Maize populations as sources of favorable alleles to improve cold-tolerant hybrids

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Executive summary

Low spring temperatures are one of the main limiting factors for maize (*Zea mays* L.) growth in the European Atlantic coast. The objective of the present study was to identify cold-tolerant maize populations with favorable alleles to improve cold tolerance and agronomic performance in early sowing of two cold-tolerant hybrids. Improved versions of the cold-tolerant inbred parents could be developed from crosses among the inbreds F7 or Z78007 and the populations Puenteareas, Rojo de Tolosa or Silver King, but the simultaneous improvement of yield and cold tolerance is not straightforward.

Abstract

Low spring temperatures are one of the main limiting factors for maize (*Zea mays* L.) growth in the European Atlantic coast. Several breeding programs have been carried out to obtain cold-tolerant maize genotypes, but the few cold-tolerant inbreds and hybrids available need further improvements for cold tolerance and agronomic performance. The objective of the present study was to identify cold-tolerant maize populations with favorable alleles to improve cold tolerance and agronomic performance in early sowing of two cold-tolerant hybrids. The parental inbred lines of two cold-tolerant hybrids were crossed to nine cold-tolerant populations. Tests were carried out in the cold chamber and in the field in two years at two locations. The populations Rojo de Tolosa and Puenteareas were the most promising sources of new favorable alleles for transferring cold tolerance to the hybrids EP80 × F7 and EP80 × Z78007, respectively. The populations Puenteareas and Silver King were the most outstanding donors to improve the agronomic performance of EP80 × F7 and EP80 × Z78007, respectively. Summarizing, some improved versions of the cold-tolerant inbred parents could be developed from crosses between F7 or Z78007 and Puenteareas or, alternatively, Rojo de Tolosa or Silver King could be used as donors of favorable alleles, but the simultaneous improvement of yield and cold tolerance is not straightforward.

Abbreviations: $lp\mu'$, relative number of loci containing favorable alleles in a population and absent in a hybrid; NI , net improvement; PNG_{agg} , probability of a net gain of favorable alleles when partial dominance and/or complementary epistasis is prevalent.

Cold temperature is one of the main stresses limiting maize performance in temperate regions. Chilling temperatures reduce emergence, seedling vigor, and early vigor, and produce chlorotic leaves (Miedema, 1982; Revilla et al., 2005). In the European Atlantic coast, maize cannot be sown before late May, because average and minimum temperatures are usually below 15 °C, and 10 °C, respectively.

Most studies on cold tolerance have been carried out in a cold chamber or in the field, but seldom in both. Menkir and Larter (1985) reported that cold tolerance in the cold chamber is not correlated with performance in the field. Similar results were obtained by other authors (Hope et al., 1992; Revilla et al., 2000; Rodríguez et al., 2007). These authors recommended both methods of evaluation to select genotypes tolerant to low temperatures and with high agronomic performance. Certainly, evaluation of cold tolerance in the cold chamber allows precise control of cold temperatures, but does not predict plant growth under field conditions. Early sowing in the field is the only practical procedure to evaluate cold tolerance, but it does not guarantee cold conditions and suffers high climatic variability and strong fluctuations over time. Cold chamber evaluations could be used for screening for cold tolerance potential, while field evaluation provides information about the actual performance of the cold-tolerant genotypes in the field.

Many efforts have been dedicated to increase cold tolerance, to advance sowing dates and to permit cultivation of cultivars with longer cycles and higher yield (Revilla et al., 2003, 2005; Ordás et al., 2006). Mosely et al. (1984) achieved an improvement of 16.6 % in the rate of germination of the population BS3 in early sowing under cold conditions. Landi et al. (1992) employed full-sib divergent recurrent selection based on the highest and the lowest value of germination under cold and optimum conditions, and achieved an increase in percent germination and a reduction in days to germination in one population of maize.

