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ORIGINAL PAPER

Genetic mapping of QTL for maize weevil resistance in a RIL population of tropical maize

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Abstract
Key message **A tropical RIL maize population was subjected to phenotypic and genotypic analysis for maize weevil resistance during four seasons, and three main genomic areas were detected as main QTLs.**
Abstract The maize weevil (*Sitophilus zeamais*) (MW) is a common and important pest of stored maize (*Zea mays*) worldwide, especially in tropical areas. Quantitative trait loci (QTLs) associated with the MW have been analyzed previously in an F_2 maize population. In this work, new germplasm-based F_6 recombinant inbred line (RIL) families, derived from the cross of Population 84 and Kilima, were analyzed using insect bioassays during four seasons. The parameters analyzed for MW resistance were grain weight losses (GWL), adult progeny (AP), and flour production (FP). Composite interval mapping identified a total of 15 QTLs for MW parameters located on six chromosomes, explaining between 14 and 51 % of phenotypic variation (σ^2_p) and 27 and 81 % of genotypic variation (σ^2_g). The QTL obtained for GWL was located in bin 2.05, which explained 15 % of σ^2_p. For AP and FP, the QTLs were located on regions 1.09 and 2.05, explaining 7 and 15 % of σ^2_p, respectively. Comparative analysis between F_2 and F_6 families showed similarities in QTL localization; three main regions were co-localized in chromosomes 4.08, 10.04, and 10.07, where no resistance-associated genes have been reported previously. These regions could be used for a marker-assisted selection in breeding programs for MW resistance in tropical maize.

Abbreviations

GWL	Grain weight losses
FP	Flour production
AP	Adult progeny
MW	Maize weevil
QTL	Quantitative trait locus
RIL	Recombinant inbred line

Introduction

Insect postharvest losses associated with maize (*Zea mays* L.) grain production are a growing constraint of food security, especially in tropical areas of developing countries, where maize has a fundamental role as food and feed. Persistent infestations of the maize weevil (*Sitophilus zeamais* [Motsch.]) (MW) are common in maize stores of subsistence farmers in Latin America, Africa, and Asia because of poor postharvest management. Global poor farmers often experience grain weight losses of 30 % in average during storage (Pingali and Pandey 2001). Infestations often start in the field and are brought into the grain store, where severe damage and losses have been observed, with more than 80 % damage and between 20 and 60 % weight losses (García-Lara and Bergvinson 2007).

Because plant breeding in the tropics has largely focused on improving agronomic traits such as yield and disease

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resistance (Xu 2010), low emphasis has been placed on postharvest pest resistance. Different efforts have been made to reduce postharvest grain losses as a primary part of an integrated pest-management strategy (Dobie 1977; García-Lara and Bergvinson 2007). Sources of resistance to the MW have been identified from maize accessions originating from Mexico, the Caribbean Islands, and Africa (García-Lara and Bergvinson 2013, 2014). Resistance mechanisms have been related with grain hardness and pericarp cell wall components of maize (Santiago et al. 2013; Ayala-Soto et al. 2014). Biochemical resistance is based on phenolic compounds, which act in two ways: through mechanical resistance and antibiosis in the pericarp and aleurone layer, respectively (Arnason et al. 1994; García-Lara et al. 2004, 2007; McMullen et al. 2009), which are also compatible with biological control (Bergvinson and García-Lara 2011).

The genetic inherence of MW resistance is known to be mainly maternally inherited based on the pericarp (Dhliwhayo and Pixley 2003; Dhliwayo et al. 2005). Previously, quantitative trait loci (QTLs) were detected for MW resistance in an F_2 population using simple sequence repeats (SSRs) and restriction fragment length polymorphism molecular markers (García-Lara et al. 2009). Twenty-six chromosomal regions were declared as putative QTLs associated with MW resistance, and main associations were co-located with grain biophysical properties. Additionally, when QTLs of phytochemical grain composition were added to the F_2 map, close genomic relationships with structural cell wall components, key enzymes, and phenolic acid biosynthesis pathways were identified. QTLs were observed in bins 1.10, 3.06, 3.09, 4.10, 6.00, 6.05, 7.03, and 10.06 (García-Lara et al. 2010).

