

CROPS AND SOILS RESEARCH PAPER

QTL analysis of resistance to Mal de Río Cuarto disease in maize using recombinant inbred lines

N. C. BONAMICO^{1*}, M. A. DI RENZO¹, M. A. IBAÑEZ¹, M. L. BORGHI¹, D. G. DÍAZ²,
J. C. SALERNO² AND M. G. BALZARINI³

¹Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Agencia No 3, 5800 Río Cuarto, Argentina

²Instituto de Genética 'Ewald A. Favret', Instituto Nacional de Tecnología Agropecuaria, cc 25, 1712 Castelar, Argentina

³Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba and CONICET (National Council of Scientific and Technological Research), cc 509, 5000 Córdoba, Argentina

(Received 20 January 2011; revised 8 October 2011; accepted 1 December 2011;
first published online 11 January 2012)

SUMMARY

Mal de Río Cuarto (MRC) is a devastating disease that reduces yield, quality and economic value of maize in Argentina. The objective of the present study was to map quantitative trait loci (QTL) for reactions to MRC from recombinant inbred lines (RILs). Reactions to the endemic MRC disease were evaluated in 145 advanced F_{2:6} lines, derived from a cross between a resistant (BLS14) and a susceptible (Mo17) line, at four environments in the temperate semi-arid crop region of Argentina. The evaluations of disease score (SCO), disease incidence (INC) and disease severity (SEV) were carried out on each individual RIL. Low heritability estimates were found across environments for SCO (0.23), INC (0.27) and SEV (0.22). A genetic map of simple sequence repeat (SSR) markers covering a total genetic distance of 1019 cM was built. QTL for resistance to MRC disease were found on different maize chromosomes. Four significant QTL, each explaining between 0.08 and 0.14 of the total phenotypic variation, were located on chromosomes 1, 4 and 10. Two QTL specific to the INC, and one specific to SEV, may be involved in different mechanisms of resistance to MRC. Although MRC reaction is highly affected by environmental effects, the QTL × environment interaction for INC and SEV was low. Most of the QTL for reaction to MRC detected in the present study were mapped to regions of the maize genome containing genes conferring resistance to various pathogens. The significant QTL across environments are good candidates to select for MRC resistance.

INTRODUCTION

Mal de Río Cuarto (MRC) disease, which was found to be associated with reovirus-like particles early in the 1980s (Nome *et al.* 1981), has become a significant disease problem in maize in several regions of Argentina. The worst epidemic of MRC occurred during 1996/97 and 2006/07, causing great economic losses. In 1997, the epidemic affected 300 000 ha with estimated losses of US\$120 million (Lenardón *et al.* 1998).

The MRC virus (MRCV) cytopathology has similarities with other viruses from the genus *Fijivirus*, family *Reoviridae* (Arneodo *et al.* 2002). The reovirus is naturally transmitted in a persistent, propagative manner by the planthopper *Delphacodes kuscheli*

Fennah (Homoptera: *Delphacidae*) (Ornaghi *et al.* 1993). Vector transmission complicates the disease epidemiology: MRC epidemics occur when large populations of *D. kuscheli* migrate from winter cereals to the emerging maize crop. Early planting has been used to avoid peak vector populations during the highly susceptible coleoptile stage (Ornaghi *et al.* 1999). Studies of the spatial pattern of the virus vector can provide relevant information to develop programmes for monitoring the vector abundance and epidemiology of MRC (Garat *et al.* 1999). Applications of systemic insecticides and removing of weedy gramineae, which constitute vectors and virus reservoirs, can reduce the disease. However, the most economical, environmentally sustainable and effective means for controlling viral diseases is to deploy resistant germplasm.

Assessing MRC severity in the field is difficult. Breeding for resistance has been hampered by the

* To whom all correspondence should be addressed. Email: nbonamico@ayv.unrc.edu.ar

obligate transmission of MRCV by the planthopper, and by environment-to-environment fluctuations in viral disease pressure. Field inoculations in the Río Cuarto region, where the disease is endemic, were used to partially overcome these difficulties. Previous studies in an early-generation $F_{2:3}$ (Di Renzo *et al.* 2002; Kreff *et al.* 2006) demonstrated that resistance to MRC is a quantitative trait that involves a relatively small number of genes. The type of action of the MRC resistance genes ranged from partial dominance to additivity and the heritability estimates were moderate (Presello *et al.* 1995; Di Renzo *et al.* 2002). The progress in breeding for MRC resistance using traditional methods can be slow because of strong environmental effects, the high cost of field evaluations and the few resistance sources available. Marker assisted selection (MAS) has been proved to increase the rate of genetic gain significantly when compared with conventional breeding and thus would help to alleviate some of these problems. Mendelian genetics applied to crops has had a major impact on crop improvement, including breeding for disease and pest resistance. Traditional genetic approaches, however, are labour intensive and time consuming. The advent of molecular genetics provided new opportunities for mapping and tracking genes of agronomic interest, leading to more efficient marker-assisted selection (Lucas 2010). The development of DNA-based markers provides a powerful alternative method for the dissection of complex traits, including reaction to MRC in maize. The identification and mapping of quantitative trait loci (QTL) associated with virus resistance in maize have been reviewed by Redinbaugh *et al.* (2004) and Redinbaugh & Pratt (2009).

