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Maize participatory breeding in Portugal: Comparison of farmer's and breeder's on-farm selection

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Abstract

"VASO" is a Portuguese participatory maize breeding project (1984), where several maize landraces such as "Pigarro" have been selected both by a farmer's (phenotypic recurrent selection) and a breeder's approach (S2 lines recurrent selection). The objectives of this study were to determine the phenotypic and genotypic responses to participatory selection using these two different approaches, to clarify to which extent both selection methods preserve genetic diversity, and conclude what is the preferred method to apply in sustainable farming systems. The results, obtained via ANOVA, regression analyses and molecular markers, indicate that for both selection methods, genetic diversity was not significantly reduced, even with the most intensive breeder's selection. Although there were some common outputs, such as the determinated versus indeterminated ears, cob and ear weight ratio per ear and rachis 2, specific phenotypic traits evolved in opposite directions between the two selection approaches. Yield increase was only detected during farmer selection, indicating its interest on PPB. Candidate genes were identified for a few of the traits under selection as potential functional markers in participatory plant breeding.

KEYWORDS
fasciation, genetic diversity, landraces, maize, participatory breeding, Pigarro, SSR, *Zea mays* L.

1 | INTRODUCTION

Since its introduction, more than five centuries ago, maize has transformed the Portuguese agricultural panorama with many locally adapted maize landraces (Moreira, 2006). In the 1960s, the Portuguese maize breeders, conscious of the threat to this unique national maize germplasm caused by diffusion of hybrids, started a regional collection of maize germplasm. More than 3,000 accessions were collected and stored at the national plant germplasm bank, BPGV (Pêgo, 1996), providing the basis for much of the national maize breeding achievements. Some of these achievements were attained through the participatory maize breeding "VASO" Project (Sousa Valley Project, initiated in 1984), implemented to answer to small farmers' concerns, such as how to increase yield without losing quality for bread production or ability for production in sustainable polycropping systems. The "Pigarro" landrace was one of the landraces improved within this project, showing a strong ear fasciation expression. Fasciation can influence yield, being quite common among Portuguese traditional maize landraces (Vaz Patto, Moreira, Carvalho, & Pêgo, 2007).

PPB can be considered a dynamic multistakeholder collaboration where a common vision of concepts, methods and means orientates

breeding goals towards new food systems, based on the strong interrelationships between multidisciplinary scientific knowledge and the know-how of practitioners. Research actions are performed jointly from conception to dissemination (Ceccarelli & Grando, 2007; Chable, Rey, & Mendes-Moreira, 2014). Participatory plant breeding (PPB) has provided solutions for climate changes (Ceccarelli, Galie, & Grando, 2013), diversity conservation (Maxted, Guarino, Myer, & Chiwona, 2002), organic and low input agriculture (Serpolay-Besson, Giuliano, Schermann, & Chable, 2014) and polycrop and agroecologic systems (Machado, Nass, & Machado, 2011). PPB encourages interaction among plant breeders, other researchers and farmers, with the objective of developing cropping systems that better meet local needs (Cleveland, Daniela, & Smith, 2000). Several selection approaches, with different levels of farmers' involvement, can be found in PPB projects. In the case of "Pigarro" participatory breeding, two selection approaches were applied: a farmer's phenotypic recurrent selection and a breeder's recurrent S2 lines selection.

In the previous study, we compared the evolution of "Pigarro" morphological response to farmer's (FS) and breeder's selection (BS) approaches, assessing just a few cycles of selection evaluated during 2 years of field trials (Mendes Moreira, Pêgo, Vaz Patto, & Hallauer, 2008). At the molecular level, response to selection was assessed only at FS cycles (Vaz Patto, Moreira, Almeida, Satovic, & Pêgo, 2008). A more detailed comparative evaluation of the responses to selection at the phenotypic and genotypic levels is lacking. To fulfil this gap, we conducted two more years of comparative FS versus BS cycles field trials. Molecular screening was also applied to the breeder selection cycles allowing a detailed comparison of both selection methods at agronomic, phenotypic and molecular levels.

The objectives of this study were to determine (i) whether "Pigarro" initial population (from 1984) changed significantly, at phenotypic and molecular levels, during this long-term PPB; (ii) whether the two selection methods led to the same or different breeding outputs; (iii) whether any of the two selection methods significantly changed genetic diversity; and (iv) which of the two selection methods is the most useful for supporting PPB in sustainable farming systems.

