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Breeding potential of early-maturing flint maize germplasm adapted to temperate conditions

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Abstract

Early-maturing flint maize germplasm could be an important alternative for the development of new early-maturing commercial hybrids adapted to cooler summer regions. Our objective was to evaluate the performance of flint populations in crosses with four testers from different heterotic groups, as sources of new potential inbred lines. Ten flint maize populations were crossed to four inbred testers representing Reid Yellow Dent, Lancaster Sure Crop and two Spanish flint heterotic groups. Topcrosses were evaluated in four environments in north eastern Spain in 2001 and 2002. The main type of gene action expressed was additive. All populations except Enano Norteño/Vasco and Hembrilla Norteño/Vasco, had a higher specific combining ability in crosses with Reid Yellow Dent or Lancaster Sure Crop testers. Average yields were better when populations were crossed with dent lines rather than with the flint inbred line EP42. These results could be useful in the development of new inbred lines that would increase genetic variability present in commercial European flint maize.

Additional key words: general combining ability, heterotic patterns, specific combining ability, testcrosses, *Zea mays*.

Resumen

Potencial para la mejora de germoplasma de maíz precoz liso adaptado a condiciones de clima templado

La evaluación de germoplasma liso precoz de maíz podría ser importante como una alternativa para el desarrollo de nuevos híbridos comerciales precoces adaptados a regiones templadas y potencialmente útiles para producir grano de alta calidad. Nuestros objetivos fueron evaluar el potencial de poblaciones lisas precoces y estimar el rendimiento de este germoplasma en cruzamientos con probadores de diferentes grupos heteróticos para el desarrollo de híbridos precoces de maíz grano. Diez poblaciones de maíz grano se cruzaron con cuatro probadores que representan Reid Yellow Dent, Lancaster Sure Crop y dos grupos heteróticos europeos. Los cruzamientos fueron evaluados en cuatro ambientes en el noreste de España durante 2001 y 2002, siendo la expresión génica expresada aditiva. Todas las poblaciones cruzadas, excepto Enano Norteño/Vasco y Hembrilla Norteño/Vasco, mostraron una mayor aptitud combinatoria específica en cruzamientos con probadores Reid Yellow Dent o Lancaster Surecrop. El mejor rendimiento medio se produjo cuando todas las poblaciones se cruzaron con las líneas dentadas en lugar de con la línea lisa EP42. Estos resultados podrían ser útiles para desarrollar nuevas líneas puras que incrementen la variabilidad presente en el maíz liso comercial europeo.

Palabras clave adicionales: aptitud combinatoria específica, aptitud combinatoria general, patrones heteróticos, probadores, *Zea mays*.

Introduction

The development of successful maize hybrids requires the establishment of heterotic patterns, defined as the cross between known genotypes that express a high level of heterosis (Carena and Hallauer, 2001). The most exploited heterotic pattern is the cross between Iowa Stiff Stalk Synthetic (ISSS, type Reid) and the Lancaster Sure Crop heterotic groups (Barata and Carena, 2006). The Reid × Lancaster cross is a common scheme used in hybrid production for Spain and other areas of southern Europe. Reid Yellow Dent and Lan-

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Abbreviations used: GCA (general combining ability), SCA (specific combining ability).

caster Sure Crop are open-pollinated cultivars that provide most of the germplasm used to develop early inbred lines that are used for commercial hybrid seed production. Crosses among inbred lines that derive from unrelated heterotic groups are known to have better grain yield than those crosses among lines of the same group (Melchinger, 1999). Most varieties of cultivated maize in southern Europe are single crosses between European flint and United States dent germplasm, which combine early vigour, earliness, resistance to stem lodging and resistance to drought stress of European inbreds with the high grain yield of American inbreds (Ordás, 1991). European germplasm presents an important amount of variability, principally for key traits for maize adaptation to European conditions, which are cold tolerance, early vigour and early maturity. Spain has maize germplasm with most variability among European countries (Rebourg *et al.,* 2003; Revilla *et al.,* 2006). Reif *et al.* (2005) stated that genetic variation within and among maize varieties in central Europe has decreased significantly over the last fifty years. Flint kernels have a better ability than other kind of maize kernels for the development of high-quality flour. These combinations permitted the expansion of hybrid maize into central Europe.