In a previous study, DNA markers linked to genes governing MRC resistance were identified with $F_{2:3}$ lines using traditional QTL mapping (Di Renzo *et al.* 2004; Kreff *et al.* 2006) and with recombinant inbred lines (RILs) using discriminate analysis (Bonamico *et al.* 2010). Because of the complex genetic nature of MRC disease, the identification of QTL for resistance is not always consistent. Therefore, validation of these QTL for reaction to MRC (MRC-QTL) is important before implementing marker-assisted selection. The RIL population used in the present study was derived from the $F_{2:3}$ population mentioned above (Di Renzo *et al.* 2004). Comparisons between the two different populations should allow comparison of the regions associated with MRC reaction detected in early and late selfing generations. Despite the inability to measure dominance effects of QTL, RILs are efficient

and powerful tools for QTL detection because of the increased homozygosity and augmented recombination. The main purpose of the present study was to map MRC-QTL from a RIL population.

MATERIALS AND METHODS

Plant materials

Two homozygous inbred lines, BLS14 and Mo17, were used as the parental material. The resistant parent BLS14, a flint maize line, was selected from selfed plants of the open-pollinated, locally adapted, Argentine cultivar 'Colorado La Holandesa'. Mo17, an American dent maize inbred line derived from the Lancaster Sure Crop population, was the susceptible parent. Mean yield of Mo17 is half that of the resistant parent. A total of 145 RILs derived from a BLS14 × Mo17 cross were developed by self-pollinating a random sample of F_2 plants through single seed descent method until the $F_{2:6}$ generations. RIL families together with the parents, used as controls, were evaluated for reaction to the endemic MRC disease in the temperate semi-arid crop region of Argentina at four field environments. The field trials were carried out during two growing seasons, at Río Cuarto (64°20'W, 33°8'S, 334 m asl) and Sampacho (64°42'W, 33°19'S, 510 m asl), Argentina. Each location–season combination was used to define four environments: Río Cuarto 2005 (R5) and 2006 (R6), and Sampacho 2004 (S4) and 2005 (S5). The parents and RILs were grown under natural infection in the four environments. The experimental design at each environment was a randomized complete block design with two replications of single-row plot 0.70 m apart and 4 m long. Plants were thinned to a distance of 0.20 m and weeds were controlled with herbicides. Hand weeding was performed as necessary in all plots. Each trial was conducted under natural infection establishing the plots where the preceding crop was winter oat, which constitutes a vector and virus reservoir.

Description of variables

A total of 15 plants in the central rows of each plot were individually evaluated for symptoms at initial male flowering (2 months after planting). The plants at the end of each plot were not rated, to avoid possible border effects. Symptoms were measured visually on each plant using a scale based on the rating system proposed by Ornaghi *et al.* (1999): 0=no symptoms;

1 = mild symptoms; 2 = severe symptoms; 3 = maximal development of the MRC disease. This rating allowed quantification of the reaction to MRC by means of three variables on a family-mean basis. Such variables are disease score (SCO) or mean rating of all plants in the family, disease incidence (INC) or proportion of plants presenting symptoms, and disease severity (SEV) or mean SCO of the plants presenting symptoms.

Data analysis

The experimental data were analysed for each variable (SCO, INC and SEV) by ANOVA using the MIXED procedure of SAS software (SAS Institute 2002). On a family-mean basis, the total phenotypic variation was partitioned as follows:

$$Y = \mu + E + B(E) + G + G \times E + e$$

where Y is the response variable, μ is the overall mean, E is the environmental effect, $B(E)$ is the block within environment effect, G is the genotype (RIL) effect, $G \times E$ is the genotype by environment interaction effect, and e is an error term. G and $G \times E$ terms were regarded as random and the other model terms as fixed. Restricted maximum likelihood (REML) was considered for estimating genotypic (σ_g^2), $G \times E$ interaction (σ_{ge}^2) and error (σ_e^2) variance components. The Shapiro–Wilks test (Shapiro & Francia 1972) was used to check the normality of the residual distributions. Further logarithmic transformations were required for SCO and SEV. Broad sense heritability (h^2) estimates on a family-mean basis were assessed for each environment and across the four environments according to Hallauer & Miranda (1981). Exact 95% confidence intervals of h^2 were calculated from Knapp *et al.* (1985). Spearman (rank) correlation coefficients (r_s) were calculated for each pair of variables at each environment and for each variable to correlate line rankings in different environments (Yan & Rajcan 2003).

A mixed-model approach was used for assessment of RIL and parental genotypic effects, regarded as random and fixed, respectively. The means of best linear unbiased predictions (BLUP) of random RIL effects at each environment were compared with the parental means at the same environment by means of t test ($P \leq 0.05$).

Population genotyping and marker linkage analysis

DNA was extracted from healthy leaves in order to determine the simple sequence repeat (SSR) genotype

of a mapping population of 145 RIL families and parents for MRC-QTL detection. Tissue from leaf samples were lyophilized and ground to a fine powder. The isolation of total DNA was performed following the procedures described by Saghai-Marooif *et al.* (1984). DNA was quantified using the spectrophotometric readings at A_{260} and A_{280} , and concentration was calculated according to Sambrook *et al.* (1989). A total of 140 SSR primer pairs, whose sequences were downloaded from the MaizeGDB website (<http://www.maizegdb.org>; verified 9 November 2011) and synthesized by Alpha DNA (<http://www.alphadna.com>; verified 9 November 2011), were screened for useful polymorphisms. Primers that were polymorphic in the parental inbred lines BLS14 and Mo17 were chosen as markers and used for testing against the whole population. Prior to linkage analysis, a chi-square analysis was performed for each RIL marker locus segregation ratios (1:1 for RIL). A linkage map was constructed using the program MapMaker/EXP 3.0 (Lander *et al.* 1987). Recombinant frequencies between marker loci were estimated by a multi-point analysis that was performed by the 'order' command, and transformed into map distances (centimorgans: cM) by using the Kosambi mapping function. For declaration of significant linkage between two markers, a \log_{10} likelihood of the odds (LOD) threshold of 3.0 and maximum distance of 30 cM was used.

