Due to the low genetic correlations estimated, genetic mechanisms involved in determining the grade of severity of the disease MRC may not be the same for different environments (Table 1). This complicates selection of genotypes from these RILs.

However, estimated h^2 showed high values (Table 2), which indicates that the grade of the disease is controlled by a high proportion of genes with additive effect and some independence with the medium. Since the *E* had values <1 (Table 1), the direct selection strategy in each environment represents the best alternative and the indirect selection strategy may not have good prospects.

Table 2. Heritability (h^2) in five environments of evaluation for the grade of severity of MRC in 111 RILs of maize.

	Environment [a]				
Parameter	R4	S4	R5	S5	R6
Heritability [b]	0.70	0.45	0.56	0.60	0.66

[a]R4 = Río Cuarto 2004, S4 = Sampacho 2004, R5 = Río Cuarto 2005, S5 = Sampacho 2005 and R6 = Río Cuarto 2006

[b]$h^2 = (\sigma^2_g)/[(\sigma^2_g)+(\sigma^2_e/r)]$

Diallel analysis of Mal de Río Cuarto tolerance and yield components in maize

--Borghi, ML; Ibañez, MA; Bonamico, NC; Dallo, MD; Kandus, MV; Salerno, JC; Di Renzo, MA

Mal de Río Cuarto (MRC) is a devastating disease of maize in Argentina. The disease reduces grain yield (GY) and causes several symptoms, such as enations, reduced root systems, shortened superior internodes, flattened stems, leaves with small leaf areas, atrophic tassels, "hokey pole" ears and small ears with few or no kernels. The identification of heterotic patterns plays an important role in the selection of germplasm to develop hybrids. Analysis of diallel crosses provides preliminary data about heterotic relationships (Hallauer and Miranda Filho, Quantitative Genetics in Maize Breeding. Iowa State Univ. Press, Ames, IA, 1981; Hallauer and Miranda, Quantitative Genetics in Maize Breeding, 2nd Edition. Iowa State Univ. Press, Ames, IA, 1988). Our objective for this study was to estimate general (GCA) and specific combining ability (SCA) (Sprague and Tatum, J. Am. Soc. Agron. 34:923-932, 1942) in twelve lines of maize evaluated through diallel crosses, without reciprocals, for tolerance to MRC, grain yield and its components. The maize lines were BLS14, BLS1, BLS16, BLS61, BLS76, BLS91, BLS96, BLS101, BLS104, LP109, LP521 and LP125R.

The lines and their 66 crosses were planted on 21 November 2007 through a complete randomized block experimental design with two replications at Río Cuarto, Argentina (33°8´S 64°20´W). All plants were artificially infested with viruliferous insect vectors of MRC (*Delphacodes kuscheli* Fennah). Data were collected for the grade of severity of MRC disease (GS), number of kernel/m^2 (KN), unit weight of kernels (WK), and grain yield, standardized to 14.5% moisture (GY). Data were subjected to an ANOVA analysis using Griffing´s method 2 model II (Griffing, Aust. J. Biol. Sci. 9:463-493, 1956), by means of a diallel computer program (Magari and Kang, J. Hered. 85:336, 1994). Significance was estimated with *t* tests. The relative importance of general and specific combining ability on progeny performance was estimated as the ratio: $2\sigma^2_{ACG} / (2\sigma^2_{ACG} + \sigma^2_{ACE})$ (Baker, Crop Sci. 18:533-536, 1978) where σ^2_{ACG} and σ^2_{ACE} are the variance components for GCA and SCA. A value of 1 indicates that all genetic variance is additive. Analysis of variance revealed that mean square values for GCA were highly significant ($p \leq 0.01$) for the traits studied, with the exception of grain yield. The variations due to SCA were highly significant ($p \leq 0.01$) for all traits studied. The ratios $2\sigma^2_{ACG} / (2\sigma^2_{ACG} + \sigma^2_{ACE})$ were 0.15, 0.08, 0.19 and 0 for GS, NK, WK and GY, respectively, indicating that non-additive effects predominated in the expression of these traits. Marino and Teyssandier (Congreso Anual de la Sociedad Argentina de Genética, BuenosAires, 1982) reported the same results for tolerance to MRC, and Bhatnagar et al. (Crop Sci. 44:1997-2005, 2004) and Srdic et al. (Maydica 52:261-264, 2007), indicated that SCA effects were highly significant for GY. In our scoring, negative effects on combining ability are associated with tolerance to disease and positive effects with susceptibility. For GS, the highest GCA values were observed for line BLS1 (-0.39), followed by BLS16 (-0.2), and for WK the highest values were observed for line LP109 (0.03), followed by BLS104 (0.02) (Table 1). These parental lines presented highly significant GCA effects

