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Full Length Research Paper

Levels of control of *Chilo partellus* stem borer in segregating tropical Bt maize populations in Kenya

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In Kenya, stem borers destroy an estimated 400,000 metric tons, or 13.5%, of farmers' annual maize harvest costing about US$80 millions. *Bacillus thuringiensis* (Bt) maize controls stem borers without harming humans, livestock and the environment and was sown to 140m ha^{-1} globally in 2009. Two public Bt maize lines of cry1Ab::ubi gene (Event 216 and Event 223) were crossed with two non-Bt maize inbred lines, CML144 and CML159. The efficacy in the control of *Chilo partellus* stem borers in the parents, F_1 and $F_{2:3}$ successive generations were studied in a biosafety level 2 greenhouse. The Bt-gene effectively reduced stem borer damage with lower values for number of exit holes, tunneling length, proportion of stalk tunneled, number of larvae and number of pupae than the non Bt-maize and the check cultivars. The F_1 generations values for all damage parameters studied were comparable to those for the Bt-maize inbred lines as expected. The $F_{2:3}$ generations showed a spread of damage parameters from resistant to susceptible. These results suggest that the Cry1A(b) genes in the study was inherited following the Mendelian segregation.

Key words: Bt maize, *Chilo partellus,* Bt δ-endotoxins, biosafety, greenhouse.

INTRODUCTION

Maize (*Zea mays* L.) is the most important staple food crop in Kenya, and its shortage is equated with famine. The high incidence and damage by the spotted stem borer (*Chilo partellus* Swinhoe) and the African stem borer (*Busseola fusca* Fuller) are among the causes of reduced maize grain yield loss estimated annually at about 400,000 metric tons equivalent to $91 million (DeGroote, 2002).

Several options for managing the damaging effects of these stem borers on maize exists but each option has its own limitations. Chemical control methods are the most effective but are expensive to small scale farmers and pose risks to humans, livestock, and the environment. Biological control methods are efficient, cost-effective and environmentally safe; but are often insufficient in maintaining the pest populations below economic injury levels (Kfir et al., 2002; Mailafiya et al., 2009). Cultural control methods are easy to use and may not involve operational costs per se, but require skills in timing and have limited mode of application, and may not be easily applicable to large scale farms. Host plant resistance using conventional methods is easy to adapt and use by farmers but its development is limited due to the polygenic nature of inheritance of the insect resistance traits (Ajala, 1992; Andre et al., 2003).

Host plant resistance presents little risks to the environment and is compatible with other pest management approaches (Ampofo and Saxena, 1989). Host plant resistance developed through conventional breeding me-

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Abbreviations: Bt, *Bacillus thuringiensis*; **BGHC,** biosafety level 2 greenhouse complex; **KARI,** Kenya Agricultural Research Institute; **KEPHIS,** Kenya Plant Health Inspectorate Service; **NBC,** National Biosafety Committee.

thods and through genetic engineering, especially Bt maize, has potential to help resource-poor farmers combat stem borer damage.

Bt maize developed from codon modified genes derived from the soil bacterium *Bacillus thuringiensis* (Bt) and that encode δ-endotoxins; proteins has found use in a way that reduces the limitations associated with other methods of stem borer control (James, 2009; Mugo et al., 2005; Tabashnik et al., 2009). Bt transgenic plants containing insecticidal proteins have featured prominently in agricultural systems in both developed and developing countries. The global area of approved transgenic crops in 2009 was 140 m ha^{-1} with 21.2 m ha grown to transgenic maize varieties (James, 2009). The benefits accruing to farmers growing Bt maize are substantial across a number of geographies and economic strata, especially in developing countries. These benefits include increased crop yields, reduced pesticide use, less environmental damage, less fungal contamination, and reduced labor (James, 2009; Tabashnik et al., 2009).

Genetic transformation using *gna* gene was first reported in tobacco for resistance targeted to aphid (Hilder et al., 1995), later on in rice targeted to brown plant hopper (Jairin et al., 2009; Sudhakar et al., 1998), and extensive work in maize for resistance to stem borers (Dutton et al., 2005; Mugo et al., 2002). Other biotic stress resistance genes (*wasabi defensin, potato proteinase inhibitor, cpTi etc*) have been introduced into rice, maize, and other crops for resistance to diseases and pests (Duan et al., 1996; Kanzaki et al., 2002). However, stable integration and expression of transgenes is of great concern in crop plants (McGauchey and Whalon, 1992). Dutton et al. (2005) suggested that the stability of transgene expression is essential for transgenic crops to become an integral part in agricultural systems. The effectiveness and sustainability of Bt-transgenic technology in the control of target stem borers will depend on the levels of expression of Bt δ-endotoxins (Kranthi et al., 2005; Olsen et al., 2005). These levels should be in sufficient quantities in appropriate plant parts at the requisite time in successive generations (Kranthi et al., 2005; Olsen et al., 2005).