In this work, we extend our previous studies to evaluate MW resistance parameters with an advanced (F_6) recombinant inbred line (RIL) population. Using a constructed molecular map in a tropical population developed to the level of RIL, the main goals of this work were to (i) generate phenotypic data on MW resistance parameters during four seasons, and (ii) perform an analysis and localization of QTLs for the MW.

Materials and methods

Mapping population

Maize resistance to the storage pest population was developed to the level of RIL using the parent lines derived from Population 84 (P84) and Kilima. P84 was developed from Caribbean germplasm and was selected based on its resistance to *Prostephanus truncatus* (Kumar 2002; García-Lara and Bergvinson 2014), whereas Kilima was developed with accessions from Tanzania and was selected based on its resistance to *Sitophilus zeamais* (MW). The cross was developed using the following parental lines: P84 C1 F27-4-3-3-B-1-B and Kilima ST94A-30/MSV-03-2-10-B-2-B-B. The mapping population after the cross was developed by selfing during six successive growing environments, until RIL was reached, under subtropical conditions at Tlaltizapan, Morelos, Mexico (18°41′N lat., 940 masl), during the winter and summer environments from 2001 to 2004. Additional seed increases for bioassays were generated at CIMMYT's Agua Fria experimental station located in the state of Puebla, Mexico (19°N lat., 60 masl), from 2004 to 2006. At the F_4 level of recombination, 397 families were evaluated for MW resistance using insect bioassays. An index of resistance for the MW was developed based on different susceptibility parameters (Dobie 1977). This index was used to select the 15 % most susceptible families and 15 % resistant families (120 in total) of the mapping populations for the MW and used for the development of RIL lines according to the extreme analysis procedure (Mansur et al. 1993). Out of these 120 RIL lines 100 were selected for QTL mapping.

Molecular marker mapping

Leaf tissue from the parental lines and RIL families' plants were harvested at the six-leaf stage of development, placed in perforated paper bags, and stored at −80 °C until lyophilization. Dried leaf tissue was ground to fine powder and stored at −20 °C until DNA extraction. Genomic DNA was extracted using the CTAB procedure (Hoisington et al. 1994). A linkage map was developed using SSRs. Primers were chosen on the basis of chromosome position and bin location to provide uniform coverage of the entire maize genome [Maize Genetics and Genomics Database, MaizeGDB (www.maizegdb.org)]. A total of 1128 SSR probes were used for screening the parental lines. Five hundred twenty-nine of the SSRs showed polymorphism between parent lines. Effective polymorphisms were observed for 407 SSR markers, with 360 being used to screen 22 families to analyze partial segregation. Finally, 170 SSRs were used to genotype the entire 100 RIL population. Analyses of SSRs, PCR amplifications, and gel electrophoresis were made after the procedures described by CIMMYT (2005). The group of Maize Microsatellite Consortium probes were the most polymorphic (65.3 %), whereas the North Carolina group showed the lowest polymorphism (20 %). Three hundred thirty-eight SSRs were selected as positive polymorphic for 22 families screening, which was enough to cover the entire maize genome.

HyperMap data were used to capture molecular marker data, and segregation was tested for deviations from expected Mendelian segregation ratios in a RIL population

(1:1) using a standard χ^2 test. The linkage map was constructed from 170 molecular markers by Haldane's mapping function with the software package MAPMAKER (Lander et al. 1987). Recombinant frequencies between adjacent markers were transformed into centiMorgans (cM). A LOD (log10 of the likelihood odds ratio) value of 3.0 was used as a critical threshold value to declare a linkage between two markers. One hundred RIL families were selected based on polymorphism for SSR mapping and QTL analyses. The molecular marker linkage map generated from the cross P84 × Kilima was based on 169 SSRs. The molecular markers spanned a map distance of 1974.8 cM, with an average marker distance of 11.68 cM. SSR markers covered 95 % of the bins within the maize genome. In chromosomes 1, 3, 6, 7, and 9 marker intervals exceeding 35 cM were present; however, the bin area was covered with at least one marker.

