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ORIGINAL PAPER

Use of mutant-assisted gene identification and characterization (MAGIC) to identify novel genetic loci that modify the maize hypersensitive response

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Abstract The partially dominant, autoactive maize disease resistance gene *Rp1-D21* causes hypersensitive response (HR) lesions to form spontaneously on leaves and stems in the absence of pathogen recognition. The maize nested association mapping (NAM) population consists of 25 200-line subpopulations each derived from a cross between the maize line B73 and one of 25 diverse inbred lines. By crossing a line carrying the *Rp1-D21* gene with lines from three of these subpopulations and assessing the F_1 progeny, we were able to map several novel loci that modify the maize HR, using both single-population quantitative trait locus (QTL) and joint analysis of all three populations. Joint analysis detected QTL in greater number and with greater confidence and precision than did single population analysis. In particular, QTL were detected in bins 1.02, 4.04, 9.03, and 10.03. We have previously termed this technique, in which a mutant phenotype is used as a "reporter" for a trait of interest, Mutant-Assisted Gene Identification and Characterization (MAGIC).

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Introduction

Potentially useful, naturally occurring genetic variation is often difficult to identify, as the effects of individual genes may be subtle and difficult to observe. To address this problem, we have used a simple yet effective method named MAGIC (for Mutant-Assisted Gene Identification and Characterization), to discover and characterize naturally occurring variation present in plant germplasm (Chintamanani et al. 2010; Johal et al. 2008). MAGIC is a forward genetics screen that uses the readily scorable phenotype of a mutant gene affecting the trait of interest as

a reporter to discover and analyze relevant, interacting genes present naturally in diverse germplasm. Mutant lines are crossed into diverse germplasm and the mutant progeny are evaluated for transgressive changes (both suppressed and enhanced) in the phenotype(s). This concept is further expounded and explored elsewhere (Chintamanani et al. 2010; Johal et al. 2008).

The hypersensitive response (HR) in plants is a common and very important plant defense response. It is characterized by a rapid, localized cell death around the point of attempted pathogen penetration, usually leading to resistance. Genetically, HR is controlled by so-called "R" (resistance) genes, which have two functions, namely the recognition of specific pathogen-derived or pathogen-associated molecules or molecular events and the subsequent elicitation of a defense response, usually including the HR (Bent and Mackey 2007). R-genes generally encode proteins carrying both a nucleotide binding site (NBS) domain and a leucine-rich repeat (LRR) domain. *Rp1-D21* is an aberrant R gene at the complex *Rp1* locus on maize chromosome 10. It was formed by unequal crossing over between two R gene paralogs that resulted in a chimeric R gene with autoactivity (Collins et al. 1999; Smith et al. 2010). The Rp1-D21 protein is autoactive, the recognition function and the elicitation function are partially uncoupled, causing the spontaneous formation of HR lesions on the leaves and stalks of the plant in the absence of the pathogen. *Rp1-D21* exhibits its HR phenotype in a partially dominant and developmentally dependent manner (Hu et al. 1996; Smith et al. 2010). The strength of the HR phenotype of *Rp1-D21* is dependent on, among other things, genetic background (Chintamanani et al. 2010).

In our previous work, we used a maize line in which *Rp1-D21* was introgressed into the background of the H95 maize line (designated *Rp1-D21*-H95). Hybrids of *Rp1-D21*-H95 with the inbred B73 produce a suppressed HR response while hybrids between *Rp1-D21*-H95 and the inbred Mo17 had a more severe HR phenotype. *Rp1-D21*-H95 plants heterozygous for *Rp1-D21* were crossed to 233 lines from the maize IBM advanced intercross recombinant inbred line (RIL) mapping population (Lee et al. 2002; Sharopova et al. 2002). The F_1 families were assessed for several phenotypes associated with HR severity and QTL associated with variation in *Rp1-D21* severity were identified. A key QTL with strong effect, which we named *Hrml*1 (for HR modulating locus-1), mapped on chromosome 10, bin 10.03 (Chintamanani et al. 2010). Our data clearly suggested that *Hrml*1 did not overlap with the *Rp1-D21* locus which is also located on chromosome 10.

This initial proof of concept encouraged us to examine the genetic architecture controlling HR in maize in a more comprehensive manner. The maize nested association mapping (NAM) population is a 5,000-member RIL population consisting of 25 200-line subpopulations each derived from a cross between the commonly used maize line B73 and one of 25 other diverse inbred lines, referred to as NAM founder lines (McMullen et al. 2009). The large size, combined with the high-density genotypic information and sophisticated quantitative analysis techniques available mean that QTL can be identified in the NAM population with unprecedented accuracy and precision, often down to the single gene level (Kump et al. 2011; Tian et al. 2011). From crosses of *Rp1-D21*-H95 with NAM founder lines, it was evident that a great deal of diversity controlling the severity of the *Rp1-D21*-conferred HR response is present in these lines (Chintamanani et al. 2010). In the present study, we crossed *Rp1-D21*-H95 to three of the NAM sub-populations: the B97 × B73, Ki3 × B73, and Tx303 × B73 sub-populations (here referred to, respectively, as the B97, Ki3, and Tx303 subpopulations). The B97 genome suppresses the *Rp1-D21* phenotype relative to most other genomes while Tx303 and Ki3 backgrounds enhance the phenotype (Chintamanani et al. 2010). By assessing the resulting F_1 families, we identified several new QTL associated with variation in the hypersensitive response in addition to previously identified *Hrml1*. Significant novel QTLs were identified on chromosomes 1, 5, and 9 and a previously identified QTL on chromosome 10 was also detected. Since the three populations shared a common parent, joint as well as separate analyses of the populations were performed. Joint analysis resulted in more narrowly defined QTL positions with higher LOD (log of odds) likelihood scores.

Materials and methods

Plant materials

The *Rp1-D21*-H95 line was generated as described previously (Chintamanani et al. 2010). Briefly, the *Rp1-D21* variant was crossed to the maize inbred line H95, and subsequently backcrossed to the H95 parent four times, while selecting for the HR phenotype indicated by the spontaneous formation of cell death lesions. Since *Rp1-D21* homozygotes in the H95 background are often unable to set seed, this stock is maintained in heterozygous condition by repeatedly crossing it as a male to the H95 inbred.

