

## Combining ability of flint corn inbred lines: Mal de Río Cuarto disease tolerance and grain yield

### Aptitud combinatoria para tolerancia a Mal de Río Cuarto y rendimiento en grano en líneas flint de maíz

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**Abstract.** Mal de Río Cuarto (MRC) disease is a significant threat to corn production in Argentina. Sixty six hybrid combinations derived from the cross of twelve flint maize (*Zea mays* L.) inbred lines were evaluated using a completely randomized block design with two replications. MRC disease severity grade (SG) and grain yield (GY) were the analyzed traits. Our objectives were to estimate the general (GCA) and specific (SCA) combining abilities for these traits among twelve corn inbred lines, and to identify potential heterotic relationships among them. Diallel crosses were performed and these results were compared using Griffing's method 2 model I and diallel GGE biplot (genotype main effect plus genotype x environment interaction). Highly significant effects for GCA and SCA for SG were observed, suggesting that both additive and non-additive genetic effects contribute to MRC tolerance. At the same time, GY showed significant effects only for SCA, hence non additive genetic effects are more important determinants of grain yield. Using both analysis methodologies, lines with good GCA to MRC tolerance and also heterotic crosses for this trait and GY were identified. These promising new crosses need further evaluation to assess their agronomic performance in larger field trials and establish whether they remain heterotic throughout an array of different productive environments, before they are included in future breeding efforts.

**Keywords:** *Zea mays* L.; Diallel crosses; GGE biplot; Combining ability.

**Abbreviations:** MRC: Mal de Río Cuarto; MRCV: Mal de Río Cuarto Virus; CA: combining ability; GCA: general combining ability; SCA: specific combining ability; SG: severity grade; GY: grain yield; GGE: genotype main effect plus genotype x environment interaction; PCA: principal component analysis; ATC: average tester coordinate.

**Resumen.** El Mal de Río Cuarto (MRC) es la enfermedad más importante del maíz en Argentina. En el presente estudio 66 híbridos derivados del cruzamiento de 12 líneas de maíz (*Zea mays* L.) fueron evaluados mediante un diseño experimental de bloques completos al azar con dos repeticiones. Los objetivos del trabajo fueron estimar la aptitud combinatoria general y específica (ACG y ACE) para el grado de severidad de la enfermedad (GS) y el rendimiento de grano (RG) entre doce líneas de maíz e identificar relaciones potenciales heteróticas entre ellas. El análisis dialélico se realizó mediante el método 2, modelo I de Griffing y el biplot GGE (efecto principal de genotipo más interacción genotipo x ambiente). Los efectos de ACG y ACE fueron significativos para GS, lo que sugiere que este carácter está determinado por acción génica aditiva y no aditiva. El RG sólo presentó efectos significativos de ACE, por lo que la expresión del carácter se debe a acción génica no aditiva. Con los dos métodos de análisis utilizados se identificaron líneas con buena ACG para tolerancia a MRC y además, cruzamientos heteróticos para este carácter y RG. Estos cruzamientos deben ser evaluados para conocer su comportamiento agronómico y observar si mantienen la expresión heterótica en relación con diferentes ambientes, con la finalidad de incluirlos en futuros programas de mejoramiento.

**Palabras clave:** *Zea mays* L.; Cruzamientos dialélicos; Biplot GGE; Aptitud combinatoria.

**Abreviaturas:** MRC: Mal de Río Cuarto; MRCV: Virus del Mal de Río Cuarto Virus; CA: aptitud combinatoria; GCA: aptitud combinatoria general; SCA: aptitud combinatoria específica; SG: severidad de la enfermedad; GY: rendimiento de grano; GGE: efecto principal de genotipo más interacción genotipo x ambiente; PCA: análisis de componentes principales; ATC: coordenada del tester promedio.

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## INTRODUCTION

Mal de Río Cuarto (MRC) disease is the most prevalent threat to corn production in Argentina, causing significant and frequent yield losses in susceptible hybrids (Lenardón et al., 1998). The disease has caused losses of approximately 5 to 60% in the Río Cuarto area (Córdoba state), where the disease is endemic (March et al., 1995). The worst epidemic occurred during the 1996/97 agricultural season, affecting about 300000 ha of corn, and a significantly high incidence of the disease was observed during 2006/07. According to the disease tetrahedron reported by Francl (2001), the MRC continues producing severe outbreaks in maize when MRC epidemics take place during the simultaneous occurrence of abundant natural virus reservoirs, high density and mobility of vector populations, early growth stage of susceptible genotypes, and suitable environments.