Bioassay data and analysis

One hundred RIL families were evaluated for MW resistance using three resistance parameters, namely, grain weight losses (GWL), adult progeny (AP), and flour production (FP). Resistance parameters were evaluated in F_6 families and parental lines during four different seasons. Experimental model included three independent replicate experiments conducted in each season. From each, seed bulk corresponds to one row of 5 m with density of 25 plants. The ANOVA model includes three independent experiments with three reps for bioassays, and analyzed during four seasons. For each sample, three replicates containing 30 g of maize kernels were infested with 25 adults that were 0–7 days old and were removed 1 week later following the method proposed by Dobie (1977). After 2 months of total bioassay incubation, the samples were stored at 4 °C for 72 h. Next, grain was sieved using mesh sieves (#6 and #18, Standard Testing Sieve, VWR, USA) and weighed to calculate GWL (initial weight minus final weight) and FP. AP were separated and counted from the sample (García-Lara et al. 2009). Phenotypic and genetic correlation coefficients were calculated from adjusted entry means across environments for each parameter. Genetic correlations were calculated according to Bernardo (2002). Estimates of variance components were calculated by equating the mean squares of their expected values (García-Lara et al. 2009). Broad sense heritability was estimated according to Hallauer and Miranda (1981).

QTL mapping analysis

QTL analysis was performed for GWL, FP, and AP using 100 RIL lines that were a subset of these 397 RIL lines. Composite interval mapping (CIM) was employed to map QTLs in a joint analysis using four environments (Jansen and Stam 1994; Jiang and Zeng 1995). Three different models were applied in the analysis. Three different models were applied in the analysis according to Zeng (1994). Model III, which corresponds to simple interval mapping, was used for the selection of cofactors. Model II was subsequently fitted with the selected markers as cofactors as long as they were unlinked to the genomic regions of interest. Finally, Model I was used to confirm the QTL detected in model II. Model I used all selected cofactors plus markers flanking the target interval with a minimum map distance (window size) of 20 or 30 cM.

The threshold values used for QTL detection for four environments, with df = 4, were LR = 21.03 with $\alpha = 0.00317$, LR = 18.46 with $\alpha = 0.00925$, and LR = 15.89 with $\alpha = 0.0266$, obtained also from a χ^2 distribution. The presence of a QTL was declared whenever the LR value exceeded the threshold in model II of the three applied models, and a peak was also significant in model III using both window sizes (0.3 and 0.2). The phenotypic variance (σ_p^2) explained by a QTL was obtained by the coefficient of determination (R^2). The explained genotypic variation (σ_g^2) considering all putative QTL simultaneously was obtained by dividing the multiple coefficient of determination by the heritability. Parent who contributed with the allele that conferred resistance to MW was specific by indicating which QTL/s were contributed by which parent, whereas the QTL × environment (QTL × E) interaction was considered significant whenever the value given by the program exceeded 12.5. Finally a co-localized QTL was declared among traits and between populations when its location in bin comparison was positive.

Results

Resistance trait analysis

Means of parental lines (susceptible and resistant) differed significantly ($p < 0.05$) for each of the three traits (GWL, AP, and FP). Means of F_6 RIL families were above the resistant parent (Table 1). The population mean of F_6 RIL families for GWL, FP, and AP was significantly ($p < 0.05$) greater than the mid-parent mean. The phenotypic distribution of RIL families' traits followed a Gaussian distribution with transgressive segregation in both directions. However, RIL population selected by the extreme values showed two tales in a normal distribution with transgressive segregation in both directions. Components of variance σ_g^2 and σ_{gxe}^2 were both highly significant ($p < 0.001$) for all traits. Heritability was high for GWL and FP (>0.63) and intermediate for AP (0.55). Phenotypic and genotypic correlations found among four environments with GWL, AP, and FP for the MW were

Table 1 Mean, estimates of variance components and heritabilities for resistance parameters to maize weevil *Sitophilus zeamais* combined across 2 years for susceptible (S) and resistant (R) parents and RILs (F_6) families from the cross P84 × Kilima

