

## **Heterotic relationships among European maize inbreds**

P. Revilla, R.A. Malvar, M.E. Cartea, P. Soengas & A. Ordás.

Misión Biológica de Galicia, CSIC, Apartado 28, E-36080 Pontevedra, Spain.

Received \_\_\_\_\_.

**Key words:** *Zea mays*, maize, European flint, combining ability

## **Abstract**

European flint maize (*Zea mays* L.) cannot be considered an uniform group of germplasm based on its origin and area of adaptation. However, maize breeders have not taken full advantage of the variability within the European flint germplasm. The objective of this work was to study the heterotic relationships among European maize inbreds from different origins. Nine European flint inbreds were crossed in a diallel that was evaluated in three environments in northwestern Spain. The variability within the European flint germplasm and the agronomic value of some inbreds could be utilized for maize breeding programs as an alternative to the systematic introduction of U.S. dent germplasm that is narrowing the germplasm base of breeding programs even in places where it is poorly adapted. Some European flint inbreds may also be valuable sources of earliness (F7 and EP42), resistance to root lodging (EA1070), and yield (EP42). These results suggest that, within the European flint germplasm, there could be some heterotic patterns, such as ‘north-central Europe × southern Europe’, which could provide an alternative to the heterotic pattern ‘European flint × U.S. dent’.

## Introduction

Heterosis among European flint and U.S. dent maize (*Zea mays* L.) is widely used by European maize breeders (Moreno-González, 1988; Misevic, 1989; Ordás, 1991; Sinobas & Monteagudo, 1996; Garay *et al.*, 1996a, b). Alternative patterns are also used in Europe. Specifically, U.S. hybrids are well established in the south of Europe and ‘dent × dent’ commercial hybrids, after selection for early maturity, could progressively replace ‘flint × dent’ hybrids in the early maize regions of Europe (Moreno-González *et al.*, 1997).

European breeders have not taken full advantage of the variability within the European flint germplasm. Indeed, European flint maize cannot be considered a uniform group of germplasm based on its origin and area of adaptation. Heterosis among European flint populations has been found by several authors (Misevic, 1989; Ordás, 1991; Radovic & Jelovac, 1995). European flint inbreds have revealed some differences based on their combining ability in crosses to inbreds from different U.S. heterotic groups (Moreno-González, 1988; Cartea *et al.*, 1999) and on molecular markers (Messmer *et al.*, 1992, 1993).

European breeders have been releasing inbreds from local maize populations, but have developed very few inbreds with good agronomic performance. Second cycle inbreds with higher agronomic performance have been produced from crosses between inbreds from U.S., Europe, or ‘U.S. × European’ germplasm. Cartea *et al.* (1999) concluded that European flint inbreds are appropriate to improve European inbreds in crosses to American germplasm; whereas, the American inbreds may be used to improve a specific cross. However, the rule is not absolute because there are European flint inbreds having high combining ability with European, U.S.-Reid, U.S.-Lancaster, or other heterotic groups. Combining ability differences among European flint inbreds indicates that an improved understanding of heterotic groups within European flint maize germplasm would be advantageous to maize breeders using this germplasm. The objective

of this study was to define the heterotic relationships among European flint maize inbreds from different origins.

## Materials and Methods

Nine European flint inbreds were crossed in a diallel (Table 1). The Spanish inbreds EP1, EP42, EP44, EA1070, and EA2000, had been obtained from typical populations representative of the varieties previously grown in the main maize producing areas of Spain. The remaining four inbreds come from maize populations that were grown in France and Italy. Two commercial hybrids from the company Dekalb Ag. Research, DK-485 (dent × dent) and DMB 15-70 (flint × dent), were included for the evaluation of hybrids.

The hybrids were evaluated in three environments in northwestern Spain: Pontecaldelas (300 m above sea level) in 1997 (first environment), Pontevedra (20 m above sea level) in 1998 (second environment), and Pontecaldelas in 1998 (third environment). The dates of sowing were 30 May 1997, 12 May 1998, and 4 June 1998, respectively. Both locations have a humid climate with an annual rainfall of about 1600 mm. The field arrangement was a randomized complete block design with three replications in all experiments. Each two-row plot consisted of 13 hills per row with two kernels per hill. Rows were spaced 0.80 m apart, and hills were spaced at 0.21 m. When the plants had four to five leaves the plots were thinned to one plant per hill for a final plant density of approximately 60 000 plants ha<sup>-1</sup>. Traits measured were d to mid-pollen shedding (number of days from planting to 50% of plants producing pollen), d to mid-silking (number of days from planting to 50% of plants exhibiting silks), plant height (centimeters from the soil to the top of the tassel), root lodging (percent of plants leaned more than 45°), grain moisture at harvest (% of water on grain weight), and yield (Mg ha<sup>-1</sup>) adjusted to a water content of 140 g kg<sup>-1</sup>.