Table 1. General combining ability (GCA) effects of each parental line for different characters.

Line	GS		NK		WK (g)	
BLS61	0.02	ns	57.85	ns	-0.01	ns
BLS91	0.07	ns	-69.54	ns	0.01	ns
BLS101	0.00	ns	-181.68	ns	0.01	ns
BLS76	-0.09	ns	32.95	ns	-0.01	ns
BLS96	-0.08	ns	-36.04	ns	-0.00	ns
BLS104	0.37	**	-74.64	ns	0.02	**
BLS16	-0.2	**	103.85	ns	-0.03	**
BLS14	0.04	ns	195.38	ns	-0.01	ns
BLS1	-0.39	**	119.1	ns	-0.01	ns
LP109	0.08	ns	-29.68	ns	0.03	**
LP521	0.09	ns	-16.93	ns	0.00	ns
LP125R	0.07	ns	-100.61	ns	0.00	ns

* Significant at 5% and ** significant at 1% probability level. GS=grade of severity of MRC disease, NK=number of kernel/m^2 and WK=unit weight of kernel.

for GS and WK, while the effects for NK were not significant. The highest SCA effects were observed for hybrids BLS101 x BLS104, LP109 x LP125R, BLS91 x BLS16 and LP109 x LP125R for GS, NK, WK and GY, respectively. The hybrid that manifested the best behavior for GS also presented good performance for the other traits, in contrast to the hybrid that manifested the greatest SCA for GY, which displayed negative effects for GS. We conclude that the lines with high general combining ability would be a valuable source of germplasm to develop hybrids that combine tolerance to MRC and good yield.

RIO CUARTO, ARGENTINA
Universidad Nacional de Río Cuarto
CORDOBA, ARGENTINA
Universidad Nacional de Cordoba
CASTELAR, ARGENTINA
Instituto Nacional de Tecnología Agropecuaria

Discriminant analysis to identify molecular markers associated with Mal de Río Cuarto (MRC) resistance

--Bonamico, NC; Arroyo, AT; Balzarini, MG; Ibañez, MA; Borghi, ML; Díaz, DG; Salerno, JC; Di Renzo, MA

In Argentina, the production of maize for grain is highly affected by MRC-disease, which is caused by a virus of the family *Reoviridae*, genus *Fijivirus* and transmitted by the planthopper

Delphacodes kuscheli Fennah (Homoptera: *Delphacidae*) vector (Nome et al., Phytopathologie Zhurnal 101:7–15, 1981). Traditional mapping of QTL for resistance to MRC disease has been reported using early-generation $F_{2:3}$ (Di Renzo et al., J. Agric. Sci., Cambridge 142:289–295, 2004). A series of agricultural applications of discriminant analysis (DA), involving recombinant inbred lines (RILs), is another way to identify meaningful markers associated with phenotypic performance (Capdevielle, MS Thesis, Louisiana State University, Baton Rouge, LA, 2001; Zhang et al., Theor. Appl. Genet. 110:721–729, 2005). Discriminant analysis was first used to identify RAPD markers associated with disease resistance in rice (Capdevielle et al., P. 216 in Proc. Fourth Internatl. Rice Genetics Symp., International Rice Research Institute, Los Baños, Philippines, 2000). It has been extended to other marker types such as SSR markers (Zhang et al., 2005) and AFLP markers (Capdevielle, 2001; McCharo et al., Euphytica 144:125–132, 2005; Miano et al., Euphytica 160:15–24, 2008).

