

## **Combining maize base germplasm for cold tolerance breeding**

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## **Abstract**

Early sowing increases yield and avoid stresses, but require cold tolerance. A limited number of cold tolerant maize genotypes have been reported. The objective of this study was to identify broad × narrow genetic base combinations as breeding populations for improving cold tolerance. Nine cold tolerant populations were crossed to five inbred lines, and evaluated in cold chamber and in the field. None of the maize inbred lines or populations was entirely cold tolerant. None of the inbred × population combinations fulfil all requirements for early sowings. The crosses between the inbred line EP80 and northwestern Spanish populations are the most promising base germplasm for further breeding programs for cold tolerance. Particularly, EP80 × Puenteareas showed the largest yield and good performance at first stages of development under cold conditions, either in cold chamber and field. The cross EP80 × Rebordanes had better performance at first stages of development under cold conditions, but poorer agronomic characteristics at early planting. In addition, EP80 and Puenteareas showed the most favorable GCA for most traits. Early vigor would be the most suitable trait to select maize genotypes with superior cold tolerance during emergence and post-emergence stages, as it was the only trait for which differences among genotypes were observed in both the cold chamber and the field.

Key words: *Zea mays*, cold tolerance, germplasm, combining ability.

In areas with cold springs, maize sowing must be delayed until late May and only genotypes of medium or short cycles can be sown, which results in reduction of potential grain yield. Early sowings allow cultivating later genotypes, which have more yield potential than early ones (Shawn, 1988; Lauer et al. 1999). According to Mock and Pearce (1975), there is an increase of yield in early sowing due to the coincidence of grain-filling stage with the period of potential maximum photosynthesis. Gupta (1985) reported that early sowing is highly recommended in temperate areas because some field-drying of maize can occur, allowing greater profit margin. Revilla et al. (1999, 2000) concluded that breeding for cold tolerance should be a priority to improve maize yields in areas with short growing season, leading to the development of longer cycle genotypes with cold tolerance.

Most breeding studies on cold tolerance of maize have focused on germination and crop establishment under cold conditions. Cold tolerant genotypes at first stages of development are required for early sowing since these stages are more sensitive to low temperatures than mature stages (Greaves, 1996). Several studies have been carried out to select cold tolerant genotypes at germination in laboratory (Semuguruka et al., 1981; Lee et al., 2002; Revilla et al., 2003) and field (Mosely et al., 1984; Verheul et al. 1996), but few studies included both types of evaluation (Revilla et al, 2000). Menkir and Larter (1985) pointed out that emergence related traits determined under controlled environment conditions were not correlated with those recorded in the field. Therefore, both the laboratory and the field evaluations are necessary for choosing the best genotypes, because cold can be guaranteed in a cold chamber, while field trials provide the real conditions (Revilla et al, 2005). Besides, as the main objective in any maize breeding program is to improve the productivity and quality of genotypes, yield and other agronomic traits should be evaluated to know the breeding potential of materials.

A limited number of reports have presented cold tolerant maize inbred lines and populations around the World, of which few field corn inbred lines and populations could be adapted to the European Atlantic conditions (Revilla et al., 2005). The collection preserved at the

Misión Biológica de Galicia (CSIC) contains only three inbred lines, belonging to the European flint group, and nine populations with superior cold tolerance, although all of them with low agronomic performance. Base populations are often made by crossing inbred lines, or open-pollinated populations. The use of open-pollinated populations as base germplasm strongly limits the potential gain through selection and reduces the chances of obtaining elite genotypes due to inappropriate initial values. Crossing inbred lines results in narrow genetic base populations that limit the gain per cycle of selection, particularly when few or related inbreds are available. The amount of variability obtainable from crosses among inbred lines is limited and depends on the genetic relationship among inbred lines (Butrón et al., 2003). Tabanao and Bernardo (2005) concluded that elite maize inbred lines supply nonadditive gene effects valuable for maintaining genetic variance, and that the use of multiple parents is important for retaining genetic variability during selection. Inbred  $\times$  open-pollinated populations crosses allow capitalizing the advantages of broad-low performing and narrow-high performing combinations. Genes involved in cold tolerance present important additive effects (Eagles and Hardacre 1979, Eagles 1982, Mahajan et al. 1993, Revilla et al. 2000), but hybrid performance under cold conditions could not always be predicted from the performance of its parental inbreds (Aidun et al. 1991). An inbred  $\times$  population cross would be useful as breeding population for cold tolerance when both parents have favorable GCA values for cold tolerance and yield-related traits and the expectation of its specific combining ability when crossed to tester inbreds was high. The objective of this study was to identify broad  $\times$  narrow genetic base combinations as breeding populations for improving cold tolerance.