Due to a severe bird attack shortly after emergence in the third location, many hills were lost. For this reason, missing data were calculated and the corresponding degrees of freedom of the error term were corrected accordingly. Yield was adjusted for missing hills using the formula proposed by Leng and Finley (1957) as stated by LeClerc (1966):

Adjusted yield =  $(1 + 0.6 \times (\text{number of missing hills} / \text{number of hills per plot})) \times \text{yield}$ .

The number of lost plants for hybrids involving inbred EA1070 was so high in the third environment, that hybrids involving this inbred had to be eliminated from the analysis of the third location and from the combined analyses over the three locations. Therefore, the analyses of variance were combined over two locations for the nine inbreds, and over three locations for eight inbreds.

Individual and combined analyses of variance were calculated for each trait using SAS (SAS Institute Inc., 1989). The commercial hybrids were included in the analysis for comparisons of means, but they were not used in the analyses of general (GCA) and specific (SCA) combining abilities. GCA and SCA were analyzed for yield and plant height in order to detect relationships among the inbred lines, while the remaining traits were used solely for comparing the agronomic performance of the inbreds and new hybrids. Combining abilities, their standard errors, and differences among them were estimated according to Griffing (1956) method 4, Model I (fixed effects) using the diallel analysis software of Burow & Coors (1994). The hybrids and hybrid interaction sums of squares were partitioned into GCA and SCA sums of squares.

## Results

The analyses of variance combined over environments showed significant hybrid  $\times$  environment, GCA  $\times$  environment, and SCA  $\times$  environment interactions for yield (data not shown). Therefore, the analysis of variance of yield was made for each environment separately. For plant height the hybrid  $\times$  environment, GCA  $\times$  environment, and SCA  $\times$  environment interactions were significant in the analyses combined over environments (data not shown), but were not significant for the analyses combined over the first two environments. Therefore, the first two environments were combined in one analysis and the third environment was analyzed separately for plant height.

Hybrids differed significantly for all traits. For d to mid-pollen and mid-silking, hybrids including F7 and Z77016 were the earliest (Table 2). Hybrids involving EA1070 had the lowest root lodging and were comparable to the commercial check DK-485. The tallest hybrids were the commercial checks along with crosses involving inbreds EP42 and PB60. The hybrids with less moisture content at harvest involved EP42. The late-maturing check DK-485 had the highest yield ( $8.89 \text{ Mg ha}^{-1}$ ), but early-maturing hybrids including EP42 were among the highest yielders.

Considering the GCA estimates for yield that were significantly different from zero in the first environment, GCA was highest for EP42 followed by PB97, and lowest for Z77016, followed by EP1 (Table 3). In the second environment, GCA was highest for EA2000 followed by EP42, and lowest for Z77016, followed by F7 and EP1. In the third environment, the highest GCA was for EP42, followed by EA2000, and the lowest for Z77016 and F7. The inbreds with the best GCA for yield across environments were EP42 and EA2000, and those with the lowest GCA were Z77016 and F7.

SCA for yield in the first environment was highest for the hybrids EP1  $\times$  PB60 and EP44  $\times$  F7, followed by EA1070  $\times$  PB97 and EP44  $\times$  Z77016, and lowest for EP1  $\times$  Z77016 (Table 3).

SCA's for crosses among PB60, EP44, and EA2000 were negative. SCA was also negative for crosses among EP1, Z77016, and F7, except for the hybrid EP1 × F7. Crosses among inbreds from the previous groups had a positive SCA except for EP1 × EA2000.

SCA for yield in the second environment was highest for F7 × PB60, followed by EP44 × Z77016 and EA2000 × Z77016 (Table 3). SCA was negative for crosses among EP42, EP44, EA2000, PB60, and PB97, except for EP44 × PB60. SCA was also negative for crosses among EP1, F7, and Z77016, except for EP1 × F7. Crosses among inbreds from these groups were positive except for EP1 × PB60 and EP44 × F7. Inbred EA1070 had relatively low SCA value with other inbreds except with PB97.

SCA for yield in the third environment was highest for PB97 × Z77016, followed by F7 × PB60, EP42 × EP44, and EA2000 × Z77016. The lowest SCA was for EA2000 × F7, followed by EP44 × PB97, EP42 × PB97, and PB60 × Z77016.



## Discussion

From the SCA figures of the first environment, we can postulate two heterotic groups: south of Europe (PB60, EP44, and EA2000), and north-central Europe (EP1, Z77016, and F7). The inbreds PB97, EA1070, and EP42 did not fit in any of these groups. The heterotic groups inferred from the results of the second environment are equivalent to those suggested from the first environment. Although the relationships among inbreds were not clear for the third environment, two groups could be formed. The first composed by EP1, EP42, PB60, and Z77016, and the second comprising EP44, EA2000, F7, and PB97.