The maize NAM population consists of 25 sub-populations comprised of 200 RILs each (McMullen et al. 2009; Yu et al. 2008). The three NAM sub-populations used in this study, B97 × B73, Ki3 × B73, and Tx303 × B73, are referred to here as the B97, Ki3, and Tx303 sub-populations. F_1 families were derived by crossing 2–3 plants of each RIL from these sub-populations to *Rp1-D21*-H95 mutant individuals. Crosses to 194, 126, and 188 RILs were successfully made for the B97, Ki3, and Tx303

sub-populations, respectively. As the *Rp1-D21*-H95 line is heterozygous for *Rp1-D21*, all F_1 families segregated 1:1 for wild type to mutant plants.

Genome scan

A genome scan of H95 and the *Rp1-D21*-H95 line was performed using Illumina's high-throughput MaizeSNP50 v1 BeadChip which interrogates 56,110 markers derived from the B73 reference genome. The 'Infinium HD Ultra' assay (Steemers et al. 2006) was executed on 200 ng each maize genomic DNA sample at a concentration of 50 ng/μl and hybridized to BeadChips. Sample intensities were detected on Illumina's iScan array scanning instrument and genotypes called with Illumina's GenomeStudio v2009.2 data analysis software. A total of 984 'Intensity Only' SNPs, 1,319 SNPs with a cluster separation <0.25, and 99 SNPs with an AB R Mean <0.25 were removed from the analysis.

Field trials

All F_1 families from the three populations were evaluated for HR severity (see below), mutant to wild type height ratio, and mutant to wild type stalk width ratio (SWR) in field experiments at the North Carolina State University Central Crops Research Station located in Clayton, NC in 2010 (CL10) and at the Purdue Agronomy Center for Research and Education (ACRE) in West Lafayette, IN in 2010 (IN10). Trials were planted in an augmented lattice design with a single replication at each location.

In Clayton ten seeds per plot were planted in each plot and rows were not thinned. One plot of inbred border was planted on all sides of the experiment. Overhead irrigation was applied as needed to ensure satisfactory plant growth. Standard fertilizer and herbicide regimes for central North Carolina were used. Plots were 2 m in length with a 0.6 m alley at the end of each plot. Inter-row spacing was 0.97 m. In West Lafayette, IN, 18 seeds per row were planted in 6 m rows spaced 0.76 m apart. No thinning was done and no irrigation was used.

Phenotypic data collection

Lesion severity (LES) At both locations lesion severity scores were assigned based on 1–10 scales, 1 = no lesion, and 10 = completely dead plant. All populations were scored six times at CL10 for LES at approximately weekly intervals. In IN10, the Ki3, Tx303, and B97, subpopulation F_1 crosses were scored three, four, and five times, respectively, at approximately weekly intervals. Scoring started at around the time of flowering.

We scored an aberrant defense response rather than disease in this case, but the phenotype observed is generally similar and we therefore used the widely accepted average standardized area under disease progress curve (sAUDPC) nomenclature. LES was calculated for each environment in the following way: the average value of two consecutive ratings was obtained and multiplied by the number of days between the ratings. Values were then summed over all intervals, and then divided by the total number of days of evaluation to determine the weighted average (Campbell and Madden 1990; McMullen et al. 2009; Shaner and Finney 1977).

Mutant to wild type height ratio (HTR) Plant height data were collected after flowering when the plant had reached its full height from three randomly selected mutant F_1 individuals and from three randomly selected wild type F_1 individuals within each F_1 family. Height means were calculated for each class within each family and the HTR was calculated by dividing the average mutant type height to the average wild type height.

Mutant to wild type SWR Stalk width immediately above the ear was measured from three randomly selected mutant F_1 individuals and from three randomly selected wild type F_1 individuals within each F_1 family. Then SWR was calculated by dividing the average mutant type stalk width to the wild type average stalk width.

Data analysis

PROC CORR in SAS version 9.2 (SAS Institute, 2002–2008) was used to estimate the Pearson correlation coefficients between the traits for each population at each environment and for combined population over environments. For LES, the sAUDPC value was used and for HTR and SWR simple means were used to calculate correlation coefficients.

QTL analysis

The LES, HTR, and SWR values were used for QTL mapping. Average values were calculated for each line across both environments for the average over-environment analyses. We mapped QTL for each trait (LES, HTR, SWR) in each environment (CL10, IN10) and for the over-environment average (defined as LES AV, HTR AV, SWR AV) using the NAM consensus genetic map developed using 1054 loci available at http://www.maizegdb.org (accessed December 20, 2011). QTL mapping was conducted using MCQTL4.0 (Jourjon et al. 2005). The genome-wide LOD threshold level for each trait at $\alpha = 0.01$ and $\alpha = 0.05$ were determined by permutation analysis (Churchill and Doerge 1994), 1,000 permutations in each case. The automated iterative QTL mapping (iQTLm) procedure (Charcosset et al. 2000) was used to detect QTL for each trait using the 5 cM walking speed option.

A two-LOD support interval, corresponding to a conservative 95% confidence interval in a RIL population, were used to limit the region around each QTL (Ooijen 1992). Significant QTL were declared when LOD scores exceeded the genome-wide $\alpha = 0.05$ threshold level. For each trait global R^2, individual R^2 and allelic effects at each QTL were estimated.

Quantitative trait locus for all the three traits were mapped in three ways: (1) for each of the three RIL populations separately, QTL were identified in each environment separately and using over-environment averages; (2) joint population analysis using all three populations simultaneously with the connected additive model of MCQTL at each of the environments and over both environments. The Connected Additive Model assumes that the founder parental allele effects are consistent across populations, so that four allele effects were estimated (with three degrees of freedom) at each QTL (Blanc et al. 2006); (3) joint population analysis using all three populations simultaneously with the Disconnected Additive Model of MCQTL at each of the environments and over both environments. The Disconnected Additive Model analyzes the three populations jointly in a single analysis but allows founder allele effects to vary across populations, so six allele-by-population effects are estimated for each QTL. The final connected and disconnected additive models for each trait were compared using the modified Schwarz's Bayesian information criterion (BIC) calculated using the following formula (Bogdan et al. 2004): $\mathrm{BIC} = n\log\left(\frac{\mathrm{RSS}}{n}\right) + k\log n$, where n is the sample size, RSS is residual sum of squares, and k is the number of parameters (Bogdan et al. 2004). The better model has the smaller BIC value.

