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Quantitative trait loci for resistance to spotted and African maize stem borers (*Chilo partellus* and *Busseola fusca*) in a tropical maize (*Zea mays* L.) population

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Maize (*Zea mays* L.) is the staple food in Kenya, and mapping the qualitative trait loci (QTL) associated with resistance to maize stem borer pest is important towards marker assisted breeding for this quantitative trait. The objective of this study was to identify any QTL associated with resistance to *Chilo partellus* and *Busseola fusca*, the two important stem borer pests in maize production in Kenya. A total of 203 $F_{2:3}$ individuals from a cross between CML442, a stem borer susceptible maize inbred line and CKSBL10026, a stem borer resistant maize inbred line; and 152 SNPs were used for mapping the QTL. Data were collected on leaf damage, stem borer exit holes and stem tunneling length as putative stem borer damage traits. A likelihood odds ratio (LOD) scores of 3.0 and maximum recombination frequency of 0.50 were used to declare linkage. LOD scores between 2.5 and 2.9 were considered strong indications of a QTL. Resistance QTL for the three putative traits were detected on chromosomes 1-7 and 9 for both individual locations and stem borer species analysis. In *B. fusca* sites, one QTL for reduced stem tunnelling was revealed on chromosome 4 while in the *C. partellus* sites, one QTL for reduced stem tunnelling was identified on chromosome 4 and another for reduced stem borer exit holes was identified on chromosome 5. Phenotypic variances explained ranged from 6 to 10%, suggesting a need to validate these QTL using a larger population and in different environments.

Key words: *Busseola fusca*, *Chilo partellus*, mapping, quantitative trait loci (QTL), resistance, single nucleotide polymorphisms (SNPs), stem borer.

INTRODUCTION

Insect pests affect 46% of global maize growing area causing about 24.5% of world maize loss annually. In economic terms, 52 million tons of grain valued at $5.7 billion is lost, and US $550 million worth of insecticide is

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used annually to curb losses (Mugo et al., 2012). Annual losses due to pests in Africa are about 17%. Maize stem borer species are the primary field pests that feed on leaves, cob and pith resulting in stem damage and grain yield losses in the entire world wherever maize is grown (Mihm, 1997; Samayoa et al., 2015a). The stem borer pest is also one of the most important maize field pests in sub-Saharan Africa (Kfir et al., 2002; Smale et al., 2011; Calatayud et al., 2014). Increased maize productivity beyond the current two (2) tons per hectare is critical for food security in sub- Saharan Africa. The stem borers are some of the major pests that account for the low maize production, with lepidopteran stem borers, including the African stem borer (*Busseola fusca* Fuller), the spotted stem borer (*Chilo partellus* Swinhoe) and the pink stem borer (*Sesamia Calamistis* Hampson) being the most damaging pests in eastern and southern Africa, where they cause 13 to 40% yield losses (De Groote, 2002; Mailafiya et al., 2011). In Kenya, the major maize stem borer species are *C. partellus*, *B. fusca* and *S. calamistis. C. partellus* is found in the warmer and lower areas, *B. fusca* is predominant in the cooler and higher altitudes areas while *S. calamistis* is found in low densities in all ecologies in Kenya (Ong'amo et al., 2006).

Lepidopteran maize stem borers are serious pests in sub-Saharan Africa region because besides the reduced grain cereal yields, they cause direct losses through loss of photosynthetic leaf area, results in dead hearts which leads to lodging from damaged stems. Plants also suffer from increased ear rots and are predisposed to infections by *Aspergillus flavus* and contamination with mycotoxins (Kfir et al., 2002; Mugo et al., 2012; Muturi et al., 2012). The lepidopteran stem borers, therefore, poses major threats to sustained food sufficiency in sub-Saharan Africa region causing annual yield losses of approximately 15%, and particularly in Kenya where they cause losses estimated at 13.5% (De Groote, 2002). The recommended control methods which include cultural, chemical and biological have not been successful. The most recently recommended control method has been by use of *Bt* (*Bacillus thuringiensis*) gene, however, its use has not been authorised in Kenya and in majority of the sub-Saharan African countries. The *Bt* control solution further remains elusive because recent studies have reported reduced efficacy of *Bt* transgenes as some of the important pests have already developed resistance since its registration in 1996 (Campagne et al., 2013; Jiménez-Galindo et al., 2017). Natural levels of resistance in elite maize varieties remain insufficient to manage the stem borer pest and detection of resistance QTL could enhance breeding for this trait through marker-assisted breeding or genomic selection (Samayoa et al., 2015a). Host plant resistance could be the most economically feasible and ecologically sound method as it is technically and socially acceptable.

The stem borer resistance is quantitatively inherited and progress in breeding for resistance through conventional methods has been slow (Jampatong et al., 2002). Stem borer resistance using conventional breeding methods has been elusive due to limited genetic variation, the difficulty in maintaining a quantitative trait, and having to deal with two organisms; pests and hosts (Mugo et al., 2002). The trait is controlled by many genes of small effects, thus, there has not been any immune inbred lines developed for its control this far. International Maize and Wheat Improvement Center (CIMMYT) has, however, endeavoured to continue developing inbred lines with high resistance levels and commits much resource in maintaining and improving them. Marker assisted selection for this trait might fast track the breeding process for the many regions in sub-Saharan Africa region where maize stem borers remain a threat to food security.

