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Effect of three cycles of recurrent selection for yield in four Spanish landraces of maize

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Abstract Landraces of maize (*Zea mays* L.) have a variability not found in the elite hybrids due to the bottleneck during selection. There is a renewed interest in using that variability, incorporating alleles for specific traits, or widening the general variability of elite germplasm. In Europe, there is also an interest in the direct use of local landraces by farmer's associations seeking agriculture that preserves crop diversity. Pre-breeding programs are aimed to reduce the yield gap among landraces and improved materials. In the Misión Biológica de Galicia we have carried out three cycles of a S_1 recurrent selection program for yield on four local landraces from Northwestern Spain with the objective of obtaining improved populations that might be cultivated by farmers and that could be used as sources of superior inbred lines. One hundred plants were selfed in each landrace and the S_1 families were evaluated in a 10×10 simple lattice. The 20 S_1 families with the highest grain yields were randomly intercrossed to form the selected population of each landrace. After three cycles of selection, each population and its cycles of selection were evaluated in three locations for two years. All the populations had a consistent yield gain in the first cycle of selection. However, the response in later cycles differed among the populations: one population had a continuous increase of 21% per cycle while two populations had a reduced yield in the last cycles. Our results highlight the need to include secondary traits to avoid unintentional indirect effects. We conclude that, in pre-breeding programs, it could be worthy to use large effective numbers to prevent inbreeding depression, particularly when the objective is the direct use of improved landraces. Another conclusion is that recurrent selection does not seem to be as efficient on European germplasm as it is on Corn Belt varieties. Finally, obtaining valuable new germplasm requires a sustained, long-term effort.

Keywords Maize · Recurrent selection · European germplasm · Prebreeding

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Introduction

The first maize (*Zea mays* L.) planting in the Old World was probably done in Seville (Southern Spain) in 1494 (Brandolini 1970). Various authors (Rebourg et al. 2003; Revilla et al. 2003) have studied the expansion of cultivation in Europe. Hybrid maize began to spread throughout Western Europe through an FAO-sponsored trial network in the 1950s and local open-pollinated varieties, also known as landraces, quickly disappeared from cultivation. The adoption of hybrids was slower in those areas where the hybrids, based initially on Corn Belt germplasm, showed poor adaptation.

Most of the maize varieties presently grown in the developed, temperate areas of the world are single-cross hybrids that are based on a very small proportion of the total germplasm of the species. Butruille et al. (2015) give a summary of the North American situation pointing out that only five open-pollinated varieties derived from one race (Corn Belt Dent) of the available 250–300 races constitute the source of the majority of modern U.S. germplasm. These authors also indicate that genetic diversity is not necessarily a commercial goal, but a need for long-term risk management. White et al. (2020) point out that in the U.S. nearly all commercial breeding occurs in the private sector. They studied the inbreds mostly used by large commercial companies, namely Corteva Agriscience (former Pioneer Hi-Bred), Bayer Crop Science, and Syngenta, and found that the top five most frequently used inbreds, based on Plant Variety Protection certificates through 2017, came from the germplasm groups Stiff Stalk and Iodent/Oh43. Given that Stiff Stalk and Iodent are mostly derived from the old Reid Yellow Dent variety, the narrow genetic base of the germplasm used by breeders is alarming. The genetic uniformity of the varieties grown by farmers can be catastrophic. The notorious Southern Corn Leaf Blight epidemic of 1970 led to an overall financial loss of \$1.0 billion at that time (≥ \$6.0 billion by 2015 standards) (Bruns 2017). This author cites Ullstrup's advice of 1972 "Diversity must be maintained in both the genetic and cytoplasmic constitution of all important crop species".