Results

Genome scan of the *Rp1-D21*-H95 line

We used the illumina MaizeSNP50 v1 BeadChip to interrogate 56,110 markers, comparing the original H95 line with and the *Rp1-D21*-H95 line (see "Materials and methods") to characterize the extent of our introgression. Our analysis identified eight regions where H95 and *Rp1-D21*-H95 differ (Table 1). Altogether, the original *Rp1-D21* genome comprised about 6% of the genome in the introgression line (121.5 Mb of the total 2,045 Mb interrogated). The introgressions included the region around *Rp1-D21* on chromosome 10 but not the region around *Hrml*1 on the same chromosome (Chintamanani et al. 2010). As expected, all the introgression regions were heterozygous.

Field trials

Three different traits were measured in field trials in North Carolina and Indiana: Lesion severity (LES), mutant:wild type height ratio (HTR) and mutant:wild type SWR (Fig. 1). All of these traits measure aspects of the strength of the *Rp1-D21*-conferred phenotype in a particular F_1 cross. HTR and SWR also have the advantage of comparing the mutant to the wild type phenotype within an F_1 family, controlling for variation in the levels of heterosis between crosses. The SWR trait was distributed in an approximately normally fashion, while for both LES and HTR the distributions were markedly skewed towards lower levels of *Rp1-D21* phenotype expression (lower scores for LES and higher ratios for HTR, see Fig. 1).

Pearson correlation coefficients between the three measured traits within each environment and between environments were generally high with all the overall (i.e. across sub-population) values being between 0.47 and 0.85 and all being highly significant ($p < 0.01$,Table 2). Correlations between the LES phenotype and the ratio phenotypes were negative because a higher number indicates a more extreme phenotype for LES while for the other two phenotypes a lower number indicates a more extreme phenotype. Overall Pearson correlation coefficients for the three phenotypes between populations were 0.67, 0.70, and 0.54 for LES, HTR, and STW, respectively. This reflects a general trend that correlations involving STW were usually

Table 1 Introgressions present in the *Rp1-D21*-H95 line relative to H95

Chromosome	Introgression start (bp)	Bin	Introgression end (bp)	Bin	Introgression size (bp)
2	20,690,005	2.03	21,516,461	2.03	826,456
3	23,379,781	3.04	70,222,548	3.04	46,842,767
3	87,801,539	3.04	91,749,060	3.04	3,947,521
3	106,401,218	3.04	143,778,703	3.05	37,377,485
5	164,235,540	5.04	177,458,131	5.05	13,222,591
7	168,557,020	7.06	170,723,217	7.06	2,166,197
8	155,907,955	8.06	164,053,560	8.06	8,145,605
10	0	10.00	9,023,706	10.02	9,023,706

Introgression start and end points are given in base pairs (bp) according to the B73 Refgen v1 positions (see http://www.maizegdb.org; Andorf et al. 2010)