Mapping of quantitative trait loci (QTL) associated with stem borer resistance would be an important step towards improving efficiency in breeding using marker assisted breeding (MAB). To date, there are several molecular markers available and coupled with the completion of sequencing of the sorghum genome (Bedell et al., 2005) provides opportunities to exploit advances in genomics and genetics for resistance breeding. Such markers especially when tightly linked to resistance loci can support the introgression and selection of associated traits in early generations of breeding, thus minimizing the need for extensive and expensive phenotypic analysis (Drinic et al., 2004). Quantitative trait loci (QTL) for insect resistance in some temperate and tropical maize germplasm against various maize stem-borer species have been detected and documented (Bohn et al., 2000; Samayoa et al., 2015b; Jiménez-Galindo et al., 2017). Such results lead to the conclusion that QTL too can be found for resistance to tropical stem borers including *C. partellus* and *B. fusca* and could underpin MAB in the future. It should also be noted that marker-assisted breeding is an expanding breeding frontier to improve the efficiency of plant breeding through the transfer of specific genomic regions of interest and accelerating the recovery of the elite parent background (Robyn, 2008).

Several methods for QTL mapping have been used and include simple interval mapping, composite interval mapping (fairly similar to multiple QTL mapping) and association mapping (Toure et al., 2000). Both simple interval mapping and composite interval mapping are mainly based on maximum likelihood regression and calculate the most likely position of a QTL within a certain interval between two flanking markers. However, though composite mapping is quite similar to simple interval mapping it possesses improved power because it includes additional genetic predictors, called 'cofactors' that represent QTL elsewhere in the genome and which absorb background genetic noise (Van Eeuwijk et al., 2010). Multiple QTL mapping (MQM) method was used in this study because theoretically, it reduces the error

Table 1. Location and description of six test sites where the testcrosses were evaluated during the March to September, 2011 rainy season.

Site name	Longitude	Latitude	Max. (°C)	Min (°C)	Rainfall (mm)	Altitude (masl)	Soils
KALRO Kiboko	37.75'E	2.15'S	35.1	14.3	530	950	Sandy clays
KALRO Kakamega	34.45'SE	0.16'N	28.6	12.8	1915	1585	Sandy loam
KALRO Mtwapa	39.219'E	4.347'S	29.0	12.8	965	30	Sandy
KALRO Embu	37.412'E	0.449'S	25.0	14.1	1200	1510	Clay loam
Kirinyaga University (KYU)	37.19'E	0.34'S	24.0	18.0	1500	1282	Clay loam
Bukura	34.36'E	0.15'N	22.0	20.0	1800	1397	Sandy loam

variance and increases the power for detecting QTL. Multiple QTL mapping (MQM) is a mapping method that has advantages above other QTL mapping methods as it reduces linkage by considering cofactors to obtain a higher power when mapping QTLs. It applies a backward model selection procedure using an analysis of deviance approach. The use of co-factors and employing a backward model selection can help identify previously unknown locations underlying complex traits (Scott et al., 1966; Arends et al., 2010).

While association mapping (linkage disequilibrium mapping) is a recent and more reliable method of locating putative QTL, the method does not deal with a fixed population like interval and multiple QTL mapping but is based on a random and larger population (Yan et al., 2011). Because of the fixed nature of the 203 $F_{2:3}$ populations used in this study, multiple QTL mapping was applied for locating putative QTL. The objective of this study was to map the QTL associated with resistance to *C. Partellus* and *B. fusca* stem borer species in a tropical maize population using stem tunnelling, number of stem borer exit holes and leaf damage score as putative stem borer-resistance traits.

MATERIALS AND METHODS

Field trials for phenotypic data

The $F_{2:3}$ mapping population was developed from the cross between CIMMYT's highly susceptible inbred line CML442 and multiple borer resistant (MBR) inbred line CKSBL10026. These two parents, both developed by CIMMYT were genetically divergent and had great differences for the resistance traits of interest (leaf damage score, cumulative stem tunnel length and number of stem borer exit holes). The F_2 and F_3 progenies were developed by self pollinating previous F_1 and F_2 materials, respectively. Concurrently, three male rows of single cross tester CML395 x CML444 were sown preceding the female $F_{2:3}$ rows. These families were used for the purpose of harvesting leaves for molecular analysis and were also crossed with the single cross tester for seed increase to enable multi locational phenotyping. Leaf samples for molecular analysis were collected from the $F_{2:3}$ generation. Tender leaves from 15 representative plants were picked at seedling stage and transferred to Biosciences east and central Africa (BecA) laboratory in Nairobi and preserved at -80°C. The testcross ears were harvested and a population of 203 selected based on amount of seeds achieved after the hand pollination. These ears were shelled for the purpose of multi-environmental phenotyping.

In March 2011, the 203 $F_{2:3}$ testcrosses were planted for phenotyping across six environments across Kenya that included Kenya Agricultural Livestock Research Organization (KALRO) Kiboko, KALRO Mtwapa, Kirinyaga University, KALRO Kakamega, Bukura and KALRO Embu sites (Table 1). The α- lattice design replicated three times was used in a 2 × 5 m rows plot spaced at 75 cm between rows and 25 cm between plants. *B. fusca* stem borer larvae were used for infestation at Kakamega, Bukura and Embu sites, while *C. partellus* larvae were used for Mtwapa, Kiboko and Kirinyaga University sites. Ten plants in each plot were each artificially infested with five stem borer neonates three weeks after planting. The remaining plants were concurrently treated with an insecticide (Bulldock® 25 EC = 25 g/l Beta-Cyfluthrin - AI) to act as a control. The trials were grown under rain-fed conditions but supplemental irrigation was applied as needed. Fertilizers were applied at the rate of 60 kg/ha N and 102 kg/ha P_2O_5 at planting. The crop was top-dressed at the rate of 48 kg N/ha 30 days after planting. Planting, weeding, harvesting and shelling operations were performed manually.

