Inheritance of cold tolerance at emergence and during early season growth in maize

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Abbreviations: GCA, general combining ability; SCA, specific combining ability; COLOR, seedling color; DEMER, d from planting to 50% emergence; DLIG, d from 50% emergence to 50% of the plants having a ligule on the first leaf; EMERG, percent emergence; MORTALITY, percent emerged plants that where dead by harvest time; SCORE, emergence score; and VIGOR, seedling vigor.

ABSTRACT

Adaptation of maize (*Zea mays* L.) to early planting dates requires the improvement of cold tolerance, which implies high percent emergence and vigorous seedling growth under cold temperatures. The objectives of this work were to evaluate the combining ability of elite European maize inbreds for cold tolerance and to study the inheritance of cold tolerance. Five maize inbreds, differing in sensitivity to cold temperatures, were crossed using a diallel design. Hybrid seed was obtained at two production environments. Hybrids were planted on trays filled with sterilized peat in a cold chamber at four minimum temperatures, and these hybrids were also grown in field trials at two locations in northwestern Spain. The most cold-tolerant inbreds, according to previous unpublished inbred evaluations in the cold chamber, F7 and EA2087, produced the most cold-tolerant hybrids. Inbred F7 performed slightly better in hybrid combinations than EA2087 for emergence-related traits in the cold chamber, and EA2087 was superior in hybrid combinations to F7 for seedling growth. The inbred F7 may contribute cold tolerance at emergence, whereas EA2087 contributed cold tolerance for both emergence and seedling growth. In the field, inbreds F7 and H104W were the best parent for cold tolerance hybrids. Percent emergence was not related to the other traits. Generally, the genetic regulation of cold-tolerance traits conformed to an additive-dominance model, and it should be possible to combine both high percent emergence and vigorous seedling growth. A promising source of new cold-tolerant inbreds is the cross between EA2087 and F7.

Maize is presently grown at 55º latitude in both the northern and southern hemispheres, even though it is widely considered a warm weather crop (Shaw, 1988). As generally accepted, maize was domesticated in warm areas from where it was moved to cooler regions. In some areas of the world, such as the European Atlantic coast, with cool and humid springs, it would be useful to have cold-tolerant inbreds (with high emergence and rapid growth under low spring temperatures) for early plantings. Cold-tolerant inbreds could be planted early to promote early pollination and harvest and, therefore, to avoid summer drought and pests (Mock and McNeill, 1979). Early plantings would also permit longer growing cycles, thus higher yields (Dugan, 1944; Mock and McNeill, 1979).

The adaptation of maize to early planting requires high percent emergence and vigorous seedling growth under cool temperatures. Although there is genetic variation for such cold tolerance in adapted maize germplasm (Mock and Eberhart, 1972; Mock and McNeill, 1979), exotic populations may provide greater cold tolerance than Corn Belt Dent populations (Eagles and Brooking, 1981). European maize, particularly the Spanish germplasm, came primarily from Central and North America and has been adapted to temperate conditions during the last four centuries (Revilla et al., 1998b). Furthermore, maize grown on the European Atlantic coast as well as in central and northern Europe should have some cold tolerance during early development to be able to stand the cool and wet springs. Few laboratory (Maryam and Jones, 1983) or field (Verheul et al., 1996) studies have dealt with cold tolerance of European germplasm, although Revilla et al. (1998a) showed that European maize is a promising source of cold-tolerance.

Several authors (Hodges et al., 1995a, 1997; Revilla et al., 1998a) suggest that the ability to germinate and survive under cold conditions may be necessary, but these characteristics, by themselves, do not ensure early vigor. Hodges et al. (1995b, 1997) stated that most studies evaluate emergence either in the laboratory or in the field, but not both, and few studies have evaluated seedling growth under laboratory conditions.

The inheritance of cold tolerance is poorly understood. Maryam and Jones (1983) found that the performance of hybrids could be predicted from the inbred parents. Hodges et al. (1997) found that germination and seedling growth may be under the control of different genetic factors and that it is not possible to reliably predict hybrid maize cold tolerance from knowledge of the parental inbreds' responses. McConnell and Gardner (1979) found that epistatic, additive, and dominance gene effects were significant for germination under cool conditions, while seedling vigor was predominantly conditioned by additive and dominance effects in crosses among three warm-season and three cool-season inbreds. Eagles (1982) concluded that additive and dominance effects were present for rate of seedling growth.

In a previous screening of inbreds under cold conditions (unpublished), the inbreds F7 and EA2087 had high percent emergence. Moreover, EA2087 had more rapid seedling growth than F7. Inbreds EP40 and H104W had low percent emergence, but H104W had more rapid seedling growth than EP40. Other inbreds, such as EA2841, had intermediate sensitivity to cold stress. Available reports suggest that the genetic and physiological mechanisms for high percent emergence and vigorous seedling growth at low temperatures could be different. Given the differences among these inbreds, it seems likely that they may have different genetic and physiological mechanisms controlling emergence and seedling growth. The objectives of this work were to evaluate the combining abilities of five inbreds differing for cold sensitivity and to study the inheritance of cold tolerance during emergence and seedling growth.

