

Quantitative Trait Loci Mapping in Maize for Resistance to Larger Grain Borer

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Abstract

Storability of maize grain is constrained by the larger grain borer (LGB) (*Prostephanus truncatus*). Host plant resistance is the most feasible way to manage LGB among smallholder farmers. Breeding for resistance to this pest in maize is dependent on understanding genetic mechanisms underlying the resistance. The objective of this study was to map quantitative trait loci (QTL) associated with LGB resistance in tropical maize. A mapping population of 203 $F_{2:3}$ derived progenies was developed from a cross between susceptible and resistant inbred lines. The $F_{2:3}$ progenies were crossed to a tester and testcrosses evaluated across six environments followed by screening for resistance to LGB. Data was collected on husk cover tip length, and grain texture in the field. Biochemical traits were analyzed on the maize grain. Harvested grain was evaluated for resistance and data recorded on grain damage, weight loss and number of insects. Grain hardness was measured as a putative trait of resistance. Univariate analysis of variance for all the traits was done using the general linear model of statistical analysis system. Genetic mapping was done using Joinmap 4, while QTL analysis was done using PLABQTL. The QTL for resistance were mapped to 6 out of the 10 chromosomes. QTL for resistance traits were located in chromosomes 1, 5 and 9. Chromosome 1 had a common QTL linked to protein content, grain hardness and husk cover tip length. Additive genetic effects were prevalent in all detected QTL. Overall, the studies show that breeding for resistance to LGB is possible.

Introduction

Diseases and insect pests are key biotic factors leading to low yields in maize production. Insect pests destroy approximately 14% of all potential food production, including maize, despite the yearly application of more than 3,000 million kilograms of pesticides (Pimentel, 2007). Losing crops to insect pests constitutes a great constraint to realization of food security worldwide. Postharvest insect pests cause serious losses to cereals in both quality and quantity and in most cases pre-dispose stored grain to secondary attack by disease causing pathogens (Evans, 1987; Kankolongo et al., 2009). The larger grain borer (LGB) infests maize grain before and after harvest. Farmers in African countries experience grain weight losses as high as 80% during storage (Tapondjou et al., 2000). Host plant resistance through crop breeding remains a cornerstone of an integrated pest management strategy to minimize storage losses and impact on grain quality. Genetic variation for resistance to postharvest insect pests has been reported (Mwololo et al., 2010). Studies that have used germplasm with more diversity confirmed the heritability of insect pest resistance

(Derera et al., 2001). Antibiosis and non-preference were found to be important mechanisms of resistance to postharvest insect pests in maize grain (Derera et al., 2001). Biochemical and physical characteristics have been associated with resistance to the maize weevil (Arnason et al., 1994). The biochemical components of grain that have been reported to confer resistance to insects or diseases through antibiosis include phenolic acids, hydroxyproline-rich glycoproteins, sugar content, soluble peroxidase and protein inhibitors. Grain hardness is a physical factor which has been closely associated with resistance to grain storage pests by conditioning non-preference (García-Lara et al., 2004).

A quantitative trait locus (QTL) is a region associated with a particular phenotypic trait, and these QTL are often found on different chromosomes (Edwards et al., 1987). The main goals of QTL mapping in plants are to (a) increase our biological knowledge of the inheritance and genetic architecture of quantitative traits, and (b) identify markers that can be used as indirect selection tools in breeding (Bernardo, 2008). Linkage analysis and association mapping are the two most commonly used methods for QTL mapping. Linkage analysis requires

biparental population whereas association mapping requires collections of germplasm to find genes underlying quantitative traits (Yu et al., 2006). Linkage analysis is critical for preliminary location of QTLs and association mapping for precise location (Mackay, 2001).

