated from this two cross. Unlike the parents, the rudimentary ear of these hybrids had exposed kernels attached to a central rachis or cob (Eubanks, 1995, 1997, 2001). If according to the proponents of the theory such hybrids once occurred naturally then the evolutionary puzzle of the origin of maize and its unparalleled architecture could be solved. However, there is no documented evidence to demonstrate that these two grasses were successfully hybridized and in addition, no phylogenetic, cytological or molecular evidence exists in support of the Tripsacum-zea diploperennis hypothesis (Eubanks, 1995, 1997, 2001).
Beadle (1939), proposed the teosinte hypothesis indicating that maize was a domesticated form of teosinte (Zea mays ssp. parviglumis). The theory proposed that through artificial selection by ancient populations, several small mutations with large effects may have transformed teosinte into maize. Scientists have pinpointed that teosinte, Zea mays ssp. parviglumis, is the most likely progenitor. Because sub-species parviglumis is the closest living relative of maize (ssp. mays), proponents of this theory reason that maize arose through changes induced by artificial selection for specific traits of interest. There is phylogenetic, cytological and molecular evidence to support the teosinte hypothesis (Doebley et al., 1994; Matsuoka et al., 2002). Most maize geneticists and evolutionists agree with the teosinte hypothesis on the origin of maize and there is little doubt that maize is a domesticated derivative of the wild Mexican grass teosinte (Zea mays ssp. parviglumis) (Bennetzen et al., 2001).

2.2 Maize taxonomy
Maize (Zea mays L.) is an extremely variable species belonging to the grass family, Gramminae and the tribe Maydae (Tripsaceae) whose member species are monoecious. Maize is further organized in the genus Zea, a group of annual and perennial grasses native to Mexico and Central America. The genus Zea includes the wild taxa, known collectively as teosinte (Zea ssp.), and domesticated maize (Zea mays L. ssp. mays) (Beadle, 1980; Mangelsdorf, 1974; Iltis and Doebley, 1980; Iltis and Benz, 2000).

Sanchez et al., (1998) provided further characterization of genus Zea based on the morphological characteristics and geographic delineations and established five species of Zea that are currently recognized: (i) Zea diploperennis, a perennial, diploid teosinte found in very limited regions of the highlands of western Mexico, (ii) Zea perennis, a
perennial tetraploid teosinte, also with a very narrow distribution in the highlands of western Mexico, (iii) *Zea luxurians*, an annual teosinte found in the more equatorial regions of southeastern Guatemala and Honduras, (iv) *Zea nicaraguensis*, closely related to *Zea luxurians* and found in mesic environments in Nicaragua and (v) *Zea mays* L., a highly polymorphic, diploid annual species, including both wild teosinte and cultivated maize (Beadle, 1980; Mangelsdorf, 1974; Ittis and Doebley, 1980; Ittis and Benz, 2000).


A classification of maize based on endosperm characteristics distinguishes five types which form the foundations of today’s modern hybrids (Goodman, 1965; Mangelsdorf, 1974; Doebley, 1994); namely pop maize, flint maize, floury maize, dent maize and sweet maize.

Pop maize is the original domesticated type, consisting of a small spherical grain with floury (soft) starch core and a flinty (hard) endosperm shell (Ittis and Benz, 2000). Moisture trapped in the floury starch expands upon heating and bursts through the hard shell, creating the popular confection. The pop type represents less than one percent of commercial production. Flint maize is similar to pop maize but with larger grain. Flinty
maize was probably developed from pop types by selection for grain size and greater yield. This type is produced in areas where cold tolerance is required or where storage and germination conditions are poor. Flint maize accounts for 14% of commercial production.

Floury maize’s discovery and selection was a key step in widespread development and adoption of a number of maize-based food staples (Goodman, 1965; Mangelsdorf, 1974; FAO, 1992; Doebley, 1994). Floury maize is the most preferred form for direct human consumption, because of its soft starch that is easily ground to produce meal that can be consumed directly, or as a flat bread, dumpling or beverage. Floury maize accounts for 12% of commercial production. Dent maize consists of a floury starch core with lateral inclusions of flinty starch. Because the crown of the kernel consists of floury starch, moisture loss from this area upon kernel maturation causes a slight collapse in volume that produces a characteristic dent. Globally, dent maize accounts for 73% of commercial production, and is used for direct human consumption, for livestock feed and for industrial manufactures (starch, sugar, syrup, oil, cellulose and ethyl alcohol) in developed economies (FAO, 1992).

Sweet maize’s endosperm consists of soluble sugar, little starch and an intermediate form of sugar polymer called phytoglycogen. Commercial production is negligible (<1%). It has a high cash value as a processed vegetable in industrial economies (Goodman, 1965; Mangelsdorf, 1974; Doebley, 1994).

2.3 Maize botany

Maize has fibrous root systems. There are three types of root systems in maize namely; (i) seminal roots developing from the radicle of the embryo, (ii) adventitious roots
developing from the lowest node and are usually 3-4 cm below the soil surface irrespective of the sowing depth and (iii) brace or prop roots produced in a whorl by two or more lower nodes close to the ground level (Aldrich et al., 1975; Singh, 1987).

Maize stems are solid, usually 2-4 cm in diameter, while the stalk may attain a height of 2-4 meters depending on the variety, production environment and crop management levels (Singh, 1987). The relative length of the various internodes varies and is used in classification of the maize varieties (Singh, 1987).

Each maize leaf is made up of a blade and a sheath tightly clasped on the stem. Leaves vary in length, width, thickness, pubescence, colour pigmentation angle and venation patterns. Maize bears its inflorescence in form of spikelets. The male flowers are borne terminally on a tassel while the female flowers are borne laterally on the ear (Randolph, 1955; Weatherwax, 1955).

The spikelets are arranged in a branched panicle. The central spike is thick with a higher condensation of spikelets than the primary and secondary branches. The pistillate or female spikelets are borne on a thick axis called a cob, which is fully covered by husk leaves. Each spikelet has two flowers and frequently the upper one is fertile (Randolph, 1955; Weatherwax, 1955; Goodman, 1965; Singh, 1987; Doebley, 1994). In maize, protandry is common, and on average count of ovules, 95% of ovules are cross pollinated except about 5% which are self pollinated. Pollen shedding starts from spikelets located on the central spike, 2-3 cm from the tip of the tassel and proceeds downwards in central spike and the primary and secondary branches of the tassels (Russell et al., 1980; Singh, 1987).
Depending on the variety, temperature and relative humidity, pollen shedding may continue 3-6 days and silks remain receptive for 3-8 days. A healthy, vigorously growing maize plant produces over 18-25 million pollen grains (Weatherwax, 1955; Randolph, 1955; Goodman, 1965; Singh, 1987). Under normal favorable cool conditions, pollen grains remain viable for 4-16 hours after shedding; however, pollen viability is lost very rapidly when temperature exceeds about 30°C at low relative humidity (Russell et al., 1980). Fertilization is completed in 24-36 hours (Singh, 1987).

A maize kernel is a caryopsis and at maturity it has three major components: (i) pericarp, which is an outermost cover of the seed, (ii) germ, which is a product of hybrid origin and (iii) endosperm, which is a product of the triple fusion and stores the food material for developing the seedling.

### 2.4 Maize breeding

Inbred lines are important for the production of hybrids. They are formed by selfing plants from another hybrid, a narrow based synthetic, a population or a landrace. Self-fertilization is the most rapid method to arrive at homozygosity. Each cycle of selfing increases homozygozity by 50%. At the seventh generation of selfing, 99% homozygozity is reached. Superior inbred lines are selected for their vigor, standability, freedom from disease, and other desirable characteristics. Development of inbred lines can be done and followed through pedigree selection or backcrossing methods.

Open-pollinated varieties (OPV’s) are superior in stability, resistance to biotic and abiotic stresses and consumption qualities. They grow well without high inputs because they have been selected under organic conditions (Pixley and Banziger, 2004). OPV’s are still attractive for three reasons: (i) they are relatively easy to develop, (ii) seeds are easy and
economical to produce and (iii) seeds can be recycled for replanting (Pixley and Banziger, 2004).

Hybrid seeds are the first generation offsprings of two distant and distinct parental lines of the same species. Hybrid seed is also known as "high response" seed. These seeds require fertilizers, herbicides, pesticides and lots of water to achieve their high yields potentials. The number and genetic composition of the parents determines the type of maize hybrid. The common ones in use are single cross hybrids, three-way cross hybrids, double cross hybrids, double top cross hybrids and varietal cross hybrids.

2.5 Maize production in Kenya

Maize is an important crop in Kenya. Its production is limited by many constraints including abiotic and biotic factors (Chitere and Omolo, 1993). The abiotic constraints affecting maize production are low and declining soil fertility, low and unreliable rainfall and low soil pH with associated nutrient deficiencies and toxicities (Pixley et al., 1997). The biotic factors that constraint maize production are diseases (turcicum leaf blight, grey leaf spot and maize streak virus) and insect pests (stem borers maize streak leafhoppers, chafers, cutworms, wireworms, grain weevils, larger grain borer). Stem borers are the major field insect pests in Kenya (De Groote 2002). The major stem borer species are C. partellus and B. fusca as they cause great field damage and grain yield losses in maize depending on the pest population density and phenological stage of the crops at infestation (Warui and Kuria, 1983; Ampofo and Saxena, 1989; Songa et al., 2002).
2.5.1 Abundance and distribution of stem borers in Kenya

Stem borers are a complex of species with overlapping spatial and temporal distributions in nearly all countries in eastern and southern Africa (Pingali, 2001; James, 2003). All stem borers may be indigenous except *C. partellus* which invaded Africa from Asia before 1930 and was first found in Malawi (Tams, 1932). *C. partellus* has spread to most countries in eastern and southern Africa and become the most damaging stem borer of maize and sorghum, particularly in warmer lowland areas (Nye, 1960, Van Hamburg, 1979).

Surveys conducted between 1995 and 2002 in all maize and sorghum growing areas of Kenya identified five stem borer species namely; *C. partellus* (about 49%), *B. fusca* (about 43%), *S. calamistis* (5%), *C. orichalcociliellus* (about 2%) and *E. saccharina* (about 1%) (Overholt et al., 1994; Khan et al., 1997; Songa et al., 2002; Mulaa et al., 2005). *C. partellus* predominates in the wetter lowlands and mid altitude areas, while *B. fusca* predominates in the highlands (Figure 3). However, both insect species occur in the mid-altitude regions of (Songa et al., 2002).

2.5.2 Economic importance of stem borers

A single stem borer larva is estimated to cause between 3-7% yield loss per plant (Showers et al., 1989). *C. partellus* and *B. fusca* cause the greatest damage resulting in maize grain yield losses of up to 53% (Warui and Kuria, 1983; Ampofo and Saxena, 1987; Songa et al., 2002). Lepidopteran stem borers cause foliar damage therefore reduces the photosynthetic leaf area. The damage caused by stem borers result into ‘dead hearts’, lodging and stem tunneling therefore interfering with translocation of water and nutrients in the plants. The damage also increases ear rots, aflatoxins and fumonisins due
to secondary infections by pathogens and subsequently reduced grain yields and quality (IITA, 1998; Hell et al., 2000; Schulthess et al., 2002; Ako et al., 2002).

Various methods for the management of stem borers have been suggested namely;

2.5.2.1 Cultural control

Cultural control refers to the deliberate alteration of the production system, either the cropping system itself or specific crop production practices, to reduce pest populations or avoid pest injury to crops (Ashdown, 1977). Cultural control can be an effective tool in the suppression of arthropods pests in agro-ecosystems. It depends on two basic
approaches: (i) to make the environment less favorable to the pest, and (ii) to make the environment more favorable to the pests’ natural enemies (Glass, 1975; Schulthess et al., 1997).

These control practices include crop-field sanitation, habitat management (management of the landscape and structures and the factors that allow pest situations to develop), companion cropping (planting of certain combinations of species close together for mutual benefit), manipulation of planting dates and destruction of dry stems and stubble, ‘push-pull system’ or “stimulo-deterrent diversionary strategy” which involves intercropping and trap cropping strategies using crops such as leguminous silver leaf Desmodium uncinatum, Molasses grass (Melinis minutiflora), fodder sorghum (Sorghum bicolor x Sorghum halepense), Napier grass (Pennisetum purpureum), and Sudan grass (Sorghum vulgare sudanese) (Liu et al., 1997; Kfir et al., 2002; Khan et al., 2006). These grasses attract greater oviposition by stem borer moths. However, these practices have limited application due to the mode of application, non-applicability on large-scale farms, difficulty in timing, and the cryptic nature of stem borers (Kfir et al., 2002; Khan et al., 2006).