A similar situation occurs in Western Europe with the flint germplasm which is very important in this area because of the frequent use of the heterotic pattern "Corn Belt Dent × European Flint" for the production of hybrids. Broadly speaking, the dent inbred contributes productivity to the hybrid, while the flint line brings, among other characteristics of adaptation to specific environments, early vigor, a trait of great importance in the cool and humid springs of Western Europe. The initial sources for developing inbred lines to generate commercial hybrids were, as in the U.S., landraces that were selfed for several generations until homozygosity was attained. These first-cycle inbreds are homozygous genotypes obtained from those source populations. Obtaining the first-cycle inbred is a process of sampling genes from the original variety. Subsequent selfing in the same population will repeat the sampling and the probability of getting a better inbred will be limited if there is no change in allelic frequencies in the original population. As the parents of hybrids originated from few landraces, recycling these lines for the development of the second cycle inbreds has led to narrowing the flint heterotic pool (Hölker et al. 2019a).

Several researchers around the world, aware of the danger posed by the loss of genetic variability associated with the abandonment of landraces, made efforts to collect them before their total disappearance. In the 1950s, two collections of local varieties of maize were established in Spain, one in the Maize Improvement Center (Alcalá de Henares, Madrid) and the other in the Misión Biológica de Galicia (Biological Mission of Galicia). The first collection consisted of 360 open-pollinated varieties and was the basis for the creation of the Spanish races (Sánchez-Monge 1962). The second collection was lost after the maize breeding program was discontinued in the 1960s.

The Misión Biológica de Galicia (MBG) is a research institute located in Pontevedra in the northwestern corner of the Iberian Peninsula that belongs to the Spanish National Research Council (CSIC). When the MBG maize improvement program was restarted in 1974, the first effort was aimed at redoing a collection of Spanish germplasm, with special emphasis on Galicia (Northwest Spain) since maize improvement for this region was the initial objective of the center. Collecting landraces in Galicia was facilitated because this region, due to its climatic and socio-economic peculiarities, was the only Spanish maize area at that time where hybrids had not yet been introduced fully, with many areas still occupied by landraces. To the collection of Galician landraces, we added a set of Spanish varieties from the VIR of St. Petersburg, populations collected in Spanish areas outside Galicia, and the Spanish races (Sánchez-Monge 1962), giving rise to a total collection of 216 landraces of field maize and popcorn from all over Spain.

We implemented a program of selfing to obtain first-cycle inbreds. The success was, as expected, quite limited and we obtained only a few inbreds with good combining abilities. Most of the inbreds showed, however, a good early vigor, usually transmitted to their hybrids, when compared to inbreds and hybrids of similar maturity from Minnesota or Wisconsin. Early vigor, as told before, is a necessary trait for hybrids grown in Galicia because of its usual cool and humid springs. The lack of this trait had retarded the expansion of hybrids based on Corn Belt germplasm in this region.

In addition, although open-pollinated varieties seem to have mostly disappeared from cultivation in Europe, the real situation is somewhat different. There is an increasing demand for them, and in some cases, they have come back replacing hybrids. For instance, two farmers' associations started reintroducing two maize landraces (Marano and Sponcio) in 1999 in the Veneto region of Italy (Fenzi and Couix 2021). A similar reintroduction of landraces came about in Aquitaine (France): a farmers' association (AgroBio Périgord), which works to develop organic agriculture, started bringing back 11 landraces from Guatemala in 2000. This initial reintroduction of landraces was continued in the following years with populations from Latin America and Europe (Fenzi and Couix 2021). M. Robin Noël, from Agrobio Perigord, has informed us that since 2000 they have given the seed of landraces to more than 1,000 farmers across France, but also in Italy, Portugal, Switzerland, and Belgium. In addition to Agrobio Perigord, there are some 15 other structures of rural development in France located in Poitou–Charentes, the Basque Country, the Landes, Région Centre, Loire, Haute-Saône… where farmers gather around those questions of seed autonomy, preservation of cultivated biodiversity, and breeding programs. It should be added that in the last years, there have been considerable research efforts on maize landraces at both the University of Hohenheim and the Technical University of Munich to get new germplasm useful to develop commercial varieties (Böhm et al. 2017; Hölker et al. 2019b; Mayer et al. 2020; Melchinger et al. 2017).