MATERIALS AND METHODS

Diallel analysis

Growth Chamber trial. Inbreds F7, EA2087, EP40, H104W, and EA2841 were crossed in a diallel design. Seed of the 10 hybrids was produced in two environments (two different fields and planting dates) in 1996. Planting dates were 15 May for the first field and 5 June for the second. The first field suffered some flooding during the spring, the second field suffered some drought during the summer. Seed from each hybrid and production environment was harvested in October.

The 20 hybrid × production environment combinations were planted in trays filled with sterilized peat in a cold chamber. The cold chamber has four pairs of shelves, each pair at a different height. Each experimental plot consisted of 15 kernels. Sowing depth was 2 cm. Kernels were planted in rows spaced 5 cm apart with 2 cm between kernels. Four plots were planted in a tray, thus each replication consisted of five trays placed on a shelf. Conditions were set at 14 h with light at 14 ºC and 10 h without light at 8 ºC. Due to a gradient of ventilation within the cold chamber, the top pair of shelves were at a minimum temperature of 7 ºC, the next pair of shelves below were at 7.5 ºC, the next pair of shelves were at 8.5 ºC, and the bottom pair of shelves were at 9.5 ºC. Therefore, there was a gradient of minimum temperatures in the cold chamber resulting in two replications at each minimum temperature. Temperature variation within a replication and variation between the two replications at the same height was assumed negligible, but the differences in temperatures among pairs of replications situated at different heights were actually measured with a thermometer. The eight replications were classified in four pairs of replications according to the minimum temperatures.

We measured the following traits in the cold chamber: percent emergence (EMERG), emergence score (SCORE), d from planting to 50% emergence (DEMER), d from 50% emergence to 50% of the plants having a ligule on the first leaf (DLIG), vigor (1

= vigorous to 5 = weak) (VIGOR), and color (1 = dark green to 5 = pale green) (COLOR). Emergence score was calculated using the formula:

$$\frac{100 \times \Sigma(\text{number of plants emerged at time i / time from planting})}{\text{time from planting to end of emergence}}$$

where time is recorded in d, and i varied from 9 to 20 d for this study.

The experiment was analyzed as a randomized complete block design with four minimum temperatures, two replications per minimum temperature, 10 hybrids, and two seed production environments. Sources of variation were minimum temperature, replications within minimum temperature, production environment, hybrids, and the appropriate interactions. Production environments, replications, hybrid × production environment, minimum temperature × production environment, and hybrids × minimum temperature × production environment interactions were considered random effects while hybrids, and hybrids × minimum temperature interaction were considered fixed effects. The reasons for considering production environments as random effects were that they were not selected a priori and no conscious differences between them can be assumed. When differences among hybrids were significant, GCA and SCA analysis were made according to Model I, Method 4 of Griffing (1956). Combining abilities and standard errors were computed using the program Diallel (Burow and Coors, 1994). Analyses of variance and comparisons of means were performed for each trait using the procedure GLM of SAS (SAS Institute Inc., 1989). For the analyses of field trials, locations and replications were considered random effects while hybrids was considered a fixed effect.

Field trial. The hybrids were planted at two locations in northwestern Spain on 4 April 1997 in Cotobade (400 m above sea level) and on 24 April 1997 in Pontevedra (20 m above sea level) in an experiment arranged as a randomized complete block design with three replications per location. Both locations have a humid climate with annual rainfall about 1600 mm. The main stresses in Cotobade were cold temperatures at emergence and frosts during early season growth, while Pontevedra provided cold temperatures at emergence and drought at flowering and during the grain-filling period. Each two-row experimental plot consisted of 10 hills per row with two kernels per hill. Seeds were hand planted at a depth of 5 cm. Rows were spaced 0.80 m apart, and hills were spaced 0.21 m apart. Hills were thinned to one plant with a final plant density of approximately 60 000 plants ha^{-1}.

We measured seedling vigor (VIGOR) as in the cold chamber at the two and four-leaf stages, d from planting to 50% of the plants shedding pollen, d from planting to 50% of the plants having silks emerged, moisture content of kernels at harvest in g kg^{-1}, and grain yield in Mg ha^{-1} at 140 g kg^{-1} grain moisture. Statistical analyses of field trials were made following the model of the cold chamber trial except that the source of variation for minimum temperatures was not included in the model. The analysis was made for the two environments separately and combined. Discussion was based on the combined analysis, however, when the genotype × environment interaction was significant, the individual environment analysis was discussed.

Generation mean analysis

From the results of the diallel, inbreds EA2087, F7, and H104W were chosen, due to their different performance under cold temperatures, to evaluate genetic effects in crosses F7 × EA2087, F7 × H104W, and EA2087 × H104W. The F_1 crosses were obtained in 1996. The F_2 and the backcrosses to both parents were obtained for each hybrid in 1997. The experiment included the parental (P_1 and P_2) inbreds, the six F_1 crosses, the six F_2 generations, and the 12 backcrosses to P_1 (BC_1) and P_2 (BC_2). The 27 entries were evaluated in 1998 in a cold chamber under the same conditions used for the evaluation of the diallel with one difference: each plot had a different number of rows depending on the genotype (15 plants in one row for each inbred or hybrid, 30 plants in two rows for each backcross, and 45 plants in three rows for each F_2). Sixteen trays were used for each replication. Each pair of shelves at the same height was a replication, thus there were four replications. Differences in temperature among shelves were included in the replication term.