2.5.2.2 Chemical control

In Kenya, some farmers apply insecticides such as trichlorophon, fenitrothion, permethrin etc which effectively reduce the stem borer populations if applied at the correct time (Mathez, 1972; Warui and Kuria, 1983). Insecticide costs limit their use for most resource-constrained farmers (Saxena et al., 1989; Mohammed and Underwood, 2004).
2.5.2.3 Biological control

Biological control methods continue to play significant role in the management of target insect pests (Overholt et al., 1994; Sanvido et al., 2007). These methods are however efficient, cost-effective, environmentally safe and safe to the humans and livestock in the long term (Songa, et al., 2002; Sanvido et al., 2007). The commonly used biological control agents for the management of cereal stem borers are *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), *Cotesia* (Cameron) (Hymenoptera: Braconidae), *Denticasmias busseolae* Henrich (Ichneumonidae) and *Trichogramma* spp. These control agents attack different stages of the stem borer larvae. However, the major challenges with these methods are that they are insufficient to maintain the pest populations below economic injury levels (Polaszek and Walker, 1991; Overholt et al., 1994; Songa et al., 2002; Sanvido et al., 2007).

2.5.2.4 Host-plant resistance

Host plant resistance through conventional plant breeding is an acceptable approach for protecting the plants against insect pests by farmers compared to the use of pesticides and biological control (Klun and Brindley; 1966; Metcalf et al., 1982; Mugo et al., 2007). It provides inherent control without environmental concerns and that it is generally compatible with other pest management approaches (Mugo et al., 2007).

Conventional breeding of plants for stem borer tolerance has proved challenging due to the polygenic control of the trait (Kennedy et al., 1987; Khush and Brar, 1991) and the knowledge that no gene for host plant resistance has been mapped for stem borer tolerance trait (Bennett et al., 1997). The inadequate understanding of the genetic basis of
the trait makes development of tolerant varieties by conventional breeding to take long and unpredictable durations (Kennedy et al., 1987).

CIMMYT developed several maize inbreds and open pollinated maize varieties with enhanced levels of host plant resistance to *C. partellus* for the lowland tropics (Dabrowski and Nyangiri, 1983; Ajala and Saxena, 1994; Songa et al., 2002). CIMMYT's multiple borer resistance (MBR) populations which include inbred lines Mp 706, Mp 707, CML67, CML123, CML139, 1CS1-cm (CIMMYT population 27) and 1CZ-cm (CIMMYT population 22) are a source of resistance to *C. partellus* (Ampofo, 1988; Van Rensburg et al., 1995; Gethi et al., 2001; Smith et al., 1994, Mugo et al., 2001, Mugo et al., 2007).

It requires a systematic evaluation and introgression of the genes for multiple borer resistance into locally adapted maize genotypes (Gethi et al., 2001; Mugo et al., 2007). Efforts to incorporate of resistance to the spotted stem borer into maize with good agronomic backgrounds has been undertaken during the past few decades (Gethi et al., 2001, Mugo et al., 2007).

2.5.2.5 Integrated Pest Management

In agriculture, integrated pest management (IPM) is an effective and environmentally sensitive approach to pest management that relies on a combination of common-sense practices. IPM programs use an ecological approach and apply current, comprehensive information on the life cycles of pests and their interaction with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means and with the least possible hazard to people, property, and the environment (Mulaa et al., 1999; 2005). An example of such
approaches is host-plant resistance combined with the use of multi-lines or multi-blends consisting a mixture of inbred isogenic lines (Cox et al., 1986; Khan et al., 1997; Lynch et al., 1999), use of natural enemies (Bonhof et al., 1997; 1998; Songa et al., 2002; Schulthess et al., 1997), use of cultural control methods (KARI, 2002) in integrated pest management (IPM), can effectively reduce the buildup of resistance by target insect pests.

2.5.3 Bt-based insecticidal biopesticides

*Bacillus thuringiensis* based insecticidal biopesticides have been formulated from crystal proteins isolated and purified from *Bacillus thuringiensis*, usually a mixture of 3-7 different toxins. They are widely used in both conventional and organic farming operations (Masson et al., 1998; Wu et al., 2003). Despite their use in commercial Bt formulations, they have several disadvantages under field conditions (Cohen 1991) such as photoinactivation by UV-light along with temperature, dew or rain are major environmental factors affecting stability and efficacy of these Bt toxins (McGuire and Shassha, 1990; Van Frankenhuyzen, 1993; Llewellyn et al., 1994; Roush, 1996). Repeated applications of biopesticides become necessary. These applications are expensive and typically used only on high value crops especially vegetables (Llewellyn et al., 1994; Roush, 1996). Resistance to Bt-based insecticidal biopesticides in most areas of the tropics has been reported in Diamond backmoth (*P. xylostella*), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and some strains of bruchids (*C. maculatus*) (Dick and Credland, 1986; Tabashnik et al., 1993, 1994, 2008; Iqbal et al., 1996; Perez, 1997; Roush, 1996, 1997; Yong-Biao et al., 2000; Zhaom et al., 2001).
There are concerns that the spores of *Bacillus thuringiensis* sprayed on crops may damage people's health through inadvertent inhalation. French researchers found that inhalation of spores from certain *Bacillus thuringiensis* strains can cause lung inflammation, internal bleeding and even death in laboratory mice (New Scientist, 1999). However, when Bt is genetically engineered into crops to confer resistance against insect pests no such effects are observed (Croft, 1990; Mendelsohn, *et al.*, 2003; Sanvido *et al.*, 2007).

2.6 Biotechnology and transgenic Bt technology

2.6.1 Opportunities in modern biotechnology

Over the last decade, farmers have consistently increased the global area under crops developed using biotechnology tools by double-digit growth rates every single year since these crops were first commercialized in 1996 (Brookes *et al.*, 2006).

In 2006, the first year of the second decade (2006-2015) of commercialization of crops developed using biotechnology tools the global area of biotechnology derived crops continued to increase for the tenth consecutive year at a sustained double-digit growth rate. In 2007, global area of biotechnology derived crops grew 12 percent or 12.3 million hectares to reach 114.3 million hectares, the second highest area increase in the past five years (Brookes *et al.*, 2006; James, 2008). Transformed maize lines carrying Bt genes is part of the 102 million hectares global area biotechnology derived crops, grown by 12 million farmers in over 22 countries in 2007 (James, 2008). This unprecedented high adoption rate of biotech crops and Transformed maize lines carrying Bt genes in particular since 1996 reflects the trust and confidence of millions of farmers in crop
biotechnology in both developed and developing countries (James, 2005a, b, Brookes et al., 2006).

Applications of modern biotechnology have led to routine production of transgenic plants for an increasing number of cereal and other crop species. Important traits such as; insect resistance (De Groote et al., 2005), herbicide resistance, drought and salt tolerance, improved colours in fiber and flower crops, resistance to water logging, high nutritional value such as high vitamin A rice, and longer shelf lives have been incorporated into many plant species through transformation (Müller et al., 1987; Fujimoto et al., 1993; Wünn et al., 1996; Ghareyazie et al., 1997; Cheng et al., 1998; Ye et al., 2000; De Groote et al., 2005).

Genetic engineering continues to be applied in improvement of crop quality and agronomic performance. These advances stem from relationships among seed biotechnology industry, crop managers, extension workers, producers, biotechnologists, plant breeders and the wider clientele domain (Hallauer, et al., 1988; Wambugu, 2001). Currently, the focus is on the characterization of molecular elements that regulate foreign gene expression in transgenic cereals (Bochardt, 1992; Alstad, 1997; Daniel, 1997; Daniella et al., 2003; Dutton et al., 2005).

Genetic engineering using Bacillus thuringiensis genes as sources of resistance have been reported in various crops and genotypes (Gallagher, 1992; Sharma, 1993). Plants transformed using Bacillus thuringiensis genes offer effective pest exposure, reduced exposure of farm workers to insecticides, elimination of pesticide application costs and
reduced environmental impacts on biodiversity resulting from pesticide use (Delannay et al., 1989; Mendelsohn, et al., 2003; Vojtech et al., 2005).

2.6.2 Concerns about modern biotechnology

There are concerns about biotechnology, and the derived products (Conner et al., 2003; European Food Safety Authority, 2004). These range firstly, from ethical and moral concerns such as moving genes inter-species barriers and the respect to ‘sanctity of life’, cloning/patenting life forms which are likened to ‘playing God’ and animal rights concerns.

Secondly, many products and processes have been developed in the private sector and are protected by intellectual property restrictions and involve payment of royalties thus affect indigenous technical knowledge (Conner et al., 2003).

Thirdly, environmental health considerations namely the effect of transgenic crops on biological diversity especially the non-target organisms such as arthropod predators and parasitoids through crop plant-based food chains (Hilbeck et al., 1998a, b, 1999; Schuler et al., 1998, 2001; Muhammad et al., 2004; Lövei et al., 2005; O'Callaghan et al., 2005).

Additionally, the potential for transgenic crops to become ‘super weeds’ with resultant consequences of gene flow through horizontal gene transfer (Hilbeck et al., 1998a, b, 1999; Schuler et al., 1998, 2001).

Fourthly, the concerns about food safety and human health such as the propensity for allergenicity, potential toxicity, microbiological safety, alteration in nutritional values, and other unidentified effects that may result from the use of transgenics and their derivative food and feed products (Shelton, et al., 2002; Mendelsohn, et al., 2003; European Food Safety Authority, 2004).
Finally, there are concerns such as liability and redress, trade related issues of labeling and traceability and the monitoring and detection of transgenics and derived products that are encompassed in the Cartagena Protocol (Shelton, *et al.*, 2002; Mendelsohn, *et al.*, 2003; European Food Safety Authority, 2004).

Expression of transgenes depends on the genetic background (Harpster *et al.*, 1988), and the physiological and environmental conditions (Meyer *et al.*, 1992; Mahon *et al.*, 2002), but, current evidence indicates that transgenes are expressed similarly in hybrids and parent plants (Estruch *et al.*, 1997; Chèvre *et al.*, 1997; Dietz-Pfeilstetter *et al.*, 1998). The stability, inheritance and expression of transgenes in subsequent generations of breeding are of paramount importance for functional analysis as well as crop improvement (Kathuria *et al.*, 2003).

Expression of a transgene(s) after out crossing is not readily predictable and the evaluation of the consequences of gene transfer to other plants is important. However, there are questions on how the transgenes are expressed when moved inter or intra-species (Chèvre *et al.*, 1997; Dietz-Pfeilstetter *et al.*, 1998).

**Genetic transformation in plants**

Genetic transformation of plants and other organisms occurs naturally. Bacteria and viruses routinely move DNA (or RNA) into an organism. Soil bacteria such as *Agrobacterium tumefaciens* and *Agrobacterium rhizogene* are examples of natural transformation systems causing crown gall disease and hairy root syndrome respectively (Gelvin 1994; Chawla, 2002). *Agrobacterium* can transfer a section of its own DNA (known as transfer DNA). In the laboratory, the DNA of interest carrying a
valuable agronomic trait (insect resistance, herbicide tolerance, drought tolerance etc) has replaced the disease genes (Gelvin 1994; Chawla, 2002). Other methods used for transformation are biolistics or particle bombardment, which uses tungsten microprojectiles coated with DNA (Gelvin 1994; Chawla, 2002).

2.6.3 Bt δ-endotoxins

*Bacillus thuringiensis* (Bt), is a common soil bacterium that produces Bt δ-endotoxins' or 'insecticidal crystalline proteins' (ICPs) during its sporulation. The bacterium was first identified by Ishawatta first discovered Bt in Japan in 1907 and later rediscovered by Ernst Berliner in 1911 in Germany (Baum et al., 1999). Many *B. thuringiensis* strains have since then been identified to exist with each producing unique ICPs (Lerechus et al., 1993).