DA has not been hitherto applied in studies for resistance to MRC disease in maize. The objective of this work was to identify an array of SSR markers associated with common symptoms of MRC using maize RILs with distinct reactions to the disease. Identification of molecular markers associated with groups of lines differing in phenotype performance would suggest the localization of genes with small individual effects on tolerance.

Genetic materials. A susceptible dent line, Mo17, and a partially resistant red flint line, BLS14, developed at the Instituto Nacional de Tecnología Agropecuaria, Castelar, were used as parents to produce 144 RILs by self-pollinating a random sample of F_2 plants by the single seed descent method (Burr and Burr, Trends Genet. 7:55–60, 1991). This is the same cross used for studies of traditional mapping of QTL for resistance to MRC disease (Di Renzo et al., 2004).

Field evaluation. For disease evaluations, trials were grown at locations where MRC disease is endemic. The trials were conducted during 2005 at the Sampacho (64º42'W, 33º19'S, 510 masl) location and during 2006 at the Río Cuarto (64º20'W, 33º8'S, 334 masl) location. The year-location combinations were treated as different environments. The experimental design was a randomized complete block design with two plots/RIL at each environment. Each trial included entries of Mo17 and BLS14. At the beginning of male flowering, RILs were evaluated for traits related to common symptoms of MRC disease. Individual plants were evaluated and data averaged at each environment for each RIL. Plants were rated on a discrete scale the values of which increase according to the increase of the disease severity. The following traits and rating scales (in parentheses) were used: superior internodes (0=normal; 1=shortened); presence and type of enations (0=no enations; 1=mild enations; 2=enlarged veins; 3=galls); multiple ears (0=normal; 1=multiple ears; 2=no ear).

Genetic markers. Forty SSR markers described in the Maize Genetics and Genomics Database (MaizeGDB, http://www.maizegdb.org) were used.

Data analysis. RILs were assigned to one of two groups defined to represent low and high values for the traits related to symptoms of MRC disease and representing the 1st and 4th quartile of the trait distribution. Missing marker data, which were around 10-15%, were computed using the multiple imputation procedure of SAS. SSR profile variation among predefined phenotypic groups was ascertained by the AMOVA method. Before performing DA, we ran a marker selection procedure with PROC STEPDISC (SAS Institute ver. 9.1) using the forward option as selection method with the select option set to 0.15. The analytic procedure used here is fully detailed in Zhang et al. (2005). Using the selected markers, a non-parametric method (k-nearest-neighbor) of DA was performed within PROC DISCRIM (SAS Institute ver. 9.1). The linear parametric DA (Fisher 1936) is also recommended because of its high robustness with outliers and non-normal or heteroscedastic data. The percentage of correct classification was calculated from cross-validation error rates by using the cross-validate option within PROC DISCRIM. A high level of correct classification infers an association between molecular markers and agronomic data for a trait expression.

Findings. The maize RILs evaluated in this study exhibited a wide range of phenotypic variation for the three MRC symptoms evaluated. Mean values for the two extreme phenotypic groups for each trait at each environment are shown in Table 1. The phenotypic mean values of the high and low groups were significantly different for the traits in the two environments (P<0.001).