Data collection

Data was taken on leaf-damage visual-rating score two weeks after infestation on a scale of 1 to 9 on an individual plant basis, according to Tefera et al. (2011), where 1 = no visible leaf damage and 9 = plants dying as a result of leaf damage. At harvest, the numbers of stem exit holes were counted and the cumulative tunnel length (cm) was measured after splitting the maize stems. Grain yield (t/ha) was computed from shelled grain weight and standardized to 12.5% moisture content.

Phenotypic data analysis

Analysis of variance was performed using the PROC GLM procedure SAS package (2007) and the means compared using Fishers protected least significant difference test (LSD) at *"P<0.05"*. Calculation of heritability for both individual and combined sites was done using PROC mixed method of SAS 9 (BLUPS). Due to the zero heritability observed from the Mtwapa site, the site was dropped from combined analysis. A selection index based on leaf damage score, number of stem borer exit holes and cumulative tunnel length was computed by summing up the ratios between values and the overall mean and dividing by the number of damage parameters evaluated. Germplasm with selection indices values less than 0.8 were regarded as highly resistant, 0.8 to 1.0 as moderately resistant, 1.0 to 1.2 as moderately susceptible and above 1.2 as highly susceptible as described in Tefera et al. (2011).

DNA extraction and analysis

Leaf samples from 15 representative three week old seedlings for

Table 2. Heritability for the putative stem borer resistance traits generated through BLUPS (Best linear unbiased predictors).

Individual sites	Heritability		
	Leaf damage score	Exit holes (#)	Tunnel length (cm)
KALRO Kiboko	0.34	0.70	0.84
Kirinyaga University (KYU)	0.69	0.88	0.90
KALRO Kakamega	0.62	0.12	0.11
Bukura	0.93	0.39	0.37
KALRO Embu	0.01	0.23	0.85
Combined *Chilo partellus* sites (Mtwapa, Kiboko, KYU)	0.13	0.00	0.00
Combined *Busseola fusca* sites (Kakamega, Bukura, Embu)	0.02	0.11	0.09

each of the 203 $F_{2:3}$ families were collected in November 2010. DNA was extracted from the lyophilized leaf tissue from 15 $F_{2:3}$ plants of each family in August 2011. DNA extraction was done using the 96-well format high throughput protocol (Mace et al., 2003).

DNA quantity and quality check

After DNA isolation, quality and quantity checks were done using agarose gel electrophoresis and Nanodrop™ (ND-1000) quantification, respectively. The DNA was subjected to electrophoresis using 0.8% agarose gel containing 0.3 µg/mL GelRed (Biotium Inc., USA) at 100V for 45 min in 1× TAE running buffer after which the integrity and intensity of the bands were used to indicate quality and quantity of the DNA. Samples with smeared bands were re-extracted and subjected to electrophoresis once more to confirm integrity. After DNA electrophoresis, the samples were quantified using Nanodrop™ spectrophotometer. Absorbance ratios A_{260}/A_{280} ranging from 1.7 to 2.0 was considered pure with no protein contamination, while A_{260}/A_{230} ratios above 1.5 were considered to be free of salt contaminants. The isolated DNA was normalized to 50 ng/µL using 0.1X TE (10 mM Tris-HCl, pH 7.5 and 0.1 mM EDTA, pH 8.0), and 50 µL of the normalized DNA was shipped to KBiosciences (present LGC genomics) for genotyping. KASPar SNP Genotyping System (allele-specific PCR amplification of target sequences and endpoint fluorescence genotyping) was used for the SNP analyses, and the generated data were used in subsequent analysis.

Single nucleotide polymorphisms (SNPs) marker analysis

One thousand two hundred and thirty (1230) SNPs were initially screened for polymorphism between the parental lines, the F_1s and F_2s. Two hundred and seventy nine (279) out of 1230 SNPs (22.7%) were heterozygote in one or both parents, that is, nine (9) were heterozygote in parent CML442, 265 (21.5%) were heterozygous in the multiple borer resistant parent, and five (5) were heterozygote in both parents. One hundred and ninety-two (192) SNPs were homozygous and polymorphic, and 98.5% of these (184) were true to type for F_1 and F_2. Out of the 184 SNPs, 152 polymorphic SNPs (Appendix 1) were used to genotype the $F_{2:3}$ plants of the 203 individuals, because the chi-square (χ^2) test of fit revealed several markers that had high significance deviations from the 1:2:1 ratio expected for an $F_{2:3}$ populations (*"P<0.001"*), such markers were, therefore, excluded from the linkage map which reduced the markers to 152 SNPs. The linkage map was constructed with the 152 SNP markers using JoinMap 4 software package (Van Ooijen, 2006). Information on the SNPs used is available on maize panzea database website (http://www.panzea.org/database). Segregation at each marker locus was analyzed using chi-square (χ^2) goodness of fit test for the expected Mendelian segregation ratio of an F_2 population. The linkage map was developed using Kosambi's mapping function. A $\log_{10}$ of the likelihood odds ratio (LOD) value of 6.0 was used to construct linkage maps. QTL detection (mapping) was performed using MapQTL 6 (Van Ooijen, 2009). Interval mapping and multiple QTL mapping (similar to composite interval mapping) were used for QTL detection. Automatic cofactors selection function was used to set cofactors for multiple QTL mapping (MQM), a process that allowed markers used as cofactors to absorb the effects of nearby QTL and increases power and precision of QTL analyses. For declaration of linkage, a threshold LOD score of 3.0 and a maximum recombination frequency of 0.50 were used. Series of 1000 permutations were performed to determine experiment wise significance levels at "*P < 0.05*" of LOD 3.0 for both insect species. Interval mapping with LOD score of above 2.5 were assumed to be highly indicative of QTL. Gene action for each QTL was calculated using the dominance ratio using absolute additive and dominance values as described in Stuber et al. (1987). Values of 0 to 0.20 were interpreted for additive gene action, 0.21 to 0.80 as partial dominance, 0.81 to 1.20 as dominance and $\geq$1.20 as over dominance. The source of resistant allele was detected by the +/- of the additive value with reference to the resistant parent CKSBL10026 where negative values showed alleles came from the resistant parent CKSBL10026, and positive additive values showed resistance came from the sensitive parent CML442 as described in Jampatong et al. (2002).