Several studies have demonstrated a good level of heterosis between Yugoslavian and Corn Belt germplasm (Misevic, 1990; Radovic and Jelovac, 1995). Messmer *et al.* (1992) found high genetic diversity in early European maize inbreds. Ordás (1991), Álvarez *et al.* (1993) and Sinobas and Monteagudo (1996) found high heterosis in crosses between Spanish and Corn Belt germplasm for grain yield. However, most of the flint maize germplasm used came from a few flint maize lines of limited diversity (Messmer *et al*., 1993). The majority of the flint germplasm had, at least, the European elite lines EP_1, F_2 or F_7 in their pedigree (Moreno-González, 1988). Little is known about the genetic variability and genetic relationships in European maize germplasm. Ordás (1991) outlined the heterotic pattern northern Spain × southern Spain. Germplasm from northern Spain is pure flint while southern Spain germplasm is mostly semident.

At present, few commercial hybrids have been produced exclusively from European flint inbreds (Soengas *et al*., 2003b). It would be interesting to find unique flint heterotic patterns exclusively because its endosperm makes it useful to produce early maize hybrids with high quality flour. Cartea *et al.* (1999) found that a second cycle of inbred lines preserve the European flint heterotic group and the flint lines showed some differences on the basis of their combining ability in crosses with United States inbreds from different heterotic groups. Soengas *et al.* (2003a) produced a diallel cross with Spanish populations from wet and dry areas and detected good heterosis among some of them. Soengas *et al.* (2003b) also found better yields in crosses between flint germplasm, with seven dent Spanish races (Sánchez-Monge, 1962), than with European flint germplasm.

The objective of this work was to evaluate the performance of a group of 10 flint maize populations in crosses with four testers belonging to different heterotic groups, as sources of new potential inbred lines.

Material and methods

Ten populations of flint maize were chosen for this study (Table 1). Five of them, Alegia, Azkoitia, Berrobi,

Table 1. Geographical origin of Spanish flint maize populations crossed to four inbred lines

Maize population	Geographic origin	Origin
Alegia	Guipúzcoa (North east Spain)	Estación Experimental de Aula Dei, Spain
Azkoitia	Guipúzcoa (North east Spain)	Estación Experimental de Aula Dei, Spain
Berrobi	Guipúzcoa (North east Spain)	Estación Experimental de Aula Dei, Spain
Enano Norteño/ Vasco	Asturias and País Vasco (North of Spain)	Misión Biológica de Galicia, Spain
EZS20	Cantabria coast (North of Spain)	Estación Experimental de Aula Dei, Spain
EZS22	León and Asturias (North west Spain)	Estación Experimental de Aula Dei, Spain
Getaria	Guipúzcoa (North east Spain)	NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Spain
Hembrilla Norteño/Vasco	Cantabria coast (North of Spain)	Misión Biológica de Galicia, Spain
Lazkano	Guipúzcoa (North east Spain)	NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Spain
Vasco	Cantabria coast (North of Spain)	Misión Biológica de Galicia, Spain

Getaria and Lazkano, are cultivars from Guipuzcoa, Basque Country (Ruiz de Galarreta and Álvarez, 2001); three of them, Enano Norteño × Vasco, Hembrilla Norteño × Vasco, and Vasco, are Spanish races that represent germplasm formerly grown in the North of Spain (Sánchez-Monge, 1962). Populations EZS20 and EZS22 are two synthetics created from several adapted varieties from northern Spain and obtained at the Experimental Station of Aula Dei. Population EZS20 was formed from 52 mid-maturing varieties with short ears while EZS232 represents 13 early-maturing varieties with long ears. All the cultivars are adapted to the climate of north eastern Spain which is characterized by wet, cool springs and short growing seasons.

