Evaluation of European maize germplasm under cold conditions

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Abstract

Maize (*Zea mays* L.) is a tropical crop with low tolerance to cold conditions and has to be planted late in temperate areas due to low temperatures. The objective of this research was to identify new cold tolerant populations among the European germplasm useful for improving adaptation to early sowing. For that, the European Union Maize Landraces Core Collection (EUMLCC) was evaluated under cold conditions. After a preliminary screening of 95 populations the 11 populations with best germination and early growth under cold conditions were multiplied and evaluated in a cold chamber and in early field sowings at two locations during two years. The cold tolerant populations from the EUMLCC were not significantly different from the cold tolerant checks in the cold chamber. In early field sowing, some EUMLCC populations had similar emergence than the commercial checks and the coldest tolerant hybrids, and higher vigor than all the hybrids and the yield of some of the populations surpassed the yield of the cold tolerant hybrids. Altogether, Aranga1 emerged as the most promising candidate as base population for improving adaptation to early sowing.

Key words: adaptation; cold tolerance; European maize; germplasm; *Zea mays*.

Introduction

Breeding maize for early sowing requires base germplasm with the ability to germinate and grow vigorously under cold conditions (Gupta, 1985; Lauer et al., 1999; Mock and Pearce, 1975; Shawn, 1988). Several authors have identified maize genotypes appropriate for cultivation under cold conditions or early field sowings (Adetimirin et al., 2006; Lee et al., 2002; Mosely et al., 1984; Revilla et al., 2003a; Revilla et al., 2000; Semuguruka et al., 1981; Verheul et al., 1996), however, cold tolerance in elite maize germplasm is partial, and favorable genes are difficult to identify (Adetimirin et al., 2006; Rodriguez et al., 2008). We would expect that maize from northern latitudes, such as European germplasm, would perform relatively well under cold conditions.

Maize was introduced in southern Europe from tropical areas of America and later was adapted also to northern latitudes (Revilla et al., 2003b). During those movements, maize suffered natural and artificial selection for adaptation to cold temperatures. Among southern European maize, the Spanish germplasm has been largely screened for cold tolerance, and the outcome was not particularly promising, since none of the populations or inbred lines was tolerant enough for a significant advance of current sowing dates (Rodriguez et al., 2007a). In addition, none of the available cold tolerant maize varieties are completely tolerant to cold stress and have poor yield and agronomic performance (Revilla et al., 2005). Nevertheless, most of the cold tolerant populations had favorable alleles useful to improve cold tolerant hybrids (Rodriguez et al., 2007b).

The search of sources of cold tolerance has not produced completely satisfactory results so far and wider collections of germplasm should be screened. In the last decade, the European institutes conserving maize collections have developed a large collection of autochthonous varieties. Given the unmanageable magnitude of the complete European collection, those institutes have established a core collection called the European Union Maize Landrace Core Collection (EUMLCC). The EUMLCC includes representative samples of maize varieties from six countries and offers a convenient opportunity to screen the variability of European maize. The objective of this research was to test the performance of European maize germplasm for early sowing and under cold conditions.

Material and Methods

EUMLCC screening

The 95 populations of the EUMLCC were screened for cold tolerance, along with 8 cold tolerant hybrids, used as checks. The 103 entries were grown during 30 days in a 20 m^3 cold chamber equipped with three selves separated 0.5 m. Conditions were set at 14 h at 14 ºC with light [provided by seven VHO (very high output) fluorescent lamps with a photosynthetic photon flux (PPF) of 228 µmol m^{-2} s^{-1}], and 10 h at 8 ºC without light. Evaluations were arranged following a randomized complete block design with three replications (one replication on each shelf). Twenty six grains from each entry were planted in 21 L trays filled with 12 L of sterilized and watered peat (Gramoflor GmbH & Co. KG, Vechta, Germany). Six cold tolerance related traits were considered: leaf color, vigor, proportion of emergence, days to emergence, emergence rating and proportion of survival. Emergence was recorded every two days up to a maximum of 30 days. Analyses of variance were performed for all traits. Sources of variation were genotypes and replications. Genotypes were considered fixed effect. Analyses were made using the GLM procedure of SAS (SAS Institute, 2007).