Data analysis and QTL mapping

The position and effect of MRC-QTL were estimated for each variable by environment and across environments. Analyses were performed on RIL data averaged across replicates and BLUP values of the 145 families. For mapping QTL, the methods of single interval mapping (SIM) and composite interval mapping (CIM) were employed using PlabQTL software (Utz & Melchinger 1996). Cofactor markers for CIM were selected by stepwise regression with the 'cov' statement in the PlabQTL software with a (LOD) score >3.0 to enter into the model.

The map was scanned at 5 cM intervals between markers and QTL controlling MRC resistance. The location of regions associated with MRC reaction corresponded to the location of peak LOD scores in the scan of the corresponding chromosome. QTL, which were designated by the corresponding chromosome bin in which they were found, are reported in relation to the nearest marker of the LOD score peak. QTL regions detected with a LOD score >2.5 were

Table 1. Means (\pm s.e.) of disease assessment variables of parents BLS14 and Mo17 and of a derived mapping population of 145 RIL families; significance of the fixed effect environment and estimates of the variance components and heritabilities with RIL data for three analysed variables across four evaluation environments

| Parameter | | Variable* | | |
|----------------------|-----------------|-----------------------|-----------------------|-----------------------|
| | | SCO (0.00–1.39 scale) | INC (0.00–1.00 scale) | SEV (0.69–1.39 scale) |
| Means | BLS14 | 0.11 (0.030) | 0.16 (0.043) | 0.69 (0.000) |
| | Mo17 | 0.99 (0.052) | 0.70 (0.076) | 1.27 (0.006) |
| | RIL | 0.81 (0.014) | 0.55 (0.011) | 1.19 (0.038) |
| Fixed effect | (Environments) | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| Variance componentst | σ_g^2 | 0.01 (0.006) | 0.01 (0.003) | 0.00 (0.001) |
| | σ_{ge}^2 | 0.12 (0.010) | 0.07 (0.005) | 0.01 (0.002) |
| | σ_e^2 | 0.03 (0.002) | 0.02 (0.001) | 0.03 (0.002) |
| Heritability | h^2 | 0.21 | 0.27 | 0.20 |
| 90% CI on h^2 | | 0.04–0.40 | 0.05–0.44 | 0.05–0.39 |

* Disease assessment. SCO, disease score; INC, disease incidence; SEV, disease severity. For SCO and SEV, the results presented refer to the data obtained by logarithmic transformation.

† σ_g^2 , σ_{ge}^2 , σ_e^2 are estimates of the variances between RIL families, of $G \times E$ interaction and within families, respectively. h^2 is the broad-sense heritability on a family-mean basis. CI, confidence interval.

identified as significant and those with a LOD score between 2.0 and 2.5 were regarded as suggestive. Bin locations are designated by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group. The multiple regression method implemented in PlabQTL software was used to determine the significance of additive \times additive epistatic interactions between QTL found to contribute to RIL data averages or BLUPs (Utz & Melchinger 1996). The total phenotypic variance explained by a single QTL of each variable was estimated by the square of the partial correlation coefficient.

RESULTS

Field variable analysis

Across environments, the resistant parent BLS14 showed a high but not complete resistance to MRC and the susceptible parent Mo17 showed heavy symptoms (Table 1). No heterogeneity of error variance was detected across environments for the log transformed data of SCO and SEV variables.

The estimated genetic variance component revealed the existence of significant differences ($P < 0.01$) in MRC reaction between RIL families (σ_g^2) for all disease variables. Heritability estimates at each environment were very high for the variables SCO and INC, which

ranged from 0.71 to 0.92, and intermediate to low for the SEV variable, which ranged from 0.12 to 0.53. Across environments (Table 1), the variance due to $G \times E$ interaction (σ_{ge}^2) was significant ($P < 0.01$) and larger than the genotypic variance (σ_g^2) for the three variables. Low heritability estimates were found averaged over all environments for SCO (0.21), INC (0.27) and SEV (0.20).

Table 2 shows Spearman correlation coefficients between the RIL rankings in different environments. Since coefficients were low (< 0.40), it was concluded that the $G \times E$ interaction, for all variables, was mostly due to RIL rank changes between environments. Such environment differences in rank of RIL families between environments, as well as high $G \times E$ variance, probably reflect the complications of evaluating MRC disease, i.e. the screening process and the effect of environment on the expression of resistance.

Phenotypic (r_p) linear correlations between variables in each of the four environments were positive and highly significant ($P < 0.01$) (Table 3). Coefficients of correlations between SCO and INC were higher than 0.90, thus only the results for INC are presented here.