Few maize inbreds and populations have been identified as base germplasm for breeding for cold tolerance at the early stages of development (Hotchkiss et al., 1997; Revilla et al., 2005; Revilla et al., 2003; Ordás et al., 2006; Rodríguez et al., 2007). Revilla et al. (2000) found that the inbred line EP80 had a high percentage of emergence and rapid seedling growth, while F7 showed a high percentage of emergence. In previous unpublished studies carried out in the cold chamber, these inbred lines, together with Z78007, were identified as cold-tolerant at the early stages of development. However, the hybrids EP80 $\times$ F7, EP80 $\times$ Z78007, and especially F7 $\times$ Z78007, showed low yield at early sowing and short growing cycle (Rodríguez et al., 2007). Cold-tolerant populations could be used as sources of favorable alleles for improving cold tolerance and agronomic performance of the current cold-tolerant inbred lines. The improved inbred lines that could result from those crosses would be subsequently used to produce cold-tolerant hybrids with higher yield, or to improve cold tolerance of elite inbred lines with longer growing cycle and higher yield.

Dudley (1987) proposed a method to identify populations with favorable alleles, absent in an elite hybrid. This method is based on the fact that, for any pair of lines, four classes of loci could be considered, classes i, j and k having favorable alleles for one or both parental lines, and class l carrying less favorable alleles for both lines. The statistic $h\mu'$ was defined as an estimate of the product of the relative number of loci not containing at least one favorable allele in the hybrid and the average frequency of favorable alleles at such loci in the population. Several statistics were developed based on Dudley's theory, some of them offer specific contributions, particularly NI (net improvement) (Bernardo, 1990) is appropriate for choosing donor populations for direct production of improved inbreds, and PNG_{ceg} (probability of a net gain of favorable alleles when partial dominance and/or complementary epistasis is prevalent) (Metz, 1994) for non dominant gene effects. These statistics were widely employed to estimate

the potential improvement of field and sweet maize hybrids (Dudley, 1988; Cartea et al., 1996; Kraja and Dudley, 2000; Trifunovic et al., 2001). Dudley originally proposed this method for using exotic populations as a source of new favorable alleles. Nevertheless, several studies have been carried out involving adapted populations (Cartea et al., 1996; Ordás et al., 2006; Revilla et al., 1998; Taller and Bernardo, 2004). In different studies, the efficiency of these statistics has been compared (Hogan and Dudley, 1991; Pfarr and Lamkey, 1992; Revilla et al., 1998). High correlations were found among these statistics; however, each author proposed a different estimator as the most appropriate in each case.

Given the poor performance of the cold-tolerant inbreds available, their direct use as sources of favorable alleles for cold tolerance to improve elite hybrids with high yield is not recommended. Alternatively, cold-tolerant populations could be used as sources of favorable alleles for both yield and cold tolerance to improve cold-tolerant inbreds. This approach should produce enhanced cold-tolerant inbreds either for use as parents of improved hybrids or as enhanced donors of favorable alleles for cold tolerance to improve elite hybrids. The objective of the present study was to identify cold-tolerant maize populations with favorable alleles to improve cold tolerance and agronomic performance in early sowing of two cold-tolerant hybrids.

Material and Methods

Nine populations were evaluated as sources of favorable alleles for improving the cold tolerance and the agronomic performance of two cold-tolerant hybrids (EP80 $\times$ F7 and EP80 $\times$ Z78007) (Table 1). In the cold chamber, the nine populations *per se*, 27 population $\times$ inbred line crosses, two cold-tolerant hybrids and three parental inbred lines were grown using a randomized complete block design with four replications. Fifteen seeds of each genotype per replication were planted in 10-liter trays with 6 liters of sterilized and watered peat (Gramoflor GmbH & Co. KG, Vechta, Germany). Sowing depth was 2 cm and seeds were planted in rows spaced 5 cm apart with 2 cm between seeds. Conditions were set at 14 h with light [provided by seven VHO (very high output) fluorescent lamps with a photosynthetic photon flux (PPF) of 228 $\mu\text{mol m}^{-2} \text{s}^{-1}$] at 14 °C, and 10 h without light at 8 °C. All trays were watered again with 300 ml of water 20 days after planting, which was enough to keep the plants turgid. Field evaluations were carried out in two locations in northwestern Spain, 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) and two years, 2003 and 2004. Both locations have a humid climate with an annual rainfall of about 1600 mm and summer drought; and the soil is acid sandy loam. Agronomic handling was made according to the local practices, including irrigation of about 60 liters m^{-2} at flowering. Minimum temperatures can fall below 0 °C during the month following planting, the mean minimum temperature being between 5 and 8 °C; and the mean maximum temperatures between 18 and 20 °C.