Parameter	Entries (#)	Grain weight loss (GWL)	Adult progeny (AP)	Flour production (FP)
		g	No	G
Means				
P(S) P84[a]	1	7.60 ± 0.34	26.00 ± 0.38	0.40 ± 0.01
P(R) Kilima[b]	1	1.58 ± 0.43	12.33 ± 1.52	0.08 ± 0.02
P[c]	2	4.59 ± 0.23	19.17 ± 0.76	0.25 ± 0.01
F_6[d]	120	10.38 ± 3.93	35.61 ± 12.36	0.30 ± 0.18
Range		1.23–20.43	4.23–68.28	0.03–0.97
σ_g^2		49.53***	390.86***	0.09***
$\sigma_g^2 \times e$		45.65***	536.42***	0.07***
σ_r^2		13.72	189.05	0.03
h^2		0.63	0.52	0.64
90 % CI of h^2		(0.65–0.58)	(0.56–0.49)	(0.67–0.60)

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

[a] S, moderately susceptible, $n = 10$

[b] R, resistance, $n = 10$

[c] P = mean of P84 and Kilima, $n = 14$

[d] F_6 = mean of F_6 lines, $n = 120$

Table 2 Phenotypic and genetic correlations among resistance parameters to maize weevil *Sitophilus zeamais* and the evaluated environments, in the RILs (F_6) families from the cross P84 × Kilima

Parameter	Season	GWL				AP				FP			
		1	2	3	4	1	2	3	4	1	2	3	4
GWL	1		0.282*	0.300*	0.101	0.940**	0.165	0.215	0.067	0.940	0.299**	0.270*	0.134
	2	0.276*		0.348**	0.279*	0.238*	0.926**	0.295*	0.228	0.307**	0.934**	0.348**	0.295*
	3	0.276*	0.342**		0.465**	0.335*	0.276*	0.970**	0.396	0.279*	0.386**	0.965**	0.534**
	4	0.095	0.279*	0.487**		0.036	0.231	0.507**	0.947	0.074	0.281*	0.466**	0.965**
AP	1	0.940**	0.235*	0.307*	0.075		0.133	0.272**	0.041	0.918**	0.262*	0.321**	0.108
	2	0.162	0.926**	0.266*	0.226	0.131		0.244	0.201	0.178	0.857**	0.278*	0.260*
	3	0.197	0.289*	0.970**	0.527**	0.249**	0.234		0.448**	0.204	0.327*	0.947**	0.574**
	4	0.063	0.230	0.410	0.947	0.038	0.199	0.462**		0.030	0.238*	0.396**	0.905**
FP	1	0.940**	0.299**	0.257*	0.070	0.918**	0.173	0.188	0.028		0.313**	0.267*	0.132
	2	0.293**	0.934**	0.368**	0.281*	0.260*	0.857**	0.312*	0.241*	0.305**		0.397**	0.292*
	3	0.248*	0.341**	0.965**	0.491**	0.294**	0.267*	0.947**	0.412**	0.247*	0.378**		0.540**
	4	0.125	0.291*	0.544**	0.965**	0.102	0.253*	0.582**	0.905**	0.124	0.289*	0.554**	

Phenotypic correlations are in the lower left diagonal, whereas genetic correlations are in the upper right diagonal of the table

GWL Grain weight losses, *AP* adult progeny, *FP* flour production; *Season 1* 2003, *2* 2004, *3* 2005, *4* 2006

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

significant at $p < 0.01$ (data not shown). For each individual season, phenotypic and genotypic resistance parameters were correlated with each other at significant levels of $p < 0.05$ and <0.01 (Table 2). The highest phenotypic correlations found were for GWL-AP, GWL-FP, and AP-FP. Between each corresponding environment (identified by number), the highest value obtained on phenotypic correlations was 0.96 for GWL3-FP3 and GWL4-FP4 ($p < 0.01$). However, when traits from different environments were tested, correlation values diminished. For genotypic correlations, a similar trend was observed. The highest genotypic correlation value obtained was 0.97 for GWL3-AP3.