2 | MATERIAL AND METHODS

2.1 | Germplasm development

"Pigarro" is a FAO 300 maturity open-pollinated variety (OPV) with white flint kernels, high levels of root and stalk lodging and high kernel-row numbers (normally between 18 and 28, but 48 rows have already been observed). Its improvement, since 1985 under the VASO Project breeding approach, focused on two main recurrent selection methodologies: FS and BS. FS corresponded to a phenotypic recurrent selection using stratified mass selection, with two parental control (stratified mass selection with parental control $c = 1.0$) in three sequential steps. This is an improved extension of the mass selection procedure commonly used by farmers (for one parental control $c = 0.5$). The farmer was advised to conduct selection under a three-step sequence (A–B–C)—the first two steps (A and B) in the field and the third one (C) at the storage facilities (Figure 1, Table 1): (A) negative selection by detasseling before anthesis (e.g., pest and disease susceptibility; weakest, undesirable ideotype plants contribute for yield but not for the seed); (B) plant and ear selection: based on stalk quality and ear size, the plants are foot-kicked at their base (first visible internodes) to evaluate their root and stalk quality. With this procedure, as an indirect measurement, the pest and disease tolerance can be evaluated. In practical terms, if the plant breaks, it is eliminated. A special selection preference is given to prolific plants; and (C) best ear selection at storage facilities is performed separately for both normal and prolific ears and always includes ear length, kernel-row number, prolificacy and the elimination of damaged/diseased ears. The selected ears are finally shelled and mixed together to form the next-generation seed. The farmer selection pressure ranged from 1% to 5% (Figure 1; Table 1).

Breeder selection (BS) correspondeds to a S2 lines recurrent selection, considering the additive component of genetic variance (i.e., $3/2[\![\sigma]\!]^2_{(a)}$ versus $[\![\sigma]\!]^2_{(a)}$, respectively, for S2 and S1 lines) (Hallauer, Carena, & Miranda Filho, 2010), organized in a four-season scheme (Figure 1; Table 1): Season (1) 1,000 S0 plants were selected and selfed, from which 500 to 600 S1's were selected at harvest; Season (2) 500 to 600 S1's were planted and selfed to obtain the S2 seed, and at harvest, the best 200 ears were selected; Season (3) the selected S2's were submitted to a yield trial in a randomized complete block design and tested for yield performance, pest and disease tolerance and stalk quality; and Season (4) using remnant S2 seed, the best 30 to 35 S2 lines (15% to 20%, selection pressure) were planted in isolation and recombined through cross pollination to form the first cycle C1(S2) seed. The same sequence was conducted until the third cycle C3(S2) was completed (Mendes Moreira et al., 2008). Both methods emphasized selection for yield, pest and disease reaction and indirectly quality for maize bread (Vaz Patto et al., 2009, 2013).

Seed from each selection cycle of "Pigarro" VASO Program, from either FS or BS selection, was stored at 4°C in NUMI (Maize Breeding Station, Braga, Portugal) cold storage facilities.

At present, "Pigarro" is under registration process.

2.2 | Phenotypic evaluation

To determine the effectiveness of both methods of selection, seed from both FS (six cycles: FSC4-88, FSC6-90, FSC9-93, FSC12-96, FSC15-99 and FSC20-04) and BS cycles (three cycles: BSC1-89, BSC2-94 and BSC3-98) and the initial "Pigarro" population (C0-84) were included in comparative field trials. Field trials were established at three locations in Portugal (Coimbra 40°13′0.22″N, 8°26′47.69″W; Montemor 40°10′4.82″N, 8°41′14.84″W and Lousada 41°14′03.43″N, 8°18′13.11″W) during 4 years, from 2005 till 2008. However, extreme drought after sowing, in 2006 at Montemor, and late thinning, in 2008 at Lousada, restricted data collection at both sites. Coimbra and Montemor are in the river Mondego irrigation

