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Mapping of QTL affecting resistance against sorghum downy mildew (*Peronosclerospora sorghi*) in maize (*Zea mays* L)

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Abstract

Sorghum downy mildew (SDM) is one of the most destructive diseases of maize (*Zea mays* L) in South-East Asia. Understanding the genetic basis of downy mildew resistance (DMR) could increase the efficiency of breeding for disease resistant germplasm. The objectives of this study were to determine the number, genomic positions and genetic effects of quantitative trait loci (QTL) conferring resistance to SDM. The study included 251 $F_{2:3}$ families derived from a cross between the two inbreds, Nei9008 (Thailand) and CML289 (CIMMYT), resistant and susceptible, respectively. Individuals in the population were genotyped for simple sequence repeat (SSR) and phenotypic resistance data were evaluated as percentage disease incidence in replicated field trials at three environments by Triple Lattice design. Heritability across environments was 94.3%. Traits were analyzed within and across environment using composite interval mapping. Nine QTLs were identified for resistance to SDM, one QTL each on chromosome 2, 3, 4, and 6, three QTLs on chromosome 5, and two QTLs on chromosome 9. Just one QTL on chromosome bin 5.07 came from the susceptible parent, all others from the resistant parent, Nei9008. The QTLs in chromosome bins 2.09 at umc1736, 5.03 at bnlg1902, and 6.01 at bnlg1867 had major effects and were consistent over all environments. A common map shows intriguing collocations of SDM QTLs with those for other disease and insect resistance QTLs from literature. As several consistent QTLs for downy mildew resistance are available now, an avenue is open for pyramiding multiple genes by marker assisted selection (MAS) that may control different mechanisms for resistance.

Keywords: QTL mapping, sorghum downy mildew, resistance, maize, marker assisted selection

Introduction

Sorghum downy mildew (SDM), *Peronosclerospora sorghi* (Weston & Uppal) CG Shaw, family *Peronosporaceae*, order *Peronosporales*, is one of the most destructive diseases of maize (*Zea mays* L) in South-East Asia. The SDM infects its host soon after the seedling emergence until about one month after planting, causing yield losses of 50-100% for susceptible cultivars. It is recognized that the genetic basis of SDM resistance must be understood for efficient breeding programs. Some QTLs of varying sizes have been identified already for downy mildew resistance (DMR) in five previous studies that have been carried out in Asian, African and North American countries with germplasm diverging in origin and in its genetic state (Agrama et al, 1999; George et al, 2003; Nair et al, 2005; Sabry et al, 2006; Pumichai et al, 2012). Although the size of DMR QTLs varied, QTLs were four times identified on chromosome 2, three times on chromosomes 1 and 9, two times on chromosome 3 and 6, and just one time in a single study on chromosomes 7 and 10. Several DNA markers linked to DMR QTLs for downy mildew, but it has been agreed upon that their uses for MAS need confirmation by QTL validation and/or fine mapping (Langridge et al, 2001). From the five studies cited above it becomes clear that different QTLs for DMR are present among resistant germplasm that may control different mechanisms for resistance. When more consistent DMR QTLs become available, linked DNA markers will be applied in MAS. The objectives of this study were to determine the number, genomic positions and genetic effects of quantitative trait loci conferring resistance to SDM in the $F_{2:3}$ population from the cross, Nei9008 × CML289.

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Materials and Methods

Population development

A survey of SDM disease in maize inbreds from three independent maize breeding research programs of the National Corn and Sorghum Research Center (Suwan Farm), Kasetsart University; Nakhon Sawan Field Crops Research Center (Nakhon Sawan), Department of Agriculture, and International Maize and Wheat Improvement Center (CIMMYT) was carried out at Suwan Farm (field station of the Kasetsart University), Nakhon Sawan (field station of Department of Agriculture), and Pacific Seed Company (field station of Pacific Seed in Thailand). Two inbred lines were selected from this study; highly resistant Nei9008 from Nakhon Sawan, and very susceptible CML289 from CIMMYT. Its cross was made and F_1 plants were selfed at Suwan Farm. By selfing an F_1 ear, 251 $F_{2:3}$ families were developed and seeds of each $F_{2:3}$ family were then increased by plant to plant sib-mating for SDM evaluation.