## Material and Methods

Nine cold tolerant populations were crossed to five inbred lines, three cold tolerant and two testers of combining ability (Table 1). The nine populations and the three inbred lines were identified as cold tolerant in previous unpublished evaluations. Each population was crossed to each inbred in 2002. Seed was produced for all genotypes in one year and in one location to reduce environmental effects on seed quality. Populations were used as males and pollen from a minimum of 40 tassels in each population was bulked and used to pollinate each inbred line. Besides, the three hybrids among the three cold tolerant inbred lines were produced, and the nine populations were multiplied in the same environment.

### *Growth Chamber Trial*

The 45 crosses, nine populations, five inbred lines, and three cold tolerant hybrids (used as checks) were planted in trays filled with 6 l of sterilized peat and 1.2 l of water and arranged in a cold chamber following a randomized complete block design with four replications. The cold chamber has four pairs of shelves, each pair at a different height. Trays containing the same replication were placed at the same height. The experimental plot consisted in a row of 15 grains. Sowing depth was 2 cm and grains were planted in rows spaced 5 cm apart with 2 cm between grains. Conditions were set at 14 h with light at 14 °C and 10 h without light at 8 °C. All trays were watered with 300 ml of water 20 days after planting. Data were recorded on five traits related to early development in a cold environment: color (1=albino to 9=dark green) and early vigor (1=weak to 9=vigorous) as visual ratings, days to emergence (days from planting to 50 % emergence), proportion of emergence (% of emerged plants over grains sown) and proportion of survival (% of plant alive over emerged plants). Analyses of variance and mean comparisons were performed for all traits. Sources of variation were genotypes and replications. Genotypes were

considered a fixed effect. Sum of squares due to genotypes were divided into crosses, populations, inbred lines and hybrids. Sum of squares due to crosses were divided into populations, inbred lines and population  $\times$  inbred line interaction. The sum of squares for populations and inbred lines correspond to the general combining ability (GCA) and sum of squares for population  $\times$  inbred line interaction to the specific combining ability (SCA). GCA and SCA were calculated according to Falconer and Mackay (1997). Comparisons of means were performed for each trait using the Fisher's protected least significant difference (LSD) at  $P = 0.05$  (Steel et al., 1997). Analyses were made using the GLM procedure of SAS (SAS Institute, 2000).

There was a gradient of air flow in the cold chamber that resulted in differences among trays within each replication for peat humidity. In order to minimize the effect of these differences in plant development data, covariance analyses were carried out using peat humidity as covariate. A sample of peat from each tray was weighted at the end of the experiment and after a period of 48 hours at 80 °C. Peat humidity was calculated as (fresh weight - dry weight) / dry weight. When covariate was significant, means were adjusted by peat humidity.

### *Field Trial*

Crosses, populations and cold tolerant hybrids were evaluated during two years (2003 and 2004) in two locations, Pontevedra (42° 24' N, 8° 38' W, 20 m above sea level) and Pontecaldelas (42° 23' N, 8° 32' W, 300 m above sea level). Both locations have a humid climate with an annual rainfall of about 1600 mm. Genotypes were evaluated in a 7  $\times$  8 lattice design with three replications. Each experimental plot consisted of two rows with 17 hills per row and two grains per hill. Rows were spaced 0.80 m apart and hills were spaced 0.21 m apart. Hills were thinned to one plant achieving a final plant density of approximately 60000 plants ha<sup>-1</sup>. Currently accepted management and cultural practices were used in all trials. Traits measured were: days to emergence, proportion of emergence, early vigor, plant height, days to silking, grain yield (Mg

ha<sup>1</sup>), grain moisture (g kg<sup>-1</sup>), ear and grain lengths, grain depth, ear rows, and 100 grain weight. Means adjusted by lattice block effects were obtained using the LATTICE procedure of SAS (SAS Institute, 2000) and were used in the combined analysis across locations and years. Sources of variation were genotypes, locations, years and their interactions. Genotypes were considered as a fixed effect and locations, years and all possible interactions were considered random effects. The pooled error mean square was calculated as reported by Cochran and Cox (1957). Sum of squares due to genotypes were divided into crosses, populations and hybrids while sum of squares due to crosses were divided into GCA of populations, GCA of inbred lines, and SCA.