The availability of a wide range of molecular markers and powerful statistical methods has significantly facilitated QTL mapping. The ability to transfer target genomic regions using molecular markers has resulted in QTL mapping experiments in economically important crops, to identify molecular markers for MAS (Xu, 1998, Semagn et al., 2006). The most common molecular markers used earlier were restriction fragment length polymorphisms, random amplified polymorphic DNA, amplified fragment length polymorphisms and microsatellites (Pinto et al., 2003). Recently, the single nucleotide polymorphism (SNPs) markers have gained preference in the scientific community as the markers of choice in molecular genetics (Slate et al., 2009). They are the most abundant type of genetic polymorphism in most genome and genotyping cost is low. Molecular markers enable breeders to exercise selection on genotypic or DNA-based differences rather than phenotypic differences (Mohan et al., 1997). They therefore have the potential to increase selection efficiency in breeding through marker-assisted selection by tagging of agriculturally important genes. Molecular markers have been used to map

of the QTL mapping and identification of candidate genes for resistance has been done for Lepidopterans particularly the corn ear worm (*Helicoverpa zea*) and European corn borer (*Ostrinia nubilalis* (Hübner)) (Byrne et al., 1997). The use of robust DNA markers that map QTL's associated with postharvest insect pests in maize is an important approach in fine mapping and marker assisted recurrent selection (MARS). The first attempt on QTL mapping for Coleopterans was on the maize weevil (García-Lara et al., 2009). There is no any QTL mapping work which has been reported for resistance to the larger grain borer in tropical maize. The objective of this study was therefore to identify QTL for larger grain borer resistance.

Materials and Methods

Germplasm and experimental design

A mapping population of 203 $F_{2:3}$ derived progenies were developed from a cross between extremely susceptible and resistant inbred parents identified from earlier studies, namely CML 444 and CKSPL10116 respectively. The $F_{2:3}$ progenies were further crossed to a tester (CML 312/CML 442) to generate F1s which were evaluated in three replicates across six environments of Kenya Agricultural Research Centers (KARI-Kiboko, KARI-Mtwapa, Kirinyaga Technical Institute (KTI), KARI-Kakamega, KARI-Embu and Bukura Agricultural Center) (Table 1).

Table 1 Characteristics of the 6 trial sites used in the evaluation of the mapping population

Site	Name	Longitude	Latitude	Elevation (masl)	Rainfall (mm)	Temp max (°C)	Temp (min)	Soil texture
1	Bukura	34° 36' E	0° 15' N	1397	1800	20	22	Orthic ferralsol
2	Kakamega	34° 44' E	0° 16' N	1530	1916	29	13	Sandy loam
3	Kiboko	37° 75' E	2° 15' S	975	530	35	14	Sandy clay
4	Embu	37° 42' E	0° 49' S	1540	1200	24	18	Clay loam
5	KTI	37° 19' E	0° 34' S	1282	1500	24	18	Clay loam
6	Mtwapa	39° 21' E	4° 34' S	30	965	29	22	Sandy soils

masl=meters above sea level; Temp=temperature; max=maximum; min=minimum; KTI-Kirinyaga Technical Training Institute

genes for insect resistance in most major crop species such as maize, rice, wheat, potato and beans. These studies examined specific plant crosses to determine QTLs affecting aspects of plant chemistry, such as maysin in corn and acyl sugars in tomato, or aspects of plant morphology (trichomes), traits already known to affect resistance to insects (Yencho et al., 1996; Byrne et al., 1996). The Br gene from mung bean, effective against several species of bruchids and the bean bug, *Riptortus clavatus* for Coleopteran resistance have been tagged (Kaga and Ishimoto, 1998). In maize, most

The experimental design for evaluation of the test cross F1s was an Alpha lattice design (35 x 6) of two rows replicated 3 times. Data was collected in the field on husk cover tip length. Harvested grains from the six locations were evaluated in the laboratory for phenotypic insect pest resistance traits for quantitative trait loci analysis namely: grain damage (GD), grain weight loss (WL), susceptibility index and numbers of insects (AP). The Grain hardness (GH) and protein content (PC) were determined as putative traits of

resistance as described in chapter 4.

Leaf samples were picked from 10 plants of each of the 203 $F_{2:3}$ families before crossing with the tester on 3 weeks old seedlings for genotyping. Genomic DNA was extracted from the leaves using the 96-well format high throughput protocol (Mace et al., 2003). Six hundred (600) single nucleotide polymorphic (SNPs) markers were screened for polymorphism between the parental lines, three hundred and forty (340) SNPs were polymorphic, and used to genotype the $F_{2:3}$ derived progenies. Genotyping was done using the K-Biosciences Competitive Allele-Specific PCR genotyping system (KASP)-(K,Biosciences Ltd Unit 7 Maple Park Essex Road Hoddesdon EN11 0EX UK).