Best linear unbiased estimation (BLUE) values of the parental lines (BLS14 and Mo17) are compared with BLUPs of the RILs for INC and SEV at each environment in Table 4. For both variables, the BLUE values of parental lines were significantly different

Table 2. Spearman (rank) correlation coefficients estimated between four evaluation environments with a 145 RIL families derived from the cross BLS14 × Mo17, for three analysed variables

| Environment† | | Variable* | | |
|--------------|----|-----------|------|------|
| | | SCO | INC | SEV |
| R5 | R6 | 0.21 | 0.20 | 0.27 |
| | S4 | 0.07 | 0.05 | 0.24 |
| | S5 | 0.18 | 0.09 | 0.28 |
| R6 | S4 | 0.08 | 0.15 | 0.38 |
| | S5 | 0.12 | 0.08 | 0.24 |
| S4 | S5 | 0.17 | 0.13 | 0.35 |

* Disease assessment. SCO: disease score; INC: disease incidence; SEV: disease severity.

† Location-season combination, R5: Río Cuarto 2005; R6: Río Cuarto 2006; S4: Sampacho 2004; S5: Sampacho 2005.

($P < 0.05$) to the expected BLUP of the RIL families, indicating that the parental reaction to MRC deviated from the population average, except for the parental line Mo17 for INC and SEV in the S4 environment. Transgressive segregation was not indicated, because only a small portion of RIL fell outside the range of the parental values.

QTL analysis

Of the 140 SSR loci, 66 (0.47) were found to be polymorphic and 58 markers, with good amplification profile and even coverage of the genome, were employed for genotyping the entire RIL mapping population. Genotypic classes of 12 loci deviated from the expected Mendelian ratios (1:1). Markers were anchored on the linkage groups on the basis of their known locations. Linkage analysis resulted in a genetic maize map consisting of eight linkage groups and two unlinked markers. These linkage groups covered a genetic distance of c. 1019 cM of the maize genome, with 0.75 of the intervals between markers being near to 20 cM. The order of SSR markers in the map was in good agreement with their bins on the chromosomes of maize (MaizeGDB website <http://www.maizegdb.org>).

The chromosomal locations of markers for the QTL analysis across environments for both RIL data averages and BLUP values, such as the effect of each QTL, are presented in Table 5. Four MRC-QTL detected as significant with a LOD score > 2.5 were located on chromosomes 1 (bins 1.01 and 1.06), 4 (bin

Table 3. Phenotypic correlation coefficients for pairwise comparisons for three analysed variables, estimated at four evaluation environments with 145 RIL families derived from the cross BLS14 × Mo17

| Environment† | Variable* | | |
|--------------|-----------|---------|---------|
| | SCO-INC | SCO-SEV | INC-SEV |
| R5 | 0.90 | 0.29 | 0.54 |
| R6 | 0.92 | 0.36 | 0.52 |
| S4 | 0.94 | 0.45 | 0.57 |
| S5 | 0.96 | 0.50 | 0.60 |

* Disease assessment. SCO: disease score; INC: disease incidence; SEV: disease severity.

† Location-season combination, R5: Río Cuarto 2005; R6: Río Cuarto 2006; S4: Sampacho 2004; S5: Sampacho 2005.

4.08) and 10 (bin 10.02). All four QTL regions were identified by the CIM method, employing four cofactor markers for INC and two for SEV. Two of these significant regions associated with MRC reaction were also identified using the SIM model. For the INC variable, the additive effects of QTL found on bins 1.01 and 1.06 were statistically significant and accounted for up to 0.08 and 0.13 of the phenotypic variation, respectively. These two QTL regions with significant additive effect were associated with alleles for MRC resistance. The QTL regions on bin 4.08 with significant additive effect were associated with alleles for MRC susceptibility which came from the resistant parent. For the SEV variable, QTL mapped to genetic bins 1.01 and 10.02 significantly contributed to variation for up to 0.06 and 0.14 of the phenotypic variation, respectively. QTL detected in bin 1.01 with significant additive effect, was associated with alleles for MRC resistance from BLS14. QTL detected in bin 10.02 did not have a statistically significant additive effect and thus, with uncertainty about the sign of the additive effect, were associated with alleles for susceptibility that probably originated from BLS14.

Significant digenic epistatic (additive × additive) interactions among the detected regions associated with MRC reaction were found between QTL mapped to genetic bins 1.01 and 4.08 for the INC variable. QTL mapped to chromosome bins 1.06 and 4.08 appeared to be specific for the INC variable, and the QTL in bin 10.02 appeared to contribute preferentially to the SEV variability. The global test for QTL × E was not significant ($P > 0.05$) for the four QTL mapped across environments, suggesting stability of QTL effects.