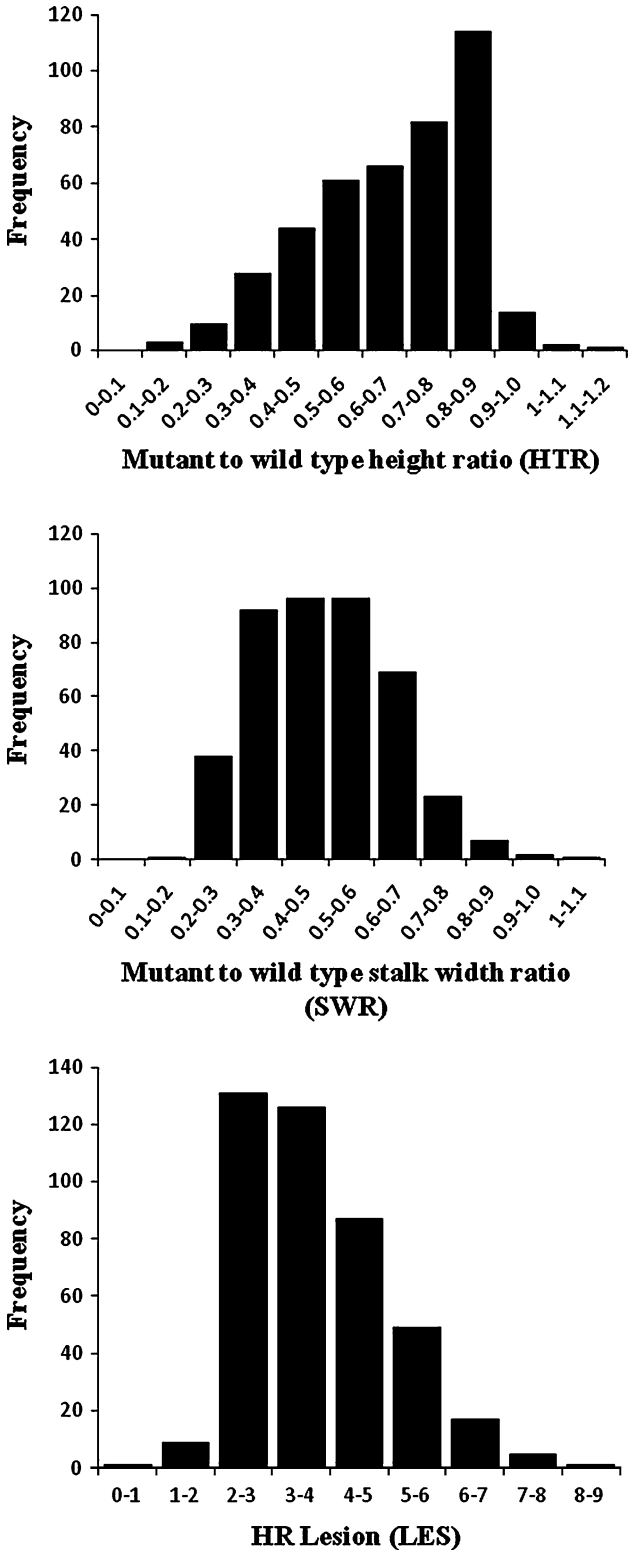

Fig. 1 Phenotypic distribution of lesion severity ratings (LES), mutant: wild type height ratio (HTR) and mutant:wild type stalk width ratio (STW) traits associated with the lesion phenotype conferred by the *Rp1-D21* gene over a population of RILs from the B97 × B73, Ki3 × B73 and Tx303 × B73 NAM sub-populations crossed to *Rp1-D21*-H95. Traits were scored in Clayton, NC and in West Lafayette, Indiana in the summer of 2010 Data from over-environment averages are shown

slightly lower than corresponding correlation values for the other traits. Comparing the three subpopulations, the Pearson correlation coefficients between traits and environments for the B97 subpopulation were somewhat lower than for the other sub-populations. This is likely due to the fact that the variation observed in the B97 subpopulation was generally lower than in the other two populations (Table 3).

QTL analysis

Table 4 shows the results of separate QTL analyses of the three individual populations within each environment and over-environments over all the three traits measured. With the exception of SWR CL10 for the Ki3 population, at least one significant QTL was detected for each trait for each environment and for each population with a maximum of four QTL detected for LES AV for the B97 population. The effects for most QTL were modest. Over all the individual population analyses, the maximum QTL effect for LES was 0.304 on the 1–10 scale employed and maximum QTL effects for HTR and SWR were 0.041 and 0.029, respectively, on an expected scale of 0–1.

As would be expected given the significant correlations between traits (Table 2), in many cases QTL for the different traits colocalized within a population. Assuming that QTL more than 10 cM distant from each other are the different QTL, most detected QTL were unique to a specific population. Only the QTL in bin 1.02 for LES and HTR and the QTL for all three traits in bin 10.03/4 were shared between the Ki3 and the Tx303 populations and between the B97 and Tx303 populations, respectively. In most cases, more QTL were detected in the Indiana compared to the North Carolina trial.

We also performed both connected and disconnected joint QTL analyses of the three populations simultaneously. We found that the number, positions, and confidence intervals of the QTL detected by both types of joint analysis did not differ significantly (Table 5 and Table S1). However, the BIC of the combined analysis was superior (i.e. had a lower value) for the connected model for almost all traits compared to the disconnected model (Table S2). For this reason, we will concentrate here on the results of the connected analysis.

For all three traits, more QTL were detected in IN10 than CL10 but every QTL detected in CL10 was also detected in IN10 with the exception of the LES CL10 QTL in bin 10.04. In the over-environment analyses, the only QTL detected for all three traits was in bin 10.03. Every other QTL indentified in the over-environment analyses was identified for two of the three traits with the exception of the LES AV QTL in bin 9.04 and the HTR AV QTL in bin 10.01.

Table 2 Pearson correlation coefficients for the three traits (lesion severity LES, height ratio HTR, stalk width ratio SWR) studied in each environment (Indiana 2010 IN10; Clayton 2010 CL10)

Trait	LES IN10	HTR CL10	HTR IN10	SWR CL10	SWR IN10
B97 × B73 pop					
LESCL10	0.31	−0.20	−0.40	−0.41	−0.10^{NS}
LESIN10		−0.25	−0.70	−0.39	−0.47
HTRCL10			0.33	0.43	0.22
HTRIN10				0.47	0.41
SWRCL10					0.32
Ki3 × B73 pop					
LESCL10	0.51	−0.76	−0.56	−0.54	−0.43
LESIN10		−0.39	−0.87	−0.12^{NS}	−0.63
HTRCL10			0.49	0.54	0.39
HTRIN10				0.23^{NS}	0.64
SWRCL10					0.35
Tx303 × B73 pop					
LESCL10	0.60	−0.58	−0.52	−0.56	−0.51
LESIN10		−0.52	−0.84	−0.42	−0.72
HTRCL10			0.58	0.49	0.49
HTRIN10				0.52	0.85
SWRCL10					0.54
Overall					
LESCL10	0.67	−0.72	−0.73	−0.61	−0.59
LESIN10		−0.60	−0.85	−0.47	−0.72
HTRCL10			0.70	0.59	0.58
HTRIN10				0.57	0.79
SWRCL10					0.54

All correlations were significant at $p < 0.01$ except where noted (NS)

Table 3 Coefficients of variation (CV) for the three traits (lesion severity LES; height ratio HTR; stalk width ratio SWR) studied in each environment (Indiana 2010 IN10; Clayton 2010 CL10)

	B97 × B73 population			Ki3 × B73 population			Tx303 × B73 population		
	LES	HTR	SWR	LES	HTR	SWR	LES	HTR	SWR
IN10	0.23	0.12	0.61	0.30	0.34	0.38	0.21	0.33	0.32
CL10	0.25	0.12	0.24	0.34	0.23	0.42	0.32	0.31	0.27
Overall	0.28	0.13	0.44	0.33	0.32	0.46	0.27	0.37	0.34

Each of the four parents (B73, B97, Ki3, and Tx303) provided alleles that both enhanced and suppressed the *Rp1-D21* phenotype, though as expected, alleles from B73 and B97 generally suppressed the phenotype and alleles from Ki3 and Tx303 generally enhanced it. The peak LODs of the QTL were generally higher and the positions were estimated more precisely in the combined connected analysis compared to the individual population analyses (Tables 4, 5; Fig. 2).

Discussion

Our previous study (Chintamanani et al. 2010) established the utility of the MAGIC technique (Johal et al. 2008) in conjunction with the *Rp1-D21* HR lesion phenotype for the identification of loci associated with the maize hypersensitive response (termed *Hrm* loci, for hypersensitve response-modulating loci). In this paper, we extend this concept and show that MAGIC can be used successfully in conjunction with the powerful maize NAM population to identify further *Hrm* loci and to achieve a more complete understanding of the genetic architecture controlling the maize defense response.