In 2000, each cultivar was crossed to each of the four inbreds lines (Table 2). The inbred lines were used as females and the cultivars as male parents. To obtain seed of each of the 40 testcrosses, 15 rows per variety and 5 rows per inbred line were sown. During pollination, pollen was collected and mixed from plants of each cultivar. A minimum of 40 tassels and a maximum of 120 tassels per population were used. Inbred lines were pollinated with each pollen bulk.

The entries in the populations cross trial were the 40 resulting testcrosses, the ten populations crossed with four inbred lines, and two checks hybrids, H125: [(A639 × A638) × W182B)] and H147: [(A239 × A251) × A635)]. These 42 entries were sown in 2001 and 2002 at two locations in Alava, Basque Country, in north eastern Spain: Arkaute (550 m above sea level) and Iturrieta (900 m above sea level). Both locations have a moist climate with an annual rainfall of about 800 mm.

A randomized complete block design, with two replicates, was used at each location. Cultural operations, fertilization, and pest and weed control followed local practices. All trials were machine-sown in mid-May in both years, at a final density of 66,000 plants ha^{-1}. Each plot consisted of two 5 m rows at 0.75 m apart. Data recorded from each plot were silking (days), early vigour 30 days from sowing (visual estimation, 1: very low vigour; 9: very high vigour), grain moisture at harvest (g H_2O kg^{-1}), lodging, estimated as the percentage of plants showing either root or stalk lodging (%) and yield (weight of grain in Mg ha^{-1}), adjusted to a kernel moisture of 140 g H_2O kg^{-1}.

Table 2. Origin and heterotic group of maize inbred lines used as testers in crosses with Spanish populations

Inbred line	Pedigree	Heterotic group
EP42	O.P. Tomiño	Spanish flint
CM105	V3 × B14[2]	Reid yellow dent
B93	(B70 × H99) × H99	Lancaster surecrop
W64A	WF9 × CI187-2	Reid yellow dent

A combined analysis of variance over locations and years was performed. Each year-location combination was considered as a random environment. Entries were assumed to be fixed effects and the sums of squares due to testcrosses were orthogonally partitioned into populations, testers and the populations × tester interaction. General combining ability (GCA) corresponds to the sums of squares for populations and testers while the sums of squares of the populations × tester interaction are related to the specific combining ability (SCA). Estimates of GCA and SCA were calculated from the means of crosses according to Falconer and Mackay (1997). Values of GCA were considered different from zero if they exceeded twice their standard error. Least significant differences were estimated for each trait. All analyses were made using the SAS package (SAS, 2000).

Results

Differences among testcrosses were significant ($P \leq 0.01$) in the combined analysis of variance for all traits, except lodging which was significant at $P \leq 0.05$ (data not shown). The source of variation due to testcrosses was subdivided into populations, testers and the interaction populations × tester. The factor of the combined analyses due to GCA of the populations was significant for all traits, while the GCA of testers was significant at $P \leq 0.01$ for early vigour and grain moisture. Grain yield and lodging were significant at $P \leq 0.05$. Pollen shedding was not significant. The interaction populations × testers was significant at $P \leq 0.01$ for yield and at $P \leq 0.05$ for early vigour, lodging and grain moisture. Analysis of variance indicated that the largest proportion of the sum of squares of testcrosses was due to GCA of populations for pollen shedding and early vigour. The GCA for testers was relatively large for lodging, grain moisture and yield. The interaction populations × tester did not show for evaluated trait the highest percentage of the sum of squares.

The main type of gene action expressed was additive, because the variation in testcrosses was mainly due to differences among GCAs of populations and inbred testers. The low percentage of the populations × testers interaction to the sum of squares of testcrosses, indicated that non additive effects were less important.