Populations, population $\times$ inbred crosses, and hybrids were evaluated along with other genotypes not used for this study, following a 7 $\times$ 8 lattice design with three replications, (Rodríguez et al., 2007). Each experimental plot consisted of two rows with 17 hills per row

and two seeds per hill. The three parental inbred lines were grown in a randomized complete-block design with four replications in an adjacent trial, to avoid interactions between genotypes, as the first trial included genotypes of similar heterozygosity and growing cycle, and the second trial included only the inbred lines. In the inbred trial, each experimental plot consisted of two rows with 15 hills per row and two seeds per hill. In both experiments, 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 60000 plants ha⁻¹. Planting dates were April 16th, 2003 and 2004 in Pontevedra and April 24th, 2003 and April 13th, 2004 in Pontecaldelas.

Traits measured in the cold chamber were: seedling vigor (1=weak to 9=vigorous) defined as a general appearance of the plant at the end of the heterotrophic period (Revilla et al., 1999), days from emergence (days from the emergence of half of the plants in each plot to 30 days after sowing, which is the time when emergence had ended for all plots), percentage of emergence (% of emerged plants), and survival percentage (% of final plants). Traits measured in the field included early vigor (at the beginning of autotrophic stage), days from silking to September 1st, plant height (cm), grain yield (Mg ha⁻¹), and grain dry matter concentration (g kg⁻¹, calculated as the proportion of dry matter left after discounting moisture).

In the population and cross trials, 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. 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). Means of each trait were used to calculate three statistics to choose the population that had more favorable alleles not present in the hybrid. These statistics were $\bar{h}\mu'$ (Dudley, 1987), the original estimator of favorable alleles, NI, the most appropriate for short term breeding (Bernardo, 1990), and PNG_{ceg} (net gain of favorable alleles if partial dominance or

complementary epistasis is prevalent), suitable for traits not following a strict dominance model (Metz, 1994). The estimator $h\mu'$ was calculated following Dudley (1987, 1988). Let I_1 and I_2 be inbred parents and P_y the donor population. NI was the maximum of $(I_1 \times P_y) - (I_1 \times I_2)$ and $(I_2 \times P_y) - (I_1 \times I_2)$ (Bernardo, 1990). PNG_{ceg} was computed as the maximum of $((I_1 \times P_y) - I_1) / (2(I_1 \times P_y) - I_1 - P_y)$ and $((I_2 \times P_y) - I_2) / (2(I_2 \times P_y) - I_2 - P_y)$ (Metz, 1994).

The standard errors of each estimator were calculated as the square root of the variance of the linear function associated with each estimator. Estimators were considered different from zero if they exceeded twice their own standard error. Values for each estimator and within each hybrid were considered significantly different when the difference between a pair of estimates exceeded twice the standard error of the difference.

Dudley (1987) also proposed a method to determine the parental inbred line to be improved. Following this method, the expression $(I_2 \times P_y) - (I_1 - P_y) + 1/2 (I_1 - I_2)$ was calculated. If this expression had positive value, the population (P_y) was crossed to inbred I_1 , and to I_2 if the expression had negative value. In addition, estimates of $j\mu$ and $k\mu$ reported information about whether to self in F_2 or to backcross prior to selfing. If I_1 is being improved, when $j\mu = h\mu'$ selfing in the F_2 would be the best approach whereas backcrossing to I_1 is advised when $j\mu > h\mu'$ and backcrossing to P_y when $j\mu < h\mu'$. If I_2 is being improved, then the appropriate comparison is between $k\mu$ and $h\mu'$.