MRC disease is caused by a *Fijivirus* (Nome et al., 1981) (*Reoviridae* family), identified as “Mal de Río Cuarto Virus” (MRCV) (Distéfano et al., 2003). MRCV is transmitted in a persistent, propagative manner through a vector insect, *Delphacodes kuscheli* Fennah (*Hemiptera*: Delphacidae) (Ornaghi et al., 1993). MRC is a monocyclic disease. The initial inoculum source consists of vector insects that mainly develop in oat crops (*Avena sativa* L.), where they acquire the virus, and then migrate to maize. The disease plants present several symptoms depending on the maize genotype, the plant's developmental stage in which the vector-mediated virus transmission occurs, and the environmental conditions in which maize grows. The greatest yield losses occur when the virus infection takes place during the early stages of crop development (March et al., 1995). A characteristic symptom of MRCV infections is swellings, referred to as enations, occurring on the underside of the leaves. Other symptoms include stunting, leaf and internodes shortening, tassel and ear malformations, dwarfing, and reduced overall yield resulting in economically significant losses.

Traditional management strategies include early planting, use of chemicals to lower *D. kuscheli* population, and use of MRC tolerant hybrids. Early planting enables the avoidance of overlapping vector population peaks with susceptible maize phenological stages (e.g. coleoptile stage) (Ornaghi et al., 1999). However, recent studies observed a significant disease incidence even in very early planted corn crops (Eyherábe, 2007). Applications of systemic insecticides, which allow good vector control, and control of weedy grasses which constitute potential virus reservoirs and hosts of vector populations, can reduce the source of the pathogen. However, the extensive use of these insecticides imposes a selection pressure on the vector populations, which might cause the appearance of resistant strains. Granular insecticide formulations applied on the seed may be useful to reduce the disease incidence and increase grain yield (March et al., 2002). This management

strategy relies on the possibility of a proper control of the vector populations during their arrival to corn crops, protecting the seedlings during the stage of greatest susceptibility (Kreff, 2010). The most effective way of disease control, nevertheless, consists of the integrated management practices of MRC. Therefore, the employment of tolerant genotypes and the use of seeds treated with insecticides, as a component of integrated management, may optimize the cost-benefit equation for farmers, and increase the sustainability of maize production in affected areas (Di Renzo et al., 2002).

Corn breeding involves the cross of inbred lines in order to obtain new hybrid genotypes. Knowledge of consistent heterosis for particular traits among different genetic combinations and across environments allows for a more efficient resource allocation, and therefore a higher chance of developing agronomically interesting combinations (Nestares et al., 1999). A flint x flint heterotic pattern would be useful in breeding programs aimed at obtaining a high yielding performance. Such hybrids would also broaden the genetic base of the commercial varieties to be developed in the near future (Soengas et al., 2003). Parental lines could be selected, considering their combining ability (CA) for important traits such as disease tolerance and grain yield. Information about CA is mostly obtained through diallel analysis. This approach has been utilized in genetic research to determine the mode of inheritance of important traits among a given set of genotypes, and to identify superior parental lines for hybrid development. Malik et al. (2004), Melani & Carena (2005), Srdic et al. (2007) and De la Cruz Lázaro et al. (2010), used diallel crosses to study the mode of inheritance of maize grain yield. Lorenzo et al. (1992) and Morata et al. (2003) also detected corn inbred lines with tolerance to MRC disease and good performance for grain yield through diallel analysis.

Conventional diallel analysis is focused on partitioning the total variation of the data into general combining ability (GCA) of each parent, and specific combining ability (SCA) of each cross (Sprague & Tatum, 1942). This model has shown to be an extremely efficient tool in quantitative genetics (Gravina et al., 2003). Alternatively, Yan & Hunt (2002) proposed the use of the principal component analysis (PCA). It was originally utilized for multi-environment trial data analysis to display the genotype main effect plus genotype x environment interaction (GGE) in a single two-dimensional figure: the GGE biplot. This graphic output allows the evaluation of relevant concepts: average value of the quantitative trait and stability of the genotypes, a rapid and effective overview of GCA and SCA effects of the populations and their performance in crosses, and the grouping of genotypes with similar behavior (Bhatnagar et al., 2004; Bertoia et al., 2006; Ibañez et al., 2006).

Combining ability for MRC disease tolerance in maize has been evaluated (Lorenzo et al., 1992; Basso et al., 1995; Presello et al., 1995). However, the information provided after the evalua-

tion in a different genetic background would be valuable in future efforts for germplasm development not only for the area where MRC disease is endemic, but also for different production areas, both in Argentina and overseas. This would be possible by making these lines available as a source of genetic resources for hybrid development. The objectives of this study were to (i) estimate GCA and SCA effects for MRC disease tolerance and GY in twelve corn inbred lines, and (ii) identify best hybrid combinations and possible heterotic relationships among these lines.

## MATERIALS AND METHODS

Twelve corn flint lines were selected based on their performance to MRC disease (Table 1). Seeds were supplied by the "Instituto de Genética" at INTA Castelar (Argentina). Flint lines were crossed in the spring of 2008, following a diallel mating design without reciprocals to create 66  $F_1$  hybrids. The ears were obtained by cross fertilization to only one tassel, and then were harvested, dried, manually shelled, and kept in a controlled environment for later use in the trials. Parental lines and 66  $F_1$  hybrids were grown in the following growing season at a Río Cuarto location in the area where the MRC disease is endemic. Trials were irrigated throughout the growing season, and weed control was performed according to normal field practices. The experiment was replicated twice in a randomized complete block design.