The traits measured were the same as for the diallel study, with the exception that DEMER, COLOR, VIGOR, and DLIG were taken on each individual plant, and additional trait, percent emerged plants that where dead by harvest time, MORTALITY, was added. MORTALITY, EMERG, and SCORE were measured on a plot basis. Due to the glossy phenotype ofF7, the scale for color was modified: 1 = pale green to 9 = dark green (glossy), in order to allow a clearer distinction of the glossy phenotype.

Analyses of variance were conducted for each trait using the MIXED procedure (SAS Institute Inc., 1996). The analysis had only two factors: replications and generations. Replications was considered a random factor and generations was considered a fixed factor. The random factors were not included in the model, and variances were obtained following the REML method (SAS Institute Inc., 1996). Since the number of plants evaluated for each entry was variable, the number of degrees of freedom used for the F tests were obtained by Satterthwaite's method (Steel and Torrie, 1980; SAS Institute Inc., 1989). The generation means were used to perform simple and joint scaling tests (Mather and Jinks, 1982). The scaling test is based on the assumption that generation means depend only on the additive and dominance effects, to test this assumption, three contrasts (A, B, and C) were used. The contrasts are represented in the following linear equations: $A=2BC_1-P_1-F_1$, $B=2BC_2-P_2-F_1$, and $C=4F2-2F_1-P_1-P_2$. Generation means were assumed independent, and variances were calculated as $V(A)=4V(BC_1)+V(P_1)+V(F_1)$, $V(B)=4V(BC_2)+V(P_2)+V(F_1)$, and $V(C)=16V(F2)+4V(F_1)+V(P_1)+V(P_2)$. A and B are contrasts of backcross means, whereas C is a contrast among parental, F_1, and F2 generation means. If the additive-dominance model is adequate quantities A, B, and C will each equal zero within the limits of sampling error. The standard errors were obtained by taking the square root of the corresponding variances.

The joint scaling test is based on estimating the mean of two lines (m), the additive gene effects (a), and the dominance gene effects (d) from the six-generation means by the method of weighted least squares. In this procedure, the weights were the reciprocal of the respective variances of generation means. Genetic parameters (additive and dominance effects), estimated from this method were used to determine the adequacy of the additive-dominance model. The goodness-of-fit to the additive-dominance model was evaluated by a weighted Chi-square (χ^2) test comparing observed and expected generation means. When non-allelic interaction was detected, the epistatic effects were also estimated. The following notation was used: m = mid-homozygote value, a = additive effects, d = dominance effects, and the non-allelic interactions: aa = additive × additive epistatic effects, ad = additive × dominance effects, and dd = dominance × dominance epistatic effects.

RESULTS AND DISCUSSION

Diallel analysis

Growth chamber trial

Minimum temperatures were a significant factor for all traits based on the combined analysis of variance of the diallel (data not shown). Production environments were also a significantly factor for all traits, and the minimum temperature × production environment interaction was not significant for any trait. Hybrids differed significantly for all traits except for SCORE, for which there was a significant hybrid × production environment interaction. The hybrid × minimum temperature interaction was not significant. When the hybrid × production environment and hybrid × production environment × minimum temperature interactions were not significant, hybrid values were expressed as the mean of production environments.

Minimum temperatures 9 and 9.5 ºC did not differ significantly except for VIGOR, though it is possible that humidity was biasing these results because the two replications at 9.5 ºC had less ventilation, and thus more residual humidity than the two replications at 9 ºC. Consequently, some traits could have been adversely affected by humidity. The changes of minimum temperatures from 9 to 7.5 ºC and from 7.5 to 7 ºC had significant effects for most traits (Table 1). As expected, the level of induced stress increases as temperatures decreases (Greaves, 1996). McConnell and Gardner (1979) also explained that at temperatures below the optimum for maize germination, small fluctuations in temperature likely have a significant effect on germination and growth.

General combining ability (GCA) was significant for all traits. Specific combining ability (SCA) was only significant for EMERG and SCORE for the second production environment (data not shown). Therefore, additive effects were generally more important than dominance effects for the genetic regulation of cold tolerance for this set of inbreds and temperatures.

The inbred F7 had the best GCA for DEMER, SCORE for the first production environment, and EMERG (Table 2). The inbred EA2087 had the best GCA for SCORE in the second production environment, COLOR, VIGOR, and DLIG. Therefore, F7 produced hybrids with the best emergence characteristics, and EA2087 produced hybrids with the most rapid growth at early stages of development and hybrids that performed well for emergence-related traits. Considering that F7 and EA2087 were previously classified as cold-tolerant, while EP40, H104W, and EA2841 were cold-sensitive (with low emergence and slow growth under cool temperatures), there was no clear relationship between inbred performance and SCA (Table 3). SCA was positive for EMERG and SCORE for the inbred EP40 in crosses to EA2087 or H104W, and for the hybrid F7 × EA2841. The hybrid EA2841 × H104W had the highest SCA for EMERG, though other hybrids were not significantly different, and EP40 × H104W had the highest SCA for SCORE (Table 3). Considering EMERG, VIGOR, and DLIG together, the most cold-tolerant hybrids were EA2087 × F7, EA2087 × H104W, and EP40 × EA2087 (Table 4). Hybrid EP40 × EA2841 had the worst EMERG. Generally, all hybrids involving EA2087 could be considered cold-tolerant (Table 4).