In the present study, we have used a small portion of the NAM population (three of 25 sub-populations) in our MAGIC approach. These populations were selected based on the seed available at the time and the fact that the parents displayed diverse phenotype when expressing *Rp1-D21*: B73 and B97 suppressed the *Rp1-D21* phenotype

Table 4 Chromosomal locations and parameters associated with the quantitative trait loci (QTL) for lesion severity ratings (LES), mutant:wild type height ratio (HTR) and mutant:wild type stalk width ratio (STW) traits associated with the lesion phenotype conferred by the *Rp1-D21* gene in population of RILs from the B97 × B73, Ki3 × B73 and Tx303 NAM sub-populations crossed to *Rp1-D21*-H95. Traits were scored in Clayton, NC and in West Lafayette, Indiana in the summer of 2010 (CL10 and IN10, respectively). QTL for individual environments as well as overall ratings averaged over environments (AV) are shown

Trait	BIN[a]	Position[b]	Nearest marker[c]	2-LOD[d]	Peak LOD[e]	R^{2f}	Additive effects[g]	
							B73	B97
B97 × B73 population								
LES CL10	5.01	13.3	phm13122	0–153	2.3	5	−0.07	0.07
	9.02	30.4	cl27880_1	20–94	3.4	8	0.082	−0.082
	10.04	47.1	cl32758_1	31–93	4	9	−0.086	0.086
HTR CL10	9.06	69.4	fad7	19–75	2.8	7	−0.013	0.013
SWR CL10	9.02	34.5	pco067521	30–71	3.1	6	−0.02	0.02
	10.04	41.1	cl37957_1	24–84	4.5	9	−0.02	0.02
LES IN10	5.00	7.2	gpm111	0–31	5.4	12	−0.13	0.13
	9.03	49.5	pzb01899	28–60	7.6	16	0.15	−0.15
	10.04	48.8	pza03196	0–88	2	5	−0.076	0.076
HTR IN10	9.03	45.9	pzb00014	25–55	5.4	12	−0.02	0.02
	10.04	48.8	pza03196	30–86	3	6	0.01	−0.01
SWR IN10	9.03	49.5	pzb01899	24–82	3.5	8	−0.02	0.02
LES AV	3.05	67.2	pza01934	64–79	5.8	13	−0.08	0.08
	5.00	7.2	gpm111	0–31	5.2	12	−0.08	0.08
	9.03	47.2	haf101b	28–57	9.8	20	0.12	−0.12
	10.04	48.8	pza03196	32–58	3.9	9	−0.07	0.07
HT RAV	9.03	41.8	pzb01110	27–69	4.3	10	−0.01	0.01
	10.04	48.8	pza03196	29–86	3	6	0.009	−0.009
SW RAV	5.00	7.2	gpm111	2–23	5	11	0.019	−0.019
	9.03	51.9	hscf1	12–107	3	7	−0.014	0.014
	10.03	33.3	pza02961	31–54	5	12	0.013	−0.013
Ki3 × B73 population								
LES CL10	1.02	37.8	pco148373a	28–64	3.7	18	−0.24	0.24
	10.07	85.3	pza02167	27–99	3.7	17	−0.23	0.23
HTR CL10	1.02	37.8	pco148373a	25–47	4	21	0.04	−0.04
SWR CL10	No QTL detected							
LES IN10	1.02	37.8	pco148373a	25–47	3.7	18	−0.28	0.28
HTR IN10	1.02	37.8	pco148373a	5–167	2.7	14	0.031	−0.031
	2.02	28.2	cl44168_1	5–40	3.4	19	−0.041	0.041
SWR IN10	9.02	26.8	pco101905	19–30	4	21	−0.029	0.029
	10.07	91.2	cl13024_1	93–101	3	18	−0.026	0.026
LES AV	1.02	37.8	pco148373a	27–47	4	20	−0.258	0.258
HTR AV	1.02	37.8	pco148373a	25–46	3.9	20	0.035	−0.035
SWR AV	10.07	91.2	cl13024_1	93–101	3	15	0.030	−0.030
Tx303 × B73 population								
LES CL10	1.02	29.9	pco082944	16–47	5.7	14	−0.304	0.304
HTR CL10	5.04	75.6	pco133463	66–78	4.4	11	0.032	−0.032
SWR CL10	1.09	144.4	phm16605	3–200	2.4	6	0.017	−0.017
LES IN10	1.02	34.3	pco094430	22–80	3.8	8	−0.129	0.129
	5.04	71.3	nfd108	64–77	5	13	−0.167	0.167
	6.05	54.7	pza02478	5–60	3	7	−0.124	0.124

Table 4 continued

Trait	BIN[a]	Position[b]	Nearest marker[c]	2-LOD[d]	Peak LOD[e]	R^{2f}	Additive effects[g]	
							B73	B97
HTR IN10	1.01	11.5	mlo1	7–46	4.4	11	0.02	−0.02
	5.04	71.3	nfd108	69–76	8.2	19	0.028	−0.028
	10.03	40.8	pco130396	26–50	4.5	12	0.021	−0.021
SWR IN10	1.01	11.5	mlo1	6.5–59	3.5	8	0.014	−0.014
	5.04	71.3	nfd108	30–81	3.4	8	0.014	−0.014
LES AV	1.02	34.5	pco094430	24–49	4.6	14	−0.195	0.195
	5.04	72.5	pza00067	48–86	3.5	7	−0.138	0.138
	6.05	55.7	cl39957_1	44–69	3.6	7	−0.139	0.139
HTR AV	1.02	29.9	pco082944	21–50	3.5	8	0.02	−0.02
	5.04	75.6	pco133463	68–77	7.2	17	0.029	−0.029
	10.03	40.8	pco130396	27–50	4.1	10	0.021	−0.021
SWR AV	1.01	11.5	mlo1	6–44	3.4	9	0.014	−0.014
	5.04	71.3	nfd108	30–86	3	8	0.013	−0.013
	10.03	40.8	pco130396	26–74	3.3	8	0.013	−0.014