The inbred grouping determined from the SCA value for yield for each environment are not identical, but there are some coincidences from which two groups could be made. One of the groups is formed by EP1, Z77016, and F7 (inbreds from the north of Spain and central Europe) while the second one comprises EP44, EA2000, PB60, and PB97 (inbreds from the south of Europe). The inbreds EP42 and EA1070 did not clearly fit in either group.

The SCA for plant height in the first two environments suggested the same two groups mentioned above EP44, EA2000, PB60, and PB97 (south), and EP1, F7, and Z77016 (north) (Table 4). Again EA1070 and EP42 did not fit in either group. In the third environment, the SCA for plant height suggested two groups: the first one formed by EP42, EP44, EA2000, PB60, and PB97, and the second one by EP1, F7, and Z77016.

The isozyme relationships (Revilla *et al.*, 1998) indicated different origins for southern and northern Spanish maize which may come from Central and North America, respectively. The isozymes also revealed differences among maize populations from southern, northern, and northeastern Spain, which agree with the differences among the inbreds EA2000 from the south, EP44 from the northeast, EP1 from the north, and EP42 from the northwest.

The variability within the European flint germplasm and the agronomic value of some inbreds could be capitalized for maize breeding programs, as an alternative to the systematic

introduction of U.S. dent germplasm which is narrowing the germplasm base of breeding programs even in places where it is poorly adapted. Within the European flint germplasm, some heterotic patterns, such as 'northern × southern' Spain (Ordás, 1991), could successfully contend with the heterotic pattern 'European flint × U.S. dent'. European flint inbreds could also be valuable sources of earliness (F7 and EP42), resistance to lodging (EA1070), and yield (EP42). These results support the heterotic pattern 'northern Spain × southern Spain' established by Ordás (1991) and suggest the existence of a heterotic pattern 'north-central Europe × southern Europe'.

#### Acknowledgements

Research supported by the Committee for Science and Technology of Spain (Project Cod. AGF98-0968) and Excma. Diputación Provincial de Pontevedra.

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Table 1. Pedigree and origin of nine inbred lines of maize crossed in a diallel.

Inbred	Pedigree	Geographical zone	Source
EP1	Lizargárate	Basque Country (N Spain)	MBG <sup>a</sup>
EP42	Tomiño	Galicia (NW Spain)	MBG
EP44	Hembrilla/Queixalet	Balearic Islands (E Spain)	MBG
EA1070	Hembrilla de Novillas	Ebro Valley (NE Spain)	CMM <sup>b</sup>
EA2000	Tremesino	Andalusia (S Spain)	CMM
F7	Lacaune O.P.	France	INRA <sup>c</sup>
PB60	Nostrano dell'Isola	Italy	EAB <sup>d</sup>
PB97	San Pancrazio	Italy	EAB
Z77016	Z27 × Z36	France	Zelder B.V.

<sup>a</sup> Misión Biológica de Galicia, CSIC, Pontevedra (Spain).

<sup>b</sup> Centro de Mejora de Maíz, INIA, Alcalá de Henares, Madrid (Spain).

<sup>c</sup> Institut National de la Recherche Agronomique (France).

<sup>d</sup> Estação Agrária, Braga (Portugal).

Table 2. Means of parental inbreds across hybrids for pollen shedding, silking, plant height, grain moisture, and yield for 36 hybrids resulting from a diallel cross of nine inbreds and two commercial checks.

Parental inbred	Pollen		Root	Plant	Grain	Yield
	shedding	Silking	lodging	height	moisture	
	———days———		%	cm	%	Mg ha <sup>-1</sup>
EP1	67.7	69.1	13.5	218.1	28.7	4.9
EP42	68.5	69.7	8.8	236.8	26.0	6.2
EP44	72.7	73.9	9.2	225.9	29.7	5.3
EA1070 <sup>a</sup>	71.4	72.7	3.7	220.8	29.8	5.2
EA2000	68.3	69.7	15.9	229.6	31.1	5.7
F7	65.6	66.7	24.8	198.7	28.7	4.8
Z77016	67.3	68.4	26.0	208.9	28.5	4.4
PB60	69.6	70.6	15.4	238.8	28.3	5.3
PB97	69.8	70.9	10.3	228.8	32.4	5.6
DK-485	78.1	78.9	1.7	257.3	31.4	8.9
DMB 15-70	73.0	74.2	9.8	256.7	25.9	7.1
LSD (5%)	2.3	2.4	16.2	11.9	2.3	1.3

<sup>a</sup> Data for inbred EA1070 come from environments 1 and 2, because data from hybrids involving this inbred were removed for environment 3.