Table 3 Parameters associated with quantitative trait loci (QTL) for grain weight losses (GWL), flour production (FP) and adult progeny (AP) by the maize weevil *Sitophilus zeamais* and their genetic effects estimated from phenotypic data obtained of RIL families from the cross P84 × Kilima

Trait	QTL		Likelihood ratio[a]			R^{2a}	Gene action[c]	P[d]
	Chr.	Marker interval	LR	LOD	QTL × E	Total	Add.	
GWL	2.05	*bnlg1909-umc1884*	20.80	2.96	4.65	15.08	−1.77	R
	3.01	*umc2071-bnlg1144*	19.33	2.67	12.22	4.97	−0.82	R
	4.08	*umc1051-umc1573*	22.17	3.22	5.41	6.92	−1.77	R
	8.05	*umc1846-umc1149*	21.77	3.14	8.33	6.13	1.30	S
	10.04	*umc1995-bmlg1526*	20.03	2.81	2.01	12.24	−1.39	R
	10.07	*umc1084*	22.89	3.36	17.22*	11.02	−0.79	R
						51.34		
FP	2.05	*bnlg1909-umc1884*	20.59	2.91	4.66	15.08	−0.08	R
	4.08	*umc1051-umc1573*	31.31	5.00	16.80*	6.97	−0.08	R
	10.04	*umc1995-bmlg1526*	23.19	3.42	2.55	12.24	−0.08	R
	10.07	*bnlg1450-umc1084*	20.11	2.82	8.05	11.17	−0.07	R
						36.46		
AP	1.09	*Phi094-umc2189*	20.23	2.84	9.69	7.53	−4.22	R
	4.08	*umc1051-umc1573*	18.50	2.51	5.61	2.47	−5.15	R
	8.05	*umc1309-umc1846*	24.14	3.61	10.07	4.24	5.43	S
	10.04	*umc1995-bmlg1526*	16.78	2.17	0.15	0.29	−5.34	R
	10.07	*umc1084*	16.84	2.18	9.54	4.19	−3.42	R
						14.2		

* QTL by environment (QTL × E) interactions significant at the 0.05 probability level

[a] Likelihood ratio was estimated under model II using markers as cofactors (grain weight loss: *bnlg1092 and bnlg1338*; flour production: *bnlg1909, umc1051, and umc1995;* adult progeny: *phi115, umc1460 and umc1995*)

[b] Phenotypic variance explained by each QTL and all putative QTL affecting the trait

[c] QTL effects where a negative value implies that the allele from the resistant parent increases the numeric value of the trait. *Add.* Additive effect

[d] P, Parents from whom the resistance genes come; moderately susceptible: P84; resistance: Kilima

Quantitative trait loci analysis

The SSR linkage molecular map based on the germplasm of subtropical maize was consistent in locus order with previously published maps (Gardiner et al. 1993; Groh et al. 1998; Khairallah et al. 1998; Davis et al. 1999), and the order of the SSR markers was in agreement with bin positions reported in the MaizeGDB (http://www.maizegdb.org/ssr.php). Using this map (Fig. 1) a total of six chromosomal regions were detected for GWL in the QTL analysis across four different seasons for the MW (Table 3). CIM detected six QTLs on chromosomes 2, 3, 4, 8, and 10 (two regions). The QTL on chromosome 2 expressed the highest value of phenotypic variation, (σ_p^2 = 15 %). A simultaneous fit with all six QTLs explained 51.3 % σ_p^2 and 81.49 % of genotypic variation (σ_g^2). Alleles associated with GWL were contributed by the resistant parent, and only one allele (8.05) was contributed by the susceptible parent. The allele localized on bin 10.07 showed a significant QTL × E interaction. Bins on regions 10.07 and 3.01 obtained the highest values of additive genetic effect for GWL.

For MW FP, four QTLs were found. These QTLs were located on chromosomes 2, 4, and 10 (two regions). The region on chromosome 2 explained the highest phenotypic variation (15 %). The total effect of the four regions explained 36.4 % of σ_p^2 and 56.96 % of σ_g^2. All alleles associated with FP were contributed by the resistant parent. The QTL localized in bin 4.08 showed a significant QTL × E interaction (Table 3).