Experimental design

All $F_{2:3}$ families were screened for SDM in three different climatic environments: 1) Suwan Farm Early Season, SWE; 2) Suwan Farm Late Rainy Season, SWL (14°24'42"N, 101°25'18"E, 360 masl elevation; 1,200 mm/year average rainfall); 3) Nakhon Sawan Rainy Season (NSR, 15°20'45"N, 100°29'4"E, 104 masl elevation; 1,200 mm/year rainfall). The field experiments were carried out during April-May and August-September at SWE and SWL; August-September at NSR. During the screening periods there were 255 mm of rainfall, with mean maximum temperature 33.1°C, mean minimum temperature 22.8°C at SWE; 319 mm of rainfall, with mean maximum temperature 30.2°C, mean minimum temperature 22.9°C at SWL: 302 mm of rainfall, with mean maximum 32.6°C, mean minimum temperature 24.5°C at NSR. The $F_{2:3}$ families were evaluated together with the parental lines, the F_1, and two checks in a 16 x 16 triple-lattice design with two-row plots at a within-row spacing of 0.20 m in 5m-long rows spaced 0.75 m apart. Each plot was planted with two seeds per hill with a total of 104 plants.

Phenotypic data evaluation

Sorghum downy mildew inoculation

The spreader-row technique was used for field inoculation of a susceptible variety (Tuxpeno-1 Sel Sequia C3) that had been planted every 16 rows and in the alley-way; its seedlings were sprayed at between one and two fully extended leaves with a spore suspension. For the spore suspension, infected leaves were collected, washed, and incubated in the dark for 8 h at 20-23°C to induce sporulation. Spores were then washed from the leaves and used for inoculation at night when air moisture was sufficient. Three weeks later, when spreader rows were showing systemic symptoms, the $F_{2:3}$ families were sown. Systemic symptoms of infected plants were accessed three weeks after sowing in the individual plants in each family, thereafter followed up by double checks one and two weeks later. Percentage disease was determined by the ratio of the total number of plants with systemic infection to the total number of plants multiplied by 100.

Phenotypic data analysis

The initial lattice analysis of variance, including parents, F_1 and checks, was done by the R program (R Core Team, 2012). Lattice-adjusted means were used as input for analyses within and across environments, excluding parents, F_1 and checks. Variance components of the $F_{2:3}$ families were estimated from linear functions of the mean squares. Broad-sense heritability (H_b) on an entry-mean basis was calculated by dividing the genotypic variance (σ^2_g) by the phenotypic variance (σ^2_p) (Hallauer and Miranda Fo, 1981). The distributions of the means of phenotypic traits were checked for normality as described by Shapiro and Wilk (1965) using R program. We used the actual data for QTL analysis.

Molecular marker genotype analysis

Genomic DNA was extracted from bulked-leaf tissue of 20 plants of $F_{2:3}$ to reconstitute the F_2 genotype by the modified method based on Rogers and Bendich (1982) and Saghai-Maroof et al (1984). To identify simple sequence repeat (SSR) markers linked to a major QTL, 760 SSR markers distributed throughout maize genome were selected from the Maize Data-

Table 1 - SDM means, standard errors, and coefficients of variation for 251 $F_{2:3}$ families from the cross of Nei9008 x CML289, parents and F_1 from individual environments and combined across environments. Values are presented as percentage disease incidence for sorghum downy mildew.