Table 3. Estimates of specific combining ability and general combining ability for yield from a diallel cross of nine inbred lines evaluated in three environments.

		Inbred								
Inbred	Env.	EP42	EP44	EA1070 <sup>a</sup>	EA2000	F7	PB60	PB97	Z77016	GCA
EP1	1	-0.09	0.50	0.73	-0.56	0.43	1.25*	-0.45	-1.81*	-0.47*
	2	0.72	0.01	0.01	-0.02	0.17	-0.14	0.43	-1.18*	-0.51*
	3	0.52	0.17	—	-0.69	0.83	-0.39	0.55	-0.99	-0.26
EP42	1		0.53	-0.38	0.66	-0.68	-0.62	-0.03	0.62	1.61*
	2		-0.03	-0.53	-0.39	0.23	-0.18	-0.24	0.41	0.49*
	3		1.38	—	0.32	0.38	-0.64	-1.24	-0.71	1.43
EP44	1			-1.56*	-1.18*	1.24*	-0.33	-0.27	1.07*	0.35*
	2			-0.15	-0.27	-0.19	0.11	-0.72	1.23*	0.24
	3			—	0.07	-0.14	0.41	-1.55*	-0.33	-0.12
EA1070	1				0.11	0.03	0.56	1.12*	-0.60	-0.34*
	2				0.62	-0.09	-0.06	0.88*	-0.67	0.01
	3				—	—	—	—	—	—
EA2000	1					0.27	-0.73	0.73	0.71	0.02
	2					0.09	-0.59	-0.51	1.07*	1.20*
	3					-1.73*	0.85	0.08	1.11*	0.54*
F7	1						0.13	-1.05*	-0.37	-0.31
	2						1.40*	0.20	-1.81*	-0.69*
	3						1.51*	-0.15	-0.69	-0.95*

PB60	1	-0.35	0.09	-0.37*
	2	-0.77	0.22	0.22
	3	-0.51	-1.22*	0.11
PB97	1		0.29	0.54*
	2		0.72	0.21
	3		2.82*	0.37
Z77016	1			-1.04*
	2			-1.17*
	3			-1.13*

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\* Significantly different from zero (5%).

LSD (5%) for GCA = 0.492 (environment 1), 0.514 (environment 2), and 0.735 (environment 3).

LSD  $[s(i,j) - s(i,k)] = 1.206$  and LSD  $[s(i,j) - s(k,l)] = 1.102$  (environment 1),

LSD  $[s(i,j) - s(i,k)] = 1.260$  and LSD  $[s(i,j) - s(k,l)] = 1.151$  (environment 2),

LSD  $[s(i,j) - s(i,k)] = 1.644$  and LSD  $[s(i,j) - s(k,l)] = 1.471$  (environment 3).

<sup>a</sup> Data for inbred EA1070 come from environments 1 and 2, because data from hybrids involving this inbred were removed for environment 3.



Table 4. Estimates of specific combining ability (SCA) for plant height from a diallel cross of nine inbred lines evaluated in three<sup>a</sup> environments.

Inbred									
Inbred	Env.	EP42	EP44	EA1070 <sup>a</sup>	EA2000	F7	PB60	PB97	Z77016
EP1	1+2	-6.52	-1.61	7.43	9.46*	4.10	8.41*	4.01	-25.28*
	3	-0.66	8.01	—	4.23	-8.83	1.90	18.56*	-23.21*
EP42	1+2		0.46	-1.49	2.70	4.84	-10.35*	0.41	9.96*
	3		-4.27	—	4.29	2.23	-13.05*	-0.05	11.51*
EP44	1+2			-13.26*	-2.73	9.74*	-3.11	-4.85	15.36*
	3			—	-11.71*	26.90*	-7.71	-16.71*	5.51
EA1070	1+2				-4.02	-7.71*	6.60	14.03*	-1.59
	3				—	—	—	—	—
EA2000	1+2					-1.68	-5.54	-8.61*	10.43*
	3					-6.21	5.51	-13.16*	17.16*
F7	1+2						6.43	2.03	-17.06*
	3						21.45*	-8.55	-26.99*
PB60	1+2							-9.16*	6.72
	3							-2.16	-5.94
PB97	1+2								2.15
	3								22.06*

\* Significantly different from zero (5%).

LSD [s(i,j) - s(i,k)] = 11.440 and LSD [s(i,j) - s(k,l)] = 10.443 (environment 1+2)

LSD [s(i,j) - s(i,k)] = 14.936 and LSD [s(i,j) - s(k,l)] = 13.360 (environment 3).

<sup>a</sup> Data for inbred EA1070 come from environments 1 and 2, because data from hybrids involving this inbred were removed for environment 3.