Results and Discussion

Differences among inbred $\times$ population crosses were significant for seedling vigor and chamber % emergence, and early vigor, grain yield, grain dry matter, and silking days in the field (Table 2). Therefore, statistics to identify donors of favorable alleles were computed only for those traits. The means used to calculate the statistics are shown in Table 2.

The populations evaluated as potential donors differed significantly only for seedling vigor in the cold chamber and for silking days in the field; the hybrids did not differ significantly for any trait; the inbred parents differed significantly for seedling vigor and chamber % emergence and for dry matter in the field, and the inbred $\times$ population crosses differed for seedling vigor, proportion of emergence, early vigor, grain yield, grain dry matter, and silking days (Table 2).

Early stages of development

High correlations between the estimators of favorable alleles evaluated in this study have been reported by many authors (Pfarr and Lamkey, 1992; Cartea et al., 1996; Taller and Bernardo, 2004). Agreeing with those authors, the populations chosen as best donors of favorable alleles based on different statistics were the same, except those selected on the base of PNG_{ceg} , the only statistic that assumes partial dominance or epistasis.

Most populations could provide new favorable alleles for improving the cold tolerance of the hybrids. Differences among populations as donors of favorable alleles for EP80 $\times$ F7 were observed for seedling and early vigor (Table 3). For seedling and early vigor, only differences among PNG_{ceg} values of the donors were significant, which is in agreement with

results obtained by other authors (Cartea et al., 1996; Malvar et al., 1997). According to the PNG_{ceg} statistic, Rojo de Tolosa had the highest value for seedling vigor, while Lalín, Rebordanes, and Silver King were the best donors of early vigor. For seedling vigor, six populations showed significant estimates for $h\mu'$, but only three of them, Amarillo de Maraón, AS-B, and Rojo de Tolosa, also showed significant values for NI. Most populations could be used to improve seedling vigor of EP80 $\times$ F7, but the improvement would be more efficient with the populations that showed significant estimates for NI. On the other hand, none of the populations showed significant estimates of NI for early vigor. Differences among donor populations for improving the percentage of emergence of the hybrid EP80 $\times$ F7 in the cold chamber were not significant for any statistic. Regarding the $h\mu'$ values, most of the populations could be used for increasing emergence of this hybrid, except Lalín and Rebordanes. However, we would not expect to obtain improved inbreds directly by self-pollinating the inbred parent $\times$ population donor because estimates for NI were not significant.

The best donors of cold tolerance at the emergence stage (cold chamber evaluation) were Lalín and Rebordanes, but they were among the least appropriate as sources of cold tolerance at the post-emergence stage (field evaluation). Rojo de Tolosa was the best potential donor for improving all traits in both the cold chamber and the field, as it had favorable alleles for improving percentage of emergence in the cold chamber, and early vigor in the field, and was the best donor for seedling vigor in the cold chamber.

For improving hybrid EP80 $\times$ Z78007, different populations were identified as the best donors of cold tolerance in the cold chamber and in the field (Table 4). In the cold chamber, all populations, except Lalín and Santiago, showed significant values of $h\mu'$ for seedling vigor. However only Amarillo de Maraón, AS-B, and Rojo de Tolosa showed

significant values for NI. Puenteareas was the best potential donor for improving early vigor, because it showed the highest values for $h\mu'$ and PNG_{ceg} and a significant estimate for NI. Therefore, Puenteareas was the most promising donor for improving seedling and early vigor of EP80 $\times$ Z78007.

Agronomic performance in early sowing

For improving yield of hybrid EP80 $\times$ F7, all donor populations showed positive and significant values of $h\mu'$, indicating that donor populations had favorable alleles not present in the hybrid (Table 5). Puenteareas was the best donor population, according to all statistics except PNG_{ceg} , followed by Silver King and Rebordanes. The statistic PNG_{ceg} was proposed by Metz (1994) as a useful statistic when partial dominance or/and epistasis are important, while the rest of estimators assume complete dominance. For traits with important dominance effects, such as yield, PNG_{ceg} is not an appropriate estimator.