Evaluation of cold tolerant populations

We selected 11 cold tolerant EUMLCC populations using an independent culling system in which populations were selected if they were not significantly different from the best one for each trait. The 11 populations were multiplied in order to produce in the same environment enough seed for further evaluations. Multiplications were made by sowing 300 plants from each population and all possible crosses were made between plants by using each plant once as male or as female.

In the cold chamber the 11 EUMLCC populations were evaluated along with 3 populations improved for cold tolerance, and 5 cold-tolerant hybrids (Table 1). The methodology and the conditions for the evaluation of these 19 varieties in the cold chamber were identical to those of the previous screening carried out for the entire EUMLCC.

In the field the 11 EUMLCC populations plus checks were evaluated in early field sowings during two years (2005 and 2006) in two locations, Pontevedra (42º 24' N, 8º 38' W, 20 m above sea level) and Pontecaldelas (42º 23´ N, 8º 32´ W, 300 m above sea level). Genotypes were evaluated in a randomized complete block design with three replications. Each experimental plot consisted of two rows with 25 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. Currently accepted management and cultural practices were used in all trials. We measured the same traits as in the cold chamber. In addition, we evaluated agronomic performance by recording stand, stem lodging, root lodging, days to silking, days to pollen shedding, plant height, plant appearance, ears per plant, grain moisture, grain yield, yield/moisture rate (yield in kg/ha divided by moisture in g/kg), ear length, 100 grain weight, and ear rows. Analyses of variance were performed for each trait, being the sources of variation locations, years, replications, varieties, and their interactions. Varieties were considered as fixed effect and locations, years and all possible interactions were considered random effects.

Results and Discussion

The EUMLCC has a range of flowering from early to medium and has been adapted to a quite wide range of climatic conditions, from the cold north to the warm Mediterranean area. Such adaptation could have been possible by increasing tolerance to cold conditions or by avoiding cold temperatures by shortening the growing period. Previous data show that early varieties grown in cold areas are not usually cold tolerant, while those varieties whose cycles imply being sown under cold conditions are more likely tolerant (Revilla et al., 1998). Accordingly, the eleven cold tolerant populations selected from the EUMLCC for having high emergence and vigour have medium to late flowering time, and those with higher yield under early sowing have also high moisture at harvest.

Cold tolerance cannot be evaluated in the field because temperature is unpredictable and fluctuates. On the other side, evaluations of cold tolerance in growth chambers are homogeneous and independent of environment, allowing precise evaluations of cold tolerance. However, the cold chamber is so diverse from the real conditions that those data have to be validated in field experiments. Indeed, in the field, climatic conditions are variable and several diseases and pests alter seed germination at the first stages of plant growth, enlarging the errors of estimates. The present results show that genotypes emerged earlier at the field than in the cold chamber, though with lower proportion, suggesting that cold tolerance was not the main limiting factor for germination in the field. Nonetheless, varieties significantly differed for most vegetative traits, except days to emergence, leaf color, and ears per plant.

Emergence in the cold chamber and in the field was not clearly related. In fact, the correlation coefficients between each trait measured in the field and in the cold chamber were not significant and below 0.3 (Data not shown). Varieties with short emergence period and high rate of emergence in the cold chamber had pale color in the field. Actually correlations were 0.62 ($P < 0.01$) between days to emergence in the field and color in the chamber, and -0.50 ($P < 0.05$) between rate of emergence in the field and color in the chamber. Considering color in the field, the correlations with emergence in the chamber followed the same pattern. Varieties with a large emergence proportion in the cold chamber, as Spin or Aranga1, had a low emergence in the field, and the opposite was true for Tuy and Rebordanes(F)C2. However, varieties as Guernika and Viseu have an acceptable emergence both in the cold chamber and at the field.