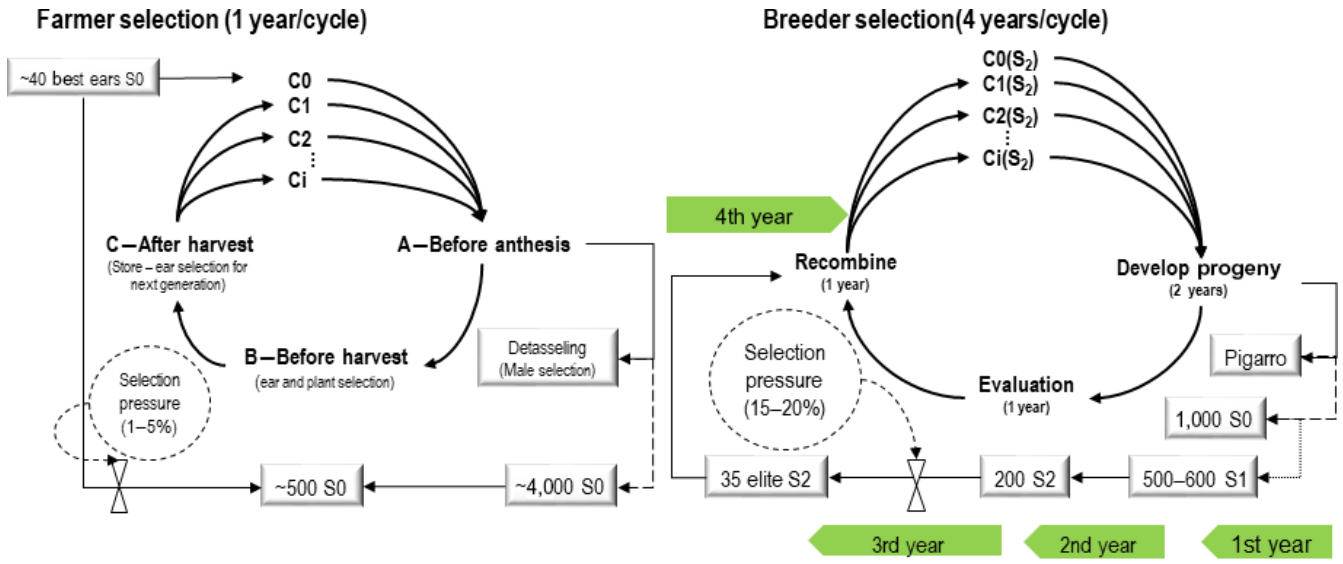

FIGURE 1 Farmer selection (phenotypic recurrent selection) and breeder selection (recurrent selection by S2) lines methodologies [Colour figure can be viewed at wileyonlinelibrary.com]

perimeter, a very high-yielding area where the average yield for maize hybrids is 14.5 Mg/ha. Lousada is located in a traditional maize production region, with an average maize hybrid production of 8 Mg/ha.

Sowing occurred in May, differing 15 days among locations, and harvests occurred in October from 2005 to 2008.

For each environment, a randomized complete block design, with three replications, was used. Each replication included two rows plot (at Lousada 6.9 m long with 0.70 m between rows, and in the other locations, 6.4 m long with 0.75 m between rows). Plots were over-planted by hand and thinned at the seven-leaf stage (Ritchie, Hanway, & Benson, 1993), for a final stand of approximately 50,000 plants per ha. Plots were mechanical and/or hand-weeded as necessary and managed following common agricultural practices for maize in the region. All the plots were harvested by hand.

Phenotypic data were collected for 43 traits and are described in Table 2. Some traits were measured per plot (traits 1–14, Table 2), such as grain yield (Mg/ha) adjusted to 15% grain moisture at harvest. All the other traits were measured on 20 plants or ears per plot, randomly selected after harvest and dried (35°C) to approximately 15% grain moisture to ensure that other conditions during measurements held constant. Following this procedure, 28 measurements were made per plant or ear (traits 15–43, Table 2) as described by Mendes Moreira et al. (2008), IBPGR (1991) and IPGRI (2000), with some minor changes. Uniformity score scales varied from one (minimum) to nine (maximum). In maize populations, average values ranged from one (minimum) to a maximum of five, being this average values six to nine used in inbred and hybrids. Cob/ear ratio at harvest was determined based on the measurement of five shelled ears.

2.3 | Phenotypic data analysis

Data analysis was conducted separately for both selection methods. Analyses of variance were computed using IBM SPSS STATISTIC 22.0 for selection cycles, environments (locations), years and the respective interactions with selection cycles. Replications were nested in environments.

Phenotypic data from 2005 and 2006 field trials and from 2005 ear traits were previously published (Mendes Moreira et al., 2008) and made available for this new comparative analysis (Table 2, Appendix S1).

To measure the selection gain of the evaluated traits along FS and BS, a linear regression model was used in the Microsoft Office Excel (version 2003), regressing observed populations means on cycle of selection (*b* = regression of trait on cycle of selection and response expressed relative to the C0 population and on a yearly basis).

2.4 | Molecular evaluations

For molecular comparison, the initial population (C0-84), the two FS (FSC9-93 and FSC20-04) and the three BS cycles (BSC1-89, BSC2-94 and BSC3-98) were used. Molecular data for C0-84, FSC9-93 and FSC20-04 were published previously by Vaz Patto et al. (2008), named as SC1984, SC1993 and SC2004, and made available for this new analysis (Table 3). For each analysed cycle, 30 individuals were randomly selected from seed stocks.