[a] Chromosome bin location of QTL peak on one of the ten chromosomes of the maize genome. Bins divide the genetic map into 100 approximately equal segments. The segments are designated with the chromosome number followed by a two digit decimal (e.g. 1.00, 1.01, 1.02 and so on) see Davis et al. (1999)

[b] Position of peak LOD value on composite NAM map available at http://www.maizegdb.org (accessed on December 20, 2011)

[c] Marker closest to the position of the peak LOD value on composite NAM map

[d] The positions that define the two LOD interval around the position of peak likelihood for the QTL

[e] The log of odds (LOD) value at the position of peak likelihood of the QTL

[f] R^2 estimates the proportion of RIL mean variance (%) explained by the detected QTL

[g] The additive effect of the QTL in terms of the scale employed for each trait. In the case of LES this is the 1–10 scale while in the case of SWR and HTR this is in terms of a ratio which is expected to vary between 0 and 1. A positive number for LES and a negative number for HTR and SWR indicates that the allele enhanced the Rp1-D21 phenotype

while Ki3 and Tx303 enhanced it (Chintamanani et al. 2010). Previously, using the IBM advanced intercross line mapping population (Lee et al. 2002; Sharopova et al. 2002) which was derived from a cross between the maize lines Mo17 and B73, the common parent of the populations used in this study, we had identified a strong *Hrm* locus, we called *Hrml*1, in bin 10.03. We identified this locus again in this study in the B97 and Tx303 subpopulations as well as in the overall combined analyses for all three traits. Furthermore, we have identified several new *Hrm* loci, notably in bins 1.02 and 5.04, in the combined analysis and additionally in bin 9.03 in the individual analyses of the B97 subpopulation.

We measured three different traits that were associated with the severity of the *Rp1-D21* phenotype: lesion severity (LES) assessed on a 1–10 scale, the ratio of the average height of mutant segregants within an F_1 family to the average height of wild type segregants (HTR) and the ratio of the average stalk width of mutant segregants within an F_1 family to the average stalk width of wild type segregants (SWR). HTR and SWR have the advantage of being entirely objective measures while LES is a somewhat subjective measure and therefore somewhat dependent on the individual assessor. This might suggest that LES would be a less reliable score than HTR or SWR. However the Pearson correlation coefficient between environments for LES was 0.67 while for HTR it was barely different at 0.7, and for SWR it was 0.54 (Table 2). For the combined analysis (Table 5), fewer QTL were detected for SWR than for the other two traits and the QTL that were detected for SWR had relatively low peak LODs compared to QTL detected for the other traits. Furthermore, the global R^2 values (i.e. the percentage of the variation explained by the QTL models) for the over-environment combined joint analyses for LES, HTR and SWR are, respectively, 30, 49, and 15. Since QTL detected for SWR were generally also detected for one of both of the other traits, it appears that the genetic architecture controlling the *Rp1-D21* response might be effectively described using just LES and HTR.

An advantage of LES over the other traits is that it can be scored several times over the season whereas HTR and SWR are scored only once at the end of the season. This means that LES could be used to identify developmental

Table 5 Chromosomal locations and parameters associated with the quantitative trait loci (QTL) for lesion severity ratings (LES), mutant:wild type height ratio (HTR) and mutant:wild type stalk width ratio (STW) traits associated with the lesion phenotype conferred by the *Rp1-D21* gene in a population of RILs from the B97 × B73, Ki3 × B73 and Tx303 × B73 NAM sub-populations crossed to *Rp1-D21*-H95. Traits were scored in Clayton, NC and in West Lafayette, Indiana in the summer of 2010 (CL10 and IN10, respectively). QTL for individual environments as well as overall ratings averaged over environments (AV) are shown. QTL were calculated using joint analysis of all the populations simultaneously using a connected additive model

Trait	BIN[a]	Position[b]	Nearest marker[c]	2-LOD[d]	Peak LOD[e]	R^{2f}	Additive effects[g]			
							B73	KI3	Tx303	B97
LES CL10	1.02	31.7	adss1	26–46	11.2	11	−0.271	0.254	0.258	−0.241
	5.04	81.7	pco136495a	30–87	4.5	5	−0.066	−0.242	0.264	0.044
	10.04	41.1	cl37957_1	27–85	5	5	−0.187	0.114	0.081	−0.008
HTR CL10	1.02	33.1	lox9	26–45	7.4	7	0.030	−0.05	−0.010	0.029
	5.04	75.6	pco133463	69–77	7.5	8	0.021	0.021	−0.040	−0.002
	10.03	40.8	pco130396	33–42	7.1	7	0.031	−0.023	−0.002	0.008
SWR CL10	10.03	40.6	pco072368	38–42	8.6	9	0.039	−0.034	0.006	−0.010
LES IN10	1.02	37.8	pco148373a	27–49	5.7	6	−0.015	0.232	0.110	−0.192
	5.04	71.3	nfd108	68–76	6.4	7	−0.144	0.005	0.216	−0.077
	6.05	45.7	AY105479	11–55	5	5	−0.178	0.266	−0.000	−0.087
	9.02	28.5	cl4939_2	27–31	7	7	0.179	−0.379	0.186	0.015
	9.03	47	pza02545	44–52	6.5	7	−0.077	0.423	−0.043	−0.303
HTR IN10	1.02	37.8	pco148373a	11–46	8.8	9	0.026	−0.038	−0.009	0.021
	2.02	29.8	phm5822	22–42	5.2	6	−0.022	0.045	−0.008	−0.015
	5.04	71.3	nfd108	69–75	8.3	9	0.017	0.006	−0.035	0.013
	9.02	28.5	cl4939_2	24–34	6.3	7	−0.023	0.044	−0.021	−0.002
	9.03	47	pza02545	46–52	6.4	7	0.015	−0.042	−0.009	0.036
	10.03	40.1	fie2	32–52	6.6	7	0.020	−0.004	−0.019	0.003
SWR IN10	1.01	13.4	phm6238	4–167	3.7	4	0.019	−0.001	−0.010	−0.000
	9.02	26.8	pco101905	12–70	4.5	5	−0.016	0.031	−0.025	0.010
	10.03	33.8	pco087321	27–-92	4.2	5	0.019	−0.010	0.000	−0.010
LES AV	1.02	37.8	pco148373a	30–45	12.6	12	−0.216	0.286	0.159	−0.228
	5.04	72.5	pza00067	64–82	6	6	−0.128	−0.013	0.190	−0.050
	9.04	53.1	pzb01358	24–59	4.3	5	−0.020	0.146	0.111	−0.237
	10.03	40.1	fie2	31–51	5.7	6	−0.166	0.174	0.018	−0.027
HTR AV	1.02	31.7	adss1	26–43	11.5	12	0.030	−0.038	−0.012	0.021
	5.04	75.6	pco133463	69–76	10.4	11	0.020	0.012	−0.033	0.001
	9.01	20.7	AY107496	16–30	6	6	−0.010	0.035	−0.033	0.008
	10.01	5.9	cl24029_1	2–8	5.8	6	−0.018	0.013	0.019	−0.014
	10.03	40.6	pco072368	32–41	14.7	14	0.033	−0.027	−0.016	0.011
SWR AV	9.02	26.8	pco101905	19–69	5.6	6	−0.015	0.023	−0.026	0.018
	10.03	40.6	pco072368	37–42	8.7	9	0.028	−0.027	0.001	−0.002