Field trials were planted on 16 April 2003 and 2004 in Pontevedra and on 24 April 2003 and 13 April 2004 in Pontecaldelas. Comparisons of means were performed for each trait using Fisher's protected least significant difference (LSD) at  $P = 0.05$  (Steel et al., 1997). Analyses were made using the GLM procedure of SAS (SAS Institute, 2000). Estimates of general and specific combining abilities were calculated according to Falconer and Mackay (1997). For each trait related to the first stages of development (days to emergence, proportion of emergence, and early vigor), simple correlations were calculated between the values recorded in cold chamber and in field trials.

## Results and discussion

### *Cold tolerance at germination and early development*

In the cold chamber, the covariate peat humidity was significant for color, early vigor and days to emergence (data not shown). Genotypes were significantly different for all traits, except for proportion of survival. Both inbred lines and crosses showed significant differences for color, early vigor and proportion of emergence, while populations were only significantly different for color. No differences among cold tolerant check hybrids were observed. In the field, genotypes were only significantly different for early vigor and differences were due to the variability among crosses. There were significant differences among GCA effects of inbreds for color, days to emergence, percentage of emergence, and early vigor in cold chamber, while GCA effects of populations were significantly different for color and early vigor both in cold chamber and in field trials. SCA effects were only significant for the percentage of emergence. Most interactions were not significant.

Menkir and Larter (1985) found significant and positive correlations between post-emergence seedling growth in controlled and field environments but not for emergence. In the present study, significant correlations were not observed between performance in cold chamber and in the field (data not shown). Nevertheless, a positive and significant correlation (0.56 \*\*) was observed between early vigor evaluated in the field and an index computed with the vigor and color evaluated in the cold chamber ( $1/2 \text{ color} + 1/2 \text{ vigor}$ ). Actually, although clear differences for early color cannot be observed at the field, color is one of the parameters affecting the estimation of early vigor at field conditions. Early vigor was recorded 30 days after sowing in both the field and the cold chamber, but plant development stage was different. Because of higher temperatures after the first week, plant development was faster in the field than in the cold chamber. Therefore, 30 days after sowing, plants were at three-four leaf stage in

the cold chamber while, in the field, plants were at five-six leaf stage. Cooper and Macdonal (1970) stated that at approximately the three- to four-leaf stage, the seed reserves are exhausted and start the photosynthetic activity. So, we can consider early vigor in the cold chamber as an emergence trait and early vigor in field as a post-emergence trait. The moderate value for the coefficient of correlation between early vigor in the field and the combination of vigor and color in the cold chamber agreed with the results obtained by Hodges et al (1997) who observed that these two stages (emergence and post-emergence) may be under the control of different genetics factors. Therefore, only a complete evaluation including both emergence and post-emergence traits give us accurate information about the cold tolerance of the genotypes.

Crosses between populations and inbred lines A661 and A641 had poor color and proportion of emergence (data not shown) as expected since A661 and A641 are not cold tolerant. The inbred line A661 was released in the University of Minnesota (Northern USA) and good performance under cold conditions could be expected; however, testcrosses to A661 performed worse than testcrosses to A641. Considering that the purpose of this work was to identify cold tolerant inbred  $\times$  population crosses as sources of new base populations, and that A641 and A661 were included solely as testers of combining ability, cold tolerance of crosses between populations and A661 and A641 was not included in the tables and will not be further discussed.