In general, open-pollinated varieties are less productive than hybrids. Recurrent selection methods have been shown to be effective in increasing yield in varieties from the Corn Belt, but data on their effectiveness in Western European populations are not available.

In 1999, we started a selection program on local landraces using S_1 recurrent selection with the objective of obtaining improved populations that might be used as sources of open-pollinated varieties for alternative modes of maize production that demand this type of variety (Fenzi and Couix 2021) and superior inbred lines. This paper reports the results of three cycles of S_1 recurrent selection with four landraces from Northwestern Spain.

Materials and methods

The maize bank of the Misión Biológica de Galicia

The collection of landraces of MBG is maintained in a cold room with low humidity. Periodically, germination tests are carried out and when a population shows germination below 70%, it is multiplied next season by planting 150 plants to be able to make plant-to-plant crosses. Each plant is used as either male or female, but never as both. At harvesting, 20 kernels are taken from each ear to form a bulk of 1000 kernels that represent at least 100 ears from the population. These kernels are used only for a new multiplication of the variety when needed. The remaining kernels from each ear are bulked to form a remaining mass that is used for any other purpose.

The program of selection

Plant material

For the present work, we chose four landraces from Galicia that represented the main types of maize, two early and two midseason, one white and one yellow in each group, grown traditionally in this region (Table 1).

Scheme of selection

The four landraces were subjected to S_1 recurrent selection. For each population, we proceeded as follows. Nine hundred kernels from the remaining mass were planted into 450 hills with two kernels per hill. When the plants were in the 4–5 leaf stage, we discarded the less vigorous plant from each hill. At flowering, we selfed the 150 earliest plants with desirable agronomic features (strong green color, absence of tillers, adequate ear settlement, no lodging, good production of pollen…). At harvest, the 100 best ears were shelled and kept in cold storage. Next year, for each of the four populations we planted a trial of the 100 S_1 families arranged in a 10×10 simple lattice.

Table 1 Populations of maize subjected to three cycles of S_1 recurrent selection

Population	Synonym	Code	Type	Year of collection
Rebordanes		ESP009-ZMV0020	Early, white	1974
Ribadumia		ESP009-ZMV0238	Midseason, white	1980
Tuy		ESP009-ZMV0205	Midseason, yellow	1975
Viana	Bibei	ESP009-ZMV0214	Early, yellow	1977

Each plot consisted of one row of 25 plants. The distance between plants in a row was 21 cm and the rows were set 80 cm apart for a plot size of 4.2 m^2 and a planting density of approximately 60,000 plants ha^{-1}. Two kernels per hill were planted and thinned to one plant per hill at the four to five leaf-stage.

Yield data were analyzed with PROC LATTICE of SAS (SAS Institute 2012). After the analysis of the data, the 20 S_1 families with the highest grain yield were randomly intercrossed by hand pollinations to form the Syn1 populations; then, a 20% selection intensity was used at each cycle of selection. The next year the Syn1 populations were selfed to start the second cycle of selection. In addition, in this same year, the Syn1 populations were randomly mated in a separate block to obtain the Syn2 populations. We repeated these processes two more times to complete three cycles of selection in each population.

Evaluation of the progress from selection

Prior to the evaluation of the efficiency of selection, we multiplied by random mating each cycle of selection in each population. Each population and its cycles of selection were evaluated in three locations for two years. Two locations were placed in Galicia (Pontevedra and Figueirido) and the other in Aragón (Ebro Valley) to sample the environments in which maize is grown in Spain: without irrigation (Figueirido), with limited irrigation (Pontevedra) and with full irrigation (Aragón).