Bioassay and biophysical data analyse

All traits were subject to analysis of variance within and across environments by the proc mixed procedure of SAS statistical package (SAS Institute, 2003). Normality test was done using Anderson-Darling test of MINITAB 14 software. Data on flour produced (%) was arcsine transformed before statistical analyses for normalization. Complete and incomplete blocks and locations were considered as random effects and entries as fixed effects when calculating the means for the mean environment. The means were used for the QTL analysis. Estimates of variance components, including genotypic variance (σ^2_g), genotype \times environment interaction ($\sigma^2_{g\times e}$), phenotypic variance (σ^2_p) and residual (σ^2_e) were calculated by equating the mean squares to their expected values (Searle, 1971). Broad-sense heritability (H) on entry mean basis was estimated according to Hallauer and Miranda, (1981). Phenotypic correlation coefficients were calculated among resistance and biophysical traits from adjusted entry means across environments for each parameter.

Genetic mapping and QTL analyses

Genetic linkage maps for the population were constructed using Joinmap 4.0 (VanOoijen and Voorrips, 2006). Segregation at each marker locus was tested against the expected Mendelian segregation ratio (1:2:1) using a Chi-square test. Grouping of the markers (loci) was done with a minimum logarithm of odds (LOD) score of 2.0. Minimum distance between loci of 5 cM and a goodness of fit jump thresh hold of 5 for removal of loci and 1 as the number of added loci after which to perform a ripple for verification of locus orders was adapted. Recombination threshold was set at 0.4 and the recombination frequencies between marker loci was estimated by multi-point analyses and transformed into centimorgans (map distance) using the Kosambi mapping function.

Quantitative trait loci mapping was performed for protein content, grain hardness, husk cover tip length, grain damage, weight loss, adult progenies and flour weight in 203 test cross progenies. Composite interval mapping with a LOD-score of 3.0 as implemented in PlabQTL was used in QTL mapping (Haley and Knott 1992Zeng, 1994; Utz and Melchinger, 1996). The QTL analyses were based on adjusted entry means of the 203 test cross progenies across six environments for the maize LGB grain damage traits and the additional traits associated with resistance. Previous studies suggest a LOD threshold value between 2 and 3 (Lander and Botstein, 1989). A thresh hold value of 2.5 has been used in other studies of QTL in maize and this minimizes the risk of type 11 error (Cardinal et al., 2003). The QTL mapping was initially carried out using simple interval mapping to identify the major QTL, a genetic model without epistasis based on the average effect of an allele substitution (Bernardo, 2002). Subsequently, composite interval mapping was employed. The cov/+select option was used to detect closely linked QTL of opposite effects.

Estimates of QTL positions were obtained at the point where the LOD score reached its maximum value in the region under consideration. Gene action was defined in relation to direction of resistance for each trait based on the additive effect value. The parameters associated with resistance after insect pest infestation (grain damage, weight loss, flour weight and adult progeny) indicate resistance if the values are smaller, therefore QTL with negative additive effect values up to +0.20 indicate additive gene action. In relation to protein content, husk cover tip length and grain hardness, greater resistance is associated with positive trait values, hence the larger the value in the positive direction the better.

Results

Analyses of biophysical and resistance traits

There were highly significant differences ($P \leq 0.001$) among the test crosses for all the traits evaluated (Table 2). The environment variance was also significant among all the traits. However the genotype \times environment interaction was not significant for all the traits. The components of variance, σ^2_g and $\sigma^2_{g\times e}$ of the test cross progenies were highly significant for all the agronomic and resistance traits evaluated, and the heritability ranged from 0.23 to 0.54 (Table 2). They were low for the grain hardness and number of insects (< 0.3) and intermediate (0.4-0.54) for the protein content, husk cover tip length, grain damage and flour weight (Table 2).