Tolerance to low temperatures of the germplasm evaluated was partial and none of the populations or inbreds had an outstanding cold tolerance (Table 2). As expected, inbred lines were more sensitive to cold temperature than populations and crosses. In general, the performance of the cold tolerant inbred line  $\times$  population crosses was comparable or even better than the performance of hybrids. The population Santiago had the darkest green color in the cold chamber, though five populations did not differ significantly. The populations were not significantly different for any other trait. Among the cold tolerant inbred line  $\times$  population crosses, EP80  $\times$  Rebordanes, F7  $\times$  Lalín, Z78007  $\times$  Amarillo de Marañón and Z78007  $\times$

Puenteareas showed the best performance in both cold chamber and field at first stages of development.

The inbred EP80 showed favorable GCA for color and early vigor in the cold chamber while F7 and Z78007 only had favorable GCA for color (Table 3). In the field, differences among the GCA of inbred lines for early vigor were not significant. Puenteareas, Rebordanes and Lalín had the best GCA for early vigor in the field and did not differ from the population with the most favorable GCA for color and early vigor in the cold chamber. Puenteareas, Rebordanes and Lalín come from northwestern Spain and were, in general, the most cold tolerant. These results agree with previous evaluations of the European Union Landrace Core Collection (unpublished data). However, Revilla et al. (1998) found that the place of origin of a variety is not a sufficient warranty for possessing high cold tolerance, because genotypes with short growing cycle escape cold temperatures when planted late. Malvar et al. (2005) pointed out that natural selection would favor cold tolerance in the full season materials, since this germplasm is typically planted earlier.

As all cold tolerant inbred lines and most populations used in this work belong to the European germplasm group, non important specific heterosis was expected in cold tolerant inbred line  $\times$  population crosses. In fact, none of the cold tolerant inbred line  $\times$  population cross had a SCA significantly different from zero for proportion of emergence in the cold chamber (data not shown).

#### *Agronomic performance in early sowing*

Most populations belong to the flint European germplasm group and performed better in crosses to A641 than to A661 (Table 4). The inbred line A641 belongs to the Reid germplasm and several studies reported high heterosis in crosses between flint European maize and Reid germplasm (Moreno-González, 1988; Soengas et al. 2003). Four populations (Amarillo de Maraño, Gallego/H. norteño, Rebordanes and Silver King) showed higher yields in crosses to A641 than

*per se*, while only Amarillo de Marañón showed higher yield in crosses to A661 than *per se*. The other populations did not show differences between *per se* performance and in crosses with tester inbred lines. Therefore, the heterotic group should not be an important criterion in the election of the best cold tolerant inbred × population cross as base population for further breeding programs. Moreover, a Reid tester would be preferable for improving specific combining ability of the resulting base population. However, as A641 is not cold tolerant enough, and appropriate cold tolerant Reid testers are not available, other potential testers should be checked.

The high potential of the populations for improving the agronomic performance of the existing cold tolerant hybrids was proved since hybrid yields were significantly surpassed by some crosses yields (Table 4). In general, crosses among EP80 and northwestern Spanish populations were among the most productive. Among cold tolerant inbred × population crosses, EP80 × Puenteareas showed the largest yield ( $7.4 \text{ Mg ha}^{-1}$ ) along with five crosses involving EP80 and two involving F7 (Table 4). In addition, EP80 × Puenteareas showed tall plants, late flowering, and low grain moisture. Although late genotypes are more productive than early ones (Shawn, 1988; Lauer et al. 1999), the genotypes with late flowering are usually more affected by stresses. Nevertheless, the cross EP80 × Puenteareas showed low grain moisture content at harvest time, which reduces damage caused by pests and fungal infections (Cartea et al. 1994; Munkvold, 2003) and problems with storage. The cross EP80 × Silver King had high productivity and showed the best values for yield components (data not shown); nevertheless it showed the highest grain moisture.

The GCA for traits related to earliness (grain moisture and silking days) and plant height, were, in general, significant for populations and for inbred lines (Table 5), agreeing with results reported by other authors, in optimum conditions (Vasal et al. 1992; Eyhéribide and González, 1997; Hede et al. 1999). These authors also found significant GCA for yield, which was not observed in the present study. No differences among SCA for any trait were found. The increase

of experimental errors due to evaluation in stress conditions could be partially responsible for this lack of significance.

