

# 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<sub>2:3</sub> derived progenies was developed from a cross between susceptible and resistant inbred lines. The F<sub>2:3</sub> 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 (KAR1-Kiboko, KARI-Mtwapa, Kirinyiga 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° 219 E | 4° 347 S | 30               | 965           | 29            | 22         | Sandy soils      |

masl=meters above sea level; Temp=temperature; max=maximum; min=minimum; KTI-Kirinyiga 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<sub>2:3</sub> 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<sub>2:3</sub> 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 ( $\sigma_g^2$ ), genotype  $\times$  environment interaction ( $\sigma_{g \times e}^2$ ), phenotypic variance ( $\sigma_p^2$ ) and residual ( $\sigma^2$ ) 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 threshold 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 1992; Zeng, 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 threshold 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,  $\sigma_g^2$  and  $\sigma_{g \times e}^2$  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 × 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        |
| $\sigma^2_l$     | 0.26**              | 7.04**                     | 4.92               | 47.82**         | 218.28**         | 3940.04**     | 0.95**           |
| $\sigma^2_g$     | 0.04**              | 0.30**                     | 9.78               | 2.19**          | 10.98**          | 228.48**      | 0.10**           |
| $\sigma^2_{gxl}$ | 0.04                | 0.14                       | 0.033              | 0.36            | 9.24             | 364.16        | 0.05             |
| $\sigma^2$       | 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             |

$\sigma^2_l$  – environmental variance;  $\sigma^2_g$ -genotypic variance;  $\sigma^2_{gxl}$ -genotype × location variance; H-broad sense heritability, h2-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**   | -0.30ns |
| 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

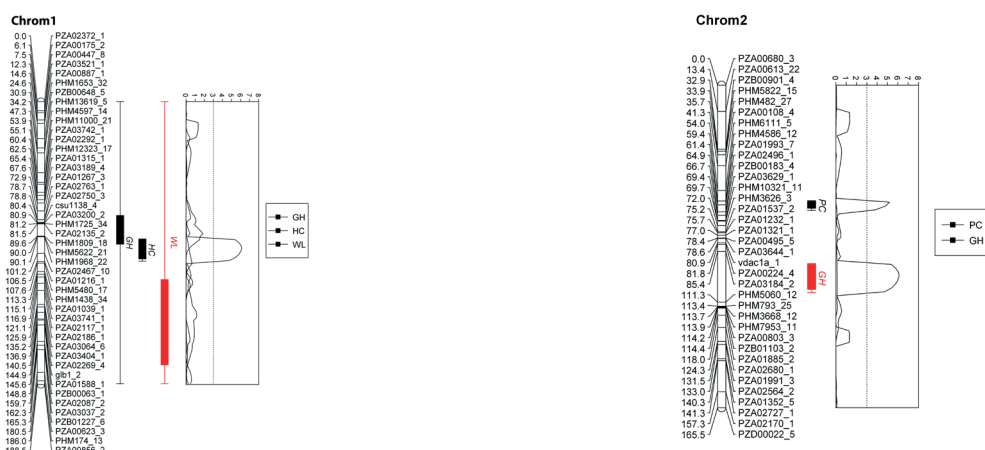
**Linkage map**

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 × 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    |