In order to increase the scope of our study and to ensure the MRC presence, each plant was infested with about seven insect vectors of MRCV that were equally dispatched between the seedlings, avoiding inoculation escape. Previous to the infestation, the insect vectors were maintained in winter oat crops, which constitute virus reservoirs and hosts of vector populations.

Disease symptoms were recorded at 60 days after planting (initial male flowering). Plots were scored for MRC disease tolerance based on the severity grade (SG) scale proposed by Ornaighi et al. (1999). Ten to fifteen plants in the centre rows of a plot were individually rated on a scale of 0 to 3. The following disease grades were established: 0 = no symptoms; 1 = mild symptoms: presence of enations; 2 = shortened superior internodes, enations and 'hockey pole' ears; 3 = severe dwarfing, enations and small ears with few or no kernels. Grain yield was evaluated by harvesting all ears from each plot. Moisture content for each seed sample was measured using an electrical hygrometer, and yield was expressed in kg/ha.

An analysis of variance (ANOVA) for combining ability (CA) was performed to have the genotypic effect partitioned into GCA and SCA, respectively, according to Griffing (1956) method 2 model I. The software package developed by Magari & Kang (1994) was utilized. Heterosis values were estimated in terms of deviation from parental averages. Student's T-test was used to assess heterosis significance, genotypic effect, and

Table 1. Origin and particular traits for each parental flint line.  
Tabla 1. Origen y características de las líneas de maíz utilizadas.

Inbred Line	Line code	Tester code	Origin	MRC tolerance	Grain colour	Cob Colour
06-805 (BLS61)	A	A	Population consisting of a mixture of commercial hybrids released in 90s along with BLS1 and BLS14 lines	HT	Reddish	Red
06-806 (BLS91)	B	B	"Colosal" open-pollinated variety	HT	Orange	White
06-807 (BLS101)	C	C	Cross between composite population "A and B" of INTA Pergamino and BLS14 line	T	Reddish	White
06-808 (BLS76)	D	D	Population consisting of a mixture of BLS1, BLS14 and BLS16 lines	HT	Reddish	Red
06-809 (BLS96)	E	E	Population consisting of a mixture of BLS1, BLS14 and BLS16 lines	HT	Orange	White
06-810 (BLS104)	F	F	F1 between N28 north american public line and BLS14 line	LT	Orange	White
06-811 (BLS16)	G	G	"Colorado La Holandesa mejorado" open pollinated variety	LT	Yellowish	White
06-812 (BLS14)	H	H	"Colorado La Holandesa mejorado" open pollinated variety	HT	Orange	White
06-813 (BLS1)	I	I	"Colorado La Holandesa mejorado" open pollinated variety	HT	Reddish	White
06-814 (LP109)	J	J	INTA Pergamino line	T	Reddish - Pale	Red
06-815 (LP521)	K	K	INTA Pergamino line	T	Orange	Red
06-816 (LP125R)	L	L	INTA Pergamino line	T	Yellowish	Red

HT: high tolerance; T: tolerant; LT: low tolerance.  
HT: tolerancia alta, T: tolerante, LT: tolerancia baja

**Table 2.** GCA effects (main diagonal), SCA effects (above diagonal) and midparent heterosis (below diagonal). Trait: SG (Severity Grade).  
**Tabla 2.** Efectos de aptitud combinatoria general en la diagonal principal, aptitud combinatoria específica por encima de la diagonal y heterosis del padre medio debajo de la diagonal para el grado de severidad de la enfermedad MRC en maíz.

Lines	BLS61	BLS91	BLS101	BLS76	BLS96	BLS104	BLS16	BLS14	BLS1	LP109	LP521	LP125R										
BLS61	0,02	ns	-0,23	**	-0,35	**	0,23	**	0,67	**	-0,32	**	-0,09	ns	-0,25	**	-0,28	**	-0,61	**	-0,23	**
BLS91	-1,52	**	0,07	ns	-0,70	**	-0,36	**	0,32	**	-0,20	**	-0,37	**	0,41	**	-0,58	**	-0,49	**	-0,48	**
BLS101	-1,89	**	-2,16	**	0,00	ns	-0,03	ns	-0,89	**	0,48	**	0,12	*	0,01	ns	-0,33	**	0,28	**	-0,57	**
BLS76	-1,71	**	-1,88	**	-2,05	**	-0,09	ns	-0,77	**	-0,32	**	-0,39	**	0,15	**	-0,61	**	0,07	ns	-0,30	**
BLS96	-0,79	ns	-1,61	**	-1,24	**	-0,08	ns	-0,71	**	-0,20	**	-0,28	**	-0,14	*	-0,21	**	-0,32	**	-0,03	ns
BLS104	-0,39	ns	-0,96	*	-2,13	**	-1,74	**	0,37	**	-0,46	**	-0,74	**	-0,48	**	0,68	**	0,10	ns	0,17	**
BLS16	-1,43	**	-1,54	**	-0,80	ns	-1,72	**	-1,58	**	-0,20	**	-0,11	*	-0,02	ns	-0,37	**	-0,26	**	-0,49	**
BLS14	-0,73	ns	-1,23	**	-0,68	ns	-1,31	**	-0,88	*	-1,38	**	0,04	ns	-0,15	**	1,34	**	-0,16	**	0,44	**
BLS1	-1,06	*	-0,60	ns	-0,95	*	-0,92	*	-1,28	**	-0,87	*	-0,52	ns	-0,39	**	-0,21	**	-0,19	**	-0,18	**
LP109	-0,88	*	-1,40	**	-1,10	*	-1,49	**	-0,78	ns	0,09	ns	1,17	**	-0,54	ns	0,08	ns	-0,29	**	0,63	**
LP521	-1,70	**	-1,80	**	-0,97	*	-1,29	**	-1,37	**	-0,97	*	-0,81	ns	-1,01	*	-0,91	*	0,09	ns	-0,29	**
LP125R	-1,10	*	-1,57	**	-1,62	**	-1,46	**	-0,87	*	-0,69	ns	-1,41	**	-0,79	ns	0,23	ns	-1,18	**	0,07	ns