RESULTS

Phenotypic data

In the *C. partellus* infested sites (Kirinyaga University, Mtwapa and Kiboko), only progeny evaluated at Kiboko showed significant differences for number of exit holes and tunnel length. In *B. fusca* infested sites, only progeny evaluated at Bukura showed significant difference for leaf damage. Heritability for resistance traits based on combined sites analysis was low for both stem borer species but high when estimated for evaluations at individual sites except at Mtwapa (Table 2). The selection index computed for all sites and both borer species identified 44 individuals that were highly resistant, 69 moderately resistant, 58 moderately susceptible and 32 highly susceptible with normal distribution frequency (Figure 1). The selection index based on individual stem

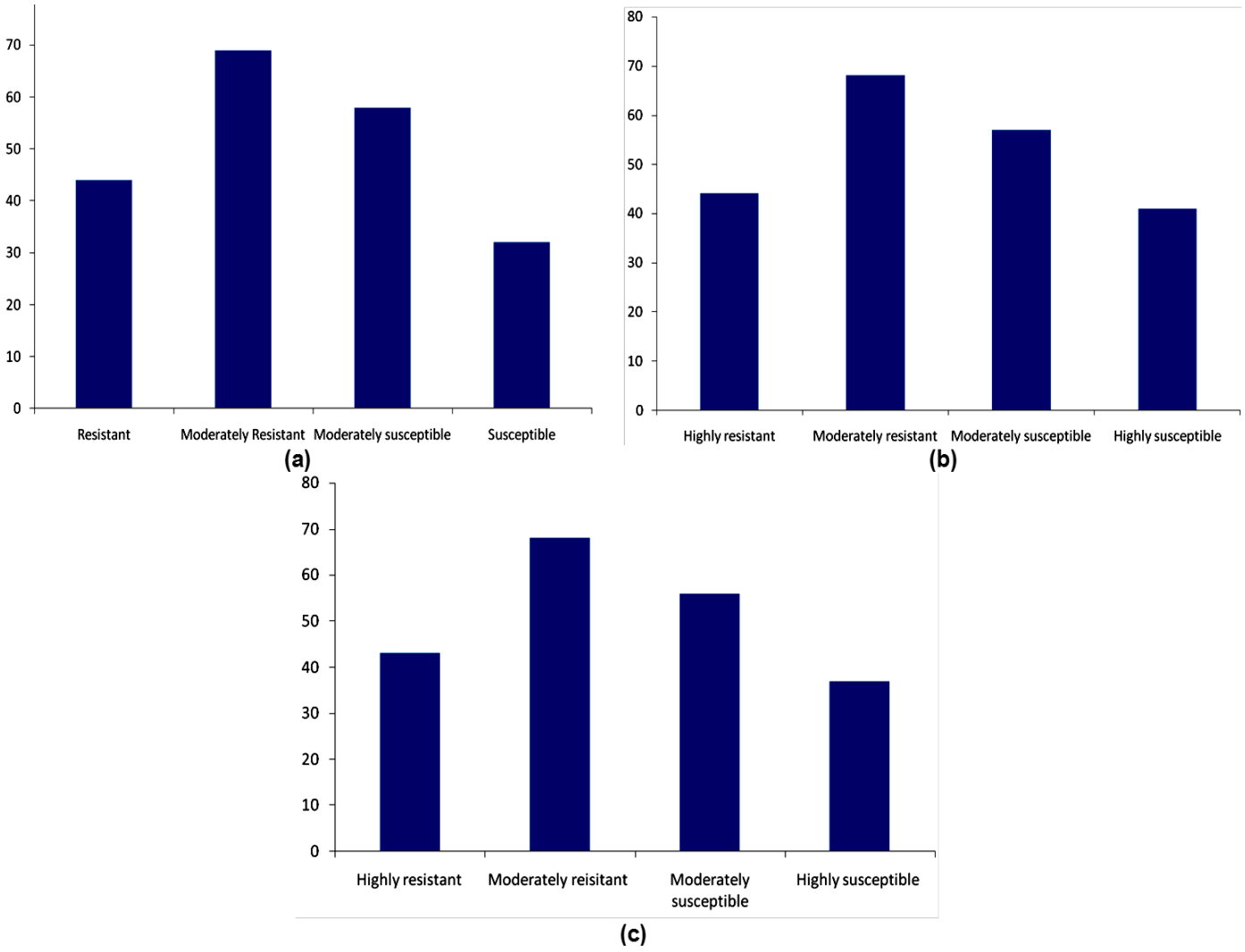

Figure 1. The distribution of genotypes according to resistance categories of the 203 $F_{2:3}$ individuals. (a) Resistance frequency of the 203 $F_{2:3}$ families from combined 6 sites against *C. partellus* and *B. fusca; (*b) Resistance frequency distribution of the 203 $F_{2:3}$ families from combined sites analysis against *C. partellus* species; (c) Resistance frequency distribution of the 203 $F_{2:3}$ families from combined sites analysis against *B. fusca* species. Y-axis represents the actual number of genotypes per category, and the X-axis shows the genotype category names.

borer species such as categorized B. fusca species as 43 progeny as highly resistant, 68 as moderately resistant, 56 as moderately susceptible and 37 as highly susceptible. In the *C. partellus* infested sites, 44 progeny were highly resistant, 68 moderately resistant, 57 moderately susceptible and 41 highly susceptible. Forty-four of the progenies were, therefore, highly resistant to both stem borer species across all locations.