Table 4. Disease incidence and severity of MRC. BLUP of RIL families and BLUE of BLS14 and Mo17 parents with probability values for the hypothesis of no differences between RIL and the parental in four evaluation environments

| Variable* | Environment† | BLUP | BLUE | | | |
|-----------------------|--------------|------|-------|----------------|------|-----------------|
| | | RIL | BLS14 | | Mo17 | |
| INC (0.00–1.00 scale) | R5 | 0.40 | 0.09 | <i>P</i> <0.01 | 0.97 | <i>P</i> <0.01 |
| | R6 | 0.41 | 0.17 | <i>P</i> <0.01 | 1.00 | <i>P</i> <0.01 |
| | S4 | 0.58 | 0.04 | <i>P</i> <0.01 | 0.59 | <i>P</i> =0.606 |
| | S5 | 0.77 | 0.33 | <i>P</i> <0.01 | 1.00 | <i>P</i> <0.01 |
| SEV (0.69–1.39 scale) | R5 | 1.21 | 0.36 | <i>P</i> <0.01 | 1.48 | <i>P</i> <0.01 |
| | R6 | 1.26 | 0.43 | <i>P</i> <0.01 | 1.58 | <i>P</i> <0.01 |
| | S4 | 1.28 | 0.51 | <i>P</i> <0.01 | 1.27 | <i>P</i> =0.109 |
| | S5 | 1.31 | 0.38 | <i>P</i> <0.01 | 1.57 | <i>P</i> <0.01 |

* Disease assessment. INC, disease incidence; SEV, disease severity.

† Location–season combination, R5: Río Cuarto 2005; R6: Río Cuarto 2006; S4: Sampacho 2004; S5: Sampacho 2005.

Table 5. Parameter estimates associated with QTL for INC and severity to MRC, in a mapping population of 145 RIL families derived from the cross BLS14 × Mo17, across four evaluation environments

| Variable* | QTL position† | LOD score‡ | Additive effect§ | Partial R ² ¶ |
|-----------------------|-----------------|--------------------|------------------|--------------------------|
| INC (0.00–1.00 scale) | 1.01 (umc1177) | 2.53 ^{ab} | –0.08 | 0.08 |
| | 1.06 (bnlg1556) | 3.36 ^a | –0.12 | 0.13 |
| | 4.08 (umc1612) | 2.78 ^{ab} | 0.13 | 0.10 |
| SEV (0.69–1.39 scale) | 1.01 (umc1177) | 2.50 ^a | –0.04 | 0.06 |
| | 10.02 (phi063) | 2.65 ^a | 0.07 | 0.14 |

* Disease assessment. INC: disease incidence; SEV: disease severity.

† Chromosomal location: bin and nearest marker.

‡ Likelihood of odds (LOD) score. *a* and *b* QTL detected using CIM and using SIM, respectively.

§ Additive effect of the QTL. A negative value of additive effects reflects that parent BLS14 contributed QTL alleles increasing resistance.

¶ Phenotypic variation explained by the QTL. Bold type indicates QTL was only detected using BLUP values, italics indicate QTL was found using only RIL data averages. All other QTL were found using both RIL data averages and BLUP values.

Table 6 shows that for the QTL mapped by environments, the range of phenotypic variation explained using both RIL data averages and BLUP values by all significant and suggestive QTL regions for each variable varied from 0.06 to 0.16. In addition to the three significant MRC-QTL identified across environments for the INC variable, three suggestive QTL regions with a LOD score between 2 and 2.5 were detected on chromosomes 1 (bins 1.04) and 4 (bins 4.03 and 4.05), and two QTL regions with a LOD >2.5 were located on chromosomes 8 (bins 8.03 and 8.08). In addition to the two significant MRC-QTL identified across environments for the SEV variable, four

suggestive QTL regions were detected on chromosomes 1 (bin 1.02), 4 (bins 4.03 and 4.05) and 8 (bin 8.3), and five significant QTL regions were located on chromosomes 1 (bins 1.03 and 1.04), 6 (bin 6.02), 8 (bin 8.08) and 10 (10.02).

The QTL regions detected on chromosome 1 (bin 1.01) accounted for variation of both variables in S4 and across environments. The QTL mapped to bin 1.06 detected in R6, S4 and across environments, and the QTL mapped on chromosome 10 (10.02) in R6 and across environments were found to be exclusively involved in the INC and SEV variation, respectively. The SSR markers identified across environments that

Table 6. Parameter estimates associated with QTL for disease incidence and severity to MRC, in a mapping population of 145 RIL families derived from the cross BLS14 × Mo17, in four environments

| Variable* | QTL position† | Environment‡ | LOD score§ | Additive effect¶ | Partial R ² # |
|-----------------------|-----------------|--------------|--------------------|------------------|--------------------------|
| INC (0.00–1.00 scale) | 1.01 (umc1177) | S4 | 2.00 ^a | −0.14 | 0.07 |
| | 1.04 (umc1811) | S4 | 2.00 ^a | 0.12 | 0.08 |
| | 1.06 (bnlg1556) | R6 | 2.26 ^a | −0.15 | 0.16 |
| | 1.06 (bnlg1556) | S4 | 2.12 ^a | −0.11 | 0.08 |
| | 4.03 (phi021) | R6 | 2.00 ^a | −0.19 | 0.10 |
| | 4.05 (nc005) | S5 | 2.00 ^a | −0.05 | 0.08 |
| | 8.03 (umc1741) | S4 | 2.50 ^a | −0.09 | 0.10 |
| | 8.03 (umc1741) | S5 | 2.50 ^a | −0.06 | 0.10 |
| | 8.08 (phi080) | S5 | 3.03 ^{ab} | −0.11 | 0.12 |
| SEV (0.69–1.39 scale) | 1.01 (umc1177) | S4 | 2.00 ^b | −0.12 | 0.06 |
| | 1.02 (bnlg1627) | R6 | 2.00 ^a | −0.02 | 0.07 |
| | 1.03 (bnlg1866) | S5 | 2.64 ^a | −0.04 | 0.11 |
| | 1.04 (bnlg1811) | R6 | 2.50 ^a | −0.10 | 0.14 |
| | 4.03 (phi021) | R6 | 2.00 ^a | −0.10 | 0.11 |
| | 4.05 (bnlg1217) | R5 | 2.10 ^a | 0.07 | 0.08 |
| | 4.05 (bnlg1217) | R6 | 2.16 ^a | 0.12 | 0.13 |
| | 6.02 (bnlg1371) | S4 | 2.50 ^a | 0.04 | 0.13 |
| | 8.03 (umc1741) | S4 | 2.09 ^a | 0.02 | 0.09 |
| | 8.08 (phi080) | S5 | 4.79 ^{ab} | −0.09 | 0.15 |
| | 10.02 (phi063) | R6 | 2.30 ^a | 0.13 | 0.16 |