Five different regions were detected for AP in the QTL analysis for the MW (Table 1). Genomic areas of QTL were located on chromosomes 1, 4, 8, and 10 (two regions). The QTL on chromosome 1 had the highest value for σ_p^2, explaining 7.5 %. The total set of QTLs associated with AP for the MW explained 14.2 % of total σ_p^2 and 27.3 % of σ_g^2. Alleles associated with AP were mainly from the resistant parent, and one allele was derived from the susceptible parent. For AP, none of the QTLs showed significant interaction with the environment. The QTL located in bin 8.05

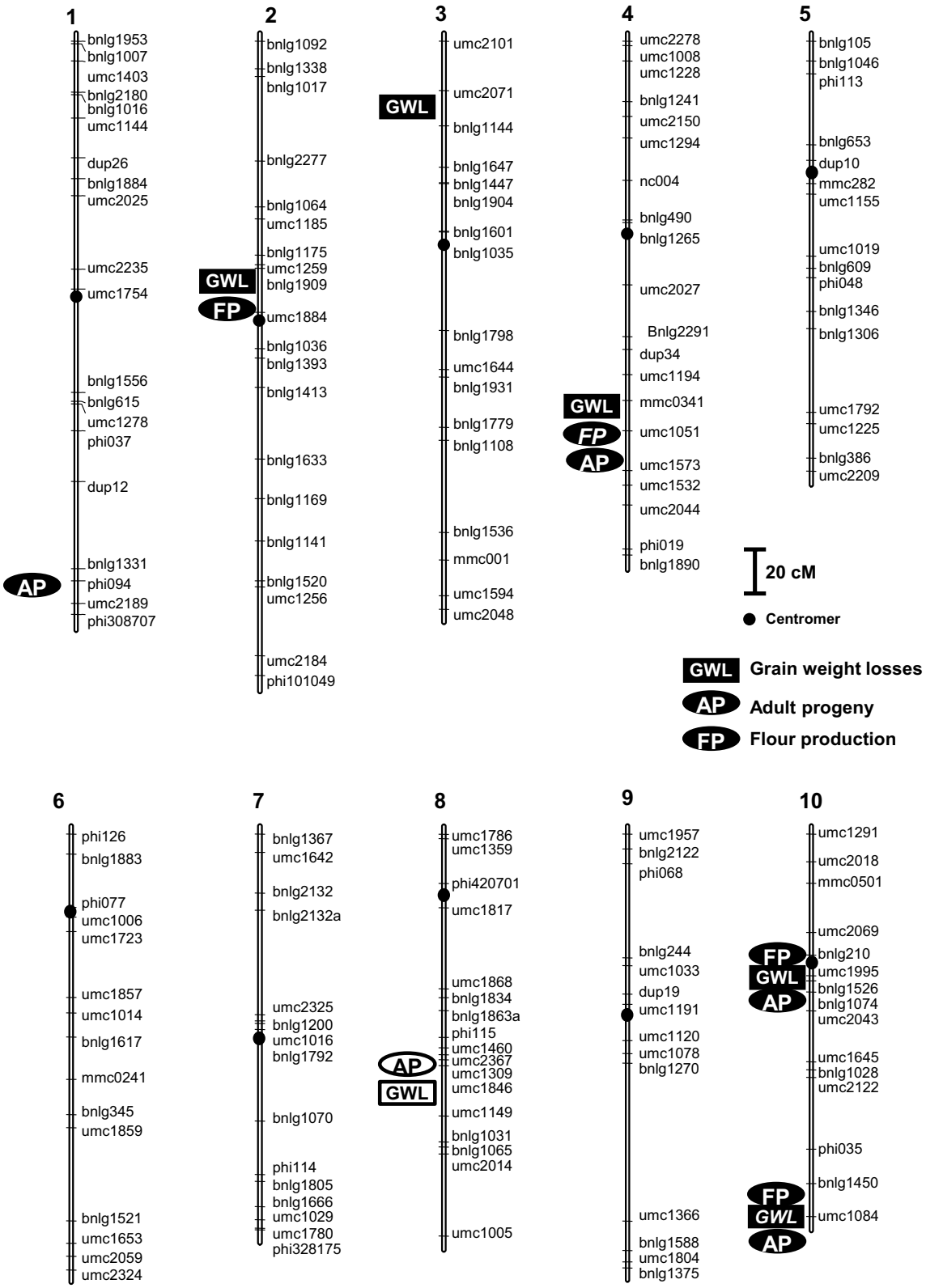

Fig. 1 Combined SSR linkage map based on 100 F6 families from the cross P84 × Kilima. Approximate QTL positions for grain weight loss (GWL), Flour production (FP), and adult progeny (AP) of maize weevil Sitophilus zeamais are shown. *Shaded (Kilima)/non-shaded (P84) shapes* indicates the parent from which the favorable allele was derived. QTL with a significant QTL × E interaction are indicating with *italics*