	Mean (%) ± SE[†]				
Environment	$F_{2:3}$ Families	Nei9008	CML289	F_1	CV‡
SWE[§]	65.0±1.32	18.3±2.48	98.2±1.81	67.9±2.75	15.7
SWL[¶]	62.5±1.34	6.8±2.22	99.0±1.01	86.6±5.25	13.1
NSR[#]	67.8±1.26	11.7±1.81	100.0±0.00	84.5±5.43	14.3
Combined Env	65.1±1.23	12.3±2.12	99.1±0.52	79.7±1.03	14.4

[†]SE: standard error of mean; [‡]CV was estimated from 256 entries in a 16 × 16 triple lattice designs; [§]SWE: Suwan Farm Early rainy season; [¶]SWL: Suwan Farm Late rainy season; [#]NSR: Nakhon Sawan Rainy season

Table 2 - Estimates of variance component and heritability based on entry means of Nei9008 x CML289 from individual environment and combined across environments (arcsine transformed value).

	Variance components†			Heritability
	σ^2_g	$\sigma^2_{g\,x\,e}$	σ^2_e	H_b‡
SWE	0.0820	-	0.0225	91.6
SWL	0.0816	-	0.0199	92.5
NSR	0.0799	-	0.0221	91.6
Combined Env	0.0748	0.0064	0.0215	94.3

†σ^2_g, $\sigma^2_{g\,x\,e}$, and σ^2_e are variance components for genotypes, genotypes × environments, and phenotypes, respectively; ‡H_b: broad-sense heritability.

base of University of Missouri at http:/www.agron.missouri.edu and http:/www.maizegdb.org for a survey of polymorphism between parent Nei9008 and CML289. The 195 SSR markers that showed polymorphism from parental screening were genotyped to the $F_{2:3}$ population. The polymerase chain reaction (PCR) consisted of 0.5 μM each SSR primer, 50-100 ng genomic DNA, and 7.5 μl GoTaq® Green Master Mix (Promega, that contained GoTaq® DNA polymerase, 1x Green GoTaq® Reaction buffer - pH 8.5, 1.5 mM $MgCl_2$, and 0.2 mM dNTP) in a final volume of 15 μl. The thermocycling program pre–denatured DNA at 94°C for 2 min, followed by 30 cycles: denaturation at 94°C for 30 sec, annealing at 50-65°C (depending on annealing temperature of each primer) for 1 min, extension at 72°C for 1 min. After the final cycle, the program was prolonged for a further 5 min at 72°C. Amplification products were resolved by electrophoresis on 4% (w/v) agarose gel (containing 2.5% Metaphor and 1.5% Seakem agarose gel) in 1x TBE buffer at 120-150 volt for 2-3 hrs. The genetic linkage map was constructed using MAPMAKER/EXP3.0 (Lander et al, 1987; Lincoln et al, 1992).

Quantitative trait locus analysis

Quantitative trait loci analyses for each individual environment and a combined one across all three environments were performed by composite interval mapping using Windows QTL Cartographer 2.5 (Basten et al, 2003). A QTL was considered significant when the LOD (log_{10} of the likelihood of odds ratio) value that derived from permutation analysis was large than 4. Additive and dominance effects for detected QTLs were estimated using the Zmapqtl procedure of QTL Cartographer. The R^2 value, the percentage of the phenotypic variance explained by marker genotype at the QTL, (coefficient of determination) was taken from the peak QTL position as estimated by QTL Cartographer. Gene action was determined by the ratio of the absolute value of the estimated dominance effect divided by the absolute value of the estimated additive effect |d| / |a| following Stuber et al (1987); (additive = 0 to 0.20; partial dominance = 0.21 to 0.80; dominance = 0.81 to 1.20; and overdominance > 1.20).

Results

Phenotypic data analysis

The means of the $F_{2:3}$ families in the individual environments ranged from 62.5 to 67.8%, the highest SDM pressure was obtained at NSR (Table 1). According to Shapiro and Wilk (1965) the distribution of phenotypic means, within and across three environments, deviated significantly from normal distribution with the majority of the lines skewing to susceptibility. No significant transgressive segregation was observed (Figure 1). Mean squares for genotypes and genotype x environment interactions were highly significant (data not shown). Broad-sense heritability estimates for DMR (Table 2) ranged from 91.6 to 92.5% from individual environments and 94.3% from across environment, indicating highly inherited genes for controlling downy mildew resistance.