We carried out six trials for each population and its cycles of selection. Four hybrid checks were included in each trial (Table 2). Two of the control hybrids were old open-formula hybrids from the University of Minnesota that we have used over the years as long-time checks for maturity. Minhybrid 6304, whose cycle is FAO 300, was included in all trials. For the evaluations of the early populations (Rebordanes and Viana), we added Minhybrid 7301 (FAO 200), while for the trials of the late ones (Ribadumia and Tuy), we included Minhybrid 5302 (FAO 400). The other two checks were elite commercial hybrids at the time of the trials. Due to the lack of availability of seed, two of the commercial hybrids had to be changed in the second year.

The experimental plots in Galicia were hand planted and measured 9.7 m^2 at a planting density of 59,500 plants ha^{-1}; in Aragón, they were machine planted and measured 7.3 m^2 at a planting density of 71,400 plants ha^{-1}. On each experimental plot, we took data on days to silking, days to tasseling, lodging (the sum of root and stalk lodging), kernel moisture at harvest, and yield of grain adjusted to 14% moisture.

Table 2 Maize hybrids used as yield and maturity checks for the evaluation of three cycles of a S_1 recurrent selection program for yield on four Spanish landraces

Name	Formula	Maturity rating (FAO)	Population trials	Notes
Minhybrid 7301	(A639 × A638) W182B	200	Rebordanes Viana	
Minhybrid 6304	(A239 × A251) A635	300	All	
Minhybrid 5302	(A437 × A556) A632	400	Ribadumia Tuy	
LG33.03	Unknown	200	Rebordanes Viana	
NK Thermo	Unknown	300	All	First-year
PR36W66	Unknown	400	Ribadumia Tuy	
DKC5276	Unknown	400	All	Second year

Statistical analyses

For each variety, we analyzed the five traits mentioned above with the PROC GLIMMIX of SAS. A comparison of means was made with Tukey–Kramer's method (Kramer 1956).

The normality of a priori non-Gaussian traits was tested with the Shapiro–Wilk statistics as implemented in the PROC SUMMARY of SAS. For checking the possible loss of variability resulting from selection, we carried out a test of covariance parameters based on the restricted likelihood for yield using the PROC GLIMMIX of SAS.

Results and discussion

Maize has been cultivated in the Old World for more than 500 years. Obviously, this period of time is not enough to bring about substantial changes in the species, but it suffices for selecting blocks of genes for adaptation to particular environments and for developing varieties for specific areas.

Kernel moisture is primarily a Gaussian trait (amount of water in the grain), but measured as a proportion may be non-Gaussian. The Shapiro–Wilk statistic was not significant for the populations Rebordanes, Ribadumia, and Tuy; it was significant only for Viana (Table 3). After applying the test individually to the six trials of Viana, we found that the Shapiro–Wilk statistic was not significant for any of them, so kernel moisture was analyzed as Gaussian. Besides, the mean and the median were very close for all the populations, including Viana (Table 3). Days to silking and days to tasseling were analyzed following a gamma distribution. Finally, for lodging, we used the binomial distribution.

Table 3 Result of the Shapiro–Wilk test for normality of kernel moisture at harvest for four populations of maize and the resulting populations after three cycles of selection grown in six environments

Population	Shapiro–Wilk		Mean	Median
	W	P < W		
Rebordanes	0.9855	0.5796	21.6	21.7
Ribadumia	0.9837	0.4758	23.2	23.3
Tuy	0.9795	0.2880	22.1	22.4
Viana	0.9376	0.0014	20.1	20.5

There have been several reports about responses to recurrent selection in maize. See, for instance, Betrán et al. (2004, pp. 314 and ff.) for a review. The Spanish germplasm maintained at MBG has been the basis of several studies conducted on its relationships with the American (Ordás 1991; Ordás et al. 1994) and French germplasm (Revilla et al 2006), and on looking for new heterotic patterns using this material (Soengas et al. 2003a, 2003b). In addition, some programs of recurrent selection have been carried out with it (Peña-Asín et al. 2013; Romay et al. 2011, 2012; Ruiz de Galarreta and Álvarez 2007; Vales et al. 2001). However, no selection program targeted at the improvement of individual landraces per se has been reported so far.