Table 4 QTL regions associated with maize resistance to MW and their relation with other important genes/QTLs

Chr.Bin	QTLs reported on RIL	QTLs reported on F_2^a	Associated QTL/gene[b]
1.09	$QTL_{AP}01$	GWL, AP, FP	Soft endosperm gene (*sen3*)
			QTL for grain weight
2.05	$QTL_{GWL}01$, $QTL_{FP}01$	GWL, AP	Peroxidase gene (*px1*)
			Soft endosperm gene (*sen5*)
			QTL for response to *Cercospora zeae-maydis*
			QTL for percent grain dry matter
3.01	$QTL_{GWL}02$	None	Soft endosperm gene (*sen1*)
			QTL for 300 kernel weight
4.08	$QTL_{GWL}03$, $QTL_{FP}02$, $QTL_{AP}02$	FP	Specific embryo genes
			QTLs for 300 kernel weight
			QTLs for response to *Cercospora zeae-maydis*
			QTLs for kernel weight
8.05	$QTL_{GWL}04$ and $QTL_{AP}03$	None	Floury grain gene (*fl*-N1163*)
			QTLs for response *to Cercospora zeae-maydis*
			QTL for dry matter percentage
			QTL for European Corn Borer response
10.04	$QTL_{GWL}05$, $QTL_{FP}03$, $QTL_{AP}04$	GWL, AP and FP	Pectin methylesterase gene (*pme1*)
			QTL for European Corn Borer response
			QTL for Sugarcane borer response
			QTL for response to *Cercospora zeae-maydis*
			QTL for Corn Earworm response
10.07	$QTL_{GWL}06$, $QTL_{FP}04$, $QTL_{AP}05$	GWL, AP and FP	QTL for leaf reductor sugars
			QTLs for response to *Cercospora zeae-maydis*

GWL Grain weight losses, *FP* flour production, *AP* adult progeny

[a] Based on information reported by García-Lara et al. (2009)

[b] Based on information available in MaizeGDB (http://www.maizegdb.org/)

obtained the highest interaction and was the only allele derived from the susceptible parent.

Common genomic regions

Regions where QTLs for different resistance traits overlapped were found in five regions, distributed on chromosomes 2, 4, 8, and 10. The principal associations were among GWL, FP, and AP, which were found in bins 4.08, 10.04, and 10.07. Overlapping QTLs for GWL-FP and GWL-AP were observed in bins 2.05 and 8.05 (Fig. 1).

Discussion

RIL genetic bases

The SSR linkage molecular map based on the germplasm of subtropical maize was used to analyze QTL for MW resistance. Fifteen QTLs associated with the three traits were identified as chromosomal regions associated with MW resistance in a RIL mapping population. This represents 50 % fewer QTLs found in our previous study (García-Lara et al. 2009) and highlights useful RILs to discover and define candidate genes (Takuno et al. 2012). Using RILs, we detected six QTLs for GWL, four for FP, and five for AP. As expected, most of the QTL alleles conferring increased resistance to the MW were derived from the resistant parent Kilima. Only two QTLs were contributed by the susceptible parent. As expected additive gene action was detected for all QTLs compared with previous studies in maize resistance against various insect pests (Bohn et al. 1997) is also present for storage pests. Genetic effects based on QTLs observed in this study are consistent with additive effects detected for quantitative inheritance of MW resistance (Dhliwhayo and Pixley 2003; Dhliwayo et al. 2005).