The inbred line EP80 showed high values for GCA for silking days and plant height, both traits positively correlated with yield (Hallauer and Miranda, 1988); while crosses to the inbred lines F7 and Z78008 had the earliest and shortest plants. The population Silver King showed, in general, favorable GCA for all traits, except for grain moisture. This population presented the worst value of GCA for grain moisture, in spite of the expectation, because dent maize has been described as having less grain moisture than flint maize at harvest time (Hunter et al. 1979). This disadvantage showed by the crosses between Silver King and European inbred lines discourages the use of this population for further breeding programs. On the other hand, Puentearreas showed the most favorable values of GCA for grain moisture and plant height.

### *Conclusion*

None of the maize inbred lines or populations could be considered completely cold tolerant and the genetic base of cold tolerant inbred lines is not large enough for allowing the release of superior second-cycle inbred lines. Therefore, inbred  $\times$  population crosses are advisable as breeding populations for cold tolerance breeding programs. Although none of the inbred  $\times$  population combinations incorporates all requirements for emergence, early vigor, and agronomic performance, the crosses between the inbred line EP80 and northwestern Spanish populations are the most promising base germplasm for further breeding programs for cold tolerance. Particularly, EP80  $\times$  Puentearreas showed the largest yield and good performance at first stages of development under cold conditions, either in cold chamber and field. The cross EP80  $\times$  Rebordanes had better performance at first stages of development under cold conditions, but poorer agronomic characteristics at early planting. Finally, early vigor was the only trait for which differences among genotypes were observed in both cold chamber and field. So,

the results of our work suggest that early vigor would be the most suitable trait to select maize genotypes with superior cold tolerance during emergence and post-emergence stages.

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Table 1. Name, origin and grain type of the maize germplasm evaluated in cold chamber and in field trials at early planting.

Name	Origin	Grain type
Populations		
Amarillo de Marañón	Northeastern Spain	Flint
AS-B	Synthetic from University of Minnesota	Dent
Gallego/Hembrilla norteño	Spanish race	Flint
Lalín	Northwestern Spain	Flint
Puenteareas	Northwestern Spain	Flint
Rebordanes	Northwestern Spain	Flint
Rojo de Tolosa	Northeastern Spain	Flint
Santiago	Northwestern Spain	Flint
Silver King	Population from University of Minnesota	Dent
Inbred lines		
A641 <sup>†</sup>	ND203 × B14	Dent
A661 <sup>†</sup>	AS-A	Dent
EP80 <sup>‡</sup>	Selection from EA2087 (Azpeitia)	Flint
F7 <sup>‡</sup>	Lacaune O.P.	Flint
Z78007 <sup>‡</sup>	(F2 × Z36)F2	Flint
Hybrids		
EP80 × F7		
EP80 × Z78007		
Z78007 × F7		

<sup>†</sup> Inbred lines used as testers of combining ability.

<sup>‡</sup> Cold tolerant inbred lines.

Table 2. Means of cold tolerant inbred line  $\times$  populations crosses, populations, inbred lines and hybrids evaluated in the cold chamber and in the field at early planting for different traits related to early development.

Genotypes	Cold chamber trial			Field trial	
	Color	Early vigor	Days to emergence	Proportion of emergence	Early vigor
	1-9 <sup>†</sup>	1-9 <sup>‡</sup>	days	%	1-9 <sup>‡</sup>
Crosses					
EP80 $\times$ Amarillo de Marañón	5.0	6.3	12	95	4.2
EP80 $\times$ AS-B	5.0	6.4	13	96	4.4
EP80 $\times$ Gallego/Hembrilla norteño	5.3	5.3	13	95	4.5
EP80 $\times$ Lalín	4.9	4.6	13	90	5.1
EP80 $\times$ Puenteareas	4.9	5.1	14	98	5.8
EP80 $\times$ Rojo de Tolosa	4.8	6.1	12	95	4.5
EP80 $\times$ Rebordanes	5.7	5.4	13	92	5.3
EP80 $\times$ Silver King	4.8	5.3	14	93	4.2
EP80 $\times$ Santiago	5.2	5.1	13	93	4.9
F7 $\times$ Amarillo de Marañón	5.2	5.0	14	90	5.5
F7 $\times$ AS-B	5.4	4.8	12	87	5.0
F7 $\times$ Gallego/Hembrilla norteño	5.1	4.3	14	94	5.2
F7 $\times$ Lalín	5.3	5.3	12	92	5.7
F7 $\times$ Puenteareas	5.2	4.4	15	92	5.8
F7 $\times$ Rojo de Tolosa	3.6	4.7	13	91	4.8
F7 $\times$ Rebordanes	4.7	4.9	13	97	5.6
F7 $\times$ Silver King	4.7	4.6	13	95	5.0
F7 $\times$ Santiago	4.9	4.3	12	95	5.3