\* p<0.05; \*\* p<0.01; ns = non-significant.  
 \* p<0.05; \*\* p<0.01; ns = no-significativo.

**Table 3.** Parent means (main diagonal), SCA effects (above diagonal) and midparent heterosis (below diagonal). Trait: Grain Yield (Gy) (kg/ha).  
**Tabla 3.** Valor promedio de las líneas en la diagonal principal, efectos de aptitud combinatoria específica por encima de la diagonal y heterosis del padre medio debajo de la diagonal para rendimiento de grano en maíz.

Lines	BLS61	BLS91	BLS101	BLS76	BLS96	BLS104	BLS16	BLS14	BLS1	LP109	LP521	LP125R												
BLS61	1366	-2610	**	6331	**	5275	**	-98	ns	367	ns	-1000	**	4479	**	-550	ns	2396	**	3056	**	3388	**	
BLS91	5596	**	3510	**	-3901	**	139	ns	-734	ns	1134	**	4406	**	4472	**	-3256	**	4814	**	3530	**	3795	**
BLS101	13144	**	600	ns	4930	**	3188	**	-4231	**	2398	**	-2618	**	-1986	**	1780	**	641	ns	1116	**	3497	**
BLS76	15035	**	7589	**	9244	**	1865	**	-3384	**	2853	**	-1579	**	-691	ns	1940	**	2647	**	576	ns	7040	**
BLS96	5344	**	2397	*	-2492	*	1301	ns	9427	**	2405	**	1726	**	-623	ns	994	*	-540	ns	4789	**	437	ns
BLS104	9856	**	8312	**	8182	**	11585	**	6821	**	936	**	5128	**	108	ns	4733	**	-2043	**	2972	**	-3137	**
BLS16	8416	**	11512	**	3094	**	7080	**	6070	**	13517	**	1754	**	3882	**	-2451	**	1867	**	2002	**	5272	**
BLS14	10326	**	8008	**	156	ns	4398	**	150	ns	4927	**	8629	**	10700	**	501	ns	-3791	**	-3442	**	-554	ns
BLS1	7603	**	2587	**	6231	**	9337	**	4076	**	11860	**	4603	**	3986	**	4630	**	-3216	**	6257	**	4851	**
LP109	12149	**	12256	**	6690	**	11643	**	4139	**	6681	**	10520	**	1292	ns	4174	**	2606	**	6290	**	8913	**
LP521	12459	**	10622	**	6815	**	9222	**	9118	**	11347	**	10305	**	1290	ns	13298	**	14930	**	3200	**	-10571	**
LP125R	14379	**	12475	**	10784	**	17274	**	6355	**	6825	**	15164	**	5766	**	13480	**	19140	**	-694	ns	0	ns

\* p<0.05; \*\* p<0.01; ns = non-significant.  
 \* p<0.05; \*\* p<0.01; ns = no-significativo

combining ability (Melani & Carena, 2005). Visualization of CA among parental lines was obtained by using principal component analysis (PCA), as proposed by Yan & Hunt (2002). The GCA and SCA effects of the entries were examined by defining an average tester coordinate (ATC). Polygon view biplots were generated, partitioning the biplot into sectors, with entries farthest from the center of the biplot representing the vertices of the polygon. Relative contribution of GCA and SCA was estimated according to Baker (1978). The MRC effect on grain yield was evaluated through coefficients of phenotypic correlation between SG and GY.

## RESULTS AND DISCUSSION

During the 2009/10 growing season, environmental conditions in Río Cuarto location were favorable for the disease development. Plants were infested with insect vectors of MRCV, allowing a strong disease pressure, and the discrimination between the different tolerance levels of the lines and the hybrids. Line BLS14 (red flint line, source of tolerance to MRC) showed good performance for both severity grade of MRC and grain yield traits. In contrast, the genotypes with low tolerance, such as line BLS104, showed a poor behavior for tolerance to MRC and grain yield (Tables 2 and 3).