Bt δ-endotoxins' are harmful proteins to susceptible insect pests (Whalon and McGaughey, 1998; Mendelssohn, et al., 2003). The Bt δ-endotoxins are present in certain Bt strains and are part of the outer membrane of the cell wall. They consist of three distinct domains (Figure 4). Domain I is a bundle of α-helices responsible for insertion into the epithelial cell membrane and pore-formation activity while domain II is responsible for toxin specificity and high-affinity receptor-binding. It consists of three β-sheets in a 'Greek key conformation' (Dean et al., 1996; Schnepf et al., 1998). Domain III consists of β sheets and it is responsible for toxin stability and binding specificity in some insects (Burton et al., 1999, Von Tersch et al., 1994; Grochulski et al., 1995).
2.6.4 Mode of action by Bt δ-endotoxins

For Bt $\delta$-endotoxins to be effective in killing a susceptible insect, a part of the plant that contains the Bt gene (not all plant parts necessarily contain the Bt $\delta$-endotoxins in equal concentrations) must be ingested, within minutes the parasporal crystalline protein inclusions bind with high affinity to receptors mainly glycoprotein’s, on the midget epithelium cells (Schnepf et al., 1998; Webb et al., 1999). Within hours, the toxins generate pores in the midgut epithelium cells; this causes a cellular osmotic imbalance. The midgut epithelium cells swell and lyses through a process called ‘colloid-osmotic lyses’ (Hofte et al., 1989; Gill et al., 1992; McGauchey et al., 1992). After cell lysis, the normal gut bacteria invade the body cavity. The insect stops feeding and dies of septicaemia as bacteria multiply in its body system (Hofte et al., 1989; Gill et al., 1992). However binding does not assure toxicity to the target pest (Whalon and McGaughey, 1998). An alteration of toxin-binding sites may interfere with normal functions of the mid-gut (Van Rie et al., 1990), and there is evidence for B. thuringiensis resistance in open field populations of the diamondback moth P. xylostella (Tabashnik et al., 1993; Groeters et al., 1994; Yong-Biao et al., 2000; Zhaom et al., 2001). In stored grain treated with Bt insecticide include the Indianmeal moth Plodia interpunctella (Hubner) (Pyralidae: Plodia interpunctella) and Cadra cautella Walker (Lepidoptera: Crambidae: Phycitinae) in laboratory studies (McGaughey and Beeman, 1988; Poppy, 2001).

### 2.7 Expression of Bt $\delta$-endotoxins

The expression of Bt $\delta$-endotoxins may be constitutive at a relatively constant dose or can be restricted to specific crop stages, tissues, or both (Husnain et al., 2002; Daniella et al., 2003; Dutton et al., 2005; O'Callaghan et al., 2005). The synthetic Bt gene for insect resistance originates from B. thuringiensis. Once the Bt cry1Ab gene is isolated and
cloned or amplified in a bacterial vector, it undergoes several modifications before it can be effectively inserted into a plant (O'Callaghan et al., 2005).

The modified form has a higher percentage of A-T nucleotide pairs compared to plants, which prefer G-C nucleotide pairs. The modification substitutes the A-T nucleotides with G-C nucleotides in the Bt gene without significantly changing the amino acid sequence (Dutton et al., 2005; O'Callaghan et al., 2005). Perlak et al., (1991) showed that guanine and cytosine (GC) content of the Bt δ-endotoxins can be increased and lead to better expression in target plants as reported by the Insect Control Groups at CIBA which made the synthetic version of cry1Ab gene with a GC content of 65% compared to the native Bt cry gene. The constructed Bt transgene contains several components namely; a selectable marker gene, a promoter sequence, a synthetic Bt gene and a termination sequence and (Figure 4).

A selectable marker gene is added to the gene "construct" to identify plant cells or tissues that have successfully integrated the transgene. This is necessary because achieving incorporation and expression of transgenes in plant cells is a rare event. Selectable marker genes encode proteins that provide resistance to agents that are toxic to plants, such as antibiotics or herbicides. Only plant cells that have integrated the selectable

![Figure 4: Illustration of a Bt gene 'construct' with its components.](image-url)
marker gene survive when grown on a medium containing the appropriate antibiotic or herbicide (Toki et al., 1992; Cornejo et al., 1993; Stoger et al., 1999).

A promoter sequence is added for the gene to be correctly expressed (i.e., translated into a protein product). The promoter is the on/off switch that controls when and where in the plant the gene will be expressed. Most promoters in transgenic crop varieties have been constitutive, causing gene expression throughout the life cycle of the plant in most tissues, while others are facultative, causing expression at certain times or in specific tissues. Ubiquitin is an example of the constitutive promoter that enables the production of *cry1Ab* protein in almost all parts of the plant (Alstad, 1997; Daniel, 1997; Husnain et al., 2002; Daniella et al., 2003; Dutton et al., 2005; O'Callaghan et al., 2005). Ubiquitin promoter directs a high level of reporter gene expression in monocot plants (Toki et al., 1992; Cornejo et al., 1993; Stoger et al., 1999).

Finally, a termination sequence which has three mRNA sequences (UGA, UAG and UAA) that do not code for an amino acid. It signals the end of protein synthesis. The synthetic Bt *cry1Ab* gene encodes for the Bt δ-endotoxins for resistance against target insects.

### 2.7.1 Approaches to expression of Bt δ-endotoxins

There are two types of approaches to expression of Bt δ-endotoxins in plants namely; ‘low and high dose’. A ‘low-dose’ approach to expression of Bt δ-endotoxins in plants aims at reducing the pest populations slightly or slowing down the larval development with five generations per year, while the ‘high-dose’ strategy consistently kills heterozygous progeny and/or the most abundant carriers of resistance (Siegfried et al., 2001; Husnain et al., 2002; Daniella et al., 2003; Dutton et al., 2005). A ‘high dose'
approach to expression of Bt δ-endotoxins ensures that inheritance of resistance is functionally recessive, however it may be compromised if sub-optimal levels of the toxin are achieved and most of the heterozygotes survive exposure (Gould, 1994, 1998a; Siegfried et al., 2001; Husnain et al., 2002). The first generation Bt plants require higher levels of expression of δ-endotoxins to achieve control of field pests such as stem borers, cotton bollworm and other pests (Daniel, 1997; Husnain et al., 2002). However, successive generations of Bt plants will demand expression of specific δ-endotoxins in specific tissues or at a particular crop stage (Alstad, 1997; Husnain et al., 2002; Daniella et al., 2003).

The antibiosis nature conferred to the Bt plant affects the reproduction of insect pests by interfering with vital life components such as longevity, oviposition rate, generation time and pre-adult mortality and it affects the insect physiology and survival (Painter, 1951; Ponti et al., 1985, 1992; Kennedy et al., 1987).

Bt resistance is single gene-based in contrast to most conventional resistance to lepidopteran pests which is polygenic and additively inherited (Khush and Brar, 1991). Mendelian inheritance applies to both types of host plant resistance (Barry, 1989; Goto et al., 1993; Wünn et al., 1996; Fearing et al., 1997; Wu et al., 2000). Introgression of target insect pest resistance into locally adapted maize genotypes can therefore, be accomplished quickly, while dominance allows the Bt gene to be utilized in the heterozygous condition in hybrid combinations (Ponti et al., 1985; Khush and Brar, 1991; Gethi et al., 2001).
A number of genetically engineered insect resistant crop species have been tested under natural conditions and many have been commercialized. These include maize (Traore et al., 2000, Andre et al., 2003), potato (Adang et al., 1993), tomato (Delannay et al., 1989), tobacco (Barton et al., 1987), cotton (Perlak et al., 1990), cowpea (Schuler et al., 1998), and rice (Fujimoto et al., 1993).

2.8 Challenges to modern biotechnology

Despite the reported success in genetic engineering for transgenic Bt technology in plant transformation, several challenges still exist. These challenges include pleiotropic effects that affect traits of economic interest, meiotic instability or transgene inactivation or transgene silencing or loss of the transgene in progenies of the transgenic plants through sexual generations as factors that may affect the transgene expression in transgenic plants (Müller et al., 1987; Spencer et al., 1992; Finnegan and Mc Elroy, 1994; Matzke and Matzke, 1995; Srivastava et al., 1996; Iyre et al., 2000; Zhang 1996, 2001; Husnain et al., 2002).

Expression of the transgene depends on the genotype (Harpster et al., 1988; Caligari et al., 1993) and the physiological and environmental conditions (Meyer et al., 1992). Current evidence indicates that transgenes are expressed similarly in hybrids and parents plants, but expression of the transgene(s) after out crossing is not readily predictable (Chèvre et al., 1997; Dietz-Pfeilstetter et al., 1998). Still, there are questions on how the transgenes are expressed and in what quantities when moved either inter or intra species and through sexual generations (Chèvre et al., 1997; Dietz-Pfeilstetter et al., 1998). The value and sustainability of the Bt technology will depend on the levels of expression of
Bt δ-endotoxins in adequate quantities, in appropriate plant parts, and at the requisite time to control major target insect pests (Kranthi et al., 2005; Wei et al., 2005).

2.8.1 Introduction of maize lines transformed using Bt genes in Kenya

Through the KARI/CIMMYT Insect Resistant Maize for Africa (IRMA) project applications to introduce maize lines transformed using Bt genes in Kenya were made to the National Biosafety Committee (NBC). Maize lines transformed using Bt genes leaves were introduced in 2001 and leaf bioassays carried out to identify the effective maize lines transformed using Bt genes events against the major stem borer species in Kenya (Mugo et al., 2005; De Groote et al., 2005). After this application to introduce maize lines transformed seeds was made to the NBC in 2003 and approved and seeds sown in May 2004 (KARI/CIMMYT, 2003).

The bioassay results indicated the effectiveness of maize lines transformed using Bt genes in controlling *C. partellus*, the coastal stem borer *C. orichalcociliellus* Strand (Lepidoptera:Pyralidae), the pink stem borer *S. calamistis* Hampson(Lepidoptera:Noctuidae), and the sugarcane borer, *E. saccharina* Walker(Lepidoptera:Crambidae), the major stem borers found in the maize growing regions of Kenya. However, *B. fusca* was not effectively controlled by the Bt-maize events tested (Mugo et al., 2005; De Groote et al., 2005).

It is with that background that this study has been proposed to determine the levels of expression and quantities of Bt δ-endotoxins through sexual generations of breeding involving crosses of tropical transformed and non-transformed maize inbred lines.
CHAPTER THREE

Materials and Methods

3.1 Research site

The study was carried out at Kenya Agricultural Research Institute (KARI), Biotechnology Centre, Kabete, Nairobi Kenya (1° 15.409’S, 36°46.410’E, 1806m above sea level) in a biosafety level 2 greenhouse complex (BGH) (Plate 1). The BGH was initially constructed by the KARI/CIMMYT Insect Resistant Maize for Africa (IRMA) project for studies on Transformed maize lines carrying Bt genes for control of stem borers in Kenya. The biosafety greenhouse was approved by the Kenya Plant Health Inspectorate Service (KEPHIS) in collaboration with the Kenya National Biosafety Committee (NBC) for research, development and dissemination of Transformed maize lines carrying Bt genes varieties and for carrying out risk assessment studies on transgenic plants (Traynor et al., 2001; Murenga et al., 2004).

Plate 1: Biosafety level II greenhouse complex at KARI NARL, Kabete
The BGH is designed to effect both genetic and material containment for transgenic plants under study. The facility serves as a biocontainment facility providing an effective means of isolation and prevention of unintended transmission of genetic material (Traynor et al., 2001; Murenga et al., 2004).

The biosafety level II greenhouse complex is consistent with international standards. It has features such as a double-entry doors system to greenhouse rooms to prevent accidental release of pollen grains and an independent drainage to prevent planting media and plant materials from being carried off through the drains. Access to this facility is restricted only to authorized personnel. In compliance with biosafety requirements by the regulatory authorities, the disposal of all planting media, plant materials and insect tissues meet special guidelines and procedures (Traynor et al., 2001; Murenga et al., 2004). Regular in-house training of staff is emphasized on approved protocols for management and operation of the biosafety greenhouse. The average temperatures in the BGH ranged between 25-32°C during the experimental period.