Regarding emergence and early vigor at the field, Tuy and Rebordanes(F)C2 had high values for both, and Lagos and Sajambre had low values for both. Menkir and Larter (1985) pointed out that emergence related traits determined under controlled environment conditions were not correlated with those recorded in the field; however, the evaluation for cold tolerance can only be guaranteed in a cold chamber, since the temperature in the field is unpredictable and variable. Therefore, both the laboratory and the field evaluations are necessary for choosing the best genotypes in order to effectively evaluate for cold conditions in the cold chamber and for field performance under natural conditions.

Altogether, the EUMLCC populations Aranga1 and Viseu were the most cold tolerant, Aranga1 and Baiao outstand for vegetative traits, and Tuy, Aranga1, Lagos, and Guernika yielded more than some cold tolerant hybrids. However, differences in growth cycle are large and, it has to be pointed out that, among the previous varieties, only Baiao has short time to flowering and low grain moisture at harvest, followed by Viseu, while Guernika, Tuy and Aranga1 are medium cycle varieties and Lagos has the longest cycle and the highest moisture. Therefore, the EUMLCC population Aranga1 was the most promising candidate as base population for improving cold tolerance in maize. Aranga1 is a medium-cycle semi-flint and yellow-kernel population from Galicia (northwest of Spain). The latest population Lagos, and the earliest one, Sajambre, performed quite poorly, while the early population Baiao had intermediate values for yield and other agronomic traits.

The relationships between cold chamber and field evaluations depends on the genotypes and the environments, and some authors report significant and positive correlations between emergence in controlled environments and at the field, or between emergence and seedling growth, while others do not find clear relationships (Menkir and Larter, 1985; Revilla et al., 1999; Rodriguez et al., 2007a). Differences between the cold chamber and the field are mainly due to the variable and unpredictable conditions of the field, while differences between emergence and early growth are due to physiological and genetic effects (Cooper and Macdonal, 1970; Hodges et al., 1997; Revilla et al., 1999). Therefore, evaluations of genotypes should involve cold chamber trials in order to actually estimate tolerance to cold conditions, as well as field trials for screening agronomic performance under real conditions. Furthermore, evaluations should involve a series of measurements of emergence and early growth in the chamber, and until grain yield in the field.

Contrarily to our expectations, in the preliminary screening of the whole collection, maize populations from the north performed worse than southern populations under cold conditions since only populations from southern countries (except for Estarvielle from France) were cold tolerant. Certainly, previous reports with reduced numbers of entries have shown that genotypes coming from colder areas are not necessarily more cold tolerant than those from warmer areas. In fact, Revilla et al. (1998) affirmed that the origin of a variety in a cold region does not warranty cold tolerance, because genotypes with short growing cycle escape cold temperatures when planted late, and Malvar et al. (2005)stated that selection would favor cold tolerance in long-cycle populations which must be sown early. Accordingly, the population performing best in the cold chamber and at early sowing was the medium-cycle Aranga1, but most medium-cycle or late populations were not so cold tolerant.

As conclusion, the EUMLCC populations belonging to the flint European germplasm group have some potential value for improving cold tolerance of maize, although they have to be improved for yield and other yield components and agronomic traits.

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Table 1. Cold tolerant maize populations from the European Union Maize Landrace Core Collection (EUMLCC) and checks evaluated in a cold chamber and early sowing.