The objective of this study was to evaluate successive generations (parents, F_1 and $F_{2:3}$) of crosses involving tropical Bt and non-Bt maize inbred lines for resistance to *C. partellus.*

MATERIALS AND METHODS

Research site and facilities

The study was conducted in 2007 and 2008 in the biosafety level 2 greenhouse complex (BGHC) located in Kenya Agricultural Research Institute (KARI), Kabete. The BGHC is consistent with international standards and 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 Bt maize varieties and for carrying out risk assessment studies on transgenic plants (Mugo et al., 2005; Murenga et al., 2004; Traynor et al., 2001). The facility serves as a bio-containment facility providing an effective means of isolation and prevention of unintended transmission of genetic material (Murenga et al., 2004; Traynor et al., 2001).

Maize parents, F_1 and F_2 generations

Plant materials for the study included the seedlings grown from the parents, F_1 and $F_{2:3}$ generations of four crosses (CML 144 x Event 216, CML 159 x Event 216, CML 144 x Event 223, and CML 159 x Event 223). Event 216 and Event 223 were obtained from CIMMYT-Mexico as BC_3S_1 lines. The two events descended from a common parent, CML216, which was transformed with a vector containing a full-length *cry1Ab* coding sequence driven by an enhanced ubiquitin (cry1Ab::ubi) (Mugo et al., 2005). CKIR6009 and H513 were used as resistant and non-resistant hybrid checks, respectively.

The multiple borer resistant inbred line (MBR C5 Bc F1-13-3-2-1-B-4-2-B) and CML216 were used as resistant and non-resistant inbred line checks, respectively. The genotypes were grown in pots filled with planting media composed of one part topsoil mixed with farm yard manure, one part sand, and one part coconut peat (Murenga et al., 2004; Traynor et al., 2001). The pots were irrigated twice a week to ensure vigorous growth. Other standard procedures for plant management at the biosafety level 2 greenhouse complex were practiced according to the laid down protocols.

F_1 generations were formed when twenty seeds each of Event 216 and 223 and CML144 and CML159 were sown in small transfer pots (7.5 x 7.5 x 9.0cm) and later transplanted into large pots (12cm x 30cm). At anthesis, plants were cross pollinated in predetermined combinations of Bt x Bt, Bt x non-Bt and non-Bt x non-Bt maize inbred lines. Bt plants and non-Bt plants were used as males and females, respectively. To ensure nicking, sowing of seed was staggered on three different dates separated by 5 days.

F_2 generations were formed by sowing twenty F_1 seed in small pots and later transplanted into large pots. During anthesis, pollen from each cross was collected and bulked, and used for pollinating an equal number of plants by sib-mating.

Experimental design and evaluation of stem borer damage

The genotypes were sown in planting media composed of one part of topsoil mixed with farm yard manure, one part sand, and one part coconut peat during two seasons of each year in 2007 to 2008 (Murenga et al., 2004; Traynor et al., 2001). Twenty seeds each of Bt maize events 216 and 223 and CML144 and CML159 were sown in small transfer pots and later transplanted into large 12 inch diameter pots. At the 4–6^{th} leaf stage, the Bt plantlets were infested with twenty first instar larvae of *C. partellus* stem borer. Foliar damage rating was based on a scale of 1–9 (1 = no leaf damage, 9 = severe leaf damage) (Table 1) (Nyhus et al., 1989). The first scoring scores were taken two weeks after infestation and repeated after three weeks. The numbers of stem borer exit holes per plant were counted at harvest. The cumulative tunnel length was measured after splitting the stems of each of the infested plants at harvest. The number of larvae and pupae recovered were counted at harvest. Susceptible seedlings were discarded while resistant ones were maintained for use in crossing.

Standard procedures were practiced according to the protocols for plant management at the biosafety level 2 greenhouse complex (Murenga et al., 2004). All tests were conducted using a randomized complete block design with four replications.

Data analysis

Plant damage parameters namely, foliar damage rating, number of

Table 1. Scale for scoring stem borer damage from seedling to whorl-stage in maize.