3.2 Plant materials and planting media
Two transformed maize inbred lines carrying events 216 and 223 both of CML126 BC₃S₁ (crylAb::ubi) and two non-transformed inbred lines; CIMMYT maize lines (CMLs) CML144 and CML159 were used in this study. Hybrid CKIR6009 and inbred line MBR were included as resistant checks with host plant resistance whereas hybrid H513 and inbred line inbred line CML 216 were included as susceptible checks.

The two transformed maize inbred lines descended from a common parent, CML126, which was transformed with a vector containing a full-length crylAb coding sequence driven by an enhanced ubiquitin. The two non-transformed inbred lines are quality
protein maize (QPM) and have double quantities of lysine and tryptophan than the normal maize varieties (Borlaug, et al., 1992; Prasanna et al., 2001; Vivek et al., 2008).

The genotypes were sown in planting media composed of one part topsoil-farm yard manure mix, one part sand, and one part coconut peat (1:1:1) (Traynor et al., 2001; Murenga et al., 2004). Fertilizer was applied fortnightly after crop establishment up to physiological maturity at the rate of 2.5 grams of urea/triple super phosphate (2:1) by volume and approximately 500 ml of NPK (20:20:0) soluble fertilizer (2 g/L of water) applied for each pot.

3.3 Formation of successive generations

Season one – Formation of F₁ generations
Twenty seeds each of the transformed maize lines carrying event 216 and 223 and the non-transformed maize lines CML144 and CML159 were sown in small transfer pots (7.5 x 7.5 x 9.0 cm) and later transplanted into large pots (30 cm x 36 cm). The transformed and non-transformed plants were used as the male and female parents, respectively. To ensure ‘nicking’, planting of males and females was staggered on three sowing dates.

Season two – Formation of F₂ generations
Twenty seeds of each F₁ generation were sown in small transfer pots and later transplanted into large pots. The plants were sib mated by pollinating half of number of plants with bulked pollen from the other half of the number of plants among the F₁ generation of the particular cross.
Season three – Formation of $F_{2:3}$ generations

The $F_{2:3}$ generation plants were the plants grown from a particular $F_2$ plant for the crosses of parental maize lines transformed using Bt genes carrying events 216 and 223, parental maize lines transformed using Bt genes carrying events 216 and 223 crossed to parental non-transformed maize lines and crosses of parental non-transformed maize lines.

3.4 Evaluation for infestation with *Chilo partellus*

This study was conducted in the KARI Biotechnology Centre’s biosafety level II greenhouse. The maize lines transformed using Bt genes carrying events 216 and 223, two non-transformed inbred lines CML144 and CML159, their $F_1$ and $F_{2:3}$ crosses of Event 216 x Event 223, CML144 x Event 216, CML144 x Event 223, CML159 x Event 216 and CML144 x Event 223 were evaluated for infestation using *C. partellus*.

The treatments consisted parents, their $F_1$ and $F_{2:3}$ crosses, and four checks. These were replicated 4 times in a 15 x 5 alpha lattice design with each plot containing 4 plants. Four seeds were sown in large pots of 30cm diameter and 36cm height. The soil media, fertilizers, and watering was done as in section 3.2 above.

Plants were infested at the 4th-5th leaf stage with twenty first instar larvae of *C. partellus* stem borer. The plants were evaluated for damage 10 days after infestation at the $V_8$ leaf stage. Foliar damage rating was carried out based on a scale of 1-9 (1= no leaf damage, 9= severe leaf damage) (Guthrie’s *et al.*, 1960). Other plant damage parameters recorded on the genotypes are plant height, number of stem borer exit holes, cumulative stem tunnel length, number of larvae recovered, and number of pupae recovered. The ratio of tunneled length to plant height was computed from the two traits.
3.5 Evaluation of genotypes using dot blots analysis

Plant leaves from all the maize lines transformed using Bt genes carrying events 216 and 223, two non-transformed inbred lines CML144 and CML159, their F₁ and F₂;3 crosses were sampled 30 days after sowing (DAS) for analysis of the levels of Bt δ-endotoxins. Three most recently fully expanded leaves from each plant in the same generations were excised from the middle of the leaf blade excluding the midrib, to standardize and minimize errors in leaf sampling (Dietz-Pfeilstetter et al., 1998). Protein was extracted from leaf tissue samples based on the procedure described in Bohovora et al., 1999 and Koziel et al., 1993.

Briefly, 20μl each of the extracted maize leaf samples was spotted and developed on a 0.2μm nitrocellulose membrane as described by Bohovora et al., 1999.

3.6 Quantification of cry1Ab protein expressed in leaf tissues

The maize leaves from which the samples used in the dot blot analysis were extracted were used to quantify cry1Ab protein using a "sandwich-type" enzyme linked immunosorbent assay (ELISA). The detection and quantification of Bt δ-endotoxins in maize leaf samples was carried out using the Bt cry1Ab/cry1Ac microtiter plate kit (Ledesma et al., 1995; Greenplate, 1999; Adamczyk et al., 2001; Walschus et al., 2002). Leaf tissues from 20 plants from each parent and all their crosses in the different generations, were sampled and ground in liquid nitrogen followed by homogenization in 5 ml of 0.1M sodium bicarbonate pH 10.01 containing 10 mM 2-mercaptoethanol, 2.5 mM EDTA, 2.5 mM EGTA, 1 mM benzamidine-HCl, 0.5 mM PMSF, 1 μg/ml pepstatin A, 40 μg/ml bestatin, 1 mM CWS, and 10% (v/v) glycerol for protein analysis. In this assay system, the standards, controls, or sample extracts were added to wells coated with
monoclonal antibodies raised against \textit{cry}1\textit{Ab} \(\delta\)-endotoxins. Any Bt \(\delta\)-endotoxins residues found in the standard or sample extracts bind to the antibodies on the wells. The "sandwich" ELISA was completed by the addition of immuno-affinity purified polyclonal goat-antibodies specific to the same Bt \(\delta\)-endotoxins as shown in Figure 5.

![Diagram of sandwich ELISA](image)

Figure 5: Illustration of the sandwich ELISA assay for quantification of Bt \(\delta\)-endotoxins in maize leaf samples

### 3.7 Data analysis of whole plant bioassays data, dot blot and ELISA assays

Data on plant damage parameters including foliar damage rating, stem tunnel length, number of exit holes, the cumulative stem tunnel length, ratio of cumulative stem tunnel length to stem length and the number of larvae recovered and pupae were subjected to analysis of variance using PROC GLM of SAS (SAS, 2003) program and LSD used for the separation of means as well as analysis using statistical contrasts.
Dot blots provided initial screening of transformed and non-transformed maize crosses from parents, F₁, and F₂₃ generations for the presence of the CryⅠAb protein. The plant damage and ELISA data were then subjected to analysis of variance (ANOVA), and the means were computed and separated using t tests (LSD), for each experimental data set at (P=0.05).
CHAPTER FOUR: RESULTS

4.0

4.1 Evaluation of genotypes for response to infestation by *Chilo partellus* larvae

4.1.1 Plant damage rating among transformed maize lines carrying events 216 and 223, non-transformed maize lines CML144 and CML159 and check cultivars.

Damage parameters recorded on the genotypes included foliar damage rating, number of exit holes, cumulative stem tunnel length, ratio of tunneled length vs stems length, number of larvae recovered and number of pupae recovered. The genotype evaluations were based on a modified scale (Guthrie *et al.*, 1960) as indicated below. The evaluations were carried out at V8 stage; hence the scores had not risen to the full 1-9 scale were it that the plant were to be evaluated at physiological maturity.

<table>
<thead>
<tr>
<th>Table 1. Modified scale for plant damage ratings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Resistant</td>
</tr>
<tr>
<td>Moderately resistant</td>
</tr>
<tr>
<td>Susceptible</td>
</tr>
</tbody>
</table>

**Foliar damage rating**

Highly significant differences (P<0.05) for foliar damage rating (FDR) scores were found among the genotypes (Table 2). The transformed maize inbred lines carrying events 216 and 223 had mean scores of 1.35 and 1.20, indicating resistance against *C. partellus* respectively. The non-transformed maize inbred lines CML144 and CML159 were susceptible and showed a mean score of 4.32 and 5.87 and LSD of 1.72 respectively. The
checks CML216, MBR, CKIR6009 and H513 had mean scores of, 5.12, 3.90, 3.45 and 5.45 and LSD (5%) of 1.72 respectively.

**Number of exit holes**

There were highly significant differences (P<0.05) for number of exit holes (EXITH) among the genotypes (Table 2). The transformed maize inbred lines carrying events 216 and 223 had 0.45 exit holes each which was an indication of low borer survivorship and infestation by *C. partellus*. The non-transformed inbred lines CML144 and CML159 were susceptible with a mean EXITH of 6.32 and 6.02 respectively. The checks CML216 and MBR had mean EXITH scores of 9.35 and 2.87 respectively. CML216 was highly susceptible than MBR. The non-transformed hybrids CKIR6009 and H513 showed a mean EXITH of 2.82 and 2.90 and were moderately resistant. The LSD (5%) value was 3.00 for EXITH.

**Table 2: Plant damage ratings among the transformed maize inbred lines carrying events 216 and 223 and check cultivars**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar damage</th>
<th>No. Exit holes</th>
<th>Tunnel length (cm)</th>
<th>TL:SL ratio</th>
<th>No. larvae</th>
<th>No. pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event 216</td>
<td>1.35</td>
<td>0.45</td>
<td>1.40</td>
<td>0.02</td>
<td>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>Event 223</td>
<td>1.20</td>
<td>0.45</td>
<td>0.50</td>
<td>0.01</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>CML144</td>
<td>4.32</td>
<td>6.32</td>
<td>10.03</td>
<td>0.13</td>
<td>1.78</td>
<td>0.20</td>
</tr>
<tr>
<td>CML159</td>
<td>5.87</td>
<td>6.02</td>
<td>13.33</td>
<td>0.22</td>
<td>2.45</td>
<td>0.62</td>
</tr>
<tr>
<td>CML216</td>
<td>5.12</td>
<td>9.35</td>
<td>21.47</td>
<td>0.51</td>
<td>3.63</td>
<td>1.77</td>
</tr>
<tr>
<td>MBR</td>
<td>3.90</td>
<td>2.87</td>
<td>7.90</td>
<td>0.086</td>
<td>1.48</td>
<td>0.20</td>
</tr>
<tr>
<td>H513</td>
<td>5.45</td>
<td>2.90</td>
<td>5.07</td>
<td>0.03</td>
<td>0.98</td>
<td>0.12</td>
</tr>
<tr>
<td>CKIR6009</td>
<td>3.45</td>
<td>2.82</td>
<td>1.75</td>
<td>0.01</td>
<td>0.40</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>3.32</td>
<td>3.90</td>
<td>7.68</td>
<td>0.13</td>
<td>1.40</td>
<td>0.38</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>1.72</td>
<td>3.00</td>
<td>7.04</td>
<td>0.13</td>
<td>1.76</td>
<td>0.88</td>
</tr>
</tbody>
</table>
Cumulative stem tunnel length (TL) (cm)
There were highly significant differences (P<0.05) for cumulative stem tunnel length (TL) among the genotypes (Table 2). Inbred lines with Bt (carrying events 216 and 223) had TL of 1.4 and 0.5 cm respectively. This was an indication of resistance to *C. partellus* since the stem borer incidence was low. The non-transformed inbred lines CML144 and CML159 showed a mean of 10.03 and 13.33 cm respectively. The checks CML216 and MBR had TL of 21.47 and 7.90 cm respectively. MBR was moderately resistant unlike CML216 which was highly susceptible. The non-transformed hybrid checks CKIR6009 and H513 had TL of 1.75 and 5.07 cm respectively, and were moderately resistant. The LSD (5%) value was 7.04.

Ratio of cumulative stem tunnel length to stem length (TL:SL)
Highly significant differences (P<0.05) were observed for mean ratio of cumulative stalk tunneled length to stem length (TL:SL) among the genotypes (Table 2). Inbred lines with Bt (carrying events 216 and 223) had TL:SL ratios of 0.02 and 0.01 respectively, indicating resistance to *C. partellus* and a low borer incidence. The non-transformed inbred lines CML144 and CML159 had a mean TL:SL of 0.13 and 0.22 respectively, indicating high susceptibility. The non-transformed hybrid checks CKIR6009 and H513 had TL:SL ratios of 0.01 and 0.03, respectively and were not different from the Bt inbred lines. The LSD (5%) value was 0.13.