Z78007 × Amarillo de Marañón	4.8	5.7	13	97	5.1
Z78007 × AS-B	4.4	5.3	12	86	4.4
Z78007 × Gal/Hembrilla norteño	5.5	4.6	15	100	4.2
Z78007 × Lalín	5.2	5.0	13	98	5.6
Z78007 × Puenteareas	4.7	5.4	13	100	5.2
Z78007 × Rojo de Tolosa	5.0	5.1	12	98	4.4
Z78007 × Rebordanes	5.4	4.4	14	98	5.5
Z78007 × Silver King	4.8	4.5	14	97	4.4
Z78007 × Santiago	5.6	5.1	13	97	5.0
LSD (5%) crosses	1.1	1.1		10	1.1
Hybrids					
EP80 × F7	5.2	5.0	13	94	4.2
EP80 × Z78007	5.0	5.0	14	92	5.0
Z78007 × F7	4.0	4.1	14	92	§
Populations					
Amarillo de Marañón	4.2	5.4	13	93	4.7
AS-B	3.4	5.7	13	92	4.7
Gallego/Hembrilla norteño	5.1	5.6	12	93	5.1
Lalín	4.5	5.2	14	93	6.6
Puenteareas	4.4	5.1	15	95	7.0
Rojo de Tolosa	3.8	6.4	11	92	4.4
Rebordanes	4.1	5.1	15	87	6.9
Silver King	4.5	5.1	14	93	5.1
Santiago	5.4	5.4	12	93	5.4
LSD (5%) populations	1.2				
Inbred lines					

EP80	5.3	3.7	17	79
F7	3.9	2.8	18	67
Z78007	4.4	2.8	20	93
LSD (5%) inbred lines	0.9	1.0		14
LSD (5%)	1.0	1.0	2	10

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† 1 to 9 score (1 = albino leaves and 9 = dark green leaves)

‡ 1 to 9 score (1 = weak plants and 9 = vigorous plants)

§ This hybrid was not evaluated in the field trials.

Table 3. Estimates of general combining ability of five inbred lines and nine maize populations for seven traits related to early development evaluated in cold chamber and in early field planting.

Genotypes	Cold chamber trial			Field trial	
	Color	Early vigor	Days to emergence	Proportion of emergence	Early vigor
	1-9 <sup>†</sup>	1-9 <sup>‡</sup>	days	%	1-9 <sup>‡</sup>
Populations					
Amarillo de Maraón	-0.2	0.3	0.2	-3.7	0.1
AS-B	0.0	0.3	0.6	0.5	-0.5*
Gallego/Hembrilla norteño	0.4	-0.4*	-0.5	-0.7	-0.2
Lalín	0.2	0.0	0.4	1.2	0.7*
Puenteareas	0.1	0.0	-0.3	2.5	0.4*
Rojo de Tolosa	-0.5 *	0.1	0.4	-1.2	-0.4*
Rebordanes	0.2	0.1	-0.2	1.5	0.4*
Silver King	-0.4	-0.4*	-0.7	-2.0	-0.5*
Santiago	0.2	-0.2	0.3	1.8	0.1
LSD (5%) populations	0.6	0.5			0.5
Inbred lines					
A641	-0.6 *	0.5 *	-0.8 *	-2.7	0.3
A661	-0.8 *	-0.2	0.1	-2.4	-0.3
EP80	0.5 *	0.3 *	0.1	1.3	-0.2
F7	0.3 *	-0.5 *	0.2	-0.3	0.3
Z78007	0.5 *	-0.2	0.4	4.1	-0.1
LSD (5%)	0.4	0.4	0.7	6.0	

<sup>†</sup> 1 to 9 score (1 = albino leaves and 9 = dark green leaves)

‡ 1 to 9 score (1 = weak plants and 9 = vigorous plants)

\* Exceeded twice the standard error.