Mapping of the quantitative trait loci

The genetic map was constructed with 152 SNP markers that spanned 1248.01 cM on 10 chromosomes of maize with an average interval length of 8.21 cM. Several QTL for resistance were detected on chromosomes 1, 2, 3, 4, 5, 6, 7 and 9 based on individual sites and different species (Figure 2). Quantitative trait loci detection varied among sites and further, more QTL were detected for *B. fusca* than for the *C. partellus*. In *B. fusca* combined sites analysis, one QTL for resistance to stem tunnelling was detected on chromosome 4 (LOD 2.86) at position 76.33 cM and accounted for 6.2% of phenotypic variation. In the *C. partellus* combined sites, two QTL for stem tunnelling on chromosome 4 (LOD 2.81) and number of stem exit holes on chromosome 5 (LOD 2.53) were detected and accounted for 6.2 and 5.6% of the phenotypic variation, respectively (Table 3).

QTL for resistance to leaf damage

Two (2) QTL affecting leaf damage feeding score were

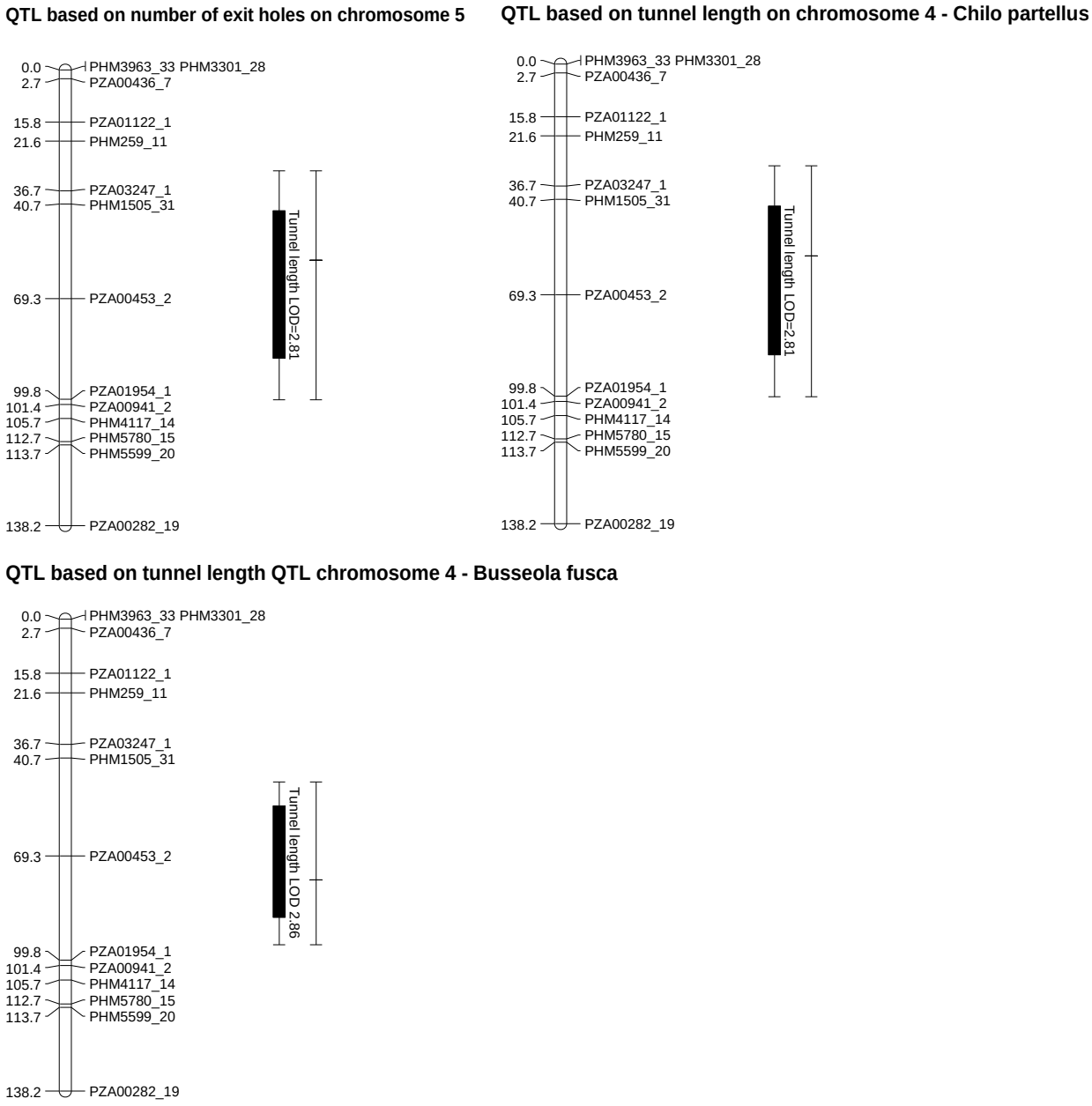

Figure 2. Linkage maps and QTL locations from specific stem borer species (*C. partellus* and *B. fusca*) analysis of the 152 SNPs on leaf damage, number borer exit holes and tunnel length. The line to the left of each QTL bar indicates the QTL peak.

detected on chromosome 2 for Embu site (LOD 3.37) and one indicative QTL on chromosome 1 for Kakamega site (LOD 2.68). The most significant was the QTL detected on the Embu site which explained 6.6% of the phenotypic variation. Gene action was due to over dominance for both QTL.