Number of recovered larvae and pupae
There were highly significant differences (P<0.05) for number of larvae recovered and number of pupae recovered among the genotypes (Table 2). Inbred lines with Bt (carrying events 216 and 223) had 0.20 and 0.25 larvae and 0.08 and 0.0 pupae indicating resistance to *C. partellus* respectively. The non-transformed inbred lines CML144 and
CML159 had a mean number of larvae and pupae recovered as 0.20 and 0.62 respectively. CML159 showed more susceptibility than CML144. The checks CML216 and MBR had mean number of larvae and pupae of 3.63 and 1.48 and 1.77 and 0.20 respectively. MBR was more resistant than CML216 since it had a lower borer survivorship. The LSD (5%) value was 1.76 and 0.88 for number of larvae and pupae recovered respectively.

4.1.2 Plant damage rating among crosses of transformed maize lines carrying events 216 and 223, their F1s and F3s.

No significant differences (P<0.15) were observed among the parents, F1s and F3 generations in this cross (Table 3) for foliar damage score, number of exit holes, tunnel length, TL:SL ratio, number of larvae, and number of pupae recovered. The results were as expected since each of the parental maize lines transformed using Bt genes carrying events 216 and 223 were insect resistance.

Table 3. Plant damage rating among crosses of transformed maize lines carrying events 216 and 223, their F1s and F3s.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar damage</th>
<th>No. Exit holes</th>
<th>Tunnel length (cm)</th>
<th>TL:SL ratio</th>
<th>No. larvae</th>
<th>No. pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event 216</td>
<td>1.35</td>
<td>0.45</td>
<td>1.40</td>
<td>0.017</td>
<td>0.20</td>
<td>0.075</td>
</tr>
<tr>
<td>Event 223</td>
<td>1.20</td>
<td>0.45</td>
<td>0.50</td>
<td>0.01</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>F1</td>
<td>1.16</td>
<td>0.90</td>
<td>0.13</td>
<td>0.00</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>F3</td>
<td>2.35</td>
<td>2.37</td>
<td>4.26</td>
<td>0.07</td>
<td>0.69</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean</td>
<td>1.52</td>
<td>1.04</td>
<td>1.57</td>
<td>0.02</td>
<td>0.35</td>
<td>0.06</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
4.1.3 Plant damage rating among non-transformed maize line CML144 and transformed maize line carrying event 216, their F₁s and F₂:₃s

Foliar damage rating

The transformed maize line carrying event 216 had a FDR of 1.35, indicating resistance against *C. partellus*. It was significantly different from the non-transformed inbred line which was susceptible with a mean score of 4.33. The F₁ had a mean score of 1.88, was not significantly different from the Bt inbred line, and the results indicated that resistance was inherited as a dominant trait. The F₂:₃ res had a mean score of 2.30, indicating moderate resistance. The F₂:₃ sus had a mean score of 4.38, was similar to the susceptible parent (CML144). The LSD (5%) value was 1.065 (Table 4).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar damage</th>
<th>No. Exit holes</th>
<th>Tunnel length (cm)</th>
<th>TL:SL ratio</th>
<th>No. larvae</th>
<th>No. pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event 216</td>
<td>1.35</td>
<td>0.45</td>
<td>1.40</td>
<td>0.02</td>
<td>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>CML144</td>
<td>4.33</td>
<td>6.33</td>
<td>10.03</td>
<td>0.13</td>
<td>1.78</td>
<td>0.20</td>
</tr>
<tr>
<td>F₁s</td>
<td>1.88</td>
<td>1.98</td>
<td>7.13</td>
<td>0.04</td>
<td>0.83</td>
<td>0.20</td>
</tr>
<tr>
<td>F₂:₃ res</td>
<td>2.30</td>
<td>3.34</td>
<td>7.63</td>
<td>0.05</td>
<td>0.79</td>
<td>~ 0.15</td>
</tr>
<tr>
<td>F₂:₃ sus</td>
<td>4.38</td>
<td>7.64</td>
<td>15.14</td>
<td>0.11</td>
<td>1.73</td>
<td>0.57</td>
</tr>
<tr>
<td>Mean</td>
<td>2.85</td>
<td>3.95</td>
<td>8.26</td>
<td>0.07</td>
<td>1.06</td>
<td>0.24</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>1.065</td>
<td>2.393</td>
<td>4.873</td>
<td>0.0526</td>
<td>0.6510</td>
<td>0.4370</td>
</tr>
</tbody>
</table>

**Number of exit holes (EXITH)**

The transformed maize line carrying event 216 had 0.45 exit holes and was significantly different from CML144 which had 6.33 exit holes. This showed that maize line carrying
event 216 had a low borer survivorship and infestation by *C. partellus* compared to CML144. The F₁ had 1.98 exit holes and was not significantly different from the transformed inbred line carrying Bt gene. This indicated that resistance was inherited as a dominant trait since F₁s were resistant. The F₂,₃ res and F₂,₃ sus had 3.34 and 7.64 exit holes and were significantly different from the Bt inbred line. The LSD (5%) value was 2.393 (Table 4).

**Cumulative stem tunnel length (TL) (cm)**
There were significant differences (P<0.05) for TL among the parents, F₁ and F₂,₃ generations in this cross (Table 4; Plate 2). The transformed maize line carrying event 216 had a tunnel length of 1.4 cm and was significantly different from CML144 which had 10.03 cm. The F₁ had TL of 7.13 which showed that resistance *C. partellus* was inherited as a dominant trait. The F₂,₃ res and F₂,₃ sus respectively had TL of 7.63 and 15.14 cm and were significantly different from the Bt inbred line. The LSD (5%) value was 4.873 (Table 4).

**Ratio of cumulative stem tunnel length to stem length (TL:SL)**
There were significant differences (P<0.05) for TL:SL ratio among the parents, F₁ and F₂,₃ generations in this cross (Table 3). The transformed maize line carrying event 216 and CML144 had a TL:SL of 0.02 and 0.13 respectively, indicating resistance and susceptibility to *C. partellus*. The F₁ and F₂,₃ res respectively had TL:SL ratios of 0.04 and 0.05 that were not significantly different from the transformed inbred line. The F₁ and F₂,₃ res respectively showed resistance to *C. partellus*. The F₂,₃ sus had a TL:SL ratio of 0.11 that was not significantly different from CML144. The LSD (5%) value was 0.0526 (Table 4).
Number of recovered larvae and pupae
There were significant differences (P<0.05) for number of larvae recovered and number of pupae recovered among the parents, F₁ and F₂:₃ generations in this cross (Table 3). The transformed maize line carrying event 216 had 0.20 larvae and 0.08 pupae, indicating resistance to *C. partellus*. The CML144 had 1.78 larvae and 0.20 pupae, indicating susceptibility to *C. partellus*. The F₁ and F₂:₃ res respectively had 0.83 and 0.79 larvae and 0.20 and 0.15 pupae and were not significantly different from the Bt inbred line. The F₂:₃ sus had 1.73 larvae and 0.57 pupae and were not significantly different from CML144. The LSD (5%) value was 0.6510 and 0.4370 for number of larvae and number of pupae recovered respectively (Table 4).

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Plate 2: Cumulative stalk tunneling by *Chilo partellus* on the transformed maize line carrying event 216, CML144, F₁ and F₂:₃ generations.
4.1.4 Plant damage rating among crosses of non-transformed maize line CML159 and transformed maize line carrying event 216, their F1s and F2:3s

**Foliar damage rating**

Highly significant differences (P<0.05) for FDR were observed among the parents, F1 and F2:3 generations in this cross (Table 5). The transformed maize line carrying event 216 had a FDR of 1.35, indicating resistance against *C. partellus*. Event 216 was significantly different from the non-transformed inbred line which was susceptible with a score of 5.88. The F1 had a mean score of 2.28 was not significantly different from the transformed inbred line, and the results indicated that resistance was inherited as a dominant trait. The F2:3 res had a mean score of 2.33 and were not significantly different from the transformed inbred line. The F2:3 sus had a mean score of 3.87, was similar to the susceptible parent (CML159). The LSD (5%) value was 0.4858.

**Number of exit holes (EXITH)**

There were significant differences (P<0.05) for EXITH among the parents, F1 and F2:3 generations in this cross (Table 5). The transformed maize line carrying event 216 had 0.45 exit holes and was significantly different from CML159 which had 6.02 exit holes. This showed that transformed maize line carrying event 216 had a low borer survivorship and infestation by *C. partellus* compared to CML159. The F1 had 2.23 EXITH and was not significantly different from the transformed maize inbred line. This showed that resistance was inherited as a dominant trait since F1s were resistant. The F2:3 res and F2:3 sus had 3.79 and 4.97 exit holes respectively and were significantly different from the Bt inbred line. The LSD (5%) value was 1.949 (Table 5).
Table 5. Plant damage rating among crosses of non-transformed maize line CML159 and transformed maize line carrying event 216, their F₁s and F₂:₃s

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar damage</th>
<th>No. Exit holes</th>
<th>Tunnel length (cm)</th>
<th>TL:SL ratio</th>
<th>No. larvae</th>
<th>No. pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML159</td>
<td>5.88</td>
<td>6.02</td>
<td>13.33</td>
<td>0.22</td>
<td>2.45</td>
<td>0.63</td>
</tr>
<tr>
<td>Event 216</td>
<td>1.35</td>
<td>0.45</td>
<td>1.40</td>
<td>0.02</td>
<td>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>F₁s</td>
<td>2.28</td>
<td>2.23</td>
<td>4.93</td>
<td>0.03</td>
<td>0.90</td>
<td>0.08</td>
</tr>
<tr>
<td>F₂:₃ res.</td>
<td>2.33</td>
<td>3.79</td>
<td>6.44</td>
<td>0.04</td>
<td>0.81</td>
<td>0.10</td>
</tr>
<tr>
<td>F₂:₃ sus.</td>
<td>3.87</td>
<td>4.97</td>
<td>11.47</td>
<td>0.08</td>
<td>1.40</td>
<td>0.35</td>
</tr>
<tr>
<td>Mean</td>
<td>3.142</td>
<td>3.492</td>
<td>7.514</td>
<td>0.078</td>
<td>1.152</td>
<td>0.248</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>0.4858</td>
<td>1.949</td>
<td>4.907</td>
<td>0.6202</td>
<td>0.7455</td>
<td>0.256</td>
</tr>
</tbody>
</table>

Cumulative stem tunnel length (TL) (cm)
There were significant differences (P<0.05) for TL among the parents, F₁ and F₂:₃ generations in this cross (Table 5). The transformed maize line carrying event 216 had a tunnel length of 1.40 cm and was significantly different from CML159 which had 13.33 cm. The F₁ had a TL of 4.93 which showed that resistance *C. partellus* was inherited as a dominant trait. The F₂:₃ res and F₂:₃ sus respectively had TL of 6.44 and 11.47 cm and were significantly different from the Bt inbred line. The LSD (5%) value was 4.907 (Table 5).

Ratio of cumulative stem tunnel length to stem length (TL:SL)
There were significant differences (P<0.05) for TL:SL ratio among the parents, F₁ and F₂:₃ generations in this cross (Table 5). The transformed maize line carrying event 216 had a TL:SL ratio of 0.02 and was significantly different from CML159 which had 0.22, indicating resistance and susceptibility to *C. partellus* respectively. The F₁ and F₂:₃ res
respectively had TL:SL ratios of 0.03 and 0.04 that were not significantly different from the Bt inbred line. The F₁ and F₂:₃ res respectively showed resistance to *C. partellus*. The F₂:₃ sus had a TL:SL ratio of 0.08 that was not significantly different from CML159. The LSD (5%) value was 0.6202 (Table 5).

**Number of recovered larvae and pupae**

There were significant differences (P<0.05) for number of larvae recovered and number of pupae recovered among the parents, F₁ and F₂:₃ generations in this cross (Table 5). The transformed maize line carrying event 216 had 0.20 larvae and 0.08 pupae, showing resistance to *C. partellus*. The CML159 had 2.45 larvae and 0.63 pupae, indicating susceptibility to *C. partellus*. The F₁ and F₂:₃ res respectively had 0.90 and 0.81 larvae and 0.08 and 0.10 pupae and were not significantly different from the Bt inbred line. The F₂:₃ sus had 1.40 larvae and 0.35 pupae and were not significantly different from CML159. The LSD (5%) value was 0.7455 and 0.2560 for number of larvae and pupae recovered respectively (Table 5).