Table 2 The mean, estimates of variance components and heritability for postharvest insect pest resistance parameters for test cross of F2:3 families from the cross CML44 x CKSPL10116

Parameter	Protein Content (%)	Husk cover tip length (cm)	Grain hardness (N)	Weight loss (%)	Grain damage (%)	Adult progeny	Flour Weight (%)
Means	10.91	5.72	160.61	14.37	43.90	207.04	2.01
Range	10.20-11.50	3.90-7.80	146.20-175.60	10.90-21.40	30.90-57.00	140.5-290.05	1.10-3.30
σ^2e	0.26**	7.04**	4.92	47.82**	218.28**	3940.04**	0.95**
σ^2g	0.04**	0.30**	9.78	2.19**	10.98**	228.48**	0.10**
$\sigma^2g \times l$	0.04	0.14	0.033	0.36	9.24	364.16	0.05
σ^2	0.42	1.58	233.76	45.93	286.66	8852.68	1.70
H	0.54	0.58	0.23	0.45	0.41	0.29	0.51

σ^2e – environmental variance; σ^2g -genotypic variance; $\sigma^2g \times l$ -genotype \times location variance; H-broad sense heritability, h^2 -narrow sense heritability

The phenotypic correlations among the resistance traits were significant ($P < 0.001$) except for the grain hardness (Table 3). The flour weight, grain damage, weight loss and the number of insects were positively correlated, but negatively correlated with the protein content and grain hardness. The protein content and grain hardness were positively correlated.

(Table 3)

observed was due to surplus of heterozygotes. Overlapping markers were excluded from the map thereby ending up with a total of 244 markers. This led to ten linkage groups corresponding to the ten chromosomes of the haploid maize genome, ranging in length from 66.7 to 188 cm. The linkage map was 1102cM in length with average marker distance of 4.5cM between loci (Figure 1). The number of markers

Table 3 Phenotypic correlations among resistance parameters to the maize weevil and larger grain borer.

	FW	GD	WL	AP	PT	GH
FW		0.70**	0.85**	0.82**	-0.30**	-0.20ns
GD		0.67		0.74**	0.74**	-0.28**
WL				0.78**	(-0.35**	-0.28ns
AP					(-0.5**	-0.25ns
PT						0.4**

Key: FW-flour weight; GD=grain damage; AP=No. of insects; PT=protein content; GH-grain hardness; WL=weight loss

Linkage map

The final linkage map comprised of a set of 249 SNPs, following exclusion of those which did not conform to the 1:2:1 Mendelian segregation ratio. The distortion

mapped per chromosome ranged from 13 to 44. The order and placement of the SNP markers were generally in a good agreement with the maize reference map. (Figure 1)

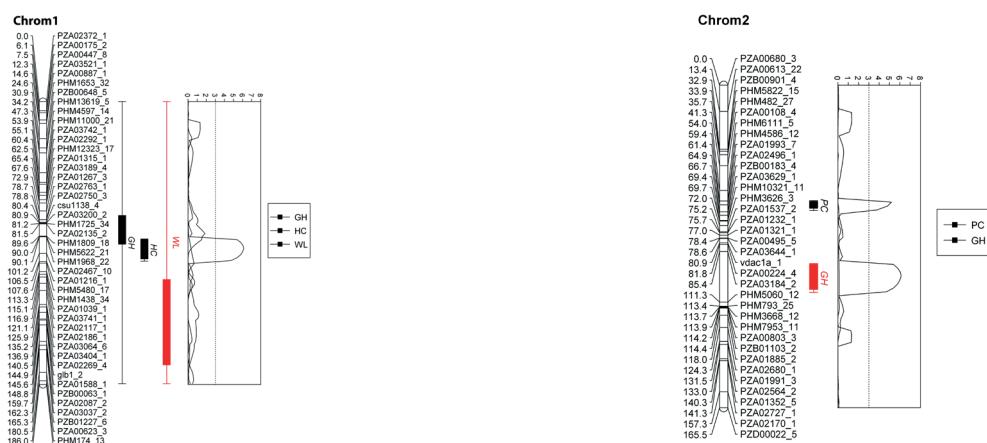
Figure 1 Quantitative trait loci (QTL) for grain hardness (GH), husk cover tip length (HC), weight loss (WL) and protein content (PC) in chromosomes 1 and 2

Table 4. Summary of QTL positions and additive effects for protein content, grain hardness and husk cover tip length as traits of resistance to postharvest insect pests in maize grain estimated from test crosses of 203 F2:3 families of the cross CML44 x CKSPL10116