QTL for resistance to number of exit holes

QTL mapped for *C. partellus* based on combined site analysis revealed 1 QTL (LOD 2.53) for number of exit holes on chromosome 4 but none for *B. fusca*. Conversely, three QTL for resistance to stem exit holes were detected in the individual sites (Kakamega, Kirinyaga University and Bukura sites). Several QTL were detected for progenies evaluated at the following sites; 1 QTL on chromosome 4 at the Kirinyaga University site (LOD 3.73) for *C. partellus* species, 1 on chromosome 9 at Bukura site (LOD 2.97) and a minor QTL (LOD 2.56) on chromosome 1 at Kakamega site for *B. fusca* stem borer species. The most important was the QTL detected

Table 3. Locations and QTL effects for *C. partellus* and *B. fusca* stem borer resistance mapped in $F_{2:3}$ families from the cross between sensitive CML442 and CKSBL10026 multiple borer resistant inbred line parents.

Evaluation sites	Trait	LOD	Chr No.	Locus	Position in cM	% variance explained	Gene effect		Gene action
							Additive	Dominance	
Embu	Leaf damage	3.4	2	PZA02890_4	106.9	6.6	-0.09	0.13	OD
	Tunnel length	2.6	7	PZA00795_1	98.01	5.8	-0.08	0.8	OD
Kakamega	Leaf damage	2.7	1	PHM14614_2	60.9	5.9	0.1	-0.11	OD
	Exit holes	2.6	6	PZA00571_1	39.02	5.5	-0.31	-0.12	PD
	Tunnel length	3	1	PZA03301_2	92.97	6.5	-0.15	-0.2	OD
Kirinyaga University (KYU)	Exit holes	3.7	4	PZA00453_2	69.33	8.1	-0.49	-0.09	A
	Tunnel length	3.2	6	PZA02478_7	57.43	7.1	-0.81	-1.17	OD
Bukura	Exit holes	3.2	9	PZA00152_1	55.41	6.5	-0.17	-0.07	PD
	Tunnel length	3.3	6	PZA00152_2	55.41	6.7	-0.41	-0.2	PD
Kiboko	Tunnel length	2.7	3	PZA03391_1	108.93	5.9	-0.24	-0.84	OD
Combined *C. partellus*	Exit holes	2.5	5	PZA01284_6-PHM13942_7	64.669	5.6	0.126	-0.668	OD
	Tunnel length	2.8	4	PHM1505_31-PZA00453_2	57.741	6.2	0.733	-0.104	PD
Combined *B. fusca*	Tunnel length	2.9	4	PZA00453_2-PZA01954_1	76.329	6.2	-0.0335	-0.837	OD

Chr, Chromosome; LOD, Log_{10} of likelihood odds ratio; OD, over dominance, PD; partial dominance, A; additive gene action.

for *C. partellus* from Kirinyaga University which explained 8% of the phenotypic variation. The QTL detected for *B. fusca* at Bukura site explained 6.5% of the total phenotypic variation while for Kakamega site; the QTL explained 5.5% variation. The gene action for both Bukura and Kakamega sites were due to partial dominance while it was additive gene action for QTL detected for Kirinyaga University site (Table 3).

QTL for resistance to stem tunnelling

Combined sites analysis for both stem borer species revealed stem tunnelling QTL on chromosome 4 (LOD 2.81 for *C. partellus* sites and LOD 2.86 for *B. fusca* sites). Five QTL for reduced tunnelling were detected on different chromosomes on the individual sites for the two stem borer species. The strongest QTL for *C. partellus* stem tunnelling resistance was detected at Kirinyaga University site on chromosome 6 (LOD 3.24), while that for *B. fusca* stem tunnelling resistance was similarly detected for Bukura site on chromosome 6 (LOD 3.33), they explained 7.1 and 6.7% of the phenotypic variation, respectively. Suggestive QTL for resistance to stem tunnelling to *B. fusca* were detected at both Kakamega (LOD 2.99) and Embu (LOD 2.61) sites. A similar indicative QTL for resistance to stem tunnelling against *C. partellus* was detected at Kiboko (LOD 2.67). Stem tunnelling QTL was conditioned by over dominance gene action except for the Bukura QTL which was due to partial dominance.

DISCUSSION

Phenotypic data

The extremely low heritability in the combined sites analysis found in this study was a probable indicator of significant genotype by environmental interactions. In a recent similar study, Jiménez-Galindo et al. (2017) reported that resistance traits are associated with high experimental error because they are affected by the plant genotype,