Several recurrent selection schemes have been described (Betrán et al. 2004) as well as some modifications (Hallauer et al. 1988). For the program reported here, we chose S_1 recurrent selection due to its simplicity and because it had already been successful with Spanish germplasm (Garay et al. 1996a, 1996b; Vales et al. 2001). We used a relatively mild selection intensity (20%), which might have caused a slow increase in the frequency of favorable alleles to improve populations as future sources of inbred lines. Higher selection intensities would have increased the frequency of favorable alleles but could have brought about some inbreeding depression in the selected populations unless very large populations were managed.

Rebordanes and Ribadumia did not respond to selection for yield whereas Tuy and Viana did (Table 4). In all populations, there is an increase in yield for the first cycle of selection. However, the increase is not maintained for subsequent cycles in Rebordanes and Ribadumia. Betrán et al. (2004) indicate that a quick fixation of alleles may reduce the long-term progress of the selection.

It should be noted that each of the first two populations had been collected from a single farmer, while the other two include samples from nine (Tuy) and 10 farmers (Viana). Thus, the lack of initial variability can make it difficult to obtain a favorable selection response.

The response to selection in the case of Tuy was quadratic, while for Viana both the linear and quadratic responses were significant (Table 5). More selection cycles following the same scheme should

Table 4 Mean grain yields of four maize populations after three cycles of selection and seven hybrid checks, grown in six environments

	Rebordanes	Ribadumia	Tuy	Viana
Population cycle	kg ha^{-1}			
Cycle 0	6062 c[a]	5831 c	5733 d	3134 d
Cycle 1	6692 c	5956 c	6653 cd	3540 cd
Cycle 2	6473 c	5386 c	6291 d	3837 cd
Cycle 3	6160 c	5538 c	6376 d	5027 c
Hybrid check (maturity)	kg ha^{-1}			
Minhybrid 7301 (FAO 200)	6955 bc[b]			8342 b
Minhybrid 6304 (FAO 300)	7898 bc	8508 b	8786 b	8853 b
Minhybrid 5302 (FAO 400)		8500 b	8401 bc	
LG33.03 (FAO 200)	7978 bc			8629 b
NK Thermo (FAO 300)	9377 ab	10,099 ab	9515 ab	10,324 b
PR36W66 (FAO 400)		11,067 a	11,476 a	
DKC5276 (FAO 400)	12,305 a	11,012 a	12,121 a	13,495 a
Mean (kg ha^{-1})	7382	7668	8067	6659
CV (%)	11.5	13.1	12.3	15.0

[a]Two means followed by the same letter within a column are not statistically different at the 0.05 probability level

[b]The data for hybrid checks correspond to the particular hybrids grown in the evaluation tests of the population indicated at the top of the table

Table 5 Regression analyses of yield for four maize varieties derived from populations after three cycles of recurrent selection grown in six environments in Spain

F-value						
Source	Num. df	Den. df	Rebordanes	Ribadumia	Tuy	Viana
Cycle of selection	3	15	0.62	1.99	5.34*	35.00*
Linear	1	15	0.00	3.05	4.40	94.22*
Quadratic	1	15	1.64	0.01	6.25*	8.11*
Lack of fit	1	15	0.21	2.91	5.36*	2.65

*Significant at the 0.05 probability level

not significantly improve Tuy, while in the case of Viana, a favorable response would still be expected. The yield gain of Tuy was 241 kg/ha or 3.7% per cycle, while those values for Viana were 631 kg/ha and 21.1% per cycle. Coors (1999, p. 231, Table 21–3), summarizing 13 studies of S_1 recurrent selection, reported gains of 182 kg/ha or 7.4% per cycle. Thus, the gains for Viana were much higher than those reported by Coors (1999), possibly due to the low initial yield of Viana.