Table 3. Average performance (Mg ha^{-1}) of maize testcrosses and checks evaluated in four Spanish environments

Maize population and checks	Inbred ester line			
	B93	**CM105**	**EP42**	**W64A**
Alegia	5.132	7.045	6.450	7.447
Azkoitia	3.875	5.075	6.225	3.525
Berrobi	5.065	8.052	5.120	5.087
Enano Norteño/Vasco	6.032	6.215	6.050	6.007
EZS20	5.112	5.872	5.205	5.547
EZS22	7.200	6.787	5.530	5.840
Getaria	5.772	7.387	5.007	5.217
Hembrilla Norteño/Vasco	4.000	4.675	4.067	7.262
Lazkano	5.135	8.952	4.940	7.802
Vasco	7.752	4.207	4.672	5.925

LSD (0.05) = 0.786 Mg ha^{-1} for means of testcrosses. Grain yield mean of check H125 = 6.700 Mg ha^{-1} and check H147 = 7.001 Mg ha^{-1}.

Table 3 shows the grain yield of testcrosses with a range of variation from 3.525 Mg ha^{-1} of W64A × Azkoitia to 8.952 Mg ha^{-1} in CM105 × Lazkano. This trait should be the main option to determine new heterotic patterns for developing new maize hybrids. The best crosses were the CM105 tester inbred line with Lazkano (8.952 Mg ha^{-1}) and with Berrobi (8.052 Mg ha^{-1}) as well as those of the W64A × Lazkano (7.802 Mg ha^{-1}) which differed significantly from the controls H125 and H147, at 7.001 Mg ha^{-1} and 6.700 Mg ha^{-1}, respectively. The general mean of testcrosses was 6.431 Mg ha^{-1} with line CM105 and 6.049 Mg ha^{-1} with the tester line W64A, and they did not differ significantly. Generally, the highest average yield was obtained when all populations were crossed with dent lines, rather than with flint line EP42.

The populations Getaria and Hembrilla Norteño/Vasco showed the highest GCA for pollen shedding 2.762 and 2.887, respectively, and the lowest value was EZS20 with –3.612 (Table 4). This population showed the best effect for early vigour with 0.984. The GCA effect for lodging was very high in the Hembrilla Norteño/Vasco population with 1.213, and Azkoitia had a low value (–0.757) for this trait. The population EZS20 had the best GCA for grain moisture, nevertheless Azkoitia showed a high value. Significant effects for grain yield ranged from –1.129 to 0.873, with the Lazkano population having the highest value.

Inbred lines CM105 and W64A had the best GCA for grain yield. The EP42 and B93 had the worst GCA for this trait (Table 5).

Table 4. General combining ability (GCA) effects of several traits for 10 flint maize populations

Maize population	Pollen shedding (days)	Early vigour	Lodging (%)	Grain moisture (g H_2O kg^{-1})	Grain yield (Mg ha^{-1})
Alegia	0.762	0.078	0.285	–0.066	0.694
Azkoitia	–1.425	–0.234	–0.757	4.173	–1.129
Berrobi	–0.800	–0.266	0.349	–0.176	0.194
Enano Norteño/Vasco	–0.425	0.328	–0.496	–0.514	0.242
EZS20	–3.612	0.984	–0.294	–3.781	–0.399
EZS22	0.762	0.203	0.025	1.805	0.505
Getaria	2.762	0.078	–0.188	0.295	0.015
Hembrilla Norteño/Vasco	2.887	–0.516	1.213	1.450	–0.835
Lazkano	–0.925	–0.359	0.077	–1.862	0.873
Vasco	0.012	–0.297	–0.213	–1.324	–0.193
LSD (0.05)	1.210	0.330	0.840	1.310	0.870

Table 5. General combining ability (GCA) effects of several traits for four maize testers evaluated in the northeaster of Spain