For grain dry matter concentration of hybrid EP80 $\times$ F7, no populations showed favorable alleles according to $h\mu'$. Only PNG_{ceg} showed positive and significant values for several populations. Nevertheless, when means of donor populations are lower than the hybrids, as in the present study (Table 2), the relative number of favorable alleles at class l can be negative and greater in absolute value than the relative number of favorable alleles at other classes of loci. In this situation, the interpretation of PNG_{ceg} would be erroneous (Cartea et al., 1996). Therefore, grain dry matter concentration is an important trait to be monitored in the breeding process because populations could provide unfavorable alleles for this trait.

Three populations, Puenteareas, Rebordanes, and Silver King, showed the best values of $h\mu'$ for yield. However, for grain dry matter concentration, Silver King showed significant

and negative values for $h\mu'$. Puenteareas was the only population with positive and significant estimates of NI for yield. This indicates that, using Puenteareas as donor of favorable alleles for improving the hybrid EP80 $\times$ F7, the probability of gaining favorable alleles is higher than the probability of losing them (Bernardo, 1990). Therefore, Puenteareas is the best donor population to obtain new improved inbred lines. This population also could provide favorable alleles for improving days from silking, plant height and most yield components (data not shown).

Few differences were observed among donor populations of agronomic performance for improving hybrid EP80 $\times$ Z78007 (Table 6). Again, all estimators except PNG_{ceg} identified Puenteareas as the best donor population, along with Silver King. For dry grain matter concentration, most populations had estimates of q_j and q_k outside the limits of 0 and 1, so it was impossible to estimate $h\mu'$ and PNG_g . Dudley (1988) pointed out that, when estimates of $h\mu'$ could not be obtained, $(I_1 \times P_y) - (I_2 \times P_y)$ values are larger than either $(I_1 \times I_2) - I_1$ or $(I_1 \times I_2) - I_2$, suggesting possible epistatic effects. When $h\mu'$ could be calculated, donor populations showed negative and significant values, indicating that unfavorable alleles for dry grain matter concentration would be provided by these populations. Puenteareas had a negative and significant estimate of $h\mu'$, so the population Silver King will be more appropriate for improving hybrid EP80 $\times$ Z78007. Besides, this population was the best donor population for all yield components (data not shown).

To determine which parental inbred line must be crossed to the donor population, only yield was considered because heterotic patterns are based on grain yield. According to Dudley (1988), in a practical breeding program, the parent to be improved would be the most closely related to the population. In the present study, populations are not significantly more related to one parental inbred line than to another, so the inbred line to be improved will, generally, be

the one with the lowest performance. For improving agronomic performance of hybrid EP80 $\times$ F7, the population Puenteareas must be crossed to inbred line F7 and backcrossed with the donor population before self-pollination, because $h\mu'$ was significantly higher than $j\mu$. Simultaneously, for improving cold tolerance, the population Rojo de Tolosa could be crossed to inbred EP80. For improving yield of EP80 $\times$ Z78007, Silver King must be crossed to Z78007 and, again, backcrossing to the donor population will be needed prior to self-pollination. Alternatively, EP80 could be crossed to the population Puenteareas.

Conclusion

Most populations are potential donors of favorable alleles for improving cold-tolerant hybrids. The population Puenteareas was the best donor for the improvement of EP80 $\times$ F7, and F7 the best receptor or, alternatively, the donor can be Rojo de Tolosa and the receptor EP80. The cross F7 $\times$ Puenteareas should be an appropriate source of inbreds with improved cold tolerance to combine with inbreds from EP80 $\times$ Rojo de Tolosa. For the improvement of hybrid EP80 $\times$ Z78007, the best donor of alleles for cold tolerance was Puenteareas, with EP80 as receptor, or Silver King for the receptor Z78007. Puenteareas and Silver King were the best donors of cold tolerance and yield, respectively.