These results suggest that the mechanisms for emergence and for growth under cold conditions could be different. The inbred EA2087 would have both high percent emergence and quick seedling growth, and F7 high percent emergence. Hodges et al. (1997) also found that germination and seedling growth may be under the control of different genetic factors and that both GCA and SCA were significant for emergence and seedling growth under cold conditions.

Field trial

In the combined analysis of variance over locations, the interaction hybrid × production environment was not significant (data not shown). Thus, the values from both production environments were averaged for each hybrid, and the analysis was performed using these means. Hybrids differed significantly for VIGOR at the four-leaf stage, d to 50% pollen shed and silking, and grain moisture (data not shown). The interaction hybrid × location was significant for VIGOR at the two-leaf stage, d to 50% pollen shed and silking, and grain moisture. Hybrids did not differ significantly for grain yield, nor was the interaction hybrid × location significant. GCA was significant for VIGOR at two-leaf stage in both locations and at four-leaf stage, and SCA was significant for VIGOR at two-leaf stage in Cotobade and at four-leaf stage (data not shown).

The GCA for VIGOR at the two-leaf stage was significant and favorable for F7 and EP40, and unfavorable for EA2087 and EA2841 (Table 2). The differences were less apparent at the second location, where only H104W had a favorable GCA. Inbreds F7 and H104W had also favorable GCA for vigor at the four-leaf stage. These results did not match with those of the cold chamber. Revilla et al. (1998a) also observed that cold-tolerant genotypes in growth chamber trials were not necessarily the more vigorous in field trials. These results are not surprising because cold stress in the field is unpredictable and due to multiple causes. The only significant favorable SCA for VIGOR at the two-leaf stage in Cotobade was for EP40 × H104W (Table 3). The most unfavorable SCA was for EA2841 × EP40 and F7 × H104W. The most favorable SCA for VIGOR at the four-leaf stage was for EP40 × H104W followed by F7 × EA2841, and the most unfavorable for EA2841 × EP40 and F7 × H104W. The GCAs and SCAs for VIGOR at the two and four-leaf stages generally agreed, so it may not be worthwhile to record VIGOR at several stages. The significant differences among hybrids for VIGOR in the field and for cold tolerance in the cold chamber did not affect differences for grain yield, since hybrids did not significantly differ for grain yield. The hybrid EP40 × H104W had the highest VIGOR in the field, while EP40 × EA2841 had the lowest vigor, though most hybrids did not differ significantly (Table 4).

Generation mean analysis

The inbred EA2087 had significantly shorter DLIG, lighter COLOR, and higher MORTALITY than F7 (Table 5). Inbred EA2087 had higher EMERG, darker COLOR, greater VIGOR, and lower MORTALITY than H104W. Last, F7 had higher EMERG, larger DLIG, darker COLOR, greater VIGOR, and lower MORTALITY than H104W. These results confirm that EA2087 and F7 are cold-tolerant compared to H104W, which was considered cold-sensitive. Inbreds EA2087 and F7 had good performance for emergence related traits, but F7 had larger DLIG than EA2087 and H104W.

Generally, F_1 hybrids had significantly shorter DEMER, and better SCORE, EMERG, and VIGOR than either parent, while the COLOR of all F_1 hybrids was intermediate to the parents, (Table 5). The F_1, EA2087 × F7 was intermediate to EA2087 and F7 for DLIG and had lower MORTALITY than EA2087. The F_1 EA2087 × H104W had greater DLIG and lower MORTALITY than EA2087 and H104W, and F7 × H104W had intermediate DLIG and MORTALITY when compared to F7 and H104W (Table 5). From previous observations, we know that F7 has a glossy phenotype due to the presence of a recessive allele. The F_1's had COLOR close to the non-glossy parent for the hybrids involving F7 and to the highest parent for the hybrid EA2087 × H104W.

The F_2 generations were above the corresponding F_1 hybrids and both parents for SCORE and similar to the F_1 hybrids for DLIG. For COLOR, the F_2 generations were between P_1 and P_2 and above the F_1 when the inbred F7 was involved, as expected given the presence of the glossy phenotype in the F_2. The BC_1 were similar to P_1 and the BC_2 were lower than P_2 and similar to F_1 for DEMER. For most traits, the comparisons between each backcross and its corresponding recurrent parent did not follow clear patterns across hybrids, therefore inheritance did not appear to have a simple basis.

For EA2087 × F7, the simple generation means χ^2 test was not significant for DEMER, DLIG, and EMERG, meaning that the additive-dominance model was satisfactory, though the contrast B significantly deviated from zero for the first two traits (Table 6). The χ^2 test and the three contrasts (A, B, and C) were significant for SCORE, thus the additive-dominance model was also inadequate. For COLOR, VIGOR, and MORTALITY, the additive-dominance model was inadequate since the χ^2 test and two of three contrasts were significant. For EA2087 × H104W the simple model was adequate for DEMER, DLIG, COLOR, VIGOR, and MORTALITY, and inadequate for SCORE and EMERG as the χ^2 tests were significant, and two of the contrasts were significantly different from zero. For F7 × H104W only DEMER fit the simple model, but contrast A significantly deviated from zero.

