

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

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

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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*, *C1880 O2O2*, Brown marker (*BM*), and 6 hybrids of maize, *W155 x BM*; *W155 fl2fl2 x BM*; *C1880 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 leaves 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%.

Only a few of the hybrid plants between maize and *Tripsacum* reached the flowering stage. They had 46 chromosomes. These were large, powerful, bushy plants. Some of them grew some years in greenhouse conditions. They were characterized by full male sterility and partial female fertility.

It has been ascertained also that if the maize has the color genes *A B Pl R*, hybrid kernels and hybrid plants have purple color. Possibly, *Tripsacum* has a gene analogous to the dominant maize gene *A1*. This fact allows the use of genetic markers for discovering apomicts among hybrids of maize and *Tripsacum*.

Megagametophyte investigation of tetraploid maize

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Tetraploid maize female gametophytes have not been investigated sufficiently. We carried out the analysis of 830 embryo sacs (*ES*) of tetraploid maize form *KrP-1* (population-1 from Krasnodar). *ES* of tetraploids, as a rule, had a structure typical for maize and consisted of a three-celled egg apparatus, the central cell with 2 polar nuclei or one central nucleus and antipodal complex. The characteristic peculiarity of tetraploids in comparison with diploids was the increase of cell, nucleus and, correspondingly, gametophyte sizes. Anomalous *ES* were discovered in 4 of 6 plants examined. The frequency of anomalous *ES* formation in tetraploids varied from 0% to 2.7%. In total, 12 anomalous *ES* were revealed. *ES* with additional polar nuclei (3-4 nuclei) and *ES* with anomalous position of polar nuclei prevailed. *ES* with egg-like synergids, and *ES* with additional nucleoli in the egg and polar nuclei were also discovered. In one ovule, the arrest of *ES* development at a one-celled stage was noted. In tetraploids, in contrast to diploids, the growth of antipodal complex cells was discovered. In one case, cells did not grow so considerably, increasing at a rate of 2-3 times. The structure and morphology of growing cells were similar to the rest of the antipodal cells. In other cases, antipodal complex cells grew considerably more, achieving 2/3 *ES* size. These growing cells were similar to central cells in their morphology. They contained large vacuoles and large nuclei, morphologically similar to polar nuclei. Growing cells always adjoined the antipodal complex. In most cases, growing cells were one-nuclear, and rarely two-nuclear. Cells with 3, 4, 6, 7, 8 and 13 nuclei were also discovered. More often, one cell, rarely two cells grew in the ovule. In one ovule, the growth of three cells was noted. The number of ovules with large growing cells varied from 3.4 to 26.4.

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Combining ability analysis for *turcicum* leaf blight (TLB) and other agronomic traits in maize (*Zea mays* L.) in the high altitude, temperate conditions of Kashmir

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Strategies for developing high-yielding cultivars resistant to *turcicum* leaf blight (TLB, *Exserohilum turcicum*; Northern Leaf blight) is one of the major objectives for our high altitude maize breeding programme. Primary breeding objectives also include: (1) earliness, due to the seasonal limitations of high altitude; (2) good performance under low moisture, critical when the temperature drops abruptly in the latter stages of crop growth; and (3) resistance to lodging, as determined by plant height and ear placement.

Three replications each of forty-five half diallel cross combinations were evaluated along with 10 parents (Table 1) at two locations, Larnoo and Khudwani, representing different altitudes with a temperate ecology. Each entry was sown in two 5 m length rows at a spacing of 60 cm. Plantings for each replication and location included 50 plants for each genotype (83333 per hectare basis). Days to 50 percent pollen shed and silking were determined on a plot basis. Plant height (cm), ear height (cm) and moisture content (%) were measured for five randomly selected plants. Grain yield (kg/plot) was adjusted to 14% moisture. The disease severity was recorded for five randomly selected plants from each plot for crosses and 10 plants for parents using a 1-9 rating scale based on the percent of the leaf area affected of adult plants: 0, 1, 10, 20, 30, 40, 60, 80 and >80 percent, respectively, per Payak and Sharma (In: Proc. Twenty Fourth Workshop of All India Coordinated Maize Improvement Project, IARI, New Delhi, 1981). Inoculations were prepared from infected leaf tissue from a farmer's field and made at the mid-silking stage. The first evaluations were made 15 days later, and thereafter, weekly for 4 weeks. Two leaves were evaluated, the ones immediately above and below the ear leaf, as these have impact on yield (Bowen and Pedersen, Plant Dis. 72:952-956, 1988). The percent disease index was calculated by using the formula suggested by McKinney (J. Agric. Res. 26:195-218, 1923). Combining ability analysis was carried out according to Model I, Method II of Griffing (Australian J. Biol. Sci. 9:463-493, 1956).

Table 1. Estimates of GCA effects for TLB and other agronomic traits in inbred lines in maize.

Parents	Pedigree	Disease severity	Grain yield	Days to 50% pollen shed	Days to 50% silking	Moisture content	Plant height	Ear placement
1	PMI-1	-1.52* (17.24)	0.29*	-1.56*	-1.64*	-0.22*	-0.14	-4.77**
2	PMI-26	-1.76** (17.24)	-0.03	-0.45**	-0.12	-0.76**	4.27**	-6.69**
3	PMI-47	-4.47** (19.89)	-0.04	0.06	0.35**	0.44**	6.04**	5.74**
4	PMI-53	2.32** (42.50)	0.26*	-0.10	-0.56**	0.19*	1.98**	-0.70**
5	PMI-83	0.61 (35.32)	-0.14	-0.21*	1.14**	-0.11	-1.01**	4.97**
6	PMI-135	1.38 (36.47)	-0.02	-1.37**	-0.97**	-0.05	8.56**	2.35**
7	PMI-198	2.56** (18.50)	0.05	1.76**	-1.64**	0.58**	1.62**	-3.98**
8	PMI-199	2.17** (40.77)	0.10	2.14**	1.43**	-0.16*	10.46**	1.66**
9	PMI-224	-1.85** (16.32)	0.25*	-0.02	-0.81**	-0.66**	15.02**	9.64**
10	PMI-401	2.48** (36.22)	0.43**	-4.00**	-2.41**	-1.48**	-34.37**	-9.62**
SE gi		0.43	0.11	0.06	0.05	0.08	0.10	0.10
SE gi-gj		0.58	0.17	0.09	0.08	0.13	0.15	0.15

Parents 1, 2, and 6 are indigenous; 9 is a local line; 3, 4, 5, 7, 8, and 10 are CIMMYT lines.

*, **significant at 5% and 1% level, respectively; parentheses (percentage disease score);