The greatest gain in yield in Tuy occurred in the first selection cycle, without achieving significant increases in the following two cycles; Viana, on the other hand, maintained a constant gain in yield throughout the selection process (Table 4). However, the grain yields of these populations are much lower than those of control hybrids (Table 4). As indicated earlier, these populations are usually highly appreciated by farmers in the targeted area compared to commercial hybrids because of their great early vigor.

Although grain yield was the primary criterion of selection, other secondary traits are also of great interest, especially the maturity of the selected populations since there is a risk that later genotypes tend to be selected, because they are usually more productive than the early ones.

There are numerous methods of determining what "maturity" is. In our case, two parameters were used: flowering date and grain moisture percentage at harvest. The flowering date (Table 6) increased significantly in Rebordanes (3.2 days) and Viana (6.8 days). The percentage of grain moisture at harvest (Table 7), on the other hand, only increased significantly in Viana, from 18.7% in the original population to 21.1% in the population obtained after the third cycle of selection. In this population, part of the yield improvement is due to the increase in flowering date and the 2.5% increase in grain moisture at harvest. Comparison with the control hybrids using the FAO system of assigning cycles of maturity, in which a lower number indicates an earlier cycle, shows that

Table 6 Means of days to silking for four maize populations after three cycles of selection and seven hybrid checks, grown in six environments

	Rebordanes	Ribadumia	Tuy	Viana
Population cycle	d			
Cycle 0	74.6 cd[a]	78.9 bc	74.0 c	62.8 f
Cycle 1	76.0 bcd	75.8 d	75.2 c	64.5 ef
Cycle 2	75.8 bcd	78.3 cd	74.4 c	66.2 e
Cycle 3	77.8 ab	78.7 bc	74.4 c	69.6 d
Hybrid check (maturity)	d			
Minhybrid 7301 (FAO 200)	76.3 abc[b]			75.6 bc
Minhybrid 6304 (FAO 300)	79.4 ab	81.2 ab	80.6 ab	79.7 a
Minhybrid 5302 (FAO 400)		80.9 ab	81.8 ab	
LG33.03 (FAO 200)	73.1 d			73.9 c
NK Thermo (FAO 300)	79.7 a	78.6 bcd	79.7 b	79.0 ab
PR36W66 (FAO 400)		84.0 a	83.0 a	
DKC5276 (FAO 400)	79.7 ab	80.5 abc	80.2 ab	80.1 a
Mean (d)	76.7	79.7	78.0	71.6
CV (%)	1.7	2.1	1.5	1.4

[a]Two means followed by the same letter within a column are not statistically different at the 0.05 probability level

[b]The data for hybrid checks correspond to the particular hybrids grown in the evaluation tests of the population indicated at the top of the table

Table 7 Means of kernel moisture for four maize populations after three cycles of selection and seven hybrid checks, grown in six environments

	Rebordanes	Ribadumia	Tuy	Viana
Population cycle	%			
Cycle 0	21.1 abc[a]	23.0 ab	22.2 b	18.7 b
Cycle 1	21.1 abc	23.7 a	22.0 b	20.0 ab
Cycle 2	21.6 abc	23.3 ab	22.2 b	20.6 ab
Cycle 3	22.8 a	22.8 ab	22.2 b	21.1 ab
Hybrid check (maturity)	%			
Minhybrid 7301 (FAO 200)	18.4 c[b]			18.9 ab
Minhybrid 6304 (FAO 300)	20.5 abc	20.3 b	21.3 b	20.5 ab
Minhybrid 5302 (FAO 400)		22.7 ab	23.8 ab	
LG33.03 (FAO 200)	19.7 bc			19.2 ab
NK Thermo (FAO 300)	21.6 abc	21.9 ab	22.0 b	21.3 ab
PR36W66 (FAO 400)		25.1 a	25.6 a	
DKC5276 (FAO 400)	22.1 ab	22.0 ab	23.0 ab	22.2 a
Mean (%)	20.9	22.9	22.7	20.1
CV (%)	7.6	6.7	5.2	6.5