**4.1.5 Plant damage rating among crosses of non-transformed maize line CML144 and transformed maize line carrying event 223, their F₁s and F₂:₃s**

**Foliar damage rating**

There were significant differences (P<0.05) for FDR observed among the parents, F₁ and F₂:₃ generations in this cross (Table 6). The transformed maize line carrying event 223 had a FDR of 1.20, indicating resistance against *C. partellus*. Event 223 was significantly different from the non-transformed inbred line which was susceptible with a mean score of had a score of 4.33. The F₁ were resistant with a mean score of 1.50 was not significantly different from the Bt inbred line, and the results indicated that resistance was inherited as a dominant trait. The F₂:₃ res had a mean score of 1.62 and were not
significantly different from the Bt inbred line. The F\textsubscript{2:3} sus had a mean score of 3.78, was similar to the susceptible parent (CML144). The LSD (5\%) value was 1.242 (Table 6).

**Table 6. Plant damage rating among crosses of non-transformed maize line CML144 and transformed maize line carrying event 223, their F\textsubscript{1}s and F\textsubscript{2:3}s**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar damage</th>
<th>No. Exit holes</th>
<th>Tunnel length (cm)</th>
<th>TL:SL ratio</th>
<th>No. larvae</th>
<th>No. pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML144</td>
<td>4.33</td>
<td>6.33</td>
<td>10.03</td>
<td>0.13</td>
<td>1.78</td>
<td>0.20</td>
</tr>
<tr>
<td>Event 223</td>
<td>1.20</td>
<td>0.45</td>
<td>0.50</td>
<td>0.01</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>F\textsubscript{1}s</td>
<td>1.50</td>
<td>0.88</td>
<td>3.35</td>
<td>0.16</td>
<td>0.70</td>
<td>0.00</td>
</tr>
<tr>
<td>F\textsubscript{2:3} res</td>
<td>1.62</td>
<td>2.80</td>
<td>4.28</td>
<td>0.03</td>
<td>0.58</td>
<td>0.06</td>
</tr>
<tr>
<td>F\textsubscript{2:3} sus</td>
<td>3.78</td>
<td>11.13</td>
<td>13.70</td>
<td>0.08</td>
<td>1.90</td>
<td>0.23</td>
</tr>
<tr>
<td>Mean</td>
<td>2.486</td>
<td>4.318</td>
<td>6.372</td>
<td>0.082</td>
<td>1.042</td>
<td>0.098</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>1.242</td>
<td>4.583</td>
<td>8.920</td>
<td>0.054</td>
<td>1.409</td>
<td>0.1924</td>
</tr>
</tbody>
</table>

**Number of exit holes (EXITH)**

There were significant differences (P<0.05) for EXITH among the parents, F\textsubscript{1} and F\textsubscript{2:3} generations in this cross (Table 6). The transformed maize line carrying event 223 had 0.45 exit holes and was significantly different from CML144 which had 6.33 exit holes. This showed that Event 223 had a low borer survivorship and infestation by *C. partellus* compared to CML144. The F\textsubscript{1} had 0.88 exit holes and was not significantly different from the Bt inbred line. This indicated that resistance was possibly inherited as a dominant trait since F\textsubscript{1}s were resistant. The F\textsubscript{2:3} res and F\textsubscript{2:3} sus had 2.88 and 11.13 exit holes respectively and were significantly different from the Bt inbred line. The LSD (5\%) value was 4.583 (Table 6).
There were significant differences (P<0.05) for TL among the parents, F₁ and F₂:₃ generations in this cross (Table 6). The transformed maize line carrying event 223 had a tunnel length of 0.50 cm and was significantly different from CML144 which had 10.03 cm. The F₁ had TL of 3.35 which showed that resistance *C. partellus* was inherited as a dominant trait. The F₁, F₂:₃ res and F₂:₃ sus respectively had tunnel lengths of 3.35, 4.28, and 13.70 cm and were significantly different from the Bt inbred line. The LSD (5%) value was 8.92 (Table 6).

**Ratio of cumulative stem tunnel length to stem length (TL:SL)**

There were significant differences (P<0.05) for TL:SL ratio among the parents, F₁ and F₂:₃ generations in this cross (Table 6). The transformed maize line carrying event 223 and CML144 had a TL:SL ratio of 0.01 and 0.13 respectively, indicating resistance and susceptibility to *C. partellus*. The F₁ and F₂:₃ res respectively had TL:SL ratios of 0.16 and 0.03 that were not significantly different from the Bt inbred line. The F₁ and F₂:₃ res respectively showed resistance to *C. partellus* The F₂:₃ sus had a TL:SL ratio of 0.08 that was not significantly different from CML144. The LSD (5%) value was 0.054 (Table 6).

**Number of recovered larvae and pupae**

There were significant differences (P<0.05) for number of larvae recovered and number of pupae recovered among the parents, F₁ and F₂:₃ generations in this cross (Table 6). The transformed maize line carrying event 223 had a mean 0.25 larvae and 0.00 pupae, showing resistance to *C. partellus*. The CML144 had 1.78 larvae and 0.20 pupae, indicating susceptibility to *C. partellus*. The F₁ and F₂:₃ res respectively had 0.70 and 0.00 larvae and 0.58 and 0.06 pupae that were not significantly different from the
transformed inbred line. The $F_{2:3}$ sus had 1.90 larvae and 0.23 pupae that were not significantly different from CML144. The LSD (5%) value was 1.409 and 0.1924 respectively for number of larvae and pupae recovered respectively (Table 6).

4.1.6 Plant damage rating among crosses of non-transformed maize line CML159 and transformed maize line carrying event 223, their $F_1$s and $F_{2:3}$s

Foliar damage rating

There were significant differences ($P<0.05$) for FDR observed among the parents, $F_1$ and $F_{2:3}$ generations in this cross (Table 7 and Plate 3). The transformed maize line carrying event 223 had a FDR of 1.20, point towards resistance against *C. partellus*. Event 223 was significantly different from the susceptible non-transformed inbred line which had a score of 5.88. The $F_1$ had a mean score of 1.63 was not significantly different from the Bt inbred line, and the results indicated that resistance was inherited as a dominant trait. The $F_{2:3}$ res had a mean score of 2.33 and were not significantly different from the Bt inbred line. The $F_{2:3}$ sus had a mean score of 3.87, was similar to the susceptible parent (CML159). The LSD (5%) value was 1.838 (Table 7).
Plate 3: Foliar damage by *C. partellus* on successive generations of crosses of CML159 x transformed maize line carrying event 223

**Number of exit holes**

There were significant differences (P<0.05) for EXITH among the parents, F₁ and F₂:₃ generations in this cross (Table 7). The transformed maize line carrying event 223 had 0.45 exit holes and was significantly different from CML159 which had 6.03 exit holes. This indicated that transformed line had a low borer survivorship and infestation by *C. partellus* compared to CML159. The F₁ had 1.75 exit holes and was not significantly different from the transformed line. The F₁:s were resistant which showed that resistance was inherited as a dominant trait. The F₂:₃ res and F₂:₃ sus had 1.55 and 5.97 exit holes respectively and were significantly different from the transformed line. The LSD (5%) value was 3.996 (Table 7).
Cumulative stem tunnel length (TL) (cm)

There were significant differences (P<0.05) for TL among the parents, F₁ and F₂:₃ generations in this cross (Table 7). The transformed maize line carrying event had a tunnel length of 0.50 cm and was significantly different from CML159 which had 13.33 cm. The F₁ had a TL of 4.05cm which showed that resistance *C. partellus* was inherited as a dominant trait. The F₂:₃ res and F₂:₃ sus respectively had tunnel lengths of 3.58 and 12.59 cm and were significantly different from the Bt inbred line. The LSD (5%) value was 9.240 (Table 7).

Table 7. Plant damage rating among crosses of non-transformed maize line CML159 and transformed maize line carrying event 223, their F₁S and F₂:₃S

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar damage</th>
<th>No. Exit holes</th>
<th>Tunnel length (cm)</th>
<th>TL:SL ratio</th>
<th>No. larvae</th>
<th>No. pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML159</td>
<td>5.88</td>
<td>6.03</td>
<td>13.33</td>
<td>0.22</td>
<td>2.45</td>
<td>0.63</td>
</tr>
<tr>
<td>Event 223</td>
<td>1.20</td>
<td>0.45</td>
<td>0.50</td>
<td>0.01</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>F₁S</td>
<td>1.63</td>
<td>1.75</td>
<td>4.05</td>
<td>0.02</td>
<td>0.45</td>
<td>0.08</td>
</tr>
<tr>
<td>F₂:₃ res.</td>
<td>1.50</td>
<td>1.55</td>
<td>3.58</td>
<td>0.02</td>
<td>0.43</td>
<td>0.05</td>
</tr>
<tr>
<td>F₂:₃ sus.</td>
<td>3.78</td>
<td>5.97</td>
<td>12.59</td>
<td>0.09</td>
<td>1.10</td>
<td>0.70</td>
</tr>
<tr>
<td>Mean</td>
<td>2.186</td>
<td>2.478</td>
<td>5.25</td>
<td>0.043</td>
<td>0.638</td>
<td>0.182</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>1.838</td>
<td>3.996</td>
<td>9.240</td>
<td>0.907</td>
<td>0.955</td>
<td>0.502</td>
</tr>
</tbody>
</table>

Ratio of cumulative stem tunnel length to stem length (TL:SL)

There were significant differences (P<0.05) for TL:SL ratio among the parents, F₁ and F₂:₃ generations in this cross (Table 7). The transformed maize line carrying event 223 had a TL:SL ratio of 0.01 and was significantly different from CML159 which had 0.22,
indicating resistance and susceptibility to *C. partellus* respectively. The F\(_1\) and F\(_{2:3}\) res respectively had TL:SL ratios of 0.02 and 0.02 that were not significantly different from the Bt inbred line. The F\(_1\) and F\(_{2:3}\) res respectively indicated resistance to *C. partellus* and that it was inherited as a dominant trait. The F\(_{2:3}\) sus had a TL:SL ratio of 0.09 that was not significantly different from CML159. The LSD (5%) value was 0.907 (Table 7).

**Number of recovered larvae and pupae**

No significant differences were observed for mean number of larvae recovered (p<0.7010) and number of pupae recovered (p<0.7435) for F\(_1\)s with a mean of 0.45 and 0.08 for larvae and pupae respectively. Among the F\(_{2:3}\) res and F\(_{2:3}\) sus, there were no significant differences (p<0.632), with a mean of 0.43 and 0.05 for larvae, and 1.10 and 0.70 for pupae respectively (Table 7). The LSD (5%) value was 0.502 (Table 7).

**4.1.7 Plant damage rating among crosses of non-transformed maize line CML144 x CML 159, their F\(_1\)s and F\(_{2:3}\)s**

**Foliar damage rating**

No significant differences (P<0.35) for foliar damage rating (FDR) scores were found among the genotypes used in this study (Table 8). The non-transformed inbred lines CML144 and CML159 had mean scores of 4.33 and 5.88, respectively and were significantly different from the Bt inbred lines. The F\(_1\) and the F\(_3\) had mean score of 3.23 and 3.38 respectively. The results for all the plant damage parameters were as expected since each of the two parents carried the no Bt gene for insect resistance.

**Number of exit holes**

No significant differences (P<0.49) for EXITH among the non-transformed inbred lines (CML 144 and CML159) used in this cross (Table 8). The F\(_1\) had 3.05 exit holes and was
significantly different from the Bt F$_1$'s. The F$_3$ had 4.60 exit holes and were significantly different from the Bt F$_3$'s.

**Cumulative stem tunnel length (TL) (cm)**

No significant differences (P<0.16) for TL among the non-transformed inbred lines (CML144 and CML159), F$_1$ and F$_3$ generations in this cross (Table 8). The non-transformed inbred lines (CML144 and CML159), F$_1$ and F$_3$ respectively had a mean tunnel length of 10.00 and 13.33 cm, 6.38 cm and 9.86 cm.