Trait	QTL	Chromosome	Position (cM)	Marker linked to trait	Supportive Interval	LOD	R2 (%)
Protein content	1	1	138	PZA03064_	134-142	4.36	11.7
	2	2	58	PHM4586_1	54-66	3.34	9.0
	3	3	98	PZB01109_	92-110	5.81	15.3
	4	8	34	PZA00908_	32-42	5.23	13.8
Husk cover	1	1	110	PHM5480_1	106-114	5.65	15.1
	2	4	26	PZA00529_	22-32	3.5	9.6
	3	7	66	PZA0284_	62-66	4.17	12.3
Grain hardness	1	1	88	PZA02135_	84-90	3.64	8.3
	2	1	120	PZA3741_	116-126	4.01	9.1
	3	2	74	PHM3626-3	72-76	3.51	7.7
Oil content	1	3	90	PZB01109_	78-98	3.92	8.5
	2	4	30	PHM5599_	28-34	7.66	16.0
	3	8	3	PZA00440_	0-4	3.38	8.1

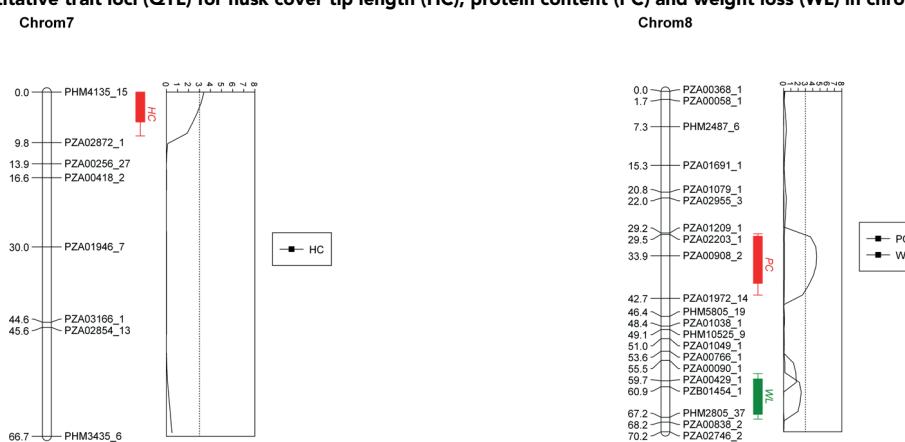
*Key: cM=centimorgan; LOD=likelihood-ratio test statistic; R2=phenotypic variation; SE=standard error; Adj σ^2 g=adjusted genetic variance

* The coefficient of determination (R2) gives the phenotypic variation explained by a given QTL

Quantitative trait loci analysis

Four QTLs for protein content were detected on chromosomes 1, 2, 3 and 8 respectively (Table 4; Figures 1, 2, 3) which explained 47.8 % of the phenotypic variation, with individual QTL accounting for 10-15 %. A simultaneous fit with all the four QTL based on the cross validation explained 75.8 % of the adjusted

in maize grains to postharvest insect pests. Three QTLs were identified in chromosome 1 (two) and 2 (one) for grain hardness, one coming from the susceptible and two from the resistant parent (Table 4; Figure 1). The phenotypic variation explained by the QTLs in chromosome 1 was 7 % and that from chromosome 2 was 5 %. The additive effects were 5.84 and -8.36 for the QTLs in chromosome 1 and 5.41 for that in

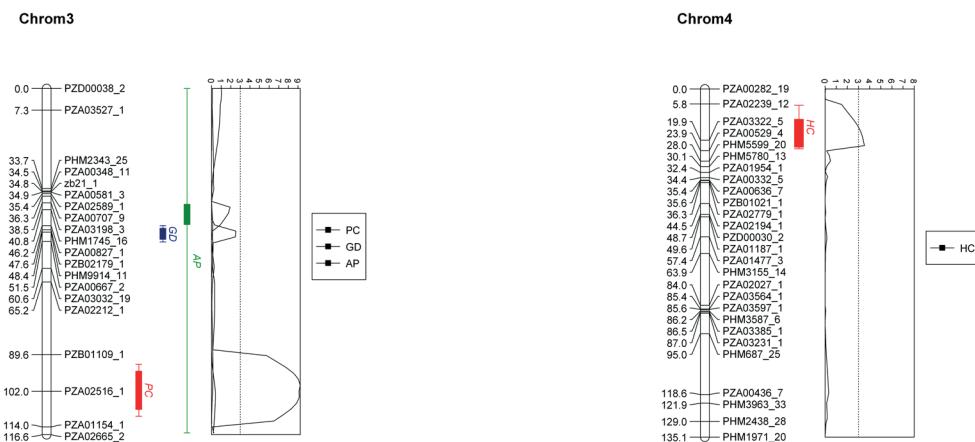
Figure 2 Quantitative trait loci (QTL) for husk cover tip length (HC), protein content (PC) and weight loss (WL) in chromosomes 7 and 8.