[a]Two means followed by the same letter within a column are not statistically different at the 0.05 probability level

[b]The data for hybrid checks correspond to the particular hybrids grown in the evaluation tests of the population indicated at the top of the table

the original landrace was around FAO 200, while that resulting from cycle 3 is around FAO 300. It seems that selection for yield in a non-adapted population like Viana has primarily targeted selection for adaptation, in this case, a selection for lateness.

One of the unfavorable characteristics of these local populations is their tendency to lodge. The comparison with the control hybrids shows that both the original populations and the selection cycles lodged much more (Table 8). Only Ribadumia had a significant increase for lodging, going from 14.9% in cycle 0 to 31.6% in cycle third. In the process of obtaining inbreds from improved populations, special attention should be paid to this character.

Another important point in a program of recurrent selection is the loss of variability caused by the process of selection. In our case, a continuous decrease is detected in the two populations that presumably had a reduced initial variation (Rebordanes and Ribadumia) as opposed to what we could call anarchic changes in the populations that initially had greater variability (Tuy and Viana) (Table 9). Therefore, there is no

Table 8 Means of lodging for four maize populations after three cycles of selection and seven hybrid checks, grown in six environments

	Rebordanes	Ribadumia	Tuy	Viana
Population cycle	%			
Cycle 0	13.9 ab[a]	14.9 bc	23.8 a	27.4 a
Cycle 1	20.3 a	27.6 ab	23.2 a	31.8 a
Cycle 2	16.0 a	35.9 a	27.3 a	31.7 a
Cycle 3	23.6 a	31.6 ab	24.9 a	26.7 a
Hybrid check (maturity)	%			
Minhybrid 7301 (FAO 200)	8.1 abc[b]			5.6 b
Minhybrid 6304 (FAO 300)	4.8 bc	2.2 d	4.6 b	3.2 bc
Minhybrid 5302 (FAO 400)		6.8 cd	7.9 b	
LG33.03 (FAO 200)	3.9 c			1.6 c
NK Thermo (FAO 300)	7.7 abc	4.8 cd	8.0 b	3.2 bc
PR36W66 (FAO 400)		2.7 d	3.6 b	
DKC5276 (FAO 400)	1.2 c	1.0 d	0.4 c	2.7 bc
Mean (%)	13.3	17.2	16.8	18.0
CV (%)	29.9	36.4	30.8	36.4

[a]Two means followed by the same letter within a column are not statistically different at the 0.05 probability level

[b]The data for hybrid checks correspond to the particular hybrids grown in the evaluation tests of the population indicated at the top of the table

Table 9 Variances[a] for cycles of selection for four populations of maize selected for three cycles of S_1 recurrent selection for yield

Cycle of selection	Population: Rebordanes	Ribadumia	Tuy	Viana
C0	1,157,586	899,820	238,003	236,536
C1	605,205	443,290	719,585	92,097
C2	422,055	342,366	34,636	541,498
	*	NS	*	NS

[a]Variances in each column are different (*) or not different (NS), as stated in the last row, according to a likelihood ratio test in a PROC GLIMMIX (SAS Institute 2012)

relationship between the assumed initial variability and the change of variance. These results, however, should be taken with caution since the values of the variances were obtained in trials carried out in different years.