SSR linkage map and QTL analysis

According to the molecular linkage map of 195 SSR markers (Figure 2), the percentage of polymorphism was 25.7. Nine QTLs intervals for resistance

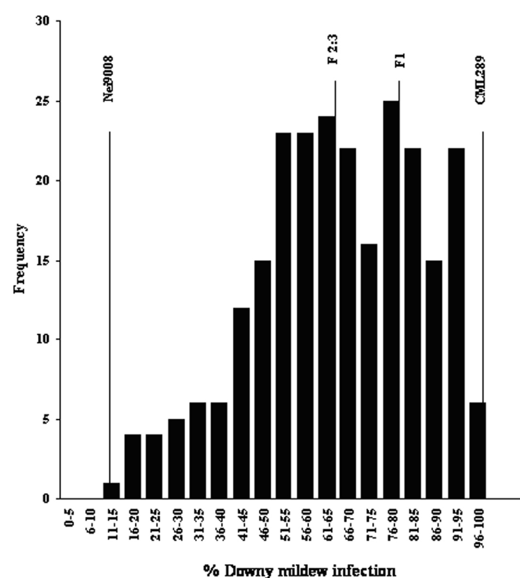

Figure 1 - Percentage of sorghum downy mildew infection frequency distribution of 251 $F_{2:3}$ families evaluated at 3 environments.