Table 4. Means of maize inbred line × populations crosses, evaluated for yield and other agronomic traits in early sowing.

Genotype	Grain	Grain	Silking	Plant
	Yield	moisture	days	heigh
	Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	days	cm
A641 × Amarillo de Marañón	5.9	200	91	215
A641 × AS-B	4.1	204	89	180
A641 × Gallego/H. norteño	5.7	217	89	192
A641 × Lalín	5.8	206	86	205
A641 × Puenteareas	7.2	211	92	237
A641 × Rebordanes	6.3	217	90	199
A641 × Rojo de Tolosa	5.7	219	93	211
A641 × Santiago	5.2	216	88	189
A641 × Silver King	5.8	215	92	214
A661 × Amarillo de Marañón	5.0	227	90	205
A661 × AS-B	3.6	230	88	182
A661 × Gallego/H. norteño	5.2	246	89	190
A661 × Lalín	5.5	215	86	188
A661 × Puenteareas	5.9	242	92	215
A661 × Rebordanes	5.7	237	89	199
A661 × Rojo de Tolosa	5.3	239	91	206
A661 × Santiago	5.3	223	87	183
A661 × Silver King	5.0	241	92	210
EP80 × Amarillo de Marañón	5.6	222	95	222
EP80 × AS-B	5.8	246	93	208
EP80 × Gallego/H. norteño	5.0	246	94	201

EP80 × Lalín	5.2	226	89	211
EP80 × Puenteareas	7.4	200	95	237
EP80 × Rebordanes	5.8	244	93	214
EP80 × Rojo de Tolosa	3.6	253	95	213
EP80 × Santiago	5.7	239	90	195
EP80 × Silver King	6.5	271	96	233
F7 × Amarillo de Marañón	4.7	222	87	181
F7 × AS-B	3.8	236	83	162
F7 × Gallego/H. norteño	4.1	233	85	180
F7 × Lalín	3.5	213	80	161
F7 × Puenteareas	5.7	230	88	193
F7 × Rebordanes	4.8	226	86	176
F7 × Rojo de Tolosa	5.5	230	84	188
F7 × Santiago	4.0	213	84	173
F7 × Silver King	5.2	249	87	186
Z78007 × Amarillo de Marañón	3.4	214	93	187
Z78007 × AS-B	3.6	211	84	184
Z78007 × Gallego/H. norteño	4.7	201	87	197
Z78007 × Lalín	3.5	222	81	189
Z78007 × Puenteareas	4.5	196	87	224
Z78007 × Rebordanes	4.2	219	85	196
Z78007 × Rojo de Tolosa	3.0	215	88	199
Z78007 × Santiago	3.9	221	84	182
Z78007 × Silver King	5.0	212	87	210
LSD (5%)	2.0	19	2	19

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Table 5. Estimates of general combining ability of five inbred lines and nine populations for yield and other agronomic traits in field early sowing.

Genotypes	Grain yield	Grain moisture	Silking days	Plant height
	Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	days	cm
Amarillo de Marañón	-0.1	- 7	2.5*	3.6
AS-B	-0.8	1	-1.4	-15.0*
Gallego/Hembrilla norteño	-0.1	4	0.1	-6.3
Lalín	-0.3	- 8*	-4.5*	-7.6
Puenteareas	1.2	- 9*	2.1*	23.0*
Rojo de Tolosa	0.3	4	-0.1	-1.5
Rebordanes	-0.4	7	1.5	5.0
Silver King	-0.2	- 4	-2.3*	-13.7*
Santiago	0.5	13*	2.1*	12.4*
LSD (5%) populations		11	2.3	13.5
A641	0.7	-14	1.1*	6.3
A661	0.2	9	0.4	-0.9
EP80	0.6	14	4.5*	16.9*
F7	-0.4	4	-3.6*	-20.4*
Z78007	-1.0	-12	-2.4*	-2.0
LSD (5%)			0.9	14.8

\* exceeded twice the standard error.