Several authors have pointed out the great value of the underused genetic variability of landraces, for instance, Dwivedi et al. (2016) and Li et al. (2018). In the specific case of maize, it is widely known that European landraces could serve as a source for traits not generally present in Corn Belt germplasm like early vigor under cold springs (Böhm et al. 2017; Hölker et al. 2019a). However, there is a yield gap of about 15% between improved and non-improved varieties, as stated by Hölker et al. (2019a), that hinders the direct use of non-improved materials in elite breeding programs. Prebreeding programs are aimed to reduce the yield gap and facilitate the incorporation of non-improved materials in breeding programs. The process of prebreeding is slow and costly, and it is usually very difficult to get long-term funding for activities whose impact may be visible only after a long time (Haussmann et al. 2004). Smith et al. (2015) indicate that public funding is needed to support prebreeding efforts and it is under this framework our work and those previously cited by the University of Hohenheim and the Technical University of Munich (Böhm et al. 2017; Hölker et al. 2019b; Mayer et al. 2020; Melchinger et al. 2017) have been framed. In addition, the utilization of exotic materials remains a challenge due to insufficient knowledge about efficient strategies for its integration into elite programs (Cowling et al. 2017; Voss-Fels et al. 2019; Wang et al. 2017). We can also point out that some works have been published recently describing the use of landraces (and even teosintes) in the search for traits of interest for maize breeding like alleles associated with root length (Li et al. 2020), saccharification and nutritive value (López-Malvar et al. 2020), and stover yield and fiber quality as target traits for dual-purpose maize (Munaiz et al. 2021). In addition, genes found in local varieties could be useful to improve adaptation to adjust crops to climate change (Campbell et al 2016).

The response of the four populations was quite different among themselves, although our results reveal some considerations that breeders must take into account when conducting prebreeding programs on local landraces and could serve as a guide to optimize the process. Given the yield gap that presently exists between commercial varieties and the old landraces, grain yield is the main trait to improve. It is a widely held fact among maize breeders that recurrent selection is an effective method of improving the yield of a population. However, our data should contribute to dampening optimism somewhat, especially with European germplasm, since a positive response was obtained in only two of the four populations. We must not forget, however, that we carried out only three cycles of selection, which does not prevent us from venturing that with more cycles or with another system of recurrent selection a positive response could be obtained in all populations.

Recurrent selection is a breeding method that was developed to rectify the limitations in inbred development by continuous selfing as the probability of fixation of favorable alleles can be increased if their frequency is increased (Bernardo 2020, p. 232). One of the objectives of intrapopulation recurrent selection is to increase the frequency of favorable alleles to have a greater probability of obtaining superior inbred lines. The populations resulting from the last selection cycle must therefore be suitable for this purpose since the self-pollination of the original populations has hardly produced valuable inbred lines, specifically only two from the populations involved in this study: EP37 (from Viana) and EP42 (from Tuy). Only the latter has had commercial importance since it was the male parent of a three-way cross widely cultivated in the 1980s. In this context, the selected populations are presently being self-pollinated to achieve inbreds with the presumably good combining ability for both yield and early vigor. The work by Hölker et al. (2019b) showed that the majority of inbreds developed from three European landraces (two from Germany and one from Spain) outperformed the commercial hybrids for early vigor. In addition, the Spanish landraces could serve as a source for traits not generally present in Corn Belt germplasm like early vigor under cold springs. For instance, the population Viana, sent to the University of Minnesota in 1975, has been used in the program GEM (Germplasm Enhancement of Maize).

In summary, our work adds new germplasm that could be useful both to farmers who are interested in growing open-pollinated varieties and to commercial companies trying to enhance the genetic basis of their hybrids. However, obtaining valuable new germplasm requires a sustained and long-term investment in prebreeding to generate genetically enhanced source germplasm. Besides, based on our results, we recommend paying attention also to secondary traits such as lodging, flowering, and grain moisture, which could change due to unintentional indirect selection worsening the adaptation to the target area. In addition, we recommend, if possible, using large effective numbers to prevent inbreeding depression, particularly when the objective is to improve open pollination varieties for direct cultivation.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article. The authors have no financial or proprietary interests in any material discussed in this article.

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