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Table 1. Name, origin, and grain type of the maize populations and inbred lines evaluated as sources and recipients of favorable alleles for improving cold tolerance.

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
Hybrids		
EP80 × F7		
EP80 × Z78007		
Inbred parents		
EP80	Selection from EA2087 (Azpeitia)	Flint
F7	Lacaune O.P.	Flint
Z78007	(F2 × Z36)F2	Flint

Table 2. Means of populations, inbred lines, hybrids, and populations $\times$ inbred crosses evaluated in the cold chamber and in the field (Data for inbred $\times$ population crosses were published by Rodríguez et al., 2007)

	Seedling	Days from	Proportion	Early	Grain	Grain dry	Silking
Genotypes	vigor	emergence	emergence	vigor	yield	matter	days [†]
Populations	1-9 [‡]	Days	%	1-9 [‡]	Mg ha ⁻¹	g kg ⁻¹	days
A. Marañón	5.4	17	93	4.7	2.3	78	92
AS-B	5.7	17	92	4.7	2.6	78	86
Gallego/H norteño	5.6	18	93	5.1	3.6	76	91
Lalín	5.2	16	93	6.6	5.4	80	81
Puenteareas	5.1	15	95	7.0	5.5	76	96
Rebordanes	5.1	15	87	6.9	3.8	77	89
Rojo de Tolosa	6.4	19	92	4.4	3.8	76	94
Santiago	5.4	18	93	5.4	4.4	79	85
Silver King	5.1	16	93	5.1	3.6	76	93
LSD (5%)	1.2	ns	ns	ns	ns	ns	2
Inbred lines							
EP80	3.7	13	79	2.1	0.8	77	99.7
F7	2.8	12	67	2.8	0.7	82	90.6
Z78007	2.8	10	93	2.6	0.5	82	92.1
LSD (5%)	1.0	ns	14	ns	ns	2	ns
Hybrids							
EP80 $\times$ F7	5.0	17	94	4.2	5.1	80	88
EP80 $\times$ Z78007	5.0	16	92	5.0	3.9	77	84
LSD (5%)	ns	ns	ns	ns	ns	ns	ns

Inbred × Population

EP80 × A Marañón	6.3	18	95	4.2	5.6	78	95
EP80 × AS-B	6.4	17	96	4.4	5.8	75	93
EP80 × G/H norteño	5.3	17	95	4.5	5.0	75	94
EP80 × Lalín	4.6	17	90	5.1	5.2	77	89
EP80 × Puenteareas	5.1	16	98	5.8	7.4	80	95
EP80 × Rojo de Tolosa	6.1	18	95	4.5	3.6	75	95
EP80 × Rebordanes	5.4	17	92	5.3	5.8	76	93
EP80 × Silver King	5.3	16	93	4.2	6.5	73	96
EP80 × Santiago	5.1	17	93	4.9	5.7	76	90
F7 × A Marañón	5.0	16	90	5.5	4.7	78	87
F7 × AS-B	4.8	18	87	5.0	3.8	76	83
F7 × G/H norteño	4.3	16	94	5.2	4.1	77	85
F7 × Lalín	5.3	18	92	5.7	3.5	79	80
F7 × Puenteareas	4.4	15	92	5.8	5.7	77	88
F7 × Rojo de Tolosa	4.7	17	91	4.8	5.5	77	84
F7 × Rebordanes	4.9	17	97	5.6	4.8	77	86
F7 × Silver King	4.6	17	95	5.0	5.2	75	87
F7 × Santiago	4.3	18	95	5.3	4.0	79	84
Z78007 × A Marañón	5.7	17	97	5.1	3.4	79	93
Z78007 × AS-B	5.3	18	86	4.4	3.6	79	84
Z78007 × G/H norteño	4.6	15	100	4.2	4.7	80	87
Z78007 × Lalín	5.0	17	98	5.6	3.5	78	81
Z78007 × Puenteareas	5.4	17	100	5.2	4.5	80	87
Z78007 × Rojo de Tolosa	5.1	18	98	4.4	3.0	78	88

Z78007 × Rebordanes	4.4	16	98	5.5	4.2	78	85
Z78007 × Silver King	4.5	16	97	4.4	5.0	79	87
Z78007 × Santiago	5.1	17	97	5.0	3.9	78	84
LSD (5%)	1.1	^{ns}	10	1.1	2.0	2	2

[†] Days from silking to the 1st of September.

