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

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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 estimate the variance and heritability of resistance to MRC disease from maize families 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). On the basis of the substantial genotype–environment interaction and the little association between variables values in the different environments, selection for an increased resistance to MRC disease would require evaluation of germplasm across multiple years and locations.

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 300000 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 win-

ter 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 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 objective of the present study was to estimate the variance and heritability of resistance to MRC disease from maize families to MRC from recombinant inbred lines (RILs). The RIL population used in the present study was derived from the $F_{2:3}$ population mentioned above (Di Renzo et al., 2004).

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 masl) and Sampacho (64°42′W, 33°19′S, 510 masl), 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 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×E is the genotype by environment interaction effect, and e is an error term. G and G×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×E interaction (σ_{ge}^2) and error (σ_e^2) variance components. The Shapiro-Wilks test (Shapiro and 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 and Miranda (1981). Exact 95% confidence intervals of h^2 were calculated from Knapp et al. (1985). Spearman (rank) correlation coefficients (r) were calculated for each pair of variables at each environment and for each variable to correlate line rankings in different environments (Yan and 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 ($P<0.05$).

RESULTS

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×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×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×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.

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×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 and 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). 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 genes. These results indicate that evaluation of RILs for disease resistance to MRC requires additional environments to obtain estimates of reaction that are predictive of the performance of lines at other environments and also explain why the breeding efforts have been so laborious and time consuming.

Table 1. Means (±SE) 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 components†				
	σ^2_g	0.01 (0.006)	0.01 (0.003)	0.00 (0.001)
	σ^2_{ge}	0.12 (0.010)	0.07 (0.005)	0.01 (0.002)
	σ^2_e	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^2_g, σ^2_{ge}, σ^2_e are estimates of the variances between RIL families, of G×E interaction and within families, respectively. h^2 is the broad-sense heritability on a family-mean basis.

CI: confidence interval.

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.

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

	Variable*		
Environment†	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.

Table 4. Disease incidence and severity of MRC. Best linear unbiased predictions (BLUP) of RIL families and best linear unbiased estimations (BLUE) of BLS14 and Mo17 parents with probability values for the hypothesis of no differences between RIL and the parental in four evaluation environments

		BLUP	BLUE			
Variable*	Environment†	RIL	BLS14		Mo17	
INC	R5	0.40	0.09	$P<0.01$	0.97	$P<0.01$
(0.00–1.00 scale)	R6	0.41	0.17	$P<0.01$	1.00	$P<0.01$
	S4	0.58	0.04	$P<0.01$	0.59	$P=0.60$
	S5	0.77	0.33	$P<0.01$	1.00	$P<0.01$
SEV	R5	1.21	0.36	$P<0.01$	1.48	$P<0.01$
(0.69–1.39 scale)	R6	1.26	0.43	$P<0.01$	1.58	$P<0.01$
	S4	1.28	0.51	$P<0.01$	1.27	$P=0.10$
	S5	1.31	0.38	$P<0.01$	1.57	$P<0.01$

* 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.

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