Inbred tester	Early vigour	Lodging (%)	Grain moisture (g H_2O kg^{-1})	Grain yield (Mg ha^{-1})
B93	–0.241	0.329	2.620	–0.325
CM105	0.147	–0.013	–1.973	0.598
EP42	–0.078	0.223	–2.463	–0.501
W64A	0.172	–0.539	1.815	0.216
LSD (0.05)	0.210	1.040	1.394	0.467

Specific combining ability (SCA) was significant ($P \leq 0.05$) for early vigour, lodging, grain moisture and was highly significant for grain yield ($P \leq 0.01$). The SCA for early vigour ranged from 1.234 in Azkoitia × EP42 to –0.916 in Alegia × B93 (Table 6). The higher SCA value for lodging was Berrobi × B93 at 2.986 and the lowest SCA was –1.970 in Hembrilla Norteño/Vasco × B93. For grain moisture SCA ranged from 3.652 in Alegia × CM105 to –4.476 in Berrobi × CM105. Table 6 shows that the highest SCA, for grain yield, was in Berrobi × CM105 from 1.429 to –1.340 in Azkoitia × W64A. The populations from Guipúzcoa, (Alegia, Berrobi, Getaria and Lazkano) had a higher SCA in crosses to Reid Yellow Dent or Lancaster Surecrop testers than to the European flint line EP42 for grain yield. Nevertheless, Enano Norteño/Vasco and Hembrilla Norteño/Vasco had a good SCA with the flint tester EP42. Population EZS20, from the Cantabria coast, and EZS22, from the northwest of Spain, had a higher SCA for grain yield in crosses with Red Yellow Dent and Lancaster Surecrop testers, respectively.

Discussion

Most of the variability for traits analyzed in this work was due to GCA effects of populations or testers, indicating a predominance of additive genetic effects. Differences among populations studied were similar to the tester for grain yield with regard to the combined analysis of variance. Sprague and Tantum (1942) found that SCA was more important than GCA for previously selected inbreds. Gutiérrez-Gaitan *et al.* (1986) found significant estimates of GCA in crosses of improved Mexican germplasm with two United States Corn Belt adapted testers. Zambezi *et al.* (1994) found variance component estimates were larger for GCA than for SCA effects. Castellanos *et al.* (1998) reported in different types of testers that the variance components of inbred lines were greater than their respective line × tester interactions. Soengas *et al.* (2003b) and Melani and Carena (2005) suggest that genetic variation among crosses was primarily additive and this is in agreement with our results. Other authors have found that both, GCA and SCA, were significant for grain yield (Eyherabide and González, 1997; Hede *et al.,* 1999). The interaction populations × testers was significant for all traits. This suggests that the relative performance of the populations in testcrosses was different for the two adapted testers.

The populations studied crossed better to dent maize than to the European flint. Other workers have shown high grain yields of hybrids between flint and dent germplasm (Radovic and Jelovac, 1995) and for maize forage (Moreno-González *et al.,* 2000). The crosses of the populations with lines CM105 and W64A (Reid Yellow Dent type) gave higher yields than crosses to EP42 (Spanish flint line). These results agree with those of Misevic (1989) with Yugoslavian populations. Sinobas and Monteagudo (1996) had the same results with Spanish and Corn Belt germplasm. Soengas *et al.* (2003b) state that their flint germplasm combined better with dent than with flint populations and it could be used to widen the genetic base of maize used in Europe, which is quite narrow at present (Messmer *et al.,* 1992).

All crossed populations except Enano Norteño/Vasco and Hembrilla Norteño/Vasco had a higher SCA in crosses with Reid Yellow Dent or Lancaster Surecrop testers. This result was expected and is in agreement with Soengas *et al.* (2003b) because the EP42 tester is a flint inbred derived from Tomiño, a local variety from North western Spain. Different heterotic patterns have been identified as 'Northern Spain × Southern Spain', 'Northern Spain × US Dent' and 'Southern Spain × US