[a] Chromosome bin location of QTL peak on one of the ten chromosomes of the maize genome. Bins divide the genetic map into 100 approximately equal segments. The segments are designated with the chromosome number followed by a two digit decimal (e.g. 1.00, 1.01, 1.02 and so on); see Davis et al. (1999)

[b] Position of peak LOD value on composite NAM map available at http://www.maizegdb.org (accessed December 20, 2011)

[c] Marker closest to the position of the peak LOD value on composite NAM map

[d] The positions that define the two LOD interval around the position of peak likelihood for the QTL

[e] The log of odds (LOD) value at the position of peak likelihood of the QTL

[f] R^2 estimates the proportion of RIL mean variance (%) explained by the detected QTL

[g] The additive effect of the QTL in terms of the scale employed for each trait. In the case of LES this is the 1–10 scale while in the case of SWR and HTR this is in terms of a ratio which is expected to vary between 0 and 1. A positive number for LES and a negative number for HTR and SWR indicates that the allele enhanced the *Rp1-D21* phenotype

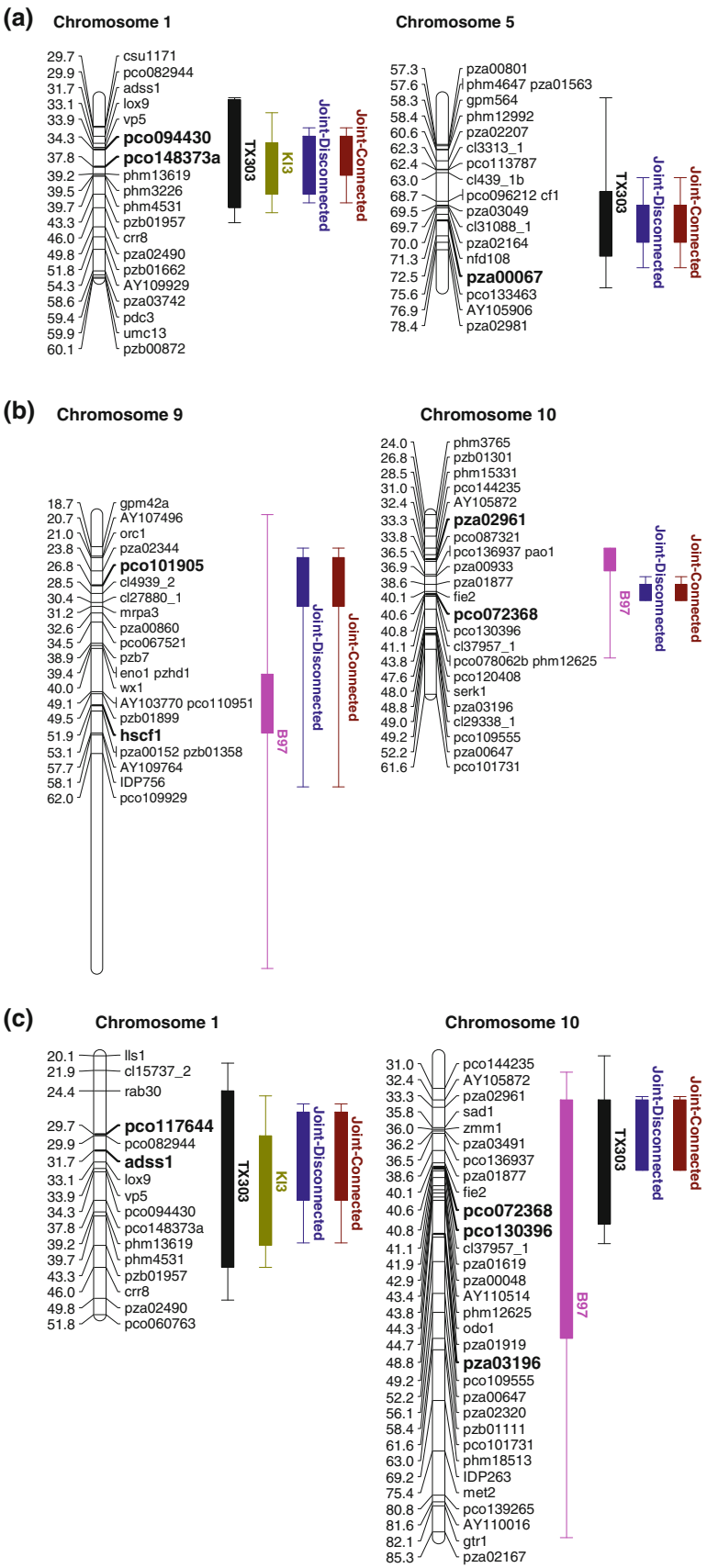

Fig. 2 Comparison of support intervals associated with QTL controlling, **a** hypersensitive response lesions (LES), **b** mutant:wild type stalk width ratio (STW) and **c** mutant:wild type height ratio (HTR) on indicated chromosomes identified by individual population analysis, additive connected joint population analysis and additive disconnected joint population analysis. The lines show 2-LOD support intervals and the boxes show 1-LOD support interval. In each case, additive connected joint population analysis narrowed the region that contains the QTL. Markers significantly associated with QTL are indicated in *bold font*

stage-dependent phenotypes. A recent study on the subjective scoring of northern leaf blight resistance by 22 different raters showed that almost identical sets of QTL are identified regardless of which rater's scores were analyzed (Poland and Nelson 2011). Much of the robustness of QTL analysis using subjective scores is likely derived from the hidden replication inherent in QTL analysis in which each marker allele is represented many times within a mapping population.