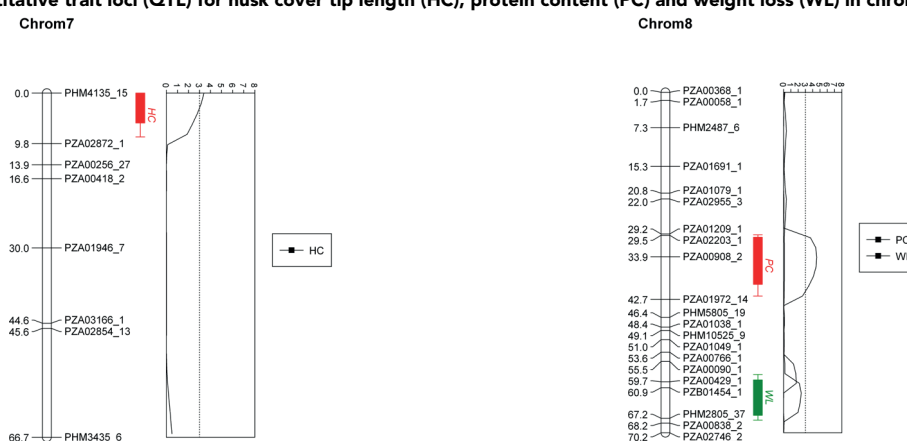
\*Key: cM=centimorgan; LOD=likelihood-ratio test statistic; R2=phenotypic variation; SE=standard error; Adj  $\sigma^2g$ =adjusted genetic variance  
 \* The coefficient of determination (R2) gives the phenotypic variation explained by a given QTL

**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

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 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

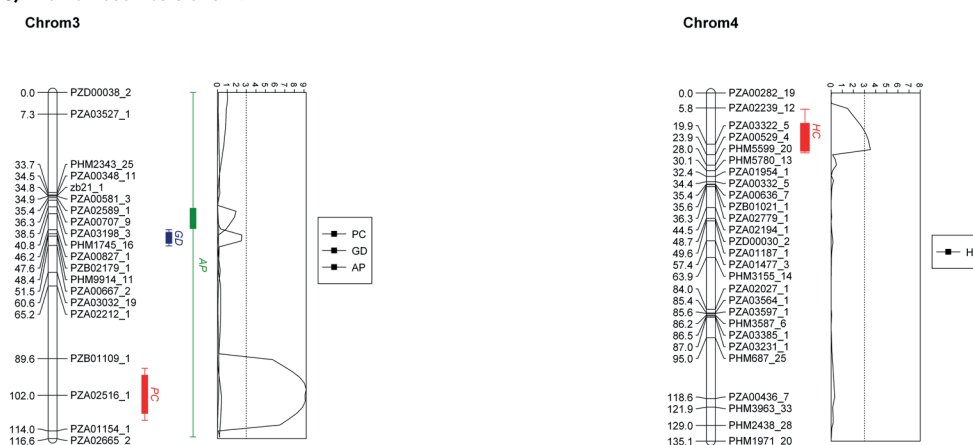
**Figure 2 Quantitative trait loci (QTL) for husk cover tip length (HC), protein content (PC) and weight loss (WL) in chromosomes 7 and 8.**



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 σ2g |
|--------------|-----|------------|---------------|-------------------------|---------------|------|------|-----------------|---------|---------|
| 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 σ2g=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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### References