genetic variance. The additive gene action ranged from 0.11 to 0.15. All the QTLs (alleles) which were from the resistant parent contributed to the increased protein content, which is a putative trait of resistance

chromosome 2.

Three QTLs on chromosomes 1, 4 and 7 respectively were detected for husk cover tip length (Table 4, Figures 1, 2 & 4). These were all stable based on the

Figure 3 Quantitative trait loci (QTL) positions for grain damage (GD), protein content (PC), number of insects (AP) and husk cover tip length (HC) in chromosomes 3 and 4.



cross validation statistics. The additive effects were -0.405, 0.291 and -0.385 respectively. This indicates that the first and third QTLs were from the susceptible whereas the second was from the resistant parent. As a result, the alleles for increase in the husk cover tip length came from the resistant and the susceptible parent. The simultaneous fit with all the three QTLs explained 21.6 % of the phenotypic variation and 37.3 % of the genetic variance.

Discussion and conclusions

There were high and significant phenotypic correlation coefficients among the traits for assessing resistance in maize grains, namely grain damage, weight loss, flour weight and number of insects. The QTL for some of the resistance traits were located in common genomic regions found on chromosomes 1, 3 and 8. Strong association was observed between grain damage, number of insects, flour weight and weight loss.

Table 5 Summary of QTL positions and additive effects for larger grain borer damage indices on maize grain estimated from test crosses of 203 F2:3 families from the cross CML44 x CKSPL10116

Trait	QTL	Chromosome	Position (cM)	Markers linked to trait	Supp interval	LOD	R2	Additive Effect	SE	Adj $\sigma^2 g$
Grain damage	1	3	20	PZA02135, PHM1745	0-52	3.51	9.7	-1.42	0.344**	43.4
	1	1	186	PHM174, PZA00856	182-188	5.25	14.7	-1.033	0.253**	
Weight loss	2	8	60	PZA00429, PZB01454	58-66	3.72	8.5	-0.823	0.239**	70.5

Key: cM= centimorgan; Supp=supportive interval; LOD=likelihood-ratio test statistic; R2=phenotypic variation; SE=standard error; Adj $\sigma^2 g$ =adjusted genetic variance.

* The coefficient of determination (R2) gives the phenotypic variation explained by a given QTL

One chromosomal region was identified as a putative QTL for grain damage by the LGB (Table 5). The QTL was on chromosomes 3 (Figures 4). The additive effect was -1.42, therefore the QTL was from the susceptible parent. The simultaneous fit with the QTL explained 9.7 of the phenotypic variation and 43.3 % of the genetic variance. Two QTLs were detected for weight loss in chromosomes 1 and 8. The additive effects ranged from -1.13 to -0.823. All the two QTLs explained 11.6 % and 70.3 % of the phenotypic and genetic variation respectively; and all alleles came from the susceptible parent (Table 5).

Chromosome 3 had QTL for grain damage, number of insects and protein content. The results are in agreement with other reports whereby most QTL for resistance were contributed by the resistant parent with few from the susceptible parent (Jampantong, 1999). Additive genetic effects were prevalent for all the detected QTL.

Resistance to insect pests in maize grain is conditioned by biophysical traits and or biochemical traits. The mapping of QTL for protein content in the same region with the resistance trait was an indication of their close association. Previous studies have reported the role