the pest pressure and the environment and, therefore, are difficult to measure. All these factors lead to insect resistance traits showing low to moderate heritability values. The phenotypic data did not reveal distinct differences in resistance levels in the different sites except at Kiboko and Bukura sites. The high heterozygosity revealed in the parents after screening for polymorphism could have compounded the phenotypic differentiation between resistance and susceptible progenies in the field. This phenomenon may have caused the low levels of trait significance for resistance traits in both individual and combined sites. High and significant differences were, however, recorded for the Kiboko site in stem borer tunnel length and exit holes number, and leaf damage at Bukura site. The selection index computed from the three resistance traits leaf damage, number of borer exit holes and cumulative tunnel length in the combined analysis revealed several individuals that were resistant to *C. partellus* or *B. fusca,* or/and both. Forty four (44) individuals were resistant to both borer species in all sites, a clear confirmation that the resistant parent carried genes for multi-borer resistance. These results agree with the findings of Mwololo et al. (2015) and Odinga et al. (2016) who reported multiple resistances in some tropical maize germplasm against both *C. partellus* and *B. fusca* maize stem borer species. The phenotypic means distribution for resistance traits exhibited a normal distribution curve for specific species sites, and combined sites analyses (Figure 1). This was in agreement with Jampatong et al. (2002) who reported similar phenotypic means distribution for European corn borer resistance mapping study. There was a high correlation between the number of stem borer exit holes and stem tunnelling in the combined sites analysis which was a strong indication of the two parameters reliability and consistence as putative measures of resistance. These findings suggest that these parameters were neither dependent on the environments nor the stem borer species.

Quantitative trait loci for resistance to stem borers

Combined mapping of QTL based on data across sites for both species mapped resistance loci to chromosome 4 for stem tunnelling at position 57.74 cM (LOD 2.81) for *C. partellus* and at position 76.33 cM (LOD 2.86). One QTL for number of exit holes (LOD 2.53) was detected on chromosome 5 for *C. partellus* at position 64.67 cM. The close proximity of these stem tunnelling QTL within 18 cM on chromosome 4 for the both stem borer species suggested that there could be a gene with significant effects on reduced stem tunnelling between positions 57.74 and 76.33 cM. In other studies of a related lepidopteran pest (the European corn borer), QTL for resistance traits occurred in clusters (Papst et al., 2005). It is thus possible that in the case of resistance to stem borer in tropical maize, similar genome setup may occur as found in this study. Quantitative trait loci for resistance to stem borers mapped based on data from individual sites were mostly inconsistent, with only two sites (Kirinyaga University and Bukura) having consistently revealed QTL on chromosome 6. These inconsistencies in QTL detection may have been due to low levels of segregation in the mapping population, or it could underscore the enormous contribution and interaction of the environmental effects on QTL detection. Several QTL may, therefore, have been undetected in this study due to the environmental effects. Similar results have been reported in other studies on the European corn borer due to environmental effects (Jampatong et al., 2002; Krakowsky et al., 2004). The phenotypic variances associated with the QTL reported in this study were fairly low (mostly slightly below 10%). This study was in agreement with other QTL mapping studies in maize that reported low phenotypic variances on both the European corn borer, and storage insect pests (Jampatong et al., 2002; Garcia-Lara et al., 2009; Samayoa et al., 2015b; Jiménez-Galindo et al., 2017). Small phenotypic variation values may suggest that the QTL have only small effects, or have larger effects but were only more loosely linked to the marker locus (Edwards et al., 1987; Bohn et al., 2000).

The detected QTL in this study were conditioned by over dominance, partial dominance and additive gene actions. In 12 of the 13 QTL detected, resistance was conditioned by over-dominance and partial dominance. Partial dominance was found on three (3) QTLs for number of exit holes and stem tunnelling whilst additive gene action accounted for 1 QTL for the number of exit holes. In maize, resistance to the European corn borer is conditioned in a similar manner, albeit with additive gene action accounting for the majority of the QTL than dominance and over dominance gene actions (Guthrie and Russell, 1989; Bohn et al., 2000; Krakowsky et al., 2004; Jampatong et al., 2002). Scott et al. (1966) showed that resistance to the European corn borer, a lepidopteran pest just like *C. partellus* and *B. fusca* was conditioned by a relatively large number of genes with small effects on chromosomes 1, 2, 4, 6 and 8. The caution is that some QTL may not have been detected and, or, were dissimilar to those reported for related stem borers species due to the low heritability of the putative traits, and differences in trait characterization (Khairallah et al., 1998; Jiménez-Galindo et al., 2017). In a similar study, Samayoa et al. (2015b) attributed such discrepancies to QTL by environment interaction effects and stressed the importance of making phenotypic evaluations in environments similar to those for which breeding materials are intended to be used.

Overall these results show the presence of QTL for maize stem borer resistance in the tropical maize population studied and thus could provide an opportunity to pyramid them into elite material as has been done for

the European corn borer (Jampatong et al., 2002). Majority of the LOD scores were below 3.0 (at *"P<0.05"*), and that was most likely due to the low heritability of the trait under study, and phenotype differences under the different environments. The probability of detecting strong QTL with small sample sizes should be comparatively low unless the QTL explains a substantial proportion of the genetic variance. Melchinger et al. (1998) also reported that with a large number of minor QTLs influencing a quantitative trait such as insect resistance, the power of QTL detection and number of common QTLs should be smaller than for a trait governed by a small number of major QTL. The low heritability for stem borer resistance which was indicative of their polygenic nature should not be considered an impediment to maize improvement breeding activities in the tropics or elsewhere (Stuber et al., 1987; Bohn et al., 2000; Garcia-Lara et al., 2009).

Conclusions

Quantitative trait loci for the three putative resistance traits were detected in the tropical maize population studied. Relative to other maize stem borer QTL mapping studies, fewer QTL were detected in this study. Among the three traits, QTL for stem tunnelling were the strongest and were the most detected in both individual and combined specific borer species environments. The variances explained by QTL-marker associations were, however, low, indicative of many QTL with small variances that could have escaped detection. Individual sites analysis revealed stronger QTL and it was noted that more QTL were detected against *B. fusca* than *C. partellus*. The low reproducibility of QTL across environments for both stem borer species underscores the need for finer mapping and need for larger populations in succeeding mapping activities in the tropics.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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Appendix 1. List of SNP markers used to generate the genetic maps.