A strong disease pressure represents a stress to assess grain yield. It implies a limitation on the scope of our results because of the difficulty to simultaneously breed for both tolerance to MRC and grain yield. Selection for grain yield under a strong disease pressure resulted in a different response of the evaluated lines and hybrids. Therefore, the same genotypes should be evaluated in other environments under optimal conditions, and without disease pressure or in areas where the disease is non endemic, for a more adequate selection for grain yield. On the other hand, factors that are of economic relevance may be related to complex or polygenic characteristics, and show a high influence of the environment. Because of this, in breeding programs, various experiments are conducted in several locations to evaluate grain yield and detect changes in the relative performance of the genotypes in different environments. This phenomenon is called genotype x environment interaction (G x E). This study was conducted at a single testing environment. It means that the study should be replicated in multiple locations to evaluate the stability of grain yield and to estimate G x E. Kandus et al. (2010) reported statistical models for evaluating the G x E interaction in materials conformed by six BLS lines and two INTA Pergamino lines. They observed G x E interaction in these materials, and suggested that their evaluation in representative conditions of the crop area is very important for identifying genotypes that are more stable and have long-term yields. Lorenzo et al. (1992) also reported G x E for resistance to MRC and grain yield in eight INTA Pergamino lines and their crosses.

This study was conducted using fixed effects models. For this reason, the following results should be taken with caution. An analysis of the variance mean squares of diallels for severity grade (SG) and grain yield (GY) was performed (data not shown). Significant differences ( $p \leq 0.01$ ) were detected among genotypes for both SG and GY. Regarding SG (MRC tolerance), significant effects ( $p \leq 0.01$ ) for GCA and SCA effects were observed, suggesting that both additive and non-additive inheritance modulate this trait. On the other hand, GY showed significant effects only for SCA ( $p \leq 0.01$ ), indicating that only non-additive genetic effects (dominance, epistasis) are responsible for its mode of inheritance.

**MRC tolerance (SG).** The variance component ratio  $2\sigma_g^2 / (2\sigma_g^2 + 2\sigma_s^2)$  discussed by Baker (1978) offers some insight into the relative importance of additive and dominance genetic variance for a given set of parents or a defined population. A value of 1 indicates that all the genetic variance is additive. For SG, this ratio was 0.15, indicating that non-additive effects predominated in the inheritance of this trait. Marino & Teyssandier (1982) and Di Renzo et al. (2002, 2004) reported similar ratios, and these authors concluded that the MRC tolerance was dominantly inherited. However, Morata et al. (2003), Presello et al. (1995) and Lorenzo (1992, 1993) concluded that additive and non-additive actions have significant effects in the inheritance of MRC tolerance.

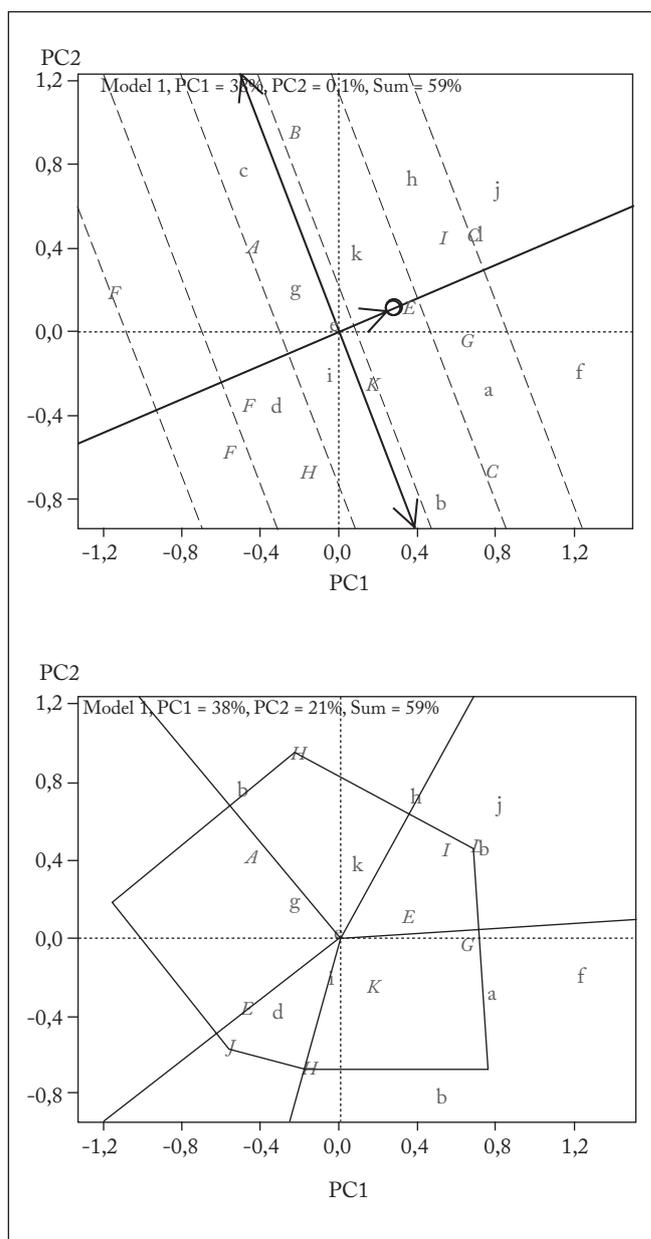
With regards to the individual effects for each line combination, the ANOVA showed significant differences among genotypes. Considering this result, GCA and SCA effects for each line and hybrid were evaluated (Table 2). Significant differences were also observed for midparent heterosis values (Table 2). Negative values of GCA, SCA and heterosis suggest tolerance to MRC, whereas positive numbers suggest susceptibility. The lines showed significant positive ( $p \leq 0.01$ ) and negative ( $p \leq 0.05$ ) GCA effects. BLS104 was the most positive line (0.37), indicating low tolerance for the disease, and BLS1 was the most negative line (-0.39), indicating a good performance for MRC and could be therefore considered in future breeding efforts for tolerance to the disease. These results are consistent with the information reported in Table 1, where lines BLS104 and BLS1 are characterized by low and high tolerance, respectively. While the GCA effects are generally not significant (Table 2), lines BLS76 and BLS96, both derived from populations consisting of a mixture of BLS1, BLS14 and BLS16 lines, showed negatives effects, supporting the expected results according to previously information presented. Di Renzo et al. (2002) observed tolerance to MRC in the line BLS14. In our study, this line showed negative values for SCA effects, indicating a good performance for severity grade of MRC. In fact, BLS14 produced superior hybrids that were more tolerant than the line itself. Line BLS16, characterized by low tolerance, showed a negative value for GCA effects. Therefore, this line together with BLS14 and BLS1 (same origin, Colorado La Holandesa improved) presented