**Table 8. Plant damage rating among crosses of non-transformed maize lines CML144 x CML159**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar damage</th>
<th>No. Exit holes</th>
<th>Tunnel length (cm)</th>
<th>TL:SL ratio</th>
<th>No. larvae</th>
<th>No. pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML144</td>
<td>4.33</td>
<td>6.33</td>
<td>10.03</td>
<td>0.13</td>
<td>1.78</td>
<td>0.20</td>
</tr>
<tr>
<td>CML159</td>
<td>5.88</td>
<td>6.03</td>
<td>13.33</td>
<td>0.22</td>
<td>2.45</td>
<td>0.63</td>
</tr>
<tr>
<td>F$_1$s</td>
<td>3.23</td>
<td>3.05</td>
<td>6.38</td>
<td>0.04</td>
<td>1.15</td>
<td>0.40</td>
</tr>
<tr>
<td>F$_3$</td>
<td>3.38</td>
<td>4.60</td>
<td>9.86</td>
<td>0.07</td>
<td>1.21</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean</td>
<td>4.21</td>
<td>5.00</td>
<td>9.90</td>
<td>0.12</td>
<td>1.65</td>
<td>0.38</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Ratio of cumulative stem tunnel length to stem length (TL:SL)**

No significant differences (P<0.90) for TL:SL ratio among the parents non-transformed inbred lines (CML144 and CML159), F$_1$ and F$_3$ generations in this cross (Table 8). The non-transformed inbred lines (CML144 and CML159), F$_1$ and F$_3$ respectively had a TL:SL ratio of 0.13, 0.22 and 0.04 and 0.07.
**Number of recovered larvae and pupae**

No significant differences (P<0.71) for number of larvae recovered and (p<0.74) for number of pupae recovered among the non-transformed inbred lines (CML144 and CML159), F₁ and F₃ generations in this cross (Table 8). The non-transformed inbred lines (CML144 and CML159), F₁ and F₃ respectively had a mean number of larvae recovered of 1.78, and 2.45, 1.15 and 1.21 and a mean number of pupae recovered 0.20, 0.63, 0.40, and 0.27 (Table 8).
4.2: Evaluation of genotypes for dot blots in various crosses of transformed and non-transformed maize inbred lines

4.2.1 Dot blots recorded for maize lines transformed using Bt genes carrying event 216 and 223 and check cultivars

The dot blots of Cry1Ab protein extracts from selected Bt and non-maize lines transformed using Bt genes leaf samples from the different parents' generation (Plate 4) shows that lane 1 with a negative standard Bt control 0μg/g Bt protein scored visually similarly in low colour intensity as the non-transformed maize extracts in lanes 2, 3 and 4 (CML144, CML159 and CML216), while the extracts in lane 5 with a positive standard Bt control 1.5μg/g Bt protein scored had a colour intensity as that of lane 6 and 7 (Event 216 and Event 223).

Plate 4: Dot blots of Cry1Ab protein extracts from transformed and non-transformed maize leaf samples from the different parents and check cultivars. The different antigens were spotted and their reactions were visually observed and reported as: - Lane 1, Bt control 0μg/g Bt protein; Lane 2, CML 144: Plant No.1: Plot 222; Lane 3, CML 159: Plant No.1: Plot 104; Lane 4, CML 216: Plant No.2: Plot 49; Lane 5, Bt control 1.5μg/g Bt protein; Lane 6, Event 223: Plant No.1: Plot 145 and Lane 7, Event 216: Plant No.1: Plot 199.

4.2.2 Dot blots for F1 generations

The dot blots of Cry1Ab protein extracts from selected leaves of F1 generations' plants (Plate 5) shows that lane 7 with a positive standard Bt control 1.5μg/g leaf tissue scored
visually similar in high colour intensity as the transformed maize and extracts from its
crosses in lane 1, Event 223 x Event 216F₁; lane 2, CML144 x Event 216F₁; lane 3,
CML144 x Event 223F₁; lane 4, CML159 x Event 216F₁ and lane 5, CML159 x Event
223F₁. Dot blots in lane 6 with the CML144 x CML159F₁ scored visually low colour
intensity comparable to lane 8, with a negative standard Bt control 0µg/g Bt protein.

Plate 5: Dot blots of Cry1Ab protein extracts from maize lines transformed carrying event
216 and non-transformed maize leaf samples in the various crosses of F₁ generations. The
different antigens were spotted and their reactions were visually observed and reported as:
- Lane 1, Event 223 x Event 216F₁: Plant No.1: Plot 157; Lane 2, CML144 x Event 216F₁:
  Plant No.1: Plot 203; Lane 3, CML144 x Event 223F₁: Plant No.1: Plot 265; Lane 4,
  CML159 x Event 216F₁: Plant No.1: Plot 159; Lane 5, CML159 x Event 223F₁: Plant No.1:
  Plot 225; Lane 6, CML144 x CML159F₁: Plant No.1: Plot 169; Lane 7, Bt control 1.5µg/ g
  leaf tissue and Lane 8, Bt control 0µg/g Bt protein.

4.2.3 Dot blots for F₂:₃ and F₃ generations
As expected at the F₂:₃ generations, variability would be observed in most of the plant
damage parameters as well as the data on the dot blots and the ELISA. These differences
may follow Mendelian segregation analyses of large samples sizes.
4.2.3.1 Dot blots for maize lines transformed using Bt genes carrying events 216 and 223 generations

The dot blots of Cry1Ab protein extracts from leaves of F_{23} generations' plants (Plate 6) shows that lane 9 with a positive standard Bt control 1.5μg/ g leaf tissue scored a high colour intensity as the Transformed maize lines carrying Bt genes Event 223 x Event 216 and their crosses in lanes 1, 3, 5 and 6, while lanes 2, 4, 7 and 8 had a slightly lesser colour intensity comparatively. No plant sample extracted from the F_{23} generation of the Transformed maize lines carrying Bt genes Event 223 x Event 216 crosses had colour intensity comparable to the negative standard Bt control 0μg/g Bt protein.

Plate 6: Dot blots of Cry1Ab protein extracts from maize lines transformed Bt genes carrying events 216 and 223 leaf samples in the various crosses of Events 223 and 216 F_{2,3} generations. The different antigens were spotted and their reactions were visually observed and reported as: - Lane 1, Plant No.1: Plot 9; Lane 2, Plant No.1: Plot 71; Lane 3, Plant No.1: Plot 67; Lane 4, Plant No.1: Plot 285; Lane 5, Plant No.1: Plot 70; Lane 6, Plant No.1: Plot 260; Lane 7, Plant No.1: Plot 193; Lane 8, Plant No.1: Plot 153; Lane 9, Bt control 1.5μg/g Bt protein and Lane 10, Bt control 0μg/g Bt protein.

4.2.3.2 Dot blots for non-transformed maize line CML144 x maize lines transformed carrying event 216 F_{2,3} generations

The dot blots of Cry1Ab protein extracts from leaves of F_{2,3} generations shows that there were differences in colour intensity formed among the crosses of the CML144 x Event 216 (Plate 7) and CML144 x Event 223 (Plate 8) and CML159 x Event 216 (Plate 9) and
CML159 x Event 223 (Plate 10). The dot blots for CML144 x Event 216 F$_{2:3}$ generations on lanes 1, 4 and 6 had high colour intensity comparable to the Bt control 1.5µg/g Bt protein in lane 9 while lanes 3, 7 and 8 had medium colour intensity. However, lanes 2 and 5 had low colour intensity comparable to the Bt control 0µg/g Bt protein in lane 10.

Plate 7: Dot blots of Cry1Ab protein extracts from maize leaf samples in the various non-transformed maize line CML144 x maize lines transformed carrying event 216 F$_{2:3}$ generations. The different antigens were spotted and their reactions were visually observed and reported as: - Lane 1, Plant No.1: Plot 132; Lane 2, Plant No.1: Plot 240; Lane 3, Plant No.1: Plot 77; Lane 4, Plant No.1: Plot 52; Lane 5, Plant No.1: Plot 2; Lane 6, Plant No.1: Plot 239; Lane 7, Plant No.1: Plot 16; Lane 8, Plant No.1: Plot 202; Lane 9, Bt control 1.5µg/g Bt protein and Lane 10, Bt control 0µg/g Bt protein.

4.2.3.3 Dot blots for non-transformed maize line CML144 x maize lines transformed carrying event 223 F$_{2:3}$ generations

The dot blots for CML144 x Event 223 F$_{2:3}$ generations on lanes 6, 7 and 8 had high colour intensity comparable to the Bt control 1.5µg/g Bt protein in lane 2 while lanes 1 and 3 had low colour intensity similar to the Bt control 0µg/g Bt protein in lane 4 (Plate 8).
Plate 8: Dot blots of Cry1Ab protein extracts from maize leaf samples in non-transformed maize line CML144 x maize lines transformed carrying event 223 F2:3 generations. The different antigens were spotted and their reactions were visually observed and reported as:
- Lane 1, Plant No.2: Plot 271; Lane 2, Bt control 1.5µg/g Bt protein; Lane 3, Plant No.1: Plot 54; Lane 4, Bt control 0µg/g Bt protein; Lane 5, Plant No.1: Plot 161; Lane 6, Plant No.1: Plot 241; Lane 7, Plant No.1: Plot 74; and Lane 8, Plant No.1: Plot 109.

4.2.3.4 Dot blots for non-transformed maize line CML159 x maize lines transformed carrying event 216 F2:3 generations

The dot blots for CML159 x Event 216 F2:3 generations on lanes 3, 4 and 7 had high colour intensity comparable to the Bt control 1.5µg/g Bt protein in lane 5 while lanes 1 and 2 had low colour intensity similar to the Bt control 0µg/g Bt protein in lane 6 (Plate 9).

Plate 9: Dot blots of Cry1Ab protein extracts from maize leaf samples in the various crosses of CML159 x Event 223 F2:3 generations. The different antigens were spotted and their reactions were visually observed and reported as: - Lane 1, Plant No. 4: Plot 17, Lane 2, Plant No. 3: Plot 36, Lane 3, Plant No. 2: Plot 136, Lane 4, Plant No. 1: Plot 196, Lane 5, Bt
control 1.5µg/g Bt protein, Lane 6, Bt control 0µg/g Bt protein, Lane 7, Plant No. 2: Plot 216.

4.2.3.5 Dot blots for non-transformed maize line CML159 x maize lines transformed carrying event 223 F_{2.3} generations

The dot blots for CML159 x Event 223 F_{2.3} generations on lanes 2, 5 7 and 10 had high colour intensity comparable to the Bt control 1.5µg/g Bt protein in lane 8. Lanes 1, 3, and 4 had medium colour intensity while lanes 6 had low colour intensity similar to the Bt control 0µg/g Bt protein in lane 9 (Plate 10).

Plate 10: Dot blots of Cry1Ab protein extracts from maize leaf samples in the non-transformed maize line CML144 x maize lines transformed carrying event 223 F_{2.3} generations. The different antigens were spotted and their reactions were visually observed and reported as: - Lane 1, Plant No.2: Plot 277; Lane 2, Plant No.1: Plot 233; Lane 3, Plant No.1: Plot 185; Lane 4, Plant No.1: Plot 129; Lane 5, Plant No.2: Plot 274; Lane 6, Plant No.1: Plot 209; Lane 7, Plant No.1: Plot 33; Lane 8, Bt control 1.5µg/g Bt protein, Lane 9, Bt control 0µg/g Bt protein and Lane 10, Plant No.1: Plot 180.
4.3: Quantification of levels of Cry1Ab protein in various generations of crosses of transformed maize lines carrying event 216 and 223 x non-transformed maize line CML144 and CML159 using ELISA

4.3.1 Mean concentration of Bt δ-endotoxins in µg/g of lyophilized leaf tissue in Bt and non-Bt inbred lines and hybrids

Highly significant differences (P<0.05) for mean concentration of Bt δ-endotoxins were found among genotypes in this study (Table 9). Inbred lines with Bt (Events 216 and 223) had 4.93 and 4.63 µg/g, respectively. The non-Bt inbred lines and hybrid checks had no Bt. The F₁ crosses of Bt and non-Bt inbred lines had high levels of Bt δ-endotoxins, similar to the Bt inbred line. The F₁ between non-Bt inbred lines had no Bt. A dose response curve of absorbance of the coloured product formed vs. concentration was generated using results obtained from Bt protein standards from the ELISA kit (Figure 6).