Molecular variance analyses found significant molecular differences between the two extreme groups for each trait. Table 2 shows the number of markers selected by the STEPDISC procedure applied before DA and the percentage of correct classifications of RILs reached with the discriminant function based on the selected markers. For evaluations in S5 environment, a high percentage of correct classifications were obtained using a maximum set of eleven SSR markers. In R6 environment, the same percentage of correct classifications were achieved using between three and nine SSR markers. For internode, a minimum set of four SSR markers were selected by DA, in both S5 and R6 environments. The results suggest an array of markers associated with traits related to symptoms of MRC disease. The rate of correct classification (obtained by cross-validation) was regularly higher than 65%.

SSR markers selected by PROC STEPDISC, which differentiate between low and high trait value groups at each environment, are shown in Table 3 and indicate chromosomes 1, 4, and 8 have regions with significant effects for MRC resistance.

Table 1. Mean scores for traits related to symptoms of Mal de Río Cuarto disease in maize for two groups of RILs at environments where Mal de Río Cuarto is endemic.

Trait	Group*	S5†	R6
Internodes	1	0.46	0.04
	2	0.95	0.74
Enations	1	0.86	0.08
	2	2.43	1.95
Multiple ears	1	0.42	0.00
	2	1.64	1.10

* Group 1: low symptoms, group 2: high symptoms.
† S5, Sampacho 2005 and R6, Río Cuarto 2006.

Table 2. Number of microsatellites pre-selected to classify maize RILs into low and high trait value groups and percent (%) of correct classification of the discriminant function at environments where Mal de Río Cuarto is endemic.

Trait	S5*		R6	
	SSR	%	SSR	%
Internodes	4	65	4	70
Enations	10	65	3	68
Multiple ears	11	81	9	72

* S5, Sampacho 2005 and R6, Río Cuarto 2006.

Table 3. SSR markers pre-selected to classify maize RILs into low and high trait value groups at environments where Mal de Río Cuarto is endemic.

Trait	S5*†	R6
Internodes	*umc1394*, 3.01	*nc004*, 4.03
	nc009, 6.04	*phi021*, 4.03
	umc1086, 4.08	*umc1177*, 1.01
	phi063, 10.02	*umc1220*, 1.11
Enations	*bnlg1371*, 6.02	*nc004*, 4.03
	bnlg1189, 4.07	*bnlg1426*, 6.01
	phi095, 1.03	*bnlg1866*, 1.03
	phi080, 8.08	
	bnlg1225, 2.06	
	bnlg1866, 1.03	
	phi021, 4.03	
	umc1612, 4.08	
	bnlg1627, 1.02	
	umc1177, 1.01	
Multiple ears	*umc1394*, 3.01	*bnlg1225*, 2.06
	nc005, 4.05	*phi076*, 4.11
	umc1086, 4.08	*umc1177*, 1.01
	phi095, 1.03	*nc004*, 4.03
	umc1169, 1.04	*umc1741*, 8.03
	umc1304, 8.02	*nc009*, 6.04
	umc1177, 1.01	*umc1394*, 3.01
	bnlg1866, 1.03	*phi095*, 1.03
	phi021, 4.03	*phi115*, 8.03
	nc004, 4.03	
	umc1021, 1.03	

* S5, Sampacho 2005 and R6, Río Cuarto 2006.

† First name-component is SSR marker, second name-component is chromosome and bin number. SSR marker order corresponds to its relative contribution to the discriminant function.

In the Sampacho 2005 environment, *umc1177*, *phi095*, *bnlg1866*, *umc1394*, *phi021* and *umc1086* SSR markers were associated with two of three traits. In the Río Cuarto 2006 environment, three traits had *nc004* in common and *umc1177* was associated with two traits. To assess consistency across environments, *umc1177*, *phi095*, and *nc004* would be useful.

Our results are consistent with the previously reported MRC-QTL mapping using the $F_{2:3}$ from the same parental cross (Di Renzo et al., 2004) and where QTL for MRC resistance were found on chromosomes 1 and 8. In a separate study, with a different $F_{2:3}$ mapping population, Kreff et al. (J. Basic Appl. Genet. 17:41–50, 2006) found regions on chromosomes 1, 4, 8 and 10 with significant effects for MRC resistance.