of proteins in maize grain for weevil and larger grain borer resistance (García-Lara et al., 2004; Mwololo et al., 2012; Siwale et al., 2009). The protein is involved in the cell wall structure strengthening together with biochemical components such as simple phenolic acids and diferulates leading to harder grains. The QTL in chromosome 3 are mapped to a genomic region associated with genes linked to cell wall sugar components in maize grain pericarp, and for whole plant fiber (Holland, 2007; Hazen et al., 2003; Cardinal et al., 2003). Quantitative trait loci for galactase, xylose and arabinose have also been mapped to chromosomes 2 and 3 (Hazen et al., 2003). Genes for peroxidase and simple phenolic compounds which are linked to maize weevil resistance were mapped to chromosome 2 (Brewbaker et al., 1985). Genes of structural proteins like HRGPs using cDNA probes, have been mapped to chromosome 2 (Davis et al., 1999). Similarly, the protein content had QTL in chromosomes 2 and 3, therefore it is closely linked with the cell wall components associated with grain resistance to insect pests. Two QTL for protein content were mapped to the same genomic region (chromosomes 1 and 8) as coumaric acid (CA) and ferulic acid (FA), found to confer resistance to storage pests (Davis et al., 1999; García-Lara et al., 2010). Extensions, which are proteins, are bound to the cell wall complex through covalent linkages with pectins by cross linking with feruloylated sugars or oxidation by peroxidase forming stable protein-protein links (Cassab, 1998).

Earlier studies have reported grain hardness as a putative trait of resistance to postharvest insect pests in maize (García-Lara et al., 2004). There were negative correlations between grain hardness and the resistance traits though low, and was positive to protein content. The QTL for grain hardness were located in chromosomes 1, 2 and 3. Earlier studies have indicated that genes linked to the kernel characteristics such as kernel size and endosperm softness are located in chromosome 1 (Gudrups et al., 2001). The three QTL correspond to those for protein content given that out of the four, two were in chromosomes 1, 2 and 3. The results from this study show links between proteins and grain hardness; and insect damage traits, suggesting common genetic basis for traits associated with resistance to postharvest insect pests. Storage proteins in food grain are responsible for the association between starch grains and endosperm matrix proteins, thus influencing the grain hardness (Hoseney, 1987; Dombrink-Kurtzman and Bietz, 1993). The presence of abundant and organized protein bodies is associated with greater adherence and better distribution of starch granules in the endosperm and, consequently,

higher physical resistance of the grain (Gibbon et al., 2003). Husk cover (bare tipped or complete husk cover) has been associated with controlling the level of field infestation of the maize cobs by postharvest insect pests including maize weevil and larger grain borer (Warfield and Davis, 1996). Three QTL were identified for husk cover tip length in chromosomes 1, 4 and 7. These genomic regions have been found to have the genomic regions for both biochemical and biophysical characteristics of maize grains (García-Lara et al., 2010). The husk cover characteristics have been reported to be under control of additive gene action with non-additive playing a minor role and this concurs with the results from this study (Brewbaker and Kim, 1979).

The results show that there was clustering of QTL for postharvest insect pest resistance with QTL for disease, lepidopteran insect resistance and structural cell wall biochemical components. Most of the QTL for resistance traits in maize are located in chromosomes 1, 3, 5 and 9. According to Papst et al., (2004), QTL for stem borer resistance were not randomly distributed in the maize genome but occur in chromosomes 1, 5 and 9. In addition, genes for lignin biosynthesis, a biochemical component associated with stem borer resistance are located in the same genomic region as those for stem borer resistance. Furthermore, the study supports the functional relationship between gene and QTL mapping for insect pest resistance, which has been previously established by other authors for insect pests of maize (Cardinal and Lee, 2005; Krakowsky et al., 2007; Meyer et al., 2007). The QTL for disease resistance, which have been mapped before and recently, map in some of the genomic areas reported in this study (Kelley et al., 2012). The clustering of QTL and genes forms an important basis for breeding for resistance. These should be prioritized in identifying markers for use in stacking multiple resistance into one variety (Bergvinson and García-Lara, 2006).

Quantitative traits loci (QTL) associated with postharvest insect resistance traits were identified. The QTL affecting LGB resistance traits in tropical maize are important in analyzing genetic variation and increasing the efficiency of maize breeding programs. The chromosomal regions containing genes involved in the synthesis of cell wall components could be associated with resistance to different insect species in maize. Marker assisted recurrent selection would be useful in transferring the QTL alleles into susceptible and promising inbred lines. The identification of QTL associated with LGB resistance in tropical maize will enable breeders to exploit the genetic variation and increase the efficiency in delivering maize lines resistant to storage pests to increase food security for subsistence farmers.

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