For the cross EA2087 × F7 additive effects were significant for DLIG, COLOR, VIGOR, and MORTALITY, dominance effects were significant for EMERG and COLOR, and additive × additive effects were significant for SCORE and COLOR (Table 6). We can assume that both EA2087 and F7 provide favorable traits related to emergence, and that the genetic regulation of DLIG, COLOR, VIGOR and MORTALITY should allow efficient improvement through selection. Therefore, the F_2 of EA2087 × F7 would be a promising base population for improving cold tolerance and developing cold-tolerant inbreds. In such a breeding program, DEMER, SCORE, and EMERG should be monitored. The improvement of EMERG may require the use of hybrid testing because dominance effects were significant, and additive effects were not. The improvement of COLOR should not use the glossy genotype of F7 because it is not clear whether fixing the glossy genotype will have any real value.

For EA2087 × H104W additive effects were significant for DEMER, EMERG, COLOR, VIGOR, and MORTALITY; dominance effects were significant for DEMER, DLIG, COLOR, VIGOR, and MORTALITY; additive × dominance effects were significant for SCORE and EMERG; and additive × additive and dominance × dominance effects were significant for EMERG. Finally, for F7 × H104W additive effects were significant for EMERG, DLIG, COLOR, VIGOR, and MORTALITY; dominance effects were significant for DEMER, SCORE, and EMERG; additive × additive effects were significant for SCORE, EMERG, COLOR, and VIGOR; and dominance × dominance effects were significant for SCORE, EMERG, and COLOR.

Additive and dominance effects were significant for DEMER, DLIG, and MORTALITY depending on the hybrid, but no significant epistatic effects were detected. SCORE would be the hardest trait to manage through selection because only dominance effects were significant for one hybrid and various epistatic effects were significant for different hybrids. EMERG was the trait with most complex genetic regulation, having significant additive, dominance and the three epistatic effects across the three hybrids. Finally, the genetic basis of COLOR and VIGOR involved significant additive, dominance, and additive × additive effects depending on the hybrid. Eagles (1982) and Eagles and Hardacre (1979) found that additive and dominance effects were present for time to emergence and percent emergence. McConnell and Gardner (1979) found that percent germination had a complex inheritance with significant epistatic effects for several hybrids.

Generally, dominance effects were larger than additive effects (Table 6), but additive effects were significant more often than dominance effects because the generation mean analysis produced larger standard errors for dominance than for additive effects. Additive effects were never significant (Table 6) when differences between P_1 and P_2 were not significant (Table 5). When a F_2 is chosen as base population for a breeding program, we can expect significant response to selection for those traits for which the parents differ. When crossed to the cold-sensitive parent H104W, the tolerant parent EA2087 apparently provided genes with additive effects for DEMER, EMERG, COLOR, VIGOR, and low MORTALITY. When crossed to the cold sensitive parent, H104W, the cold tolerant parent F7 may have contributed genes with additive effects for EMERG, COLOR, VIGOR, and low MORTALITY, while H104W contributed genes for DLIG. These results support the existence of different genetic mechanisms for high percent emergence and rapid seedling growth under low temperatures, as Hodges et al. (1997) also proposed.

These results partially support the notion that hybrid cold tolerance can be predicted from the performance of the inbred parents and that cold tolerance is not related to grain yield. However, breeders should be cautious using cold chambers to estimate cold tolerance in the field. The inbreds F7 and H104W were the best parents for producing vigorous hybrids in the field. There appear to be at least two mechanisms of cold tolerance; one for emergence and another for seedling growth. Inbred F7 exhibited a good cold tolerance at emergence, and EA2087 had rapid seedling growth in addition to emergence. A promising source of new cold-tolerant inbreds is the cross between EA2087 and F7.

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Table 1. Means of four minimum temperatures for cold tolerance-related traits from a diallel cross among five maize inbred lines evaluated in a cold chamber.

Minimum temperature	DEMER[†]	SCORE	EMERG	COLOR	VIGOR	DLIG
°C	d		%	1-5		d
7	16.9	3.91	90.2	3.19	3.50	16.0
7.5	14.1	5.75	95.8	2.96	2.79	13.6
9	11.7	8.29	95.9	2.13	2.13	10.8
9.5	12.4	7.46	96.3	2.28	2.60	11.1
LSD (5%)	1.3	1.13	5.3	0.26	0.37	0.6

† DEMER, d from planting to 50% emergence; SCORE, emergence score; EMERG, percent emergence; COLOR, 1 = dark green to 5 = pale green; VIGOR, 1 = vigorous to 5 = weak; and DLIG, d from 50% emergence to 50% of the plants having a ligule on the first leaf.

Table 2. Estimates of general combining ability for cold tolerance-related traits from a diallel cross among five maize inbred lines evaluated in a cold chamber and in the field.