Results from this work indicate that it is possible to use DA to select powerful markers that may be useful to breeders. This is a new tool for germplasm improvement providing a discriminant model to integrate the information from markers selected to classify RILs. The model can then be used to facilitate the allocation of new genotypes into groups with distinct performance for MRC resistance, as well as to identify additional markers associated with the trait. Thus far, results suggest that the complementation of DA and QTL analysis in RILs would be a good strategy to identify informative markers.

This work was supported by grants from Universidad Nacional de Río Cuarto, Agencia Córdoba Ciencia S.E. and Agencia Nacional de Promoción Científica y Tecnológica, Argentina.

SARATOV, RUSSIA
State University of Saratov

Androgenetic, matroclinic, hybrid and semi-lethal plants in progeny from cross-breeding maize and *Tripsacum*

--Zavalishina, AN; Tyrnov, VS

Tripsacum dactyloides (2n = 72) is a wild maize relative capable of apomictic reproduction, and so a cross-breeding of maize and *Tripsacum* is of interest to researchers. In our experiments 4 lines, *W23 igig, W155 fl2fl2*, *CI880 O2O2*, Brown marker *(BM)*, and 6 hybrids of maize, *W155* x *BM; W155 fl2fl2* x *BM; CI880 O2O2* x *BM; W64 fl2fl2* x *BM; (SynA O2O2* x *W64 O2O2*) x *BM; W155 O2O2* x *Tester of Mangelsdorf (TM*), were used as maternal forms, while *Tripsacum* was used as the male parent.

The maize ears were prepared two ways for pollination: 1) the husks of ears were cut, turned down and then the pistils were cut to 4 cm in length; and 2) one-third of the ear was cut together with the husks. Both pollination methods showed high seed production: 40-100%, calculating on pollinated ovaries. Dry weather seed production is better than wet weather, since traumatized ears have rot more often in wet conditions. In total, 186 ears were pollinated and 136 (73%) of them contained hybrid seeds. In all, 4566 seeds were recovered, the majority of which were little and puny. However, 37 were very large. These set on maternal forms, one of the parents of which was the line *BM.*

We did not use nutrient medium for seed germination. We put seeds in tap water for 12 hours, then removed the dense pericarp with sharp tweezers and put seeds on wet filter paper. The seedlings at the coleoptile stage were planted in pots. Then at the 3-leaf stage they were placed in soil in the greenhouse. 943 seedlings were obtained from 4566 seeds; many of them were the hybrids. The average seed germination was 21%. This quantity varied from 0% to 80% depending on genotype. 256 seeds of the maternal form *(W155 x BM)* did not germinate at all. Only one seedling among 302 seeds was found for the hybrid *(W155 O2O2* x *TM)*. The line *W23 igig* showed the highest germination--48% on average for 3 years.

Seeds that did not germinate contained endosperm without an embryo. Three matroclinic haploids were discovered among the seedlings obtained from the different maternal forms. Among the 37 large seeds, only 10 produced hybrids; others did not germinate. 12 polyembryos were discovered among the seedlings obtained from line *W23 igig*. One plant among twin seedlings was identified as an androgenetic *Tripsacum*. It had a *Tripsacum* genome and a maize cytoplasm. It developed more intensively than the hybrids.

778 seedlings among 943 perished at an early stage so they did not form roots. The rest of the 165 seedlings were planted in soil in the greenhouse. It is possible that surviving hybrids depended on maize and *Tripsacum* genome compatibility. When the maternal form was the line *BM*, 203 seedlings were produced. Among them were one matroclinic haploid, three normal hybrids and 199 semi-lethal plants. These had two small leafs during 2-6 months of their life. When a new leaf appeared, the lower one turned yellow and died off. The leaf length was about 5 cm. When the maternal form was used as a hybrid, one parent of which was *BM*, semi-lethal seedlings accounted for 50%.