Variety	Germplasm type	EUMLCC Code	Country	Kernel type	Kernel color
Aranga1	Landrace	ESP11973C03	Spain	Semi-flint	Yellow
Baiâo	Landrace	PRT00100120	Portugal	Flint	Yellow
Estarvielle	Landrace	FRA0410010	France	Flint	Yellow
Guernika	Landrace	ESP11982031	Spain	Flint	Yellow
Guetaria	Landrace	ESP0070784	Spain	Flint	Yellow
Hazas de Soba	Landrace	ESP0070127	Spain	Flint	Brown
Lagos	Landrace	PRT00100530	Portugal	Flint	Orange
Sajambre	Landrace	ESP0090300	Spain	Semi-flint	Orange
Spin	Landrace	ITA0370143	Italy	Semi-flint	Red
Tuy	Landrace	ESP0090205	Spain	Flint	Yellow
Viseu	Landrace	PRT00100394	Portugal	Flint	Yellow
Rebordanes(F)C2	Improved population	Check	Spain	Flint	White
Santiago(F)C2	Improved population	Check	Spain	Flint	Yellow
Silver King(F)C2	Improved population	Check	USA	Dent	White
EP80 x F7	Cold tolerant hybrid	Check		Flint	Yellow
EP80 x Z78007	Cold tolerant hybrid	Check		Flint	Yellow
A666 x F7	Cold tolerant hybrid	Check		Flint x Dent	Yellow
A666 x EP80	Cold tolerant hybrid	Check		Flint x Dent	Yellow
A666 x Z78007	Cold tolerant hybrid	Check		Flint x Dent	Yellow
Miguel	Commercial hybrid	Check		Dent	Yellow
Randa	Commercial hybrid	Check		Dent	Yellow

Table 2. Means and least significant difference (LSD) for the cold tolerant populations from the European Union Maize Landrace Core Collection and checks evaluated in a cold chamber

Variety	Days to emergence	Proportion of emergence	Rate of emergence	Seedling vigor	Leaf color	Proportion of survival
	days	0 – 1	Plants day^{-2}	1 - 9	1 - 9	0 - 1
Aranga1	19.0	0.92	15.7	4.0	4.7	1.00
Baiao	18.3	0.88	21.1	5.0	5.0	0.99
Estarvielle	16.3	0.92	31.4	6.7	5.0	0.99
Guernika	17.3	0.91	20.5	4.3	3.0	0.99
Guetaria	18.0	0.72	23.5	5.3	4.3	0.98
Hazas de Sobas	17.3	0.86	24.9	5.0	3.7	1.00
Lagos	17.7	0.68	23.5	5.3	4.7	0.98
Sajambre	15.7	0.85	35.0	6.3	4.7	1.00
Spin	16.7	0.95	32.1	6.3	5.3	1.00
Tuy	18.3	0.76	17.1	5.0	4.3	1.00
Viseu	17.0	0.91	29.1	6.0	5.0	1.00
Rebordanes(F)C2	17.3	0.87	25.0	6.0	3.7	0.99
Santiago(F)C2	18.7	0.91	19.6	3.3	4.0	1.00
Silver King(F)C2	18.7	0.91	17.9	4.0	3.3	1.00
EP80 x F7	18.3	0.82	18.6	4.0	4.0	1.00
EP80 x Z78007	16.7	0.93	33.8	5.7	6.0	1.00
A666 x F7	17.7	0.60	15.9	4.0	4.3	0.93
A666 x EP80	18.3	0.67	16.8	4.3	4.3	0.98
A666 x Z78007	18.0	0.80	25.6	4.3	4.3	0.98
LSD (0.05)	**_**	**0.13**	**_**	**_**	**_**	**0.03**

Table 3. Means and standard errors (SE) of stover traits for the cold tolerant populations from the European Union Maize Landrace Core Collection and checks evaluated at two locations during two years ordered by flowering time.