	Cold chamber trial							Field trial		
	DEMER†	SCORE		EMERG	COLOR	VIGOR	DLIG	VIGOR		
		Production envir						two-leaf stage		four-leaf stage
Inbred line		1	2					loc. 1‡	loc. 2	
	d			%	1-5		d	1-5		
EP40	-0.07	-0.35	-1.51*	-4.66*	0.17*	0.24*	0.16	-0.28*	0.04	0.25
EA2087	-0.13	0.70*	0.99*	2.55*	-0.29*	-0.61*	-1.52*	0.61*	0.12	-0.06
EA2841	-0.05	-0.57*	0.22	-3.42*	0.03	0.08	-0.44*	0.33*	0.43*	0.56*
F7	-0.36*	0.80*	-0.21	2.83*	0.13	0.16	1.31*	-0.44*	0.04	-0.42*
H104W	0.60*	-0.57*	0.52*	2.69*	-0.04	0.14	0.48*	-0.22	-0.40*	-0.33*
LSD (5%)	0.40	0.60	0.51	2.69	0.20	0.29	0.30	0.39	0.46	0.35

* Exceeded twice the standard error.

† DEMER, d from planting to 50% emergence; SCORE, emergence score; EMERG, percent emergence; COLOR, 1 = dark green to 5 = pale green; VIGOR, 1 = vigorous to 5 = weak; and DLIG, d from 50% emergence to 50% of the plants having a ligule on the first leaf.

‡ loc. 1= Pontevedra, loc. 2=Cotobade.

Table 3. Estimates of specific combining abilities for cold tolerance-related traits from a diallel cross of five maize inbred lines evaluated in a cold chamber and in the field.

Cold Chamber trial

EMERG†

	EP40	EA2087	EA2841	F7	H104W
EP40		3.40*	-8.54*	3.54*	1.59
EA2087			2.15	-2.43*	-3.12*
EA2841				1.87	4.52*
F7					-2.99*

LSD[s(i,j)-s(i,k)]=4.39, LSD[s(i,j)-s(k,l)]=3.10

SCORE (production environment 2)

	EP40	EA2087	EA2841	F7	H104W
EP40		0.55*	0.17	-1.76*	1.04*
EA2087			-0.30	0.46	-0.71*
EA2841				0.88*	-0.75*
F7					0.42

LSD[s(i,j)-s(i,k)]=0.72, LSD[s(i,j)-s(k,l)]=0.51

Field trial

VIGOR (at two-leaf stage) in Cotobade

	EP40	EA2087	EA2841	F7	H104W
EP40		0.19	0.47*	0.19	-0.86*
EA2087			-0.19	-0.31	0.31
EA2841				-0.36	0.08
F7					0.47*

LSD[s(i,j)-s(i,k)]=0.66, LSD[s(i,j)-s(k,l)]=0.46

VIGOR (at four leaf stage)

	EP40	EA2087	EA2841	F7	H104W
EP40		0.18	0.57*	0.13	-0.88*
EA2087			-0.29	-0.07	0.18
EA2841				-0.51*	0.24
F7					0.46*

LSD[s(i,j)-s(i,k)]=0.50, LSD[s(i,j)-s(k,l)]=0.35

* Exceeded twice the standard error.

† EMERG, percent emergence; SCORE, emergence score; VIGOR, 1 = vigorous to 5 = weak.

Table 4. Means of for cold-tolerant-related traits from a diallel cross among five maize inbred lines evaluated in a cold chamber and in the field.

	Cold chamber trial						Field trial		
							VIGOR		Grain
Hybrid	DEMER†	SCORE	EMERG	COLOR	VIGOR	DLIG	two-leaf stage	four-leaf stage	yield
	d		%	1-5		d	1-5		Mg ha^{-1}
EP40 × EA2087	13.7	6.5	95.8	2.6	2.3	11.6	2.9	3.6	3.1
EP40 × EA2841	13.3	5.3	77.9	2.9	3.0	13.1	3.5	4.6	3.5
EP40 × F7	13.4	5.0	96.3	2.9	3.2	13.7	2.8	3.2	3.7
EP40 × H104W	14.4	5.8	94.2	2.7	3.3	13.5	2.0	2.3	3.6
EA2087 × EA2841	13.8	7.0	95.8	2.4	2.3	10.4	3.5	3.4	3.6
EA2087 × F7	13.1	7.6	97.5	2.5	2.5	12.9	2.9	2.7	2.9
EA2087 × H104W	14.0	6.9	96.7	2.3	2.1	11.9	3.0	3.0	3.2
EA2841 × F7	13.4	6.8	95.8	2.7	2.9	13.9	2.7	2.8	3.0
EA2841 × H104W	14.4	5.8	98.3	2.7	3.1	12.7	3.1	3.7	2.5
F7 × H104W	14.1	6.9	97.1	2.8	2.9	14.8	2.6	2.9	2.9
LSD (5%)	0.8	0.7	5.5	0.4	0.5	1.5	1.2	0.7	1.6

† DEMER, d from planting to 50% emergence; SCORE, emergence score; EMERG, percent emergence; COLOR, 1 = dark green to 5 = pale green; VIGOR, 1 = vigorous to 5 = weak; and DLIG, d from 50% emergence to 50% of the plants having a ligule on the first leaf.

Table 5. Means and standard errors for cold-tolerant-related traits from generation mean analysis derived from the maize crosses EA2087 × F7, EA2087 × H104W, and F7 × H104W evaluated in a cold chamber.