No.	SNP-Chromosome	No.	SNP-Chromosome	No.	SNP-Chromosome	No.	SNP-Chromosome	No.	SNP-Chromosome
1	PHM13942_7Chr5	34	PZA00152_1Chr9	67	PZA03301_2Chr1	99	PZB01403_1Chr1	131	PZA00664_3Chr1
2	PHM4752_17Chr1	35	PZA00245_20Chr1	68	PZA03391_1Chr3	100	csu1171_2Chr1	132	PZA00750_1Chr3
3	PHM5794_13Chr6	36	PZA00255_14Chr5	69	PZA03409_1Chr4	101	PHM10621_29Chr1	133	PZA00795_1Chr7
4	PZA00191_5Chr5	37	PZA00273_5Chr5	70	PZA03461_1Chr6	102	PHM11946_19Chr9	134	PZA00838_2Chr8
5	PZA00424_1Chr7	38	PZA00300_14Chr5	71	PZA03527_1Chr3	103	PHM1218_6Chr9	135	PZA00910_1Chr6
6	PZA00892_5Chr3	39	PZA00418_2Chr7	72	PZA03577_1Chr2	104	PHM14475_7Chr1	136	PZA01122_1Chr4
7	PZA01257_1Chr8	40	PZA00453_2Chr4	73	PZA03605_1Chr10	105	PHM1505_31Chr4	137	PZA01210_2Chr7
8	PZA02117_1Chr1	41	PZA00498_5Chr8	74	PZB00901_3Chr2	106	PHM1511_14Chr2	138	PZA01374_1Chr2
9	PZA03713_1Chr10	42	PZA00706_16Chr8	75	PZB01009_2Chr6	107	PHM15331_16Chr10	139	PZA01462_1Chr6
10	PZD00027_2Chr3	43	PZA00942_2Chr6	76	PZB01062_3Chr1	108	PHM16125_47Chr2	140	PZA01470_1Chr8
11	PHM11114_7Chr8	44	PZA00978_1Chr1	77	PZB01647_1Chr1	109	PHM18513_156Chr10	141	PZA01542_1Chr7
12	PHM12830_14Chr7	45	PZA00986_1Chr7	78	PZD00022_5Chr2	110	PHM1968_22Chr1	142	PZA01570_1Chr5
13	PHM15449_10Chr3	46	PZA01028_2Chr7	79	sh1_12Chr9	111	PHM2518_28Chr4	143	PZA01591_1Chr6
14	PHM1932_51Chr1	47	PZA01241_2Chr10	80	PHM14614_22Chr1	112	PHM2658_129Chr6	144	PZA01642_1Chr10
15	PHM2487_6Chr8	48	PZA01246_1Chr1	81	PHM2691_32Chr7	113	PHM2691_31Chr7	145	PZA01779_1Chr5
16	PHM259_11Chr4	49	PZA01284_6Chr5	82	PHM2919_23Chr3	114	PHM3078_12Chr7	146	PZA01954_1Chr4
17	PHM2714_11Chr8	50	PZA01297_1Chr8	83	PHM3301_28Chr4	115	PHM3334_4Chr2	147	PZA01978_23Chr1
18	PHM2769_43Chr5	51	PZA01438_1Chr5	84	PHM3896_9Chr10	116	PHM4145_18Chr3	148	PZA02040_2Chr5
19	PHM3147_18Chr1	52	PZA01501_1Chr3	85	PHM3963_33Chr4	117	PHM4604_18Chr9	149	PZA02164_16Chr5
20	PHM3334_6Chr2	53	PZA01799_1Chr9	86	PHM4080_15Chr7	118	PHM4780_38Chr2	150	PZA02167_2Chr2
21	PHM3337_23Chr8	54	PZA01933_3Chr7	87	PHM5529_4Chr6	119	PHM4786_9Chr8	151	PZA02385_6Chr4
22	PHM3598_20Chr2	55	PZA02019_1Chr8	88	PHM595_30Chr1	120	PHM499_19Chr2	152	PZA02478_7Chr6
23	PHM3736_11Chr10	56	PZA02247_1Chr6	89	PZA00224_4Chr2	121	PHM5296_6Chr5		
24	PHM4117_14Chr4	57	PZA02383_1Chr5	90	PZA00282_19Chr4	122	PHM5359_10Chr5		
25	PHM4620_24Chr2	58	PZA02423_1Chr3	91	PZA00381_4Chr1	123	PHM537_22Chr10		
26	PHM4997_17Chr1	59	PZA02549_3Chr2	92	PZA00818_1Chr5	124	PHM789_16Chr5		
27	PHM5306_16Chr1	60	PZA02653_12Chr5	93	PZA00860_1Chr9	125	PHM7953_11Chr2		
28	PHM533_46Chr5	61	PZA02698_3Chr1	94	PZA00941_2Chr4	126	PZA00081_18Chr1		
29	PHM5599_20Chr4	62	PZA02769_1Chr5	95	PZA01301_1Chr8	127	PZA00111_10Chr7		
30	PHM565_31Chr5	63	PZA02872_1Chr7	96	PZA01445_1Chr1	128	PZA00256_27Chr7		
31	PHM5780_15Chr4	64	PZA02890_4Chr2	97	PZA02129_1Chr1	129	PZA00436_7Chr4		
32	PHM662_27Chr5	65	PZA02955_3Chr8	98	PZA02186_1Chr1	130	PZA00571_1Chr6		
33	PHM7584_9Chr9	66	PZA03001_15Chr1	99	PZA02328_5Chr6	131	PZA00581_3Chr3		

No., Number.