- Arnason JT, Baum J, Gale JD, Lambert R, Bergvinson D, Philogene BJ, Serratos JA, Mihm J, and Jewell DC, 1994. Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. *Euphytica* 74: 227-236
- Bergvinson D, García-Lara S, 2006. Consensus mapping for field and storage pest resistance in tropical maize. International Plant Breeding Symposium, Mexico City. S. Higman (ed.), CIMMYT, Mexico, D.F
- Bernardo R, 2002. Breeding for quantitative traits in plants. Stemma Press, Woodbury MN.
- Brewbaker J, Kim S, 1979. Inheritance of husk cover and ear insect damage in maize. *Crop Science* 19: 32-36
- Brewbaker J, Nagai C, Liu E, 1985. Genetic polymorphisms of 13 maize peroxidases. *Journal of Heredity* 76: 159-167
- Byrne PF, McMullen MD, Wiseman BR, Snook ME, Musket TA, 1997. Identification of maize chromosome regions associated with antibiosis to corn earworm (*Lepidoptera: Noctuidae*) larvae. *J. Econ. Entomol* 90: 1039-45
- Byrne PF, McMullen MD, Snook ME, Musket TA, Theuri JM, Widstrom NW, Wiseman BR, Coe EH, 1996. Quantitative trait loci and metabolic pathways: Genetic control of the concentration of maysin, a corn earworm resistance factor in maize silks. *Proceedings of National Academy of Science, USA* 93: 8820-8825
- Cardinal A, Lee M, 2005. Genetic relationships between resistance to stalk-tunneling by the European corn borer and cell-wall components in maize population B73•B52. *Theor. Appl. Gen* 111: 1-7
- Cardinal A, Lee M, Moore K, 2003. Genetic mapping and analysis of quantitative trait loci affecting fiber and lignin content in maize. *Theor. Appl. Gen* 106: 866-874
- Cassab GI, 1998. Plant cell wall proteins. *Ann. Rev. Plant Physiol. Mol Biol* 49: 281-309
- Davis G, McMullen M, Baysdorfer C, Musket T, Grant D, Staebell M, Xu G, Polacco M, Koster L, Melia-Hancock S, Houchins K, Chao S, Coe E, 1999. A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. *Genetics* 152: 1137-1172
- Derera J, Pixley V, Giga DP, 2001. Resistance of maize to the maize weevil. I. Antibiosis. *African Crop Sci. J* 9: 431-440
- Dombrink-Kurtzman MA, Bietz JA, 1993. Zein composition in hard and soft endosperm of maize. *Cereal Chemistry* 70: 105-108
- Edwards MD, Stuber CW, Wendel JF, 1987. Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics* 116: 113-125
- Evans D, 1987. The survival of immature grain beetles at low temperatures. *J. Stored Prod. Res* 23:79-83
- García-Lara S, Andrew J, Arnason J, Bergvinson D, 2010. QTL Mapping of Tropical Maize Grain Components Associated with Maize Weevil Resistance. *Crop Sci* 50:815-825
- García-Lara S, Bergvinson D, Burt AJ, Ramput AI, Díaz-Pontones DM, Arnason JT, 2004. The role of pericarp cell wall components in maize weevil resistance, *Crop Sci* 44:546-1552
- García-Lara S, Khairallah MM, Vargas M, Bergvinson DJ, 2009. Mapping of QTL associated with maize weevil resistance in tropical maize. *Crop Sci* 49: 139-149
- Gibbon BC, Wang X, Larkins BA, 2003. Altered starch structure is associated with endosperm modification in quality protein maize. *Proceedings of National Academy of Sciences, U. S. A* 100:15329-15334
- Gudrups I, Floyd S, Kling J, Bosque-Perez N, Orchard J, 2001. A comparison of two methods of assessment of maize weevil resistance to the maize weevil, *Sitophilus zeamais* Motschulsky, and the influence of kernel hardness and size on susceptibility. *J. Stored Prod Res* 37: 287-302
- Hallauer A, Miranda J, 1981. Quantitative genetics in maize breeding. Iowa State University Press, Ames
- Hazen S, Hawley RM, Davis G, Henrissat B, Walton J, 2003. Quantitative trait loci and comparative genomics of cereal cell wall composition. *Plant Physiol* 132: 263-271
- Holland J, 2007. Genetic architecture of complex traits in plants. *Curr Opin Plant Biol* 10: 156-161
- Hoseney RC, 1987. Wheat hardness. *Cereal Foods World* 32: 320-322.
- Jampantong C, 1999. Quantitative trait loci for first and second generation European corn borer resistance in maize. PhD. Dissertation (Diss. Abstr. AA199462650), University of Missouri, Columbia