Resistance mechanisms

Important relationships found between QTLs for GWL and previously reported genes are listed in Table 4. Genomic regions associated with the MW are located in bins, where

other related characteristics are clustered according to the MaizeGDB. For the chromosomal region 2.05, GWL is related to a peroxidase gene, QTL of soft endosperm gene, and QTL of percentage of grain dry matter. Grain hardness is a biophysical parameter associated with insect resistance, whereas peroxidase is involved in the formation of diferulic molecules; both are associated with mechanisms of resistance (García-Lara et al. 2004).

The QTL found in bin 10.04 contains a gene for pectin methyl-esterase and pectin content, which is related to maize cell shape and its presence in cell walls. Barrière et al. (2012) reported a QTL for lignin on this same bin, as well as a QTL of galactose content of the pericarp cell wall (Hazen et al. 2003). For FP, the QTL regions associated with the MW share bins with structural characteristics such as endosperm softness and biochemical response of peroxidase to insect attack. The QTL regions associated with AP for the MW are located in bin 1.09, which also contains a gene responsible for a soft endosperm and a QTL for grain weight; both are related to resistance mechanisms. A recent QTL study showed that eight QTLs associated with cell walls were detected in bin 4.09 and near to those identified in bin 4.08 in our study (Courtial et al. 2013).

Clusters of QTLs for MW resistance plus genes and QTLs for disease and insect resistance were also found. An interesting case is the QTL found in bin 10.04, which also contains QTLs for important field pests, such as the European corn borer, sugarcane borer, and corn earworm (Meihls et al. 2012). By an advanced intermated B73 × Mo17 population, corn borer-resistant QTLs for *Sesamia nonagrioides* were located in bins 4.08, 8.05, 10.04 (Ordas et al. 2009), which are regions associated with MW resistance in this work. Meihls et al. (2012) summarized an important number of insect–plant interactions reported previously, finding new coincidences in regions involved in resistance at different levels compared with this study. Clusters of QTLs and genes are important in delivering multiple insect resistances and should be used to stack multiple stress resistances into one variety (Bergvinson and García-Lara 2006).

Comparison of F_2 versus F_6

The three important genomic regions, where all resistant traits co-localize, were found for the MW. The three resistance parameters were located in bins 4.08, 10.04, and 10.07. Comparing QTLs from the RIL mapping population obtained in this work with previous F_2 reports on biophysical (García-Lara et al. 2009) and phytochemical (García-Lara et al. 2010) resistance parameters, the only common region found was localized in bin 10.07. This QTL for GWL overlapped in the same bin as QTLs of the three traits in this study. For phytochemical QTLs, several overlapping QTLs were found. A QTL for *trans*-ferulic acid content was reported in bin 3.01, where a QTL for GWL was found. In bin 1.09, a *p*-coumaric acid QTL was reported, which co-localizes with AP. An important case was present in bin 4.08, where we reported the existence of QTL regions for GWL, AP, and FP, as well as a QTL for a diferulic acid. Also, a total phenolic content QTL matched with a QTL for GWL in bin 3.01. Phenolic content is highly related to the grain hardness and represents a well-known defense mechanism (García-Lara and Bergvinson 2014). Secondary metabolites shared bin localizations in both studies: diferuloyl putrescine, localized in bin 10.07, with the GWL, AP, and FP analyzed, and the insoluble hydroxyproline-rich glycoprotein content of the pericarp cell wall, which was reported in bin 8.05, the same for GWL and AP.

This study identified QTLs that contribute to MW resistance that had not been discovered before so there is some unique new genetic information. If flanking markers are present in the QTL regions, then they can be used for introgressing the QTLs into susceptible backgrounds. The identification of QTLs by SSRs leads us to the recognition of three QTL genes related with MW resistance. Further studies using more dense molecular markers set such as single-nucleotide polymorphism will help corroborate the importance of these QTLs and form the basis of marker-based selection in high-throughput genotyping schemes.

Author contribution statement All authors contributed equally in this work and wrote and reviewed the latest version of this manuscript. FFC developed the bioassay analysis and QTL analysis. MaW developed the SSR maize platform to construct the maize map. DJB designed and developed the mapping population. SGL developed the genotypic and phenotypic data and constructed the SSR map.

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Conflict of interest The authors declare that they have no conflict of interest.

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