[‡] 1 to 9 score (1 = low vigor and 9 = high vigor).

^{ns} Differences were not significant.

Table 3. Estimates of $h\mu'$, NI, and PNG_{ceg} for nine populations evaluated as sources of favorable alleles to improve the first stages of development of the cold-tolerant single cross EP80 $\times$ F7 evaluated in both cold chamber and early planting in the field.

Estimator [†]	A. Marañón	AS-B	G./H. Norteño	Lalín	Puenteareas	Rebordanes	R. Tolosa	Santiago	Silver King
Seedling vigor (cold chamber)									
$h\mu'$	0.88a*	0.85a*	0.45a*	0.30a	0.38a	0.53a*	0.75a*	0.38a	0.48a*
NI	0.65a*	0.70a*	0.15a	0.15a	0.05a	0.20a	0.55a*	0.05a	0.15a
PNG_{ceg}	1.22g-j	1.82e	7.50b	3.00d	1.78ef	1.11g	9.50a	3.75c	1.38fg
Early vigor (field)									
$h\mu'$	0.62a*	0.52a*	0.62a*	0.88a*	0.93a*	0.84a*	0.46a*	0.68a*	0.51a*
NI	0.21a	-0.04a	0.06a	0.33a	0.38a	0.28a	-0.10a	0.12a	-0.02a
PNG_{ceg}	1.31cd*	1.19d*	1.40cd*	1.93ab*	1.69bc*	2.00a*	0.96d*	1.26d*	1.74ac*

* The estimate exceeded twice its own standard error. Means with the same letter within the same row do not differ significantly (LSD 5%).

[†] $h\mu'$, as defined by Dudley (1987); NI, net improvement as defined by Bernardo (1990); and PNG_{ceg} as defined by Metz (1994).

[‡] Estimate of $h\mu'$ cannot be calculated (Dudley, 1987).

Table 4. Estimates of $h\mu'$, NI, and PNG_{ceg} for nine populations evaluated as sources of favorable alleles to improve the first stages of development the cold-tolerant single cross of EP80 $\times$ Z78007 evaluated in both cold chamber and early planting in the field

Estimator [†]	A. Marañón	AS-B	G./H. Norteño	Lalín	Puenteareas	R. Tolosa	Rebordanes	Silver King	Santiago
Seedling vigor (cold chamber)									
$h\mu'$	0.98a*	0.98ab*	0.48ab*	0.23c	0.45bc*	0.85ab*	0.50ab*	0.48ab*	‡
NI	0.65ab*	0.70a*	0.15ab	0.00b	0.20ab	0.55ab*	0.20ab	0.15ab	0.05b
PNG _{ceg}	0.91e*	1.19de*	2.25bc*	3.00a*	1.00e*	2.30b*	1.78cd*	1.55d*	1.27de
Early vigor (field)									
$h\mu'$	0.73a-c*	0.48bc*	0.45d*	1.08a*	1.03a*	0.51d*	1.03ab*	0.49bc*	0.76a-c*
NI	0.43ab	0.08b	0.11b	0.68ab*	0.77a*	0.14ab	0.63ab*	0.09b	0.36ab
PNG _{ceg}	1.31de*	1.22e*	2.48b*	1.93c*	3.24a*	1.04e*	2.01c*	1.74cd*	1.26e*
Percentage of emergence (cold chamber)									
$h\mu'$	2.18a	‡	3.83a	2.98a	3.78a	3.03a	2.78a	2.18a	2.23a
NI	2.50a	2.00a	4.15a	3.30a	4.10a	3.35a	3.10a	2.50a	2.55a
PNG _{ceg}	0.90a*	0.83a	0.88a*	1.45a*	0.88a*	0.85a	0.74a	1.00a*	0.98a*

* The estimate exceeded twice its own standard error. Means with the same letter within the same row do not differ significantly (LSD 5%).