- Kaga A, Ishimoto M, 1998. Genetic localization of a bruchid resistance gene and its relationship to insecticidal cyclopeptide alkaloids and the vignatic acids in mungbean (*Vigna radiata* L. Wilczek). *Mol General Genet* 258: 378–84
- Kankolongo M, Hell K, Nawa I, 2009. Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia. *J. Food Sci Agric* 89: 1366-1375
- Kelley R, Williams, W, Mylroie, J, Boykin D, Harper J, 2012. Identification of Maize Genes Associated with Host Plant Resistance or Susceptibility to *Aspergillus flavus* Infection and Aflatoxin Accumulation. *PLoS ONE* 7:doi:10.1371/journal.pone.003689
- Krakowsky M, Lee M, Holland J, 2007. Genotypic correlation and multivariate QTL analyses for cell wall components and resistance to stalk tunneling by the European corn borer in maize. *Crop Sci* 47: 485–490
- Lander S, Botstein D, 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121: 185-199
- Mace ES, Buhariwalla, HK, Crouch JH, 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Mol Biol Reporter* 21: 459
- Mackay FC, 2001. The genetic architecture of quantitative traits. *Ann Rev Gen* 35: 303-339
- Meyer J, Snook M, Houchins K, Rector B, Widstrom N, McMullen M, 2007. Quantitative trait loci for maysin synthesis in maize (*Zea mays* L.) lines selected for high silk maysin content. *Theor Appl Gen* 115: 119-128
- Mwololo J, Mugo S, Tefera T, Okori P, Munyiri S, Semagn K, Otim M, Beyene Y, 2012. Resistance of tropical maize genotypes to the larger grain borer. *J Pest Sci* 85: 267-275
- Mwololo J, Mugo S, Okori P, Tefera T, Munyiri SW, 2010. Genetic diversity for resistance to larger grain borer in maize hybrids and open pollinated varieties in Kenya. *Second RUFORUM Biennial Meeting, Kampala, Uganda*, pp. 535–539
- Papst C, Bohn M, Utz H, Melchinger A, Klein D, Eder J, García-Lara S, 2004. QTL mapping for European corn borer resistance (*Ostrinia nubilalis* Hb.), agronomic and forage quality traits of test cross progenies in early-maturing European maize (*Zea mays* L.) germplasm. *Theor Appl Gen* 108: 1545-1554
- Pimentel D, 2007. Area-wide pest management: environmental, economic, and food issues, in: Vreysen M.J.B., R.A.S., Hendrichs (Eds), (Ed.), *Area-wide control of insect pests*. Springer, Netherlands, pp. 35-47
- SAS Institute, 2003. Statistical software system. Proceedings of the Twenty-Eighth Annual SAS® Users Group International Conference, Cary, NC, USA
- Searle L, 1971. Evidence for Composition Gradients across the Disks of Spiral Galaxies. *Astrophysical Journal* 168: 327-341
- Semagn K, Bjornstad A, Skinnes H, Maroy A, Tarkegne Y, William M, 2006. Distribution of DArT, AFLP and SSR markers in a genetic linkage map of a double haploid wheat population. *Genome* 49: 545-555
- Siwale J, Mbata K, Mrobert J, Lungu D, 2009. Comparative resistance of improved maize genotypes and landraces to maize weevil. *African Crop Sci J* 17: 1-16
- Tapondjou A, Bouda H, Fonte D, Zapfack L, Lonsi D, Sondengam B, 2000. Local plants used for traditional stored product protection in the Menoua division of the Western Highlands of Cameroon. *IOBC Bulletin* 73-77
- Utz H, Melchinger A, 1996. PLABQTL: A program for composite interval mapping
- VanOoijen J, Voorrips R, 2006. JoinMap Version 4.0, Software for the calculation of genetic linkage maps in experimental populations. *Plant Research International, Wageningen*
- Warfield C, Davis R, 1996. Importance of the husk covering on the susceptibility of corn hybrids to *Fusarium* ear rot. *Plant Dis.* 80: 208-210
- Xu S, 1998. Mapping quantitative trait loci using multiple families of line crosses. *Genetics* 148, 517-524
- Yencho GC, Bonierbale MW, Tingey WM, Plaiste RL, Tanksley SD, 1996. Molecular markers locate genes for resistance to the Colorado potato beetle, *Leptinotarsa decemlineata*, in hybrid *Solanum tuberosum* x *S. berthaultii* potato progenies. *Entomol Exp Appl* 81, 141–154
- Yu J, Pressoir G, Briggs WH, Bi I, Yamasaki M, Doebley J, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES, 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics* 38: 203-208
- Zeng Z., 1994. Precision mapping of quantitative trait loci. *Genetics* 136: 457-1468