a good performance to MRC. In contrast, genotypes LP521, LP109 and LP125R from INTA Pergamino showed a poor performance to MRC.

**Grain yield (GY).** According to our results, relative contribution of additive effects for GY inheritance was null, indicating that non-additive effects are responsible for the inheritance of this trait in the genotypes evaluated in the environment located within the area where MRC disease is endemic. Although Melani & Carena (2005) and De la Cruz Lázaro et al. (2010) found that SCA for GY was non-significant, our data match the results reported by Bhatnagar et al. (2004), Nestares et al. (1999), Malik et al. (2004) and Srdic et al. (2007), because they concluded that SCA effects were highly significant for GY. SCA effects and midparent heterosis values for GY for individual genotypes are shown in Table 3. Negative numbers for CA and heterosis suggest low GY, whereas positive values imply higher GY. Significant differences were observed between the lines for grain yield. The line BLS14 showed the highest values of parent means for grain yield (10700), and lines BLS96, BLS1 and BLS76 also presented high values of parent means for this trait (9427, 4630, and 1865, respectively). Kandus et al. (2010) reported that the BLS14 line showed a greater specific adaptation in the environment evaluated, and obtained a greater yield. Line BLS104 showed one of the lowest values of parent means (936). These results suggest a possible positive relationship between tolerance of MRC and grain yield.