Numerical scores	Visual ratings of plant damage	Reaction to resistance
0	No damage	Probable escape
1	Few pin holes	Highly resistant
2	Few shot holes on a few leaves	Resistant
3	Several shot holes on leaves (<50%)	Resistant
4	Several shot holes on leaves (>50%) or small lesions (<2cm long)	Moderately resistant
5	Elongated lesions (>2cm long) on a few leaves	Moderately resistant
6	Elongated lesions on several leaves	Susceptible
7	Several leaves with long lesions with leaf tattering	Susceptible
8	Several leaves with long lesions with severe leaf tattering	Highly susceptible
9	Plant dying due to death of growing points ('dead-hearts')	Extensively sensitive to damage

Source: Adapted from CIMMYT (1989).

exit holes, the cumulative stalk tunnel length, ratio of cumulative stalk tunnel length to plant stature and the number of larvae and pupae were analyzed by PROC GLM (SAS Institute Inc., 2003). Each cross was analyzed separately for comparison of differences in the effect of the two Bt events in the successive generations of their crosses. Genotype was fixed and the replications were random. Mean comparisons were made using Fisher's protected LSD. To study the possible relationships among the different plant damage traits, the spearman's rank correlation coefficients between the variables were computed (SAS Institute Inc., 2003).

RESULTS AND DISCUSSION

There were significant differences among the F_1 crosses of CML144 x Event 216 and CML144 x Event 223, and CML159 x Event 216 and CML159 x Event 223 for the five traits measured (Table 2 and Table 3).

Number of exit holes

Bt maize Events 216 and 223 had 0.4 number of exit holes, while CML144 and CML159 had 6.3 and 6.0, respectively. The F_1 cross of CML144 x Event 216 and CML144 x Event 223 had 1.9 and 0.9 number of exit holes, respectively. The F_1 cross of CML159 x Event 216 and CML159 x Event 223 had 2.2 and 1.8 number of exit holes, respectively (Table 2 and Table 3). The results indicated that for this trait, all the four F_1 populations had values that were lower than the mid-parent values for each cross.

In the $F_{2:3}$ population of CML144 x Event 216, entries 8 and 9 had 2.8 and 0.8 exit holes, respectively, and were similar to the resistant parent. Entries 1, 2, 3, 6, 7 and 10 had over 5 exit holes and were similar to susceptible parent. Other entries were intermediate between resistant and susceptible parent. In the $F_{2:3}$ population of CML159 x Event 216, entries 1, 3, and 9 had 2.4 1.9 and 3 exit holes, respectively. Entries 2, 4 and 7 had over 5 exit holes. In the $F_{2:3}$ population of CML144 x Event 223, entries 1, 2, 3, 4, 5, 8, 9, and 10 had less that 3 exit holes. Entries 6 and 7 had over 5 exit holes. In the F2:3 population of CML159 x Event 216, all the 10 entries had less than 3 exit holes. All the checks had less than 3 number of exit holes except CML216 with 9.3 (Table 2 and Table 3).

Stem tunnel (%)

Bt maize Events 216 and 223 had 1.4 and 0.4% stem tunneling, while CML144 and CML159 had 10 and 13.3% stem tunneling, respectively. The F_1 cross of CML144 x Event 216 and CML144 x Event 223 had 7.1 and 3.3% stem tunneling, respectively. The F_1 populations of CML159 x Event 216 and CML159 x Event 223 had 4.9 and 4% stem tunneling, respectively. Among the $F_{2:3}$ populations of CML144 x Event 216, entries 1, 2, 7, and 10 had the highest percentage of stem tunneling above 11 % while line 9 had the least percentage stem tunneling.

The $F_{2:3}$ crosses of CML159 x Event 216 had entries 2, 4, 7 and 10 with the highest percentage stem tunneling above 11%, however, line 3 had the least with 3%. CML216 had 21.5% stem tunneling among the checks (Table 2 and Table 3). The $F_{2:3}$ populations of CML144 x Event 223 had line 1 with less than 1% stem tunneling, while entries 5, 6 and 7 had above 10% stem tunneling. $F_{2:3}$ crosses of CML159 x Event 223 had entries 1, 8, and 10 with above 5% stem tunneling. Check CML216 had the highest percent stem tunneling at 21.5% (Table 2 and Table 3).