* Disease assessment. INC, disease incidence; SEV, disease severity.

† Chromosomal location: bin and nearest marker.

‡ Location-season combination, R5: Río Cuarto 2005; R6: Río Cuarto 2006; S4: Sampacho 2004; S5: Sampacho 2005.

§ Likelihood of odds (LOD) score. *a* and *b* QTL detected using CIM and using SIM, respectively.

¶ Additive effect of the QTL. A negative value of additive effects reflects that parent BLS14 contributed QTL alleles increasing resistance.

Phenotypic variation explained by the QTL. Bold type indicates QTL was only detected using BLUP values, italics indicate QTL was found using only RIL data averages. All other QTL were found using both RIL data averages and BLUP values.

can be used to select for MRC resistance in maize are shown in Fig. 1.

DISCUSSION

The present results are consistent with previous reports about the quantitative inheritance of MRC resistance (Presello *et al.* 1995; Di Renzo *et al.* 2002; Kreff *et al.* 2006), suggesting an oligogenic or polygenic genetic control with low to moderate heritability. The inconsistency of the resistance phenotype was demonstrated by a high $G \times E$ interaction variance and low correlations between data collected in different environments, resulting in a low heritability across environments. Interactions among a competent vector, a virulent pathogen, a susceptible host and a suitable environment are necessary for disease development (Redinbaugh & Pratt 2009; Lucas 2010).

Previous inheritance studies of reaction to MRC have shown the importance of additive and

non-additive genetic effects (Presello *et al.* 1995; Di Renzo *et al.* 2004; Kreff *et al.* 2006). Since the regions associated with MRC reaction were mapped using homozygous RILs in the present study, only the additive and additive \times additive effects were estimated. The current results suggest the presence of significant digenic epistatic interactions. Little evidence for epistatic interactions has been observed in other QTL mapping studies in maize (Berke & Rocheford 1999). A small proportion of the progeny showed BLUPs larger than the susceptible parent. Such a small amount of transgressive segregation could be explained by environmental effects or by experimental errors rather than by the recombination of complementary QTL.

The use of RIL integrated with the known genomic positions of SSR markers proved to be highly efficient for QTL mapping. The SSR markers were deliberately chosen to cover the genome uniformly. The map used in the present study could be improved by increasing the marker density that would enable phenotypic

MRC-resistant QTL on bin 4-02/03 linked to marker phi021 was found, and four QTL conferring resistance to grey leaf spot, southern corn rust and northern corn leaf blight have been mapped at the same position (Hoisington 1989; Bubeck *et al.* 1993; Chen *et al.* 2004). On chromosome bins 1-01 and 1-02, where two suggestive MRC-QTL linked to marker umc1177 and bnlgl1627 were mapped, one major QTL conferring resistance to northern corn leaf blight was previously identified by Hoisington (1989).

As the variables INC and SEV were not determined by the same set of genetic factors, it is possible to infer that different sets of QTL may be involved in different resistance mechanisms to MRC disease. Some authors (Pernet *et al.* 1999b; Dintinger *et al.* 2005) have argued that specific QTL to the INC in maize may be involved in several defence mechanisms that hamper the invasion of the plant by the virus, and that QTL related to the SEV may be involved in some resistance mechanisms affecting the virus multiplication rate in the plant.

In spite of the high interaction variance, no significant QTL×E interaction was observed for the four significant MRC-QTL identified in the present study.

By comparing the present results against those previously reported for MRC SEV in an early generation (Di Renzo *et al.* 2004), it was possible to contrast QTL detected in early and late selfing generations, and to demonstrate the advantage of RILs over F_{2:3} lines for QTL analysis. More QTL regions were detected in the RIL population than in the F_{2:3} lines. However, three putative QTL placed in the bins 1-03, 1-04 and 8-03 were detected in the same genomic regions in the RIL families and in the F_{2:3} lines. The agreement of findings between these two generations may be considered an indication of the existence of these QTL and may encourage the undertaking of more intensive research on these regions. In contrast, selection of a subset of RILs (e.g. genetic drift), loss of alleles during the development of RILs by insufficient population size, or natural selection can be associated with lack of consistency across generations. Other mapping studies have reported results in which few QTL were validated in different generations (Cardinal *et al.* 2001).

Although the relative efficiency of MAS in comparison with phenotypic selection is close to 1 for the studied trait (Di Renzo *et al.* 2004), the mapped MRC-QTL could improve the breeding efficiency in resistance to MRC disease since the plants can be selected at a young age. Moreover, experiments

involving planthopper vectors could be avoided and environmental effects controlled. Considering the high costs and technology input, only regions associated with MRC reaction consistently expressed across environments and different genetic backgrounds can be recommended for use in MAS.