DNA was isolated from a total of 90 individuals corresponding to the three BS cycles (using 2-week old seedling leaf samples), employing a modified CTAB procedure (Saghai-Maroof, Soliman, Jorgensen, & Allard, 1984). These individuals were subsequently screened with the same 15 SSR markers (umc1013, umc1823, umc1635, umc1907, umc1528, umc1524, umc1143, umc1229, umc1066, umc1483, umc1858, umc1279, umc1120, umc2067 and umc2021) previously used in Vaz Patto et al. (2008) to allow comparisons. SSR marker technique was performed as in Vaz Patto, Satovic, Pêgo, and Fevereiro (2004). Fragment analysis was conducted using automated laser fluorescence (ALFexpress II) sequencer (Amersham Biosciences), as in Vaz Patto et al. (2008).

TABLE 1 Years and respective cycles of selection produced with different selection methods. Multiplication of seed stocks for seed regeneration to be used for trials. For evaluation trials, some cycles were used and tested during 4 years in 2 to 3 locations. For molecular analyses, some cycles were also selected

Selection method																					
Year	1984	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	2000	01	02	03	04
Cycles of																					
Mass selection (1 year per cycle)	C0	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
Recurrent selection (S_2) (4 years per cycle)						C1(S2)					C2(S2)				C3(S2)						
Multiplication seed stock	2005				05	05	05			05	05		05		05	05					
Evaluation trials																					
Mass Selection	C0-84				C4-88		C6-90			C9-93	C12-96					C15-99					C20-04
Recurrent Selection (S_2)						C1(S2)-89					C2(S2)-94				C3(S2)-98						
Locations (with three replications)																					
Portugal (2005)[b]	3				3	3	3			3	3		3		3	3					3
Portugal (2006[a])[b]	2				2	2	2			2	2		2		2	2					2
Portugal (2007)	3				3	3	3			3	3		3		3	3					3
Portugal (2008[a])	2				2	2	2			2	2		2		2	2					2
Molecular analyses																					
Mass Selection[c]	C0-84									C9-93											C20-04
Recurrent selection (S2)						C1(S2)-89					C2(S2)-94				C3(S2)-98						

Breeding methodologies applied to Pigarro since 1984, selected cycles for trials, evaluation (locations and years) and seasons per cycle.

[a]Drought after sowing at Montemor-o-Velho location in 2006 and late thinning at Lousada in 2008 lead to data exclusion; Cx(S2)-y, where C, cycle; *x*, number of cycles; S2, if selection by S2 lines; *y*, year correspondent to cycle of selection.

[b]Data published in Mendes Moreira et al. (2008).

[c]Data published in Vaz Patto et al. (2008).

TABLE 2 Linear regression for two of breeding methodologies applied to "Pigarro" since 1984, based on field trials agronomical evaluation. Estimates of linear regression coefficient (*b*), standard errors, initial cycle prediction ($\hat{C}0$), coefficients of determination (R^2) and % of gain per year (%Gain/Y) for farmer's selection (20 cycles) and for breeder's selection (3 cycles)