Tunneling length (cm)

Bt maize Events 216 and 223 had 1.7 and 1 cm, while CML144 and CML159 had 12 and 22 cm stem tunneling, respectively. The F_1 cross of CML144 x Event 216 and CML144 x Event 223 had 4.1 and 3 tunnel length, respectively (Table 2 and Table 3). The F_1 populations of CML159 x Event 216 and CML159 x Event 223 had 3.1 and 4 tunnel length, respectively. Among the $F_{2:3}$ populations of CML144 x Event 216, entries 1, 2, 4, 6, and 7

Table 2. Means of five traits recorded on the parents, F_1 and $F_{2:3}$ populations of crosses CML144 x Event 216 and CML159 x Event 216 infested with *Chilo partellus.*

Genotype	No. exit holes	Tunneling length (cm)	Stem tunnel (%)	No. of larvae	No. of pupae
Parent					
Event 216	0.4	1.7	1.4	0.2	0.1
CML144	6.3	12.9	10.0	1.8	0.2
CML159	6.0	22	13.3	2.4	0.6
F_1 population					
CML144 x Event 216	1.9	4.1	7.1	0.8	0.2
CML159 x Event 216	2.2	3.1	4.9	0.9	0.1
$F_{2:3}$ population					
CML 144 x Event 216					
Entry 1	5.0	8.7	12.7	1.0	0.4
Entry 2	7.8	10.7	16.1	1.4	0.5
Entry 3	5.6	6.2	8.2	0.9	0.3
Entry 4	3.9	8.2	9.4	1.1	0.4
Entry 5	3.1	5.5	9.0	1.5	0.0
Entry 6	5.2	10.0	10.6	1.3	0.0
Entry 7	5.3	9.9	12.5	0.9	0.5
Entry 8	2.8	4.6	8.1	0.9	0.3
Entry 9	0.8	1.3	2.4	0.3	0.0
Entry 10	5.4	6.9	11.5	1.0	0.3
CML 159 x Event 216					
Entry 1	2.4	3.8	4.5	0.7	0.1
Entry 2	6.4	8.0	13.7	1.1	0.3
Entry 3	1.9	2.0	3.4	0.7	0.0
Entry 4	5.2	8.5	12.3	2.1	0.3
Entry 5	4.4	6.2	9.4	1.4	0.3
Entry 6	3.4	6.0	8.7	0.9	0.3
Entry 7	5.6	7.0	11.2	1.3	0.4
Entry 8	3.1	3.9	6.8	1.1	0.1
Entry 9	3.0	4.9	9.1	1.4	0.1
Entry 10	4.2	10.1	16.0	1.1	0.5
Checks					
CML216	9.3	51.4	21.5	3.6	1.8
CKIR6009	0.8	0.9	1.7	0.4	0.0
H513	2.9	3.2	5.1	0.9	0.1
MBR C5 Bc F1-13-3-2-1-B-4-2-B	2.9	8.6	7.9	1.4	0.2
Mean	**4.0**	**8.3**	**9.2**	**1.2**	**0.3**
LSD	**3.5**	**8.4**	**7.2**	**1.2**	**0.7**

had the highest tunneling above 10 cm, although line 9 had the least tunneling with 1.3 cm.

The $F_{2:3}$ crosses of CML159 x Event 216 had entries 2, 4, 7 and 10 with the highest percentage stem tunneling above 11%, however, line 3 had the least with 3%. CML216 had 21.5% stem tunneling among the checks (Table 2 and Table 3). The $F_{2:3}$ populations of CML144 x Event 223 had line 1 with less than 1 cm stem tunneling, though entries 5, 6 and 7 had above 5 cm. $F_{2:3}$ crosses of CML159 x Event 223 had entries 1, 8, 9 and 10 with above 2.5 cm of tunnel length. Check CML216 had the highest stem tunneling at 51.4 cm.

Table 3. Means of five traits recorded on the parents, F_1 and $F_{2:3}$ populations of crosses CML144 x Event 223 and CML159 x Event 223 infested with *Chilo partellus.*