As stated by Hogenhout *et al.* (2008) plant virus diseases may well increase in the future. In an increasingly unstable climate, which will have profound effects on virus vectors, pyramiding disease resistance genes from different sources of germplasm will allow provision of a more rapid response in the delivery of resistance and elevate the effectiveness by increasing the long-term stability of resistance (Lucas 2010). This approach, in combination with an integrated management, will help to set criteria for sustainable control of MRC in maize. The present study suggests that an increase of alleles controlling MRC resistance could be accomplished using a biparental recurrent selection scheme aided by MAS, and molecular markers associated with environmentally independent MRC-resistant QTL.

The authors would like to acknowledge the help of Ingrid Teich for improvements in the language editing. This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica, PICT-02231/07-BID; Agencia Córdoba Ciencia, PID-38, CONICET and Universidad Nacional de Río Cuarto, Argentina.

REFERENCES

- ARNEODO, J. D., LORENZO, E., LAGUNA, I. G., ABDALA, G. & TRUOL, G. A. (2002). Cytopathological characterization of Mal de Río Cuarto virus in corn, wheat and barley. *Fitopatologia Brasileira* **27**, 298–302.
- BERKE, T. G. & ROCHEFORD, T. R. (1999). Quantitative trait loci for tassel traits in maize. *Crop Science* **39**, 1439–1443.
- BONAMICO, N. C., BALZARINI, M. G., ARROYO, A. T., IBAÑEZ, M. A., DÍAZ, D. G., SALERNO, J. C. & DI RENZO, M. A. (2010). Association between microsatellites and resistance to Mal de Río Cuarto in maize by discriminant analysis. *Phyton* **79**, 31–38.
- BUBECK, D. M., GOODMAN, M. M., BEAVIS, W. D. & GRANT, D. (1993). Quantitative trait loci controlling resistance to gray leaf spot in maize. *Crop Science* **33**, 838–847.
- CARDINAL, A. J., LEE, M., SHAROPOVA, N., WOODMAN-CLIKEMAN, W. L. & LONG, M. J. (2001). Genetic mapping and analysis of quantitative trait loci for resistance to stalk tunneling by the European corn borer in maize. *Crop Science* **41**, 835–845.
- CHEN, C. X., WANG, Z. L., YANG, D. E., YE, C. J., ZHAO, Y. B., JIN, D. M., WENG, M. L. & WANG, B. (2004). Molecular