Variety	Days to emergence	Proportion of emergence	Early vigor	Leaf color	Stand	Stem lodging	Root lodging	Female flowering	Male flowering	Plant height	Plant appearance
	Days	0 – 1	1 - 9	1 - 9	0 – 1	0 – 1	0 – 1	days	days	cm	1 – 9
Sajambre	12.0	0.50	4.2	4.7	0.35	0.34	0.03	58	57	124	1.3
Estarvielle	12.1	0.60	5.5	4.3	0.57	0.22	0.01	62	61	157	3.0
Hazas de Sobas	12.4	0.62	4.9	4.7	0.65	0.28	0.00	67	66	170	3.7
A666 x Z78007	12.4	0.49	3.9	4.7	0.60	0.13	0.00	69	67	177	4.7
Baiao	12.3	0.74	5.9	5.7	0.82	0.10	0.02	69	68	178	4.9
A666 x F7	12.4	0.61	4.4	5.0	0.79	0.33	0.02	69	68	170	4.1
EP80 x Z78007	14.0	0.13	3.2	4.3	0.28	0.11	0.01	71	70	197	4.9
Viseu	11.8	0.64	6.4	5.0	0.75	0.28	0.01	72	70	196	5.1
Santiago(F)C2	11.8	0.67	5.6	5.0	0.85	0.14	0.01	72	70	179	4.8
Tuy	12.0	0.69	7.1	6.0	0.81	0.32	0.03	73	71	203	6.0
EP80 x F7	12.3	0.50	4.4	5.0	0.70	0.16	0.00	73	71	193	4.9
Guernika	11.6	0.63	5.8	5.3	0.76	0.44	0.02	74	71	208	6.2

Spin	11.8	0.55	4.5	5.0	0.68	0.13	0.01	75	72	199	5.3
Rebordanes(F)C2	11.9	0.74	6.9	5.3	0.86	0.19	0.03	75	73	188	5.4
Aranga1	12.1	0.60	6.7	5.3	0.78	0.18	0.02	76	74	210	6.9
SilverKing(F)C2	10.9	0.57	4.2	4.7	0.64	0.40	0.04	76	74	196	4.8
A666 x EP80	11.5	0.65	5.1	5.3	0.83	0.11	0.00	76	74	220	7.6
Guetaria	12.1	0.56	5.3	5.0	0.72	0.11	0.02	78	75	194	5.6
Miguel	11.8	0.65	3.7	4.7	0.84	0.07	0.00	80	79	226	7.7
Randa	12.6	0.59	4.0	4.7	0.83	0.06	0.00	86	84	231	8.2
Lagos	12.4	0.52	4.3	4.3	0.68	0.23	0.07	89	86	228	6.4
LSD (0.05)	**_**	**0.21**	**0.9**	**_**	**0.17**	**0.15**	**0.02**	**2**	**2**	**14**	**1.0**

Table 4. Means and standard errors (SE) of ear traits for the cold tolerant populations from the European Union Maize Landrace Core Collection and checks evaluated at two locations during two years ordered by kernel moisture at harvest.

Variety	Ears per plant	Grain moisture	Grain yield	Yield / moisture	Ear length	100-Kernel weight	Ear rows
	No.	g/kg	t/ha	%	cm	g	No.
A666 x Z78007	1.01	188	2.9	15.5	15	35	11
EP80 x Z78007	1.11	189	1.5	8.1	16	32	12
Santiago(F)C2	1.02	190	3.4	17.9	15	29	13
Hazas de Sobas	1.09	190	2.0	10.7	15	29	9
Estarvielle	1.18	194	2.4	12.5	13	35	11
Baiao	1.18	197	3.6	18.3	14	30	11
Viseu	1.11	206	3.5	17.4	17	31	12
Rebordanes(F)C2	1.03	208	4.4	20.8	18	37	11
SilverKing(F)C2	0.92	208	2.7	13.9	14	29	15
Sajambre	1.59	212	0.7	3.1	10	29	8
Miguel	1.09	214	6.0	28.2	18	30	15
Aranga1	1.11	216	4.2	19.8	16	35	13
Guernika	1.03	217	3.8	17.3	15	35	12
Spin	0.98	217	3.0	13.8	15	29	13
EP80 x F7	1.10	222	3.8	17.6	17	29	13
A666 x F7	1.02	224	4.3	19.4	16	30	12
Tuy	1.01	226	4.6	20.6	16	41	11
A666 x EP80	1.04	231	5.1	21.8	17	34	13
Randa	1.02	232	7.8	33.7	18	35	17
Guetaria	0.87	245	3.0	12.2	15	38	13
Lagos	1.04	302	4.1	13.4	18	40	12
LSD (0.05)	**_**	**23**	**1.3**	**5.8**	**2**	**3**	**1**