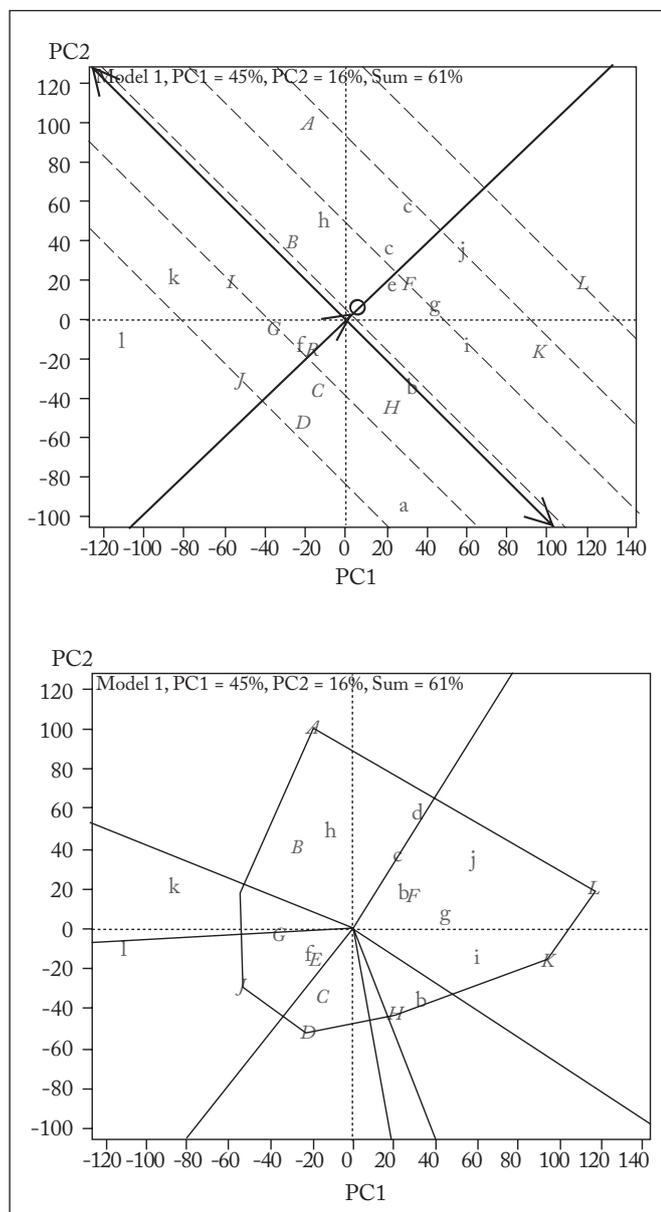
**SG-GY.** Tables 2 and 3 show crosses with significant positive ( $p \leq 0.01$ ) and negative ( $p \leq 0.05$ ) SCA effects for the traits under analysis, SG and GY. The hybrids with the highest and most significant ( $p \leq 0.01$ ) SCA effects for SG and GY were BLS101 x BLS104 (-0.89) and LP109 x LP125R (8913). The hybrid combination with the best SG performance in terms of virus tolerance also showed a significant positive effect for GY (2398\*\*), and the hybrid with the highest GY effect showed a significant positive effect for SG (0.63\*\*). Midparent heterosis values emphasized these results. Hybrids BLS101 x BLS104 (-2.13\*\*) and BLS91 x BLS101 (-2.16\*\*) presented the highest performance in terms of virus tolerance for SG, and hybrid LP109 x LP125R (19140\*\*) was the best for GY. Kandus et al. (2010) found a type of hybrid formed by INTA Pergamino lines (LP109 x LP521) with a good performance for GY, and Lorenzo et al. (1992) also reported other hybrid formed by INTA Pergamino lines with tolerance to MRC and a good performance for GY.

**GGE / Griffing analysis. SG.** GGE biplot for SG data explained 59% (38 and 21% by PC1 and PC2, respectively) of total variation (Figs. 1A and B). GCA effects for the different entries resulted as follows:  $D > I > G > C > E > B > K > A > H > L > J > F$  (Fig. 1A), which is roughly consistent with Table 2. The projections of the entries onto the ATC ordinate must



**Fig. 1.** Biplot based on diallel data of twelve maize genotypes for Severity Grade; (A) average tester ordination view, (B) polygon view. Codes of the genotypes are: A = BLS61, B = BLS91, C = BLS101, D = BLS76, E = BLS96, F = BLS104, G = BLS16, H = BLS14, I = BLS1, J = LP109, K = LP521 and L = LP125R. Genotypes are labeled with upper-case letters when viewed as entries and with lower-case letters when viewed as testers. The circle indicates the average tester.

**Fig. 1.** Biplot GGE basado en datos dialélicos de doce líneas de maíz para grado de severidad, (A) Coordenadas del tester promedio, (B) Vista del polígono. Los códigos para los genotipos son: A = BLS61, B = BLS91, C = BLS101, D = BLS76, E = BLS96, F = BLS104, G = BLS16, H = BLS14, I = BLS1, J = LP109, K = LP521, y L = LP125R. Los genotipos se indican como entradas cuando están en letras mayúsculas y como testers cuando están en letras minúsculas. En A el círculo indica el tester promedio.



**Fig. 2.** Biplot based on diallel data of twelve maize genotypes for Grain Yield; (A) average tester ordination view, (B) polygon view. Codes of the genotypes are: A = BLS61, B = BLS91, C = BLS101, D = BLS76, E = BLS96, F = BLS104, G = BLS16, H = BLS14, I = BLS1, J = LP109, K = LP521 and L = LP125R. Genotypes are labeled with upper-case letters when viewed as entries and with lower-case letters when viewed as testers. The circle indicates the average tester.

**Fig. 2.** Biplot GGE basado en datos dialélicos de doce líneas de maíz para rendimiento de grano; (A) Coordenada del Tester promedio, (B) vista del polígono. Los códigos para los genotipos son: A = BLS61, B = BLS91, C = BLS101, D = BLS76, E = BLS96, F = BLS104, G = BLS16, H = BLS14, I = BLS1, J = LP109, K = LP521, y L = LP125R. Los genotipos se indican como entradas cuando están en letras mayúsculas y como testers cuando están en letras minúsculas. En A el círculo indica el tester promedio.