Genotype	No. exit holes	Tunneling length (cm)	% Stem tunneling	No. of larvae	No. of pupae
Parent					
Event 223	0.4	1.0	0.5	0.3	0.0
CML 144	6.3	12.9	10.0	1.8	0.2
CML 159	6.0	22.0	13.3	2.4	0.6
F_1 population					
CML 144 x Event 223	0.9	1.6	3.3	0.7	0.0
CML 159 x Event 223	1.8	2.0	4.0	0.4	0.1
$F_{2:3}$ population					
CML 144 x Event 223					
Entry 1	0.6	0.3	0.4	0.4	0.1
Entry 2	1.1	2.5	3.4	1.2	0.2
Entry 3	0.3	0.9	1.5	0.1	0.0
Entry 4	1.5	4.0	5.4	0.2	0.1
Entry 5	1.8	5.0	7.9	0.6	0.1
Entry 6	7.6	6.8	11.3	1.3	0.1
Entry 7	5.9	5.6	9.5	1.3	0.1
Entry 8	1.2	1.4	2.4	0.4	0.0
Entry 9	1.3	1.1	1.7	0.2	0.0
Entry 10	2.2	3.5	5.5	1.0	0.0
CML 159 x Event 223					
Entry 1	2.2	3.2	6.4	0.4	0.0
Entry 2	1.2	1.1	2.1	0.3	0.3
Entry 3	1.1	1.4	2.0	0.3	0.0
Entry 4	0.7	1.1	1.9	0.4	0.0
Entry 5	1.3	2	3.6	0.7	0.0
Entry 6	2.6	2.2	4.1	0.6	0.2
Entry 7	1.8	1.7	3.1	0.3	0.1
Entry 8	2.9	4.6	6.0	0.6	0.3
Entry 9	0.8	2.5	3.8	0.2	0.0
Entry 10	2.0	2.8	5.0	0.4	0.1
Checks					
CML216	9.3	51.4	21.5	3.6	1.8
CKIR6009	0.8	0.9	1.7	0.4	0.0
H513	2.9	3.2	5.1	0.9	0.1
MBR C5 Bc F1-13-3-2-1-B-4-2-B	2.9	8.6	7.9	1.4	0.2
Mean	**2.5**	**5.4**	**5.3**	**0.8**	**0.2**
LSD	**4.0**	**7.7**	**8.1**	**1.3**	**0.5**

Number of larvae and number of pupae recovered

Bt maize Events 216 and 223 had no larvae recovered, while CML144 and CML159 had at least 2 larvae and pupae recovered, respectively. The F_1 crosses of CML144 x Event 216 and CML144 x Event 223, and CML159 x Event 216 and CML159 x Event 223 had at least 1 larvae or pupae recovered from them. Among the $F_{2:3}$ populations of CML144 x Event 216, all entries had at least 1 larvae and 1 pupae recovered except line 9. The

$F_{2:3}$ crosses of CML159 x Event 216 had all entries with at least 1 larvae recovered.

Among the $F_{2:3}$ populations of CML144 x Event 223, all entries had at least 1 larvae and 1 pupae recovered except entries 3, 4, and 9. The $F_{2:3}$ crosses of CML159 x Event 223 had entries 5, 6, and 8 with at least 1 larvae and no pupae recovered, respectively. Check CML216 had at least 4 larvae and 2 pupae recovered (Table 2 and Table 3).

The inbred lines that had a high number of exit holes had a higher percentage of the stem tunneling and cumulative tunnel length and vice versa. For example, CML144 had 6.3 number of exit holes and also recorded the highest percent of stem tunneling and cumulative tunneling similar to the susceptible check CML216. Overall, the Bt maize Events 216 and 223 and their crosses had a low number of exit holes, a low number of larvae and pupae recovered, a low cumulative tunnel length, a low ratio of plant height to cumulative tunnel length than the susceptible checks. These results indicated the effectiveness of the two Bt events in controlling *C. partellus* larvae. The non-Bt maize MBR and CKIR6009 bred for resistance to stem borers had low leaf damage scores than CML216 and H513. These results suggest the effectiveness of conventional breeding for stem borer resistance in these materials (Mugo et al., 2005).

Conclusions

The variations in the observations on plant damage could be attributed to differences in plant growth conditions, which has been reported as contributing factor to discrepancies in the levels of Bt δ-endotoxins in other transgenic crops (Ramachandran et al., 1998) or may be attributed to hybrid vigor and the ability of the plant to replace damaged leaf tissues as it grows. Significant differences were observed among the $F_{2's}$ of crosses of CML144 x Event 216 and CML144 x Event 223, and CML159 x Event 216 and CML159 x Event 216 for the five traits measured except plant height. The differences in these segregating generations may suggest a Mendelian segregation since Bt gene is a single dominant trait (Ramachandran et al., 1998; Tabashnik et al., 2009; Wu et al., 2002). Recent reviews indicate that Bt maize varieties under these trials in Kenya were considered to be low since the level of endotoxicity depended on the Bt maize event used. Additionally, Mugo et al. (2005) reported that the Bt maize events used in this experiments did not have fixed *Cry1Ab* genes. The segregation of Bt genes in these generations was inherited in a normal Mendelian inheritance and that the gene could be used in other maize germplasm.

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