Dent' (Ordás, 1991). Soengas *et al*. (2003a) in a diallel among 10 flint populations, found the highest heterosis in crosses between Spanish and American germplasm. A dent × dent heterotic pattern was proposed by Moreno-González *et al*. (1997) for temperate areas of Europe using populations selected for early maturity. Melani and Carena (2005) identified alternative heterotic patterns to Iowa Stiff Stalk Synthetic (BSSS)

Table 6. Specific combining ability (SCA) for 40 crosses among ten flint maize populations and four testers

Maize population	Tester	Early vigour	Lodging (%)	Grain moisture (g H_2O kg^{-1})	Grain yield (Mg ha^{-1})
Alegia	B93	–0.916	2.796	–4.261	–1.071
	CM105	0.072	–1.029	3.652	–0.048
	EP42	0.672	–1.264	0.482	0.425
	W64A	0.172	–0.502	2.129	0.706
Azkoitia	B93	–0.353	–0.329	–0.012	–0.494
	CM105	–0.366	0.013	1.014	–0.214
	EP42	1.234	–0.223	–2.328	1.095
	W64A	–0.516	0.539	1.327	–1.340
Berrobi	B93	0.053	2.986	1.572	–0.638
	CM105	0.666	–1.092	–4.476	1.429
	EP42	–0.609	–1.328	–0.116	–0.408
	W64A	–0.109	–0.566	3.021	–0.371
Enano Norteño/Vasco	B93	0.334	–0.590	2.233	0.280
	CM105	0.197	0.794	1.556	–0.458
	EP42	–0.078	–0.483	–3.665	0.477
	W64A	–0.453	0.279	–0.122	–0.287
EZS20	B93	0.178	–0.792	–3.374	0.004
	CM105	–0.117	–0.543	0.760	0.338
	EP42	0.016	–0.686	1.879	0.271
	W64A	0.016	0.076	–0.419	–0.104
EZS22	B93	0.459	–1.111	1.807	1.186
	CM105	0.072	–0.769	1.152	–0.151
	EP42	–0.703	2.121	–1.419	–0.307
	W64A	0.172	–0.242	–1.539	–0.715
Getaria	B93	–0.291	1.375	–0.952	0.247
	CM105	0.447	–0.555	–3.046	0.941
	EP42	–0.484	–1.269	1.303	–1.034
	W64A	–0.586	–1.619	2.005	–0.767
Hembrilla Norteño/Vasco	B93	–0.438	–1.970	–0.353	0.100
	CM105	–0.477	–1.686	–1.668	–0.224
	EP42	–0.141	–1.402	–0.285	0.220
	W64A	–0.180	0.549	0.637	–0.084
Lazkano	B93	–0.094	0.833	0.124	–0.366
	CM105	0.266	0.906	0.674	1.193
	EP42	–0.125	0.978	0.112	0.181
	W64A	–0.016	–0.616	–0.796	0.586
Vasco	B93	–0.156	–0.543	–0.434	0.208
	CM105	–0.528	–0.434	–1.258	–0.952
	EP42	–0.328	–0.385	–2.036	0.177
	W64A	–0.453	–0.004	–3.158	–0.010
LSD (0.05)		0.357	0.987	1.207	0.654

× Lancaster Sure Crop, among North American early-maturing dent populations.

In this work, the populations generally crossed best to CM105 and W64A testers, two inbred lines representing Reid Yellow Dent germplasm. The cultivar Lazkano produced hybrids with the highest grain yield when crossed with the CM105 tester and showed the best field performance. Revilla *et al.* (2006) found highest early vigour and high adult plant vigour when Lazkano was crossed with the Tuy population. The flint populations studied gave greater yield in crosses with dent germplasm, this could be useful to increase variability in commercial European flint maize. The Lazkano population could be the best option for breeding programs developing elite products for temperate conditions. The above average combining ability for harvest grain yield, even across heterotic groups, is sufficient evidence to use the population as source of new inbred lines. They can be exploited between this heterotic group, to produce highly productive hybrid combinations, or used as improved maize populations.

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