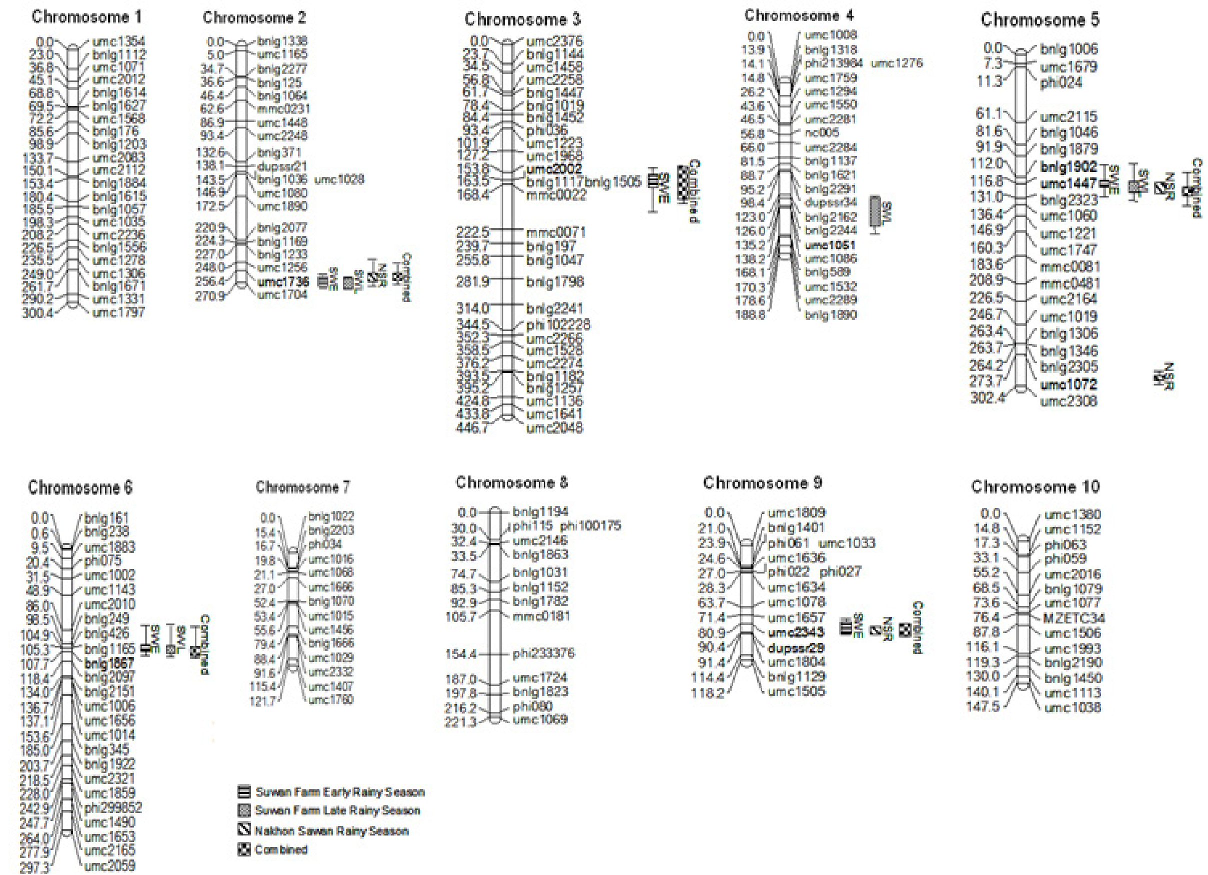

Figure 2 - Molecular linkage map of 195 SSR loci and location of QTLs in the 251 $F_{2:3}$ population from the cross, Nei9008 x CML289, for three individual environments and combined over environments. Numbers to the right of the chromosomes indicate the cumulative distance in cM. The boxplots along the chromosome indicate the QTLs region in which the LOD scored exceed 4.

to SDM were identified on chromosomes 2, 3, 4, 5, 6 and 9 (Table 3): data is provided for the five QTLs from the combined analysis across the environments and for the four QTLs that were just significant in a single environment. The following QTLs were confirmed in the combined analysis: bin 2.09 by SSR marker umc1736 a strong peak LOD score of 16.49, and was identified with an R^2 of 26.7%; bin 3.04 by SSR marker umc2002, peak LOD score of 5.39, R^2 value of 5.6%; bin 5.03 by SSR marker bnlg1902 a strong peak LOD score again of 14.70, with an R^2 value of 10.6%; at bin 6.01 by SSR marker bnlg1867, a peak LOD score of 7.23 with R^2 value of 8.4%; at bin 9.07 by SSR marker dupssr29, peak LOD score of 4.84 with an R^2 value of 0.02%.

Four further QTLs were confirmed just in a single environment: at bin 4.08 by SSR marker umc1051 in the environment SWL, a peak LOD score of 4.44 with an R^2 value of 3.1%; two others QTLs on chromosome 5 were in the environment SWL, at bin 5.03 by SSR marker umc1447, a peak LOD score of 12.46 with an R^2 value of 10.5%, and in the environment NSR, at bin 5.07 by SSR marker umc1072, a peak LOD score of 4.53 with an R^2 value of 10.6%; at bin 9.05 by SSR marker umc2343 in the environment, SWE, a peak LOD score of 4.87 with an R^2 value of 0.7%.

According to multiple regression analysis the phenotypic values accounted together for 46.5% of the total phenotypic variance.

In the combined analysis (Table 3), two QTLs at chromosome bins 3.04 and 6.01 showed additive gene action, two QTLs showed partial dominance at chromosome bins 2.09 and 5.03, and one QTL showed over dominance at chromosome bin 9.07.