- tagging and genetic mapping of the disease resistance gene RppQ to southern corn rust. *Theoretical and Applied Genetics* **108**, 945–950.
- DINTINGER, J., VERGER, D., CAIVEAU, S., RISTERUCCI, A. M., GILLES, J., CHIROLEU, F., COURTOIS, B., REYNAUD, B. & HAMON, P. (2005). Genetic mapping of maize stripe disease resistance from the Mascarene source. *Theoretical and Applied Genetics* **111**, 347–359.
- DI RENZO, M. A., BONAMICO, N. C., DIAZ, D. D., SALERNO, J. C., IBÁÑEZ, M. M. & GESUMARIA, J. J. (2002). Inheritance of resistance to Mal de Río Cuarto (MRC) disease in *Zea mays* (L.). *Journal of Agricultural Science, Cambridge* **139**, 47–53.
- DI RENZO, M. A., BONAMICO, N. C., DIAZ, D. G., IBÁÑEZ, M. A., FARICELLI, M. E., BALZARINI, M. G. & SALERNO, J. C. (2004). Microsatellite markers linked to QTL for resistance to Mal de Río Cuarto disease in *Zea mays* L. *Journal of Agricultural Science, Cambridge* **142**, 289–295.
- GARAT, O., TRUMPER, E. V., GORLA, D. E. & PEREZ-HARGUINDEGUY, N. (1999). Spatial pattern of the Río Cuarto corn disease vector, *Delphacodes kuscheli* Fennah (Hom., *Delphacidae*), in oat fields in Argentina and design of sampling plans. *Journal of Applied Entomology* **123**, 121–126.
- HALLAUER, A. R. & MIRANDA, J. B. (1981). *Quantitative Genetics in Maize Breeding*. Ames, IA: Iowa State University Press.
- HOGENHOUT, S. A., EL-DESOUKY, A., WHITFIELD, A. E. & REDINBAUGH, M. G. (2008). Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology* **46**, 327–359.
- HOISINGTON, D. (1989). Working linkage maps. *Maize Genetics Cooperation Newsletter* **63**, 141–151.
- JANSEN, R. C. & STAM, P. (1994). High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* **136**, 1447–1455.
- KNAPP, S. J., STROUP, W. W. & ROSS, W. M. (1985). Exact confidence intervals for heritability on a progeny mean basis. *Crop Science* **25**, 192–194.
- KREFF, E. D., PACHECO, M. G., DÍAZ, D. G., ROBREDO, C. G., PUÉCHER, D., CÉLIZ, A. E. & SALERNO, J. C. (2006). Resistance to Mal de Río Cuarto virus in maize: A QTL mapping analysis. *Journal of Basic and Applied Genetics* **17**, 41–50.
- LANDER, E. S., GREEN, P., ABRAHAMSON, J., BARLOW, A., DALY, M. J., LINCOLN, S. E. & NEWBURG, L. (1987). Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**, 174–181.
- LENARDÓN, S. L., MARCH, G. J., NOME, S. F. & ORNAGHI, J. A. (1998). Recent outbreak of 'Mal de Río Cuarto' virus on corn in Argentina. *Plant Disease* **82**, 448 (Abstract).
- LUCAS, J. A. (2010). Advances in plant disease and pest management. *Journal of Agricultural Science, Cambridge* **149** (Supp. 1), 91–114.
- McMULLEN, M. D. & SIMCOX, K. D. (1995). Genomic organization of disease and insect resistance genes in maize. *Molecular Plant–Microbe Interactions* **8**, 811–815.
- NOME, S. F., LENARDÓN, S. L., RAJU, B. C., LAGUNA, I. G., LOWE, S. K. & DOCAMPO, D. (1981). Association of reovirus-like particles with Enfermedad de Río IV of maize in Argentina. *Phytopathologische Zeitschrift* **101**, 7–15.
- ORNAGHI, J. A., BOITO, G., SANCHEZ, G., MARCH, G. & BEVIACQUA, J. E. (1993). Studies on the populations of *Delphacodes kuscheli* Fennah in different years and agricultural areas. *Journal of Genetics and Breeding* **47**, 277–282.
- ORNAGHI, J. A., MARCH, G. J., BOITO, G. T., MARINELLI, A., BEVIACQUA, J. E., GIUGGIA, J. & LENARDÓN, S. L. (1999). Infectivity in natural populations of *Delphacodes kuscheli* vector of "Mal de Río Cuarto" virus. *Maydica* **44**, 219–223.
- PERNET, A., HOISINGTON, D., DINTINGER, J., JEWELL, D., JIANG, C., KHAIRALLAH, M., LETOURMY, P., MARCHAND, J. L., GLASZMANN, J. C. & GONZÁLEZ DE LEÓN, D. (1999b). Genetic mapping of maize streak virus resistance from the Mascarene source. II. Resistance in line CIRAD390 and stability across germplasm. *Theoretical and Applied Genetics* **99**, 540–553.
- PERNET, A., HOISINGTON, D., FRANCO, J., ISNARD, M., JEWELL, D., JIANG, C., MARCHAND, J. L., REYNAUD, B., GLASZMANN, J. C. & GONZÁLEZ DE LEÓN, D. (1999a). Genetic mapping of maize streak virus resistance from the Mascarene source. I. Resistance in line D211 and stability against different virus clones. *Theoretical and Applied Genetics* **99**, 524–540.
- PRESELLO, D., CÉLIZ, A. & FRUTOS, E. (1995). Efectos genéticos asociados con la resistencia a la enfermedad Mal de Río Cuarto en líneas endocriadas de maíz. In *Proceedings of III Latin American and XVI Andean Zone of Maize Researchers Meeting, Tomo I*, (Eds L. G. Avila & L. M. Céspedes-P.), pp. 407–413. Bolivia: Fundación S. I. Patiño.
- REDINBAUGH, M. G., JONES, M. W. & GINGERY, R. E. (2004). The genetics of virus resistance in maize (*Zea mays* L.). *Maydica* **49**, 183–190.
- REDINBAUGH, M. G. & PRATT, R. C. (2009). Virus resistance. In *Handbook of Maize: Its Biology* (Eds J. L. Bennetzen & S. C. Hake), pp. 251–270. New York: Springer Verlag.
- SAGHA-MAROOF, M. A., SOLIMAN, K. M., JORGENSEN, R. A. & ALLARD, R. W. (1984). Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceeding of National Academy of Sciences of the United States of America* **81**, 8014–8018.
- SAMBROOK, J., FRITSCH, E. F. & MANIATIS, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. New York: Cold Spring Harbor Laboratory Press.
- SHAPIRO, S. S. & FRANCA, R. S. (1972). An approximate analysis of variance test for normality. *Journal of the American Statistical Association* **67**, 215–216.
- SAS Institute (2002). *SAS/STAT release 9.1*. Cary, NC: SAS Institute.
- UTZ, H. F. & MELCHINGER, A. E. (1996). PLABQTL. A program for composite interval mapping of QTL. *Journal of Agricultural Genomics*. Available online at: <https://www.uni-hohenheim.de/plantbreeding/software/> (verified 9 November 2011).

- WANG, G. X., CHEN, Y., ZHAO, J. R., LI, L., KORBAN, S. S., WANG, F. G., LI, J. S., DAI, J. R. & XU, M. L. (2007). Mapping of defense response gene homologs and their association with resistance loci in maize. *Journal of Integrative Plant Biology* **49**, 1580–1598.
- WELZ, H. G., SCHECHERT, A., PERNET, A., PIXLEY, K. V. & GEIGER, H. H. (1998). A gene for resistance to the maize streak virus in the African CIMMYT maize inbred line CML202. *Molecular Breeding* **4**, 147–154.
- WISSER, R. J., BALINT-KURTI, P. J. & NELSON, R. J. (2006). The genetic architecture of disease resistance in maize: A synthesis of published studies. *Phytopathology* **96**, 120–129.
- YAN, W. & RAJCAN, I. (2003). Prediction of cultivar performance based on single-versus multiple-year tests in soybean. *Crop Science* **43**, 549–555.
- ZENG, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics* **136**, 1457–1468.