In this work, we were measuring the response in F_1 hybrids and as such we have to consider the possible effects of heterosis. Using an *Rp1-D21* mutant in heterozygous condition is beneficial in this respect, in that it allows HTR to be used as a highly sensitive and easy-to-measure parameter of the HR response that, to some extent at least, mitigates against any bias that might arise as a result of different levels of heterotic vigor in different crosses. The LES trait cannot account for heterosis in the same way.

It should also be noted that we were assessing the effect of these genes in F_1 hybrids between NAM lines and the *Rp1-D21*-H95 line rather than in inbred lines—the more conventional situation in QTL analysis. Therefore, for a modifying locus to be detected, one of the parental alleles from the original NAM sub-population has to be dominant or partially dominant with respect to the allele in the *Rp1-D21*-H95 line, i.e. if both NAM parental alleles are completely recessive to the H95 allele, a QTL cannot be detected. Recessive modifiers could in theory be identified by generating additional segregating populations (see also Chintamanani et al. 2010). We are currently generating and analyzing some of these types of populations. We should also note that the fact that we are using F_1 crosses means that we need to be clear about the meaning of the calculated effect value for each QTL. In a conventional QTL study in which RILs are used, the effect value would mean " the average effect of replacing one parental allele with the other parental allele". In this case, however, it means "the effect of replacing one parental allele in a heterozygous state with the H95 allele with the other allele in a heterozygous state with the H95 allele". The effect of the H95 allele itself cannot be calculated, of course, as it is present in every F_1 cross and is therefore not segregating in the population.

We have determined that the *Rp1-D21* phenotype is very sensitive to temperature (Negeri et al. unpublished), with higher temperatures suppressing the phenotype. With this in mind, it should be noted that, during the period in which data for this study were collected, the average temperature in West Lafayette, IN was 2.1°C cooler than in Clayton, NC. This may account for the fact that fewer QTL were detected in NC as the phenotypes were relatively suppressed by the higher temperature. This is illustrated by the fact that the HR and SWR averages for each population were lower in IN10 than CL10 in every case.

For many traits in a variety of species, lines with transgressive phenotypes carry a combination of both enhancing and suppressive modifiers. A good example of this are the large fruit size alleles derived from the small-fruited relative of tomato *Lycopersiocn pimpinellifolium* (Tanksley et al. 1996). In our study, the B97 genetic background substantially suppresses *Rp1-D21* phenotype in a heterozygous condition relative to almost all the other NAM founders while the Tx303 and Ki3 backgrounds enhance the phenotype (Chintamanani et al. 2010). Consistent with these observations, 9 of the 11 QTL effects detected for B97 alleles in the joint combined over-environment analyses of RILs derived from B97 suppress the *Rp1-D21* phenotype. Similarly, most of the QTL effects derived from Ki3 and Tx303 are enhancers, although there are some exceptions, e.g., the Ki3 allele at the LES AV and HTR AV QTL in bin 5.04 has a suppressive effect. Another recent study using the maize NAM populations to characterize the genetic architecture of resistance to southern leaf blight identified alleles conferring increased resistance and alleles conferring increased susceptibility (relative to B73 reference alleles) in all the founder lines regardless of how resistant or susceptible each founder was to southern leaf blight (Kump et al. 2011).

Joint analysis assumes that the founder parental allele effects are consistent across populations, so in this case four allele effects are estimated. This assumption is not necessarily valid in every case. For instance, two linked genes might be present in B73, one of them segregating in the B97 population and the other in the Ki3 population. In this case, the joint analysis might conflate the effects of these two distinct genes. The advantages of joint analysis with connected populations (i.e. populations that share a parent) are that allele effects of a wide range of alleles can be compared. Also the larger total population size often leads to more power to detect QTL and more precise positional estimates (e.g. Coles et al. 2010) as is the case here (Tables 4, 5; Fig. 2).

The mechanisms responsible for modulation of HR and the plant defense response in general are not well understood. They are obviously crucially important for plant disease resistance and there is mounting evidence that they may play an important role in the control of plant growth in the wild and in control of yield under cultivation (e.g. Bomblies and Weigel 2007; Todesco et al. 2010). An enhanced defense response is often correlated with reduced growth/yield and there is accumulating evidence that, during evolution, environment-dependent selection has acted to select for one trait over the other depending on disease pressure (Tian et al. 2003; Todesco et al. 2010). It seems likely that this conflict may play out in crop

breeding, such that breeding for increased yield may in some cases lead to lower levels of disease resistance and vice versa. While there is anecdotal evidence for this relationship, there are also published studies which suggest there is not necessarily a link between the selection response of these two traits (Ceballos et al. 1991; Miles et al. 1981).

In the present study, we have identified several novel loci controlling the maize HR. Different loci segregated in different populations. The implication then is that the HR response and by extension the entire maize defense response may vary quantitatively between lines and that this variation may be dependent on segregation at at least four or five loci and likely many more. It is quite likely that different loci effect different process. For example, some may be associated with variation in lesion initiation and others with variation in lesion spread. We have laid the groundwork for a much more extensive elucidation of the genetic architecture controlling this trait which will involve using the entire NAM population in a conceptually similar way. This research will ultimately help to address some of these fundamental questions of plant evolution and breeding and should be useful in the breeding of higher yielding, disease resistance varieties.

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