![Figure 6: Mean concentration of Bt protein in µg/g from the standards.](image-url)
Table 9: Mean concentration of Bt δ-endotoxins (µg/g) of lyophilized leaf tissue in transformed and non-transformed inbred lines and hybrids

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mean concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transformed inbred</strong></td>
<td></td>
</tr>
<tr>
<td>Event 216</td>
<td>4.93</td>
</tr>
<tr>
<td>Event 223</td>
<td>4.63</td>
</tr>
<tr>
<td><strong>Non- transformed inbred lines</strong></td>
<td></td>
</tr>
<tr>
<td>CML144</td>
<td>0.00</td>
</tr>
<tr>
<td>CML159</td>
<td>0.00</td>
</tr>
<tr>
<td>CML216</td>
<td>0.00</td>
</tr>
<tr>
<td>MBR</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Non- transformed hybrids</strong></td>
<td></td>
</tr>
<tr>
<td>H513</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Hybrids among transformed and non-transformed inbred lines</strong></td>
<td></td>
</tr>
<tr>
<td>CML144 x Event 216F_1</td>
<td>4.24</td>
</tr>
<tr>
<td>CML144 x Event 223F_1</td>
<td>3.92</td>
</tr>
<tr>
<td>CML159 x Event 216F_1</td>
<td>5.44</td>
</tr>
<tr>
<td>CML159 x Event 223F_1</td>
<td>3.73</td>
</tr>
<tr>
<td>CML144 x CML159F_1</td>
<td>0.00</td>
</tr>
<tr>
<td>Event 223 x Event 216F_3</td>
<td>2.99</td>
</tr>
<tr>
<td>CML144 x Event 216F_2:3</td>
<td>3.90</td>
</tr>
<tr>
<td>CML144 x Event 223F_2:3</td>
<td>1.69</td>
</tr>
<tr>
<td>CML159 x Event 216F_2:3</td>
<td>1.60</td>
</tr>
<tr>
<td>CML159 x Event 216F_2;3</td>
<td>3.38</td>
</tr>
<tr>
<td>CML144 x CML159F_3</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>LSD (5%)</strong></td>
<td><strong>1.056</strong></td>
</tr>
</tbody>
</table>
5.0 CHAPTER FIVE

5.1 Discussions

Transformed maize lines carrying events 216 and 223 had low leaf damage, low number of exit holes, number of larvae and pupae recovered, and stem tunnel length compared to the non-Bt inbred lines and hybrid checks. This indicated the effectiveness of the two transformed maize lines carrying Bt genes in controlling *C. partellus*. It was also notable that inbred lines and hybrids designated to be carrying host plant resistance (MBR and CKIR6009) also had lower damage scores compared to susceptible lines (CML216, CML144, CML159 and H513). This indicates the effectiveness of conventional breeding resistance in these materials to *C. partellus* (Mugo et al., 2005; Mugo et al., 2007). The two events also showed higher colour intensity (very dark and distinct) that indicated high concentrations of Cry1Ab protein extracts (Bt δ-endotoxins) from the dot blot analysis.

The F₁s and F₃S of Event 223 x Event 216 and the F₁s and F₃S of CML144 x CML159 did not show significant differences for foliar damage, number of exit holes, cumulative stem tunnel length, ratio of tunneling vs plant height, number of larvae recovered, number of pupae recovered. The F₁s and F₃s of Event 223 x Event 216 were probably homozygous for the Bt gene alleles resistant unlike the F₁s and F₂:₃s of CML144 x CML159 which may be homozygous resistance to the susceptible for *C. partellus* stem borer.

However, among the CML144 x Event 216F₁s, CML144 x Event 223F₁s, CML159 x Event 216F₁s and CML159 x Event 223F₁s there were no significant differences in the foliar damage ratings, number of exit holes, cumulative stem tunnel length, ratio of
tunneling vs plant height, number of larvae recovered number of pupae recovered. These differences may be caused by differences in plant growth conditions, which has been reported as a contributing factor to variations in the Bt toxins levels in other transgenic Transformed maize lines carrying Bt genes lines (Ramachandran et al., 1998) or may be attributed to hybrid vigor and the ability to replace the damaged leaf tissues as the plant grows.

Among the CML144 x Event 216F2:3s, CML144 x Event 223F2:3s, CML159 x Event 216F2:3s and CML159 x Event 223F2:3s there were significant differences for the foliar damage ratings, number of exit holes, cumulative stem tunnel length, ratio of tunneling vs plant height, number of larvae recovered number of pupae recovered measured with F2:3 resistant and F2:3 susceptible observed. This probably follows Mendelian segregation since Bt resistance is a dominant trait (Fearing et al., 1997; Wu et al., 2002; Ramachandran et al., 1998). In addition, these findings conform to earlier work, which indicated that the transformed maize lines carrying Bt genes used in this research work did not have a fixed Cry1Ab gene (Mugo et al., 2001, Mugo et al., 2005).

In the dot blot analysis, the transformed maize lines carrying events 216 and 223 and their F1S showed positive colour intensity (very dark and distinct) for Bt while the non-transformed showed negative colour intensity (very light and indistinct). All the F1S crosses of transformed and non-transformed lines showed positive colour intensity for Bt δ-endotoxins while, the F2:3s showed both positive and negatives for Bt. This perhaps is due to Mendelian segregation. These differences can be attributed to the presence of the Cry1Ab protein in the crosses of transformed maize lines carrying Bt genes and non-transformed maize lines carrying Bt genes inbred lines respectively.
However the dot blot analysis for CML144 x Event 216F2:3S, CML144 x Event 223F2:3S, CML159 x Event 216F2:3S and CML159 x Event 223F2:3S there were significant differences for colour intensity. This could be attributed to Mendelian segregation. However, in certain instances it was observed for some samples that showed a medium to high colour intensity had low concentrations of the Cry1Ab protein (Adamczyk et al., 2001).

In the quantification of cry1Ab protein expressed in leaf tissues a "sandwich-type" enzyme linked immunosorbent assay (ELISA) for cry1Ab (Adamczyk et al., 2001; Walschus et al., 2002) was carried out on crosses of Event 223 x Event 216F1s and its F3s, CML144 x Event 216F1, CML144 x Event 223F1, CML159 x Event 216F1 and CML159 x Event 223F1 There were significant differences for mean concentration of Bt δ-endotoxins in the maize leaves. The transformed maize lines carrying events 216 and 223 showed highly significant differences for mean concentration of the Bt δ-endotoxins compared to the non-transformed maize inbred lines. The F1's indicated the resistance to C. partellus is inherited as a dominant trait. However, the F2:3's of the same crosses showed significant differences for mean concentration of Bt δ-endotoxins. The observations may be attributed to segregation. The relation between plant damage data and expression of Bt δ-endotoxins with the efficacy of Bt crops has been studied using ELISA and bioassays techniques (Sachs et al., 2000; Adamczyk et al., 2001). Such studies have very broad implications for dealing with pest resistance and may indicate the stability of a transgene over successive generations.

Some field trials in some cereal crops revealed certain limitations of this technology such as morphological variations in transgenic lines as compared to control plants (Shu et al., 2001).
2002; Bashir et al., 2004) and variations in Bt δ-endotoxins’ expression during the plant life cycle (Alinia et al., 2000; Bashir et al., 2004) or during several generations (Fearing et al., 1997, Wu et al., 2002, James et al., 2004).

However, the results from this study indicate that the crylAb gene appeared to be stably transmitted successive sexual generations, and the concentration of the CrylAb protein kept quantitatively stable to the F2:3 generations (Fearing et al., Wu et al., 1997). This is observed among the crosses of CML144 and CML159 and transformed maize lines carrying events 216 and 223, both at the F1s and F2:3s. Most of these crosses had resistant and susceptible plants for all the plant damage parameters. However, other studies show differential expression of Bt among varieties and plant structures to be the result of the ELISA measuring only soluble protein (Sachs et al., 1998, Greenplate et al., 2000), but Adamczyk et al., 2001 indicated that expression differences among some Bt varieties are quantifiable.

5.2 Conclusions

This study revealed that there were differences in resistance to C. partellus due to differences in the expression levels of Bt δ-endotoxins among Bt and non-Transformed maize lines carrying Bt genes inbred lines, their F1s and F2:3s.

In addition, the dot blots’ analysis indicated differences in the colour intensity for the parents, F1s and F2:3s. Generational studies of transgene expression have been conducted in cereal crops and in many other crops (Mlynárová et al., 1996; Ülker et al., 1999; Vain et al., 1999, 2002; James et al., 2004). However, more rarely have transgene expression levels been quantified over generations in plants (Mlynárová et al., 1996; Ülker et al.,
1999). These studies are important in assessing transgene behaviour in plants (Fearing et al., 1997, James et al., 2004). The conclusion, the results from this research experiment reveal that the expression of Bt δ-endotoxins appeared to be stable in the three successive generations of breeding.

5.3 Recommendations

The recommendations emerging from this study are;

- This research work could be done under field conditions using other transformed maize lines carrying events containing *cry1Ab* or any other genes that target stem borers or other major field pests, and at different agronomic conditions e.g. varied plant nutrition (especially nitrogen) and other factors contribute to the observable effects.

- At the molecular level, further work can be carried out to determine at the transcription level if there are differences in stability of expression of the Bt δ-endotoxins depending on the genotype and associated physiological and environmental conditions.

Future research may provide new transformed maize lines carrying Bt events (public or private) with more powerful resistance to insect pests that may be used as Bt source lines, however, maintenance of a healthy transgenic crop by agronomic management is important in realizing the transgenic potential of a Bt crop variety.
6.0 REFERENCES


Adamczyk, J.J. Jr., D.D. Hardee, L.C. Adams and Sumerford D.V. 2001. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of cry1Ac endotoxins in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *Journal of Economic Entomology* 94:284-290.


Bashir, K., Husnain, T., Fatima, T., Riaz, N., Makhdooom, R. and Riazuddin, S. Novel indica basmati line (B-370) expressing two unrelated genes of *Bacillus thuringiensis* is highly resistant to two lepidopteran insects in the field. *Crop Protection*, vol. 24, no. 10, p. 870-879.


performance of transgenic tomato plants expressing the *Bacillus thuringiensis* var. *kurstaki* insect control plant. *Bio/Technology* 7: 1265-1269.


Greenplate J.T. 1999. Quantification of *Bacillus thuringiensis* insect control protein *Cry1Ac* overtime in Bollgard cotton fruit and terminals. *Journal of Economic Entomology* 92:1377-1383.


aizawai, and Abamectin in field populations of Plutella xylostella from Malaysia. *Pesticides Science* 48: 89-97.


Lynch, R.E.; Wiseman B.R.; Plaisted D.; Warnick D. 1999. Evaluation of Transgenic Sweet Corn Hybrids Expressing Cry1A(b) Toxin for Resistance to Corn Earworm and Fall


Mugo S., Kanampiu F., and Diallo A. 2005. Presentation to the participants of the Maize Improvement Course held at CIMMYT-ALP Nairobi, 29 August to 10 September, 2005.


_Crop Protection_ 12: 11-34


Shu, Q., Ye, Gong-Yin; C., Hairui; C., Xiong-Ying., Xiang Y.; Wu D., Gao M., Xia Y., Hu C., Sardana R., and Altosaar I. Transgenic rice plants with a synthetic _cry1Ab_ gene from _Bacillus thuringiensis_ were highly resistant to eight lepidopteran rice pest species. _Molecular Breeding_, August 2000, vol. 6, no. 4, p. 433-439.


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Warui, C. M. and Kuria J. N. 1983. Population incidence and the control of maize stalk-borers Chilo partellus (Swinhoe) and Chilo orichalcociliellus (Stand) and Sesamia calamistis (Hampson) in Coast Province, Kenya. *Insect Science and its Application* 4, 11-18.


Wu, G., Cui HR Shu QY, Xia YW, Xiang YB, Gao MW, Cheng X, Altosaar I. 2000. Striped stem borer (*Chilo suppressalis*) resistant to yellow transgenic rice with cry 1Ab gene from *Bacillus thuringiensis* (Bt) and its rapid screening. *Journal Zhejiang University* 19: 15-18.