approximate their SCA effects, which represent the tendency of the entries to produce superior hybrids with specific testers. Entries B, F, A, D and I had the highest SCA effects, because they interacted positively with testers c, h, j, l, k and g, but negatively with themselves. Two heterotic groups are suggested by Fig. 1A, genotypes B, F, A, D and I as one group, and genotypes L, J, K, G, H and C as the other. Entry E located near the ATC abscissa, and did not appear to belong to any of the groups. With the GGE biplot was possible to define these groups, complementary information to the conventional diallel analysis. The biplot in Fig. 1B was divided into six sectors, with entries B, F, J, H, C and D as the vertex entries, and are referred to as sectors B, F, J, H, C and D, respectively. Testers k, h and c fell within sector B, indicating that entry B was the best mating partner for them. Moreover, since genotype B, as a tester, was not in sector B, all the crosses within this sector should be better than both parents. The term heterosis is used hereafter to refer to such situations. Tester g fell in sector F; tester d in sector J; tester i in sector H; testers a, f and b in sector C, and finally testers j and l fell in sector D. Consequently, eleven superior hybrids were identified: B x [k, h, c]; F x g; J x d; H x i; C x [a, f, b]; D x [j, l]. In sector B, genotype C was considered the best mating partner. Likewise, in sector C, genotype B was considered the best partner. Therefore, B and C were identified to be the best partners for each other. The same situation is verified for D and J; B x C and D x J are thus to be considered the best combinations among all the tested lines using both Griffing model I and GGE biplot. Entries B and D belong to the first heterotic group defined, and C with J to the other group defined (Fig. 1A).

The performance for these crosses can be verified from the SCA effects and midparent heterosis (Table 2). However, consistency between Griffing and GGE is not conserved across every combination. For instance, Table 2 suggests heterosis for crosses E x f and F x h. Such discrepancies are expected because 41% of total variation is not accounted for by the biplot approach. Predictions are probably more reliable than the individual observations since the biplot displays and makes predictions on the general pattern of the whole dataset (Yan & Hunt, 2002).

**GY.** GGE biplot analysis for GY data explained 61% (45% and 16% by PC1 and PC2, respectively) of total variation (Figs. 2A and B). The biplot in Fig. 2A suggests two heterotic groups for grain yield. Some entries of these groups coincides with the definite entries for heterotic groups to SG, for example entries A, B and I correspond at the first definite group and entries L, K, H and C correspond at the second definite group. The entries A, B and I interacted negatively with themselves, as SG trait, but positively with testers c, d, f, h, k and l. The biplot in Fig. 2B was divided into seven sectors, A, I, J, D, H, K and L. Testers i, g, e and j fell within sector L, suggesting that entry L was the best mating partner for these testers. Moreover, tester l is located within sector J. Crosses between

J x L and I x K could be considered as promising. Combinations H x b and A x h resulted heterotic as well. These results agree with Griffing analysis (Table 3). Entries A, B, I and J, and the entries H, L and K belong to the first and second heterotic groups defined, respectively (Fig. 1A).

Coincidences between Griffing and PCA analysis are not to be used to conclude that GGE biplot is redundant and therefore unnecessary. On the contrary, these results show that PCA is an excellent tool to visualize relationships that remain unnoticed otherwise. As an example, and with regards to GY, GGE biplot shows that lines A and L are equally fit for developing hybrid combinations with testers c and d. The biplot approach has two advantages: the first of them is its graphical presentation of the data, which greatly enhances our ability to understand the patterns of the data and the second advantage of the biplot approach is that it tries to interpret the phenotypic variation of the crosses by understanding the parents.

A significant and negative ( $r = -0.26^{**}$ ) correlation between SG and GY was observed under strong disease pressure, thus suggesting that these characters have some degree of interdependency. It also indicates that lines with low values SG (high tolerance) tended to produce higher yields than lines with high values SG (low tolerance). Morata et al. (2003) obtained similar results. They found that under a moderate disease pressure, correlation coefficient for SG and GY was -0.29; under a high disease pressure such correlation increased up to -0.65, and under low disease pressure the severity of the MRC did not influence grain yield. All these results illustrate the significance of MRC disease as a very important factor influencing GY in the study population, and this relation should be considered when breeding for MRC tolerance. This study showed that the coefficient correlation between SG and GY depends of the disease pressure as well as the genetic structure genetics, as reported by Morata et al. (2003).

In agreement with Soengas et al. (2003), our results showed heterosis for crosses involving different flint maize inbred parents. This study suggests the feasibility of developing hybrid combinations between the lines evaluated with a good performance for grain yield and favorable performance for MRC tolerance.

## CONCLUSIONS

The results of this experiment were obtained from a fixed effects model, therefore the conclusions are for the materials tested in the environment of evaluation. Both GCA and SCA effects were significant for SG, suggesting additive and non-additive type of inheritance for MRC tolerance. GCA effects were detected for GY, suggesting that only non-additive factors determine the inheritance for this trait.

The present study was successful at detecting flint corn hybrids with MRC tolerance and reasonably good GY. The crosses showing good agronomic and sanitary performance

should be further evaluated, in order to establish whether they remain heterotic across different productive environments.

It is interesting to note that both conventional diallel analysis (Griffing) and PCA methodologies were useful and complementary for detecting lines and crosses with good GCA and SCA, respectively, for MRC tolerance and grain yield.

This work attempted to identify germplasm with both MRC tolerance and good grain yield with the aid of a cross diallel design, which has enabled the generation of genetic estimates that may be considered preliminary, but extremely valuable for practical breeding.

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