Generation†	DEMER‡	SCORE	EMERG	DLIG	COLOR	VIGOR	MORTALITY
	d		%	d	1 - 9	1 - 5	%
EA2087 × F7							
P_1	21.8 ± 1.2	2.0 ± 0.8	73.3 ± 4.1	14.1 ± 0.9	5.3 ± 0.2	3.9 ± 0.2	43.4 ± 4.5
P_2	23.1 ± 1.2	1.9 ± 0.8	77.5 ± 4.1	21.9 ± 0.9	9.0 ± 0.2	3.4 ± 0.2	15.9 ± 4.5
F_1	19.6 ± 1.2	3.7 ± 0.8	96.7 ± 4.1	19.0 ± 0.8	5.5 ± 0.2	2.8 ± 0.1	14.1 ± 4.5
F_2	20.8 ± 1.1	7.6 ± 0.8	91.7 ± 4.1	18.5 ± 0.7	7.0 ± 0.1	2.9 ± 0.1	6.0 ± 4.5
BC_1	21.0 ± 1.2	5.0 ± 0.8	88.3 ± 4.1	17.2 ± 0.8	5.5 ± 0.1	3.2 ± 0.1	13.8 ± 4.5
BC_2	17.9 ± 1.2	5.9 ± 0.8	85.4 ± 4.1	18.1 ± 0.8	7.8 ± 0.1	2.6 ± 0.1	10.0 ± 4.5
EA2087 × H104W							
P_1	21.8 ± 1.3	2.0 ± 0.7	73.3 ± 4.1	13.9 ± 0.7	5.3 ± 0.2	3.9 ± 0.2	43.4 ± 5.7
P_2	25.6 ± 1.5	0.7 ± 0.7	31.7 ± 4.1	15.0 ± 0.9	3.2 ± 0.4	5.0 ± 0.3	91.3 ± 5.7
F_1	20.4 ± 1.3	2.8 ± 0.7	85.0 ± 4.1	17.1 ± 0.7	5.1 ± 0.2	2.6 ± 0.2	7.9 ± 5.7
F_2	22.4 ± 1.3	5.0 ± 0.7	72.8 ± 4.1	16.4 ± 0.6	4.4 ± 0.2	3.6 ± 0.2	30.2 ± 5.7
BC_1	19.9 ± 1.3	5.8 ± 0.7	92.5 ± 4.1	15.9 ± 0.6	5.1 ± 0.2	2.9 ± 0.2	12.1 ± 5.7
BC_2	21.9 ± 1.4	1.6 ± 0.7	29.6 ± 4.1	17.7 ± 0.7	4.3 ± 0.2	3.9 ± 0.2	38.6 ± 5.7
F7 × H104W							
P_1	23.0 ± 1.3	1.9 ± 0.7	77.5 ± 4.8	21.9 ± 0.9	9.0 ± 0.2	3.4 ± 0.2	15.9 ± 6.6

P_2	25.5 ± 1.4	0.7 ± 0.7	31.7 ± 4.8	15.1 ± 1.1	3.0 ± 0.8	5.0 ± 0.2	91.3 ± 6.6
F_1	17.1 ± 1.3	3.4 ± 0.7	80.8 ± 4.8	18.1 ± 1.0	5.0 ± 0.2	3.2 ± 0.2	34.8 ± 6.6
F_2	20.3 ± 1.3	8.1 ± 0.7	93.1 ± 4.8	20.0 ± 0.9	5.7 ± 0.1	3.1 ± 0.1	10.3 ± 6.6
BC_1	23.5 ± 1.3	3.6 ± 0.7	79.2 ± 4.8	23.4 ± 0.9	7.8 ± 0.1	3.5 ± 0.2	10.0 ± 6.6
BC_2	19.6 ± 1.3	4.0 ± 0.7	60.8 ± 4.8	17.4 ± 0.9	4.2 ± 0.2	3.8 ± 0.2	34-0 ± 6.6

† P_1 = First parent, P_2 = Second parent, $F_1 = P_1 \times P_2$, F_2 = Selfed F_1, BC_1 = Backcross to P_1, BC_2 = Backcross to P_2.

‡ DEMER, d from planting to 50% emergence; SCORE, emergence score; EMERG, percent emergence; COLOR, 9 = dark green to 1 = pale green; VIGOR, 1 = vigorous to 5 = weak; and DLIG, d from 50% emergence to 50% of the plants having a ligule on the first leaf.

Table 6. Estimates of mid homozygote (m), additive [a], dominance [d], and non-allelic interaction ([aa], [ad], and [dd]) pooled effects ± standard errors, and χ^2 test of generation mean analysis for cold tolerance traits.