Discussion

Kaneko and Aday (1980) suggested already that downy mildew resistance was governed by a polygenic system with a threshold nature i.e. the mode of inheritance of resistance to downy mildew changes from complete to partial dominance as the infection changes from slight to severe, indicating that possibly different genes and alleles can be expressed in different disease situations. Therefore standardized downy mildew expression is essential to achieve consistent uniform responses; here like in other studies a heavy disease pressure evaluates most accurately plant genotypes. The distribution of means of the $F_{2:3}$ families for DMR did not indicate any trans-

Table 3 - QTLs for sorghum downy mildew resistance for individual environments and combined over three environments for 251 $F_{2:3}$ families from the cross of Nei9008 x CML289.

chrom /bin†	Nearest markers‡	Position (cM)§	Max LOD¶	R^2 (%)#	Genetic effect&		Gene action¥		Donor DMR allele≈
					Additive	Dominance			
Suwan Farm Early Rainy Season									
2.09	umc1736	264.41	12.47	18.1	11.07	6.60	0.60	PD	Nei9008
3.04	umc2002	157.81	6.44	4.8	7.64	5.84	0.76	PD	Nei9008
5.03	bnlg1902	114.01	14.70	13.3	12.64	4.30	0.34	PD	Nei9008
6.01	bnlg1867	109.71	11.68	13.1	11.18	0.10	0.01	A	Nei9008
9.05	umc2343	80.91	4.87	0.7	4.85	11.06	2.28	OD	Nei9008
Suwan Farm Late Rainy Season									
2.09	umc1736	264.41	13.79	21.7	12.45	6.88	0.55	PD	Nei9008
4.08	umc1051	137.21	4.44	3.1	6.15	4.42	0.72	PD	Nei9008
5.03	umc1447	118.81	12.46	10.5	11.74	6.98	0.59	PD	Nei9008
6.01	bnlg1867	109.71	8.47	6.8	9.51	5.34	0.56	PD	Nei9008
Nakhon Sawan Rainy Season									
2.09	umc1736	260.41	13.70	24.1	11.51	8.67	0.75	PD	Nei9008
5.03	bnlg1902	114.01	12.90	8.9	11.49	9.51	0.83	D	Nei9008
5.07	umc1072	291.71	4.53	10.6	-8.10	8.13	1.00	D	CML289
9.07	dupssr29	90.41	4.07	0.01	2.75	14.92	5.42	OD	Nei9008
Combined									
2.09	umc1736	262.41	16.49	26.7	12.00	8.80	0.73	PD	Nei9008
3.04	umc2002	157.81	5.39	5.6	6.79	1.31	0.19	A	Nei9008
5.03	bnlg1902	114.01	14.70	10.6	11.52	7.48	0.65	PD	Nei9008
6.01	bnlg1867	109.71	7.23	8.4	8.30	0.37	0.04	A	Nei9008
9.07	dupssr29	90.41	4.84	0.02	2.70	14.97	5.53	OD	Nei9008

†Chromosome/ bin corresponded to UMC SSR map (University of Missouri - Columbia, USA); ‡Nearest markers: Position of QTL peak as indicated by SSR primer; §Position: Position of QTL peak as indicated by cumulative distance from the end of the short arm; ¶Max LOD: Likelihood of odds (LOD) scores = Likelihood ratios (LR)/4.6052. Critical thresholds of QTL were defined at LOD 4; #R^2: Percentage of the phenotypic variance explained by genotype at Max LOD peak; &Genetic effect, additive and dominance effect at QTL peak, +/- direction of additive value with reference to Nei9008; ¥Gene action was established by $|d|/|a|$: A (additive): 0 to 0.20, PD (partial dominance): 0.21 to 0.80, D (dominance): 0.81 to 1.20, and OD (over dominance) >1.20; ≈Donor DMR allele detected by +/- of additive with reference to Nei9008. Positive values showed resistance alleles came from Nei9008, negative values showed resistance alleles came from CML289.

gressive segregation; the resistant parent contained indeed almost all resistant alleles with major effects in the combined analysis. As the heritability estimate (94%) was high, the genetic basis for DMR should be strong and the environmental influence low in our germplasm. In other studies heritabilities ranged between 50 and 75% (Agrama et al, 1999; George et al, 2003; Nair et al, 2005). The high heritability estimate in the present study can be explained by the facts that DMR variation was largely influenced by major genetic effects and by an accurate uniform measurement of symptoms in all environments. High heritability estimates are corroborated by studies of Sabry et al (2006) and Phumichai et al (2012).