[†] $\hat{\mu}$, as defined by Dudley (1987); NI, net improvement as defined by Bernardo (1990); and PNG_{ceg} as defined by Metz (1994).

[‡] Estimate of $\hat{\mu}$ cannot be calculated (Dudley, 1987).

Table 5. Estimates of $h\mu'$, NI, and PNG_{ceg} for nine populations evaluated as sources of favorable alleles to improve the agronomic performance in early sowing of the cold-tolerant single cross EP80 $\times$ F7

Estimator [†]	A. Marañón	AS-B	G./H. Norteño	Lalín	Puentearcas	Rebordanes	R. Tolosa	Santiago	Silver King
Yield									
$h\mu'$	1.12*ab	0.98*ab	0.82*b	0.72*b	1.84*a	1.22*ab	0.80*b	0.97*ab	1.48*ab
NI	0.26 a	0.40 a	-0.04 a	0.06 a	1.17*a	0.38 a	0.20 a	0.30 a	0.72 a
PNG_{ceg}	0.62 b	0.71 b	0.87*b	3.35*a	0.95*b	0.80*b	1.09*b	1.15*b	0.73*b
Grain dry matter concentration									
$h\mu'$	0.26 a	-0.92 ac	-0.92 ac	0.05 ab	-0.81 ac	-0.81 ac	-1.26*bc	-0.58 ab	-2.19*c
NI	0.30 a	-0.43 a	-0.27 a	0.72 a	-0.12 a	0.09 a	-0.13 a	0.74	-1.06 a
PNG_{ceg}	1.14 ab	0.79*ab	1.08*ab	0.67 ab	1.12*ab	1.17*ab	1.33*a	1.04*ab	0.83*b

* The estimate exceeded twice its own standard error. Means with the same letter within the same row do not differ significantly (LSD 5%).

[†] $h\mu'$, as defined by Dudley (1987); NI, net improvement as defined by Bernardo (1990); and PNG_{ceg} as defined by Metz (1994).

Table 6. Estimates of $h\mu'$, NI, and PNG_{ceg} for nine populations evaluated as sources of favorable alleles to improve the agronomic performance in early sowing of cold-tolerant single cross EP80 $\times$ Z78007

Estimator [†]	A. Marañón	AS-B	G./H. Norteño	Lalín	Puenteareas	Rebordanes	R. Tolosa	Santiago	Silver King
Yield									
$h\mu'$	1.16*ab	1.28*ab	1.31*ab	1.09*ab	1.90*a	1.42*ab	0.56*b	1.32*ab	1.78*a
NI	0.85 ab	0.99 ab	0.55 ab	0.66 ab	1.76*a	0.97 ab	-0.14 b	0.89 ab	1.31*ab
PNG_{ceg}	0.72 bc	0.76*bc	0.79*c	2.71*a	1.30*bc	0.90*c	1.53*b	1.16*c	0.77*c
Grain dry matter concentration									
$h\mu'$	‡	‡	-1.49*a	‡	-1.36*a	‡	‡	‡	‡
NI	-0.68 a	-0.52 a	-1.04 a	0.26 a	-0.91 a	-0.71 a	-1.01 a	-0.54 a	-1.16 a
PNG_{ceg}	1.30*cd	1.60*bd	1.64*cd	1.18*ad	1.82*c	2.15*ab	2.29*a	1.09*d	1.36*cd

* The estimate exceeded twice its own standard error. Means with the same letter within the same row do not differ significantly (LSD 5%).

[†] $h\mu'$, as defined by Dudley (1987); NI, net improvement as defined by Bernardo (1990); and PNG_{ceg} as defined by Metz (1994).

[‡] Estimate of $h\mu'$ cannot be calculated (Dudley, 1987).