Parameters		DEMER†	SCORE	EMERG	DLIG	COLOR	VIGOR	MORTALITY
		d		%	d	1 - 9	1 - 5	%
EA2087 × F7								
m		22.01 ± 1.15	10.70 ± 3.92	76.27 ± 2.15	17.74 ± 0.90	8.61 ± 0.64	3.48 ± 0.58	6.14 ± 22.40
[a]		0.22 ± 1.12	0.03 ± 0.56	-1.08 ± 2.12	-3.07* ± 0.87	-1.87* ± 0.13	0.22* ± 0.11	13.73* ± 3.20
[d]		-3.22 ± 2.11	-5.28 ± 9.42	22.11* ± 3.98	0.81 ± 1.64	-3.42* ± 1.59	-1.68 ± 1.43	-8.47 ± 53.83
[aa]			-8.75* ± 3.88			-1.47* ± 0.63	0.16 ± 0.57	23.49 ± 22.17
[ad]			-1.89 ± 2.51			-0.93 ± 0.47	0.70 ± 0.41	-19.95 ± 14.31
[dd]			-1.69 ± 5.82			0.35 ± 1.01	0.94 ± 0.90	16.38 ± 33.26
Scaling test	A	0.72 ± 2.85	4.28* ± 1.94	6.67 ± 10.00	1.33 ± 1.94	0.10 ± 0.38	-0.20 ± 0.32	-29.91* ± 11.09
	B	-6.81* ± 2.85	6.17* ± 1.94	-3.33 ± 10.00	-4.59* ± 1.93	1.03* ± 0.36	-0.90* ± 0.32	-9.96 ± 11.09
	C	-0.64 ± 5.39	19.19* ± 3.72	22.50 ± 19.14	0.07 ± 3.59	2.60* ± 0.65	-1.27* ± 0.58	-63.36* ± 21.23
Joint-scaling test	χ^2	6.33	30.74*	2.02	7.23	21.62*	10.16*	12.87*
EA2087 × H104W								
m		23.43 ± 0.58	6.64 ± 3.32	99.44 ± 20.36	15.07 ± 0.52	4.15 ± 0.20	4.45 ± 0.18	63.55*4.95
[a]		-1.95* ± 0.57	0.64 ± 0.47	20.83* ± 2.91	-0.98 ± 0.51	1.00* ± 0.20	-0.74* ± 0.18	-24.45* ± 4.88
[d]		-3.44* ± 1.03	-2.58 ± 7.98	-92.22 ± 48.94	2.42* ± 0.85	0.86* ± 0.30	-1.95* ± 0.31	-63.20* ± 9.17
[aa]			-5.31 ± 3.28	-46.95* ± 20.15				

[ad]			7.23* ± 2.12	84.16* ± 13.01				
[dd]			-1.30 ± 4.93	77.78* ± 30.23				
Scaling test	A	-2.27 ± 3.18	6.92* ± 1.64	26.67* ± 10.08	0.80 ± 1.50	-0.28 ± 0.38	-0.73 ± 0.45	-27.11 ± 14.05
	B	-2.21 ± 3.36	-0.32 ± 1.64	-57.50* ± 10.08	3.30 ± 1.85	0.34 ± 0.61	0.22 ± 0.52	-21.98 ± 14.05
	C	1.23 ± 6.07	11.91* ± 3.15	16.11 ± 19.30	2.65 ± 2.88	-0.90 ± 0.83	0.39 ± 0.86	-29.67 ± 26.91
Joint-scaling test	χ^2	1.13	27.99*	48.48*	3.52	3.03	3.86	5.43
F7 × H104W								
m		24.29 ± 1.44	18.46 ± 3.51	146.81 ± 23.93	17.09 ± 4.38	4.81 ± 0.72	1.98 ± 0.72	6.57 ± 32.61
[a]		0.02 ± 1.41	0.62 ± 0.50	22.92* ± 3.42	3.41* ± 0.74	3.00* ± 0.43	-0.79* ± 0.14	-37.68* ± 4.66
[d]		-6.78* ± 2.60	-26.50* ± 8.44	-149.03* ± 57.52	10.71 ± 10.65	3.20 ± 1.99	3.23 ± 1.76	-13.52 ± 78.35
[aa]			-17.16* ± 3.48	-92.23* ± 23.69	1.42 ± 4.32	1.87* ± 0.58	2.23* ± 0.70	47.01 ± 32.27
[ad]			-2.05 ± 2.24	-9.17 ± 15.29	5.16 ± 2.97	1.14 ± 0.96	0.94 ± 0.51	27.47 ± 20.83
[dd]			11.44* ± 5.21	83.06* ± 35.53	-9.71 ± 6.66	-2.98* ± 1.34	-2.02 ± 1.11	41.77 ± 48.40
Scaling test	A	6.96* ± 3.14	1.83 ± 1.74	0.00 ± 11.84	6.73* ± 2.26	1.47* ± 0.39	0.37 ± 0.38	-30.65 ± 16.13
	B	-3.41 ± 3.21	3.89* ± 1.74	9.17 ± 11.84	1.57 ± 2.36	0.33 ± 0.92	-0.57 ± 0.41	-58.13* ± 16.13
	C	-1.51 ± 5.95	22.88* ± 3.33	101.4* ± 22.68	6.88 ± 4.24	0.61 ± 1.04	-2.44* ± 0.71	-135.8* ± 30.90
Joint-scaling test	χ^2	7.38	48.11*	21.53*	9.54*	17.52*	17.77*	26.05*

* Estimated effect or contrast exceeded twice the standard error, and χ^2 was above the tabulated χ^2 at P=0.05

† DEMER, d from planting to 50% emergence; SCORE, emergence score; EMERG, percent emergence; COLOR, 9 = dark green to 1 = pale green; VIGOR, 1 = vigorous to 5 = weak; and DLIG, d from 50% emergence to 50% of the plants having a ligule on the first leaf.