DMR QTLs have been identified now on nine of ten chromosomes, indicating a complex genetic situation (Figure 3). At or close to our location at bin 2.09, bins were detected between 2.04 and 2.09 (George et al, 2003; Nair et al, 2005; Sabry et al, 2006; Phumichai et al, 2012). This supports the suggestion from Sabry et al (2005), that a major region on chromosome 2 affects the response to downy mildew. A similarly important role may have chromosome 9 as we could add two more QTLs to already know ones.

A further QTL in our study at bins 3.04 matched with one reported by Nair et al (2005). Six of our QTLs in chromosome bins 4.08, 5.03, 5.07, 6.01, 9.05, and 9.07 were new QTLs, i.e. they have not been published before. On the other hand, twelve QTLs described in other studies were not found here (Agrama et al, 1999; George et al, 2003; Nair et al, 2005; Sabry et al, 2006; Phumichai et al, 2012). No explanations could be found from our results and those from literature how differences depended on the germplasm used; a cross-comparison of all available results indicated that partial dominance (PD) and additive gene action were most frequent for resistance to SDM. The major QTL of our study had a PD gene action. It could be confirmed that SDM resistance alleles in Nei9008 are a good source for a MAS supported maize breeding program.

QTLs for SDM from this study and other studies were compared to disease and insect resistance loci reported by McMullen and Simcox (1995) and Jampatong et al (2002) (Figure 3). Matches were identified with our and other SDM QTLs at bin 2.09 (European corn borer - Ecb) and bin 3.04 (Ecb, Fusarium stalk rot - Fsr, Common rust - Cr, Maize mosaic - Mm,

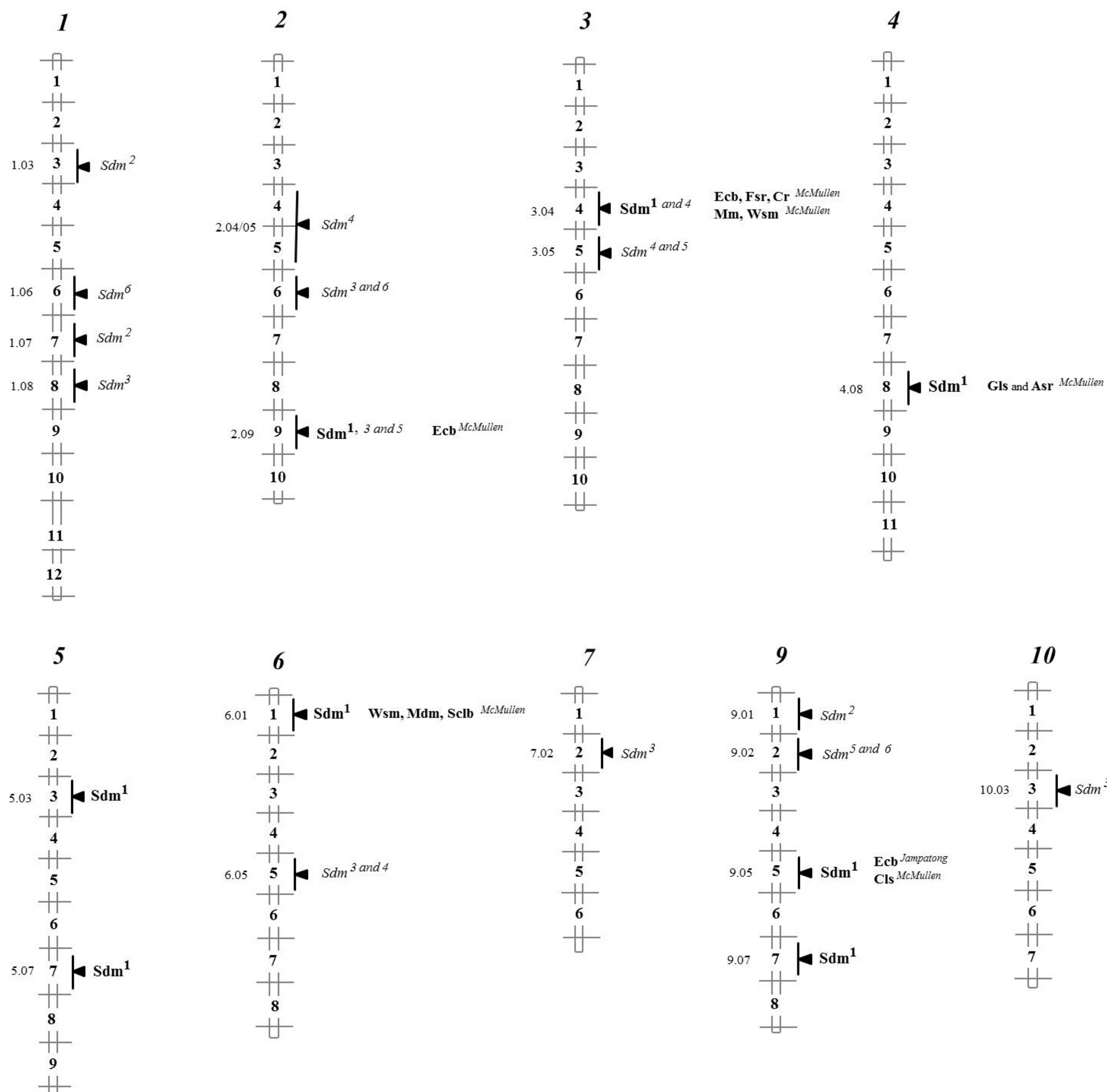

Figure 3 - SDM QTL locations and some matching QTLs for other disease and insect resistances derived from literatures. Sdm1, SDM from the present study; Sdm2,3,4,5,6, SDM from publications by Agrama et al (1999), George et al (2003), Nair et al (2005), Sabry et al (2006), and Phumichai et al (2012), respectively. Matching QTLs for other disease and insect resistances: European corn borer - Ecb; Fusarium stalk rot - Fsr; Common rust - Cr; Maize mosaic - Mm; Wheat streak mosaic - Wsm; Gray leaf spot - Gls; Antracnose stalk rot - Asr; Maize dwarf mosaic - Mdm; Southern corn leaf blight - Sclb; Carbonum leaf spot - Cls (McMullen and Simcox, 1995; Jampatong et al, 2002).

and Wheat streak mosaic - Wsm. Matches exclusively with our SDM QTLs were found at bins 4.08 (Gray leaf spot - Gls, Antracnose stalk rot - Asr); 6.01 (Wsm, Maize dwarf mosaic - Mdm, Southern corn leaf blight - Sclb); and 9.05 (Ecb, Carbonum leaf spot - Cls). These results support the concept that resistance genes for diseases and insects in maize are not randomly distributed over the genome, but located in clusters (Bohn et al, 2000).

One of the major goals of QTL mapping is to locate markers that can be broadly used for MAS in a breeding program. One major concern against using MAS has been the lack of consistency of QTLs across environments. Results from Stuber et al (1992) suggested rather little QTL × environment interaction. This was corroborated here for the QTLs at bin 2.09 and 5.03 that were detected in all three environments and the combined analysis, and partly for the QTL at bin 6.01 that was detected in two environments and the combined analysis. Schön et al (1994) reported that most likelihood peaks were identified in the same marker intervals for all environments and differed only

in the level of significance and the size of estimated genetic effects. Conclusively these three SSR markers associated with QTLs (bin 2.09 at umc1736, 5.03 at bnlg1902, and 6.01 at bnlg1867) are the favorites to be used for the transfer resistance alleles to susceptible lines. As several consistent QTLs for DMR are available, an avenue is open for pyramiding multiple genes by MAS that may control different mechanisms for resistance.

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