		Farmer's selection				Breeder's selection			
Traits	Data measurements/plot	*b*	$\hat{C}0$	R^2	%Gain/Y	*b*	$\hat{C}0$	R^2	%Gain/Y
50Fi	1	0.138 ± 0.037*	63.35	0.74	0.22	0.097 ± 0.038	63.31	0.76	0.15
50Ff	1	0.160 ± 0.040*	68.78	0.76	0.23	0.151 ± 0.037	68.53	0.89	0.22
50Mi	1	0.123 ± 0.030**	60.43	0.77	0.20	0.064 ± 0.022	60.66	0.81	0.11
50Mf	1	0.151 ± 0.032**	65.90	0.82	0.23	0.084 ± 0.032	66.15	0.78	0.13
OI	1	0.002 ± 0.003	0.43	0.04	0.37	−0.003 ± 0.002	0.49	0.52	−0.59
MO	1	0.080 ± 0.021*	27.71	0.74	0.29	−0.024 ± 0.046	27.25	0.12	−0.09
CWEW	1	0.003 ± 0.000**	0.22	0.88	1.20	0.003 ± 0.001	0.21	0.89	1.36
Yld	1	0.014 ± 0.017	6.91	0.12	0.21	−0.027 ± 0.007	6.87	0.87	−0.39
U	1	−0.001 ± 0.006	2.80	0.01	−0.04	−0.032 ± 0.006*	2.89	0.93	−1.10
N	1	0.010 ± 0.006	5.06	0.36	0.20	0.018 ± 0.011	5.02	0.58	0.36
T	1	0.011 ± 0.004*	6.39	0.64	0.18	−0.003 ± 0.019	6.50	0.02	−0.05
E	1	−0.005 ± 0.003	5.27	0.31	−0.10	−0.015 ± 0.013	5.19	0.39	−0.29
R	1	0.000 ± 0.000	0.02	0.04	0.86	0.000 ± 0.000	0.02	0.28	1.37
S	1	0.001 ± 0.001	0.06	0.21	0.98	−0.001 ± 0.001	0.07	0.44	−1.26
H	20	0.514 ± 0.309	231.45	0.36	0.22	−0.600 ± 0.516	230.03	0.40	−0.26
H1E	20	0.371 ± 0.234	138.06	0.33	0.27	−0.527 ± 0.474	134.88	0.38	−0.39
L	20	−0.052 ± 0.016*	17.37	0.66	−0.30	0.057 ± 0.044	17.59	0.46	0.33
ED1	20	0.041 ± 0.007**	5.67	0.87	0.73	0.007 ± 0.013	5.54	0.14	0.13
ED3	20	0.054 ± 0.010**	4.62	0.87	1.17	−0.005 ± 0.011	4.51	0.09	−0.12
ED2	20	0.031 ± 0.005**	5.29	0.90	0.59	0.006 ± 0.009	5.20	0.19	0.12
ED4	20	0.030 ± 0.004***	4.23	0.92	0.70	0.002 ± 0.004	4.19	0.08	0.04
R1	20	0.242 ± 0.049**	17.53	0.83	1.38	−0.042 ± 0.081	16.78	0.12	−0.25
R2	20	0.256 ± 0.048**	16.61	0.85	1.54	−0.090 ± 0.079	16.15	0.39	−0.56
Fa	20	0.064 ± 0.015**	1.94	0.78	3.31	−0.025 ± 0.022	1.80	0.39	−1.36
DI	20	−0.005 ± 0.002*	1.25	0.58	−0.43	−0.012 ± 0.000**	1.29	1.00	−0.96
CV	20	0.027 ± 0.008*	1.86	0.71	1.47	−0.008 ± 0.003	1.88	0.78	−0.41
E_CWEW	20	0.001 ± 0.000**	0.15	0.79	0.75	0.003 ± 0.000***	0.15	1.00	1.86
EW	20	1.193 ± 0.386*	190.14	0.66	0.63	0.285 ± 0.855	183.24	0.05	0.16
KW	20	0.768 ± 0.306	161.42	0.56	0.48	−0.297 ± 0.698	156.38	0.08	−0.19
CW	20	0.425 ± 0.093**	28.72	0.81	1.48	0.582 ± 0.157	26.86	0.87	2.17
Emo	20	0.005 ± 0.008	15.95	0.07	0.03	−0.024 ± 0.019	16.02	0.45	−0.15
KD	20	0.000 ± 0.000	1.01	0.05	−0.02	−0.002 ± 0.001	1.00	0.57	−0.22
SW	20	−1.606 ± 0.354**	347.78	0.80	−0.46	0.490 ± 0.512	352.55	0.31	0.14
KN	20	5.039 ± 1.369*	465.29	0.73	1.08	−1.559 ± 2.402	446.17	0.17	−0.35
KR	20	−0.048 ± 0.035	29.34	0.27	−0.16	−0.005 ± 0.043	29.36	0.01	−0.02
CD1	20	0.043 ± 0.007**	4.14	0.89	1.03	0.015 ± 0.010	4.02	0.50	0.37
CD3	20	0.054 ± 0.009**	3.18	0.87	1.68	0.000 ± 0.014	3.07	0.00	−0.01
CD2	20	0.030 ± 0.004***	3.63	0.91	0.82	0.015 ± 0.004	3.57	0.87	0.42
CD4	20	0.025 ± 0.003***	2.71	0.94	0.91	0.007 ± 0.004	2.71	0.65	0.26
M1	20	0.028 ± 0.005**	2.11	0.88	1.31	0.009 ± 0.006	2.05	0.52	0.42

(Continues)

TABLE 2 (Continued)

		Farmer's selection				Breeder's selection			
Traits	Data measurements/plot	*b*	Ĉ0	R^2	%Gain/Y	*b*	Ĉ0	R^2	%Gain/Y
M2	20	0.017 ± 0.002***	1.61	0.92	1.05	0.006 ± 0.002	1.60	0.77	0.38
Rq1	20	0.037 ± 0.006**	3.24	0.90	1.13	0.015 ± 0.006	3.14	0.77	0.49
Rq2	20	0.026 ± 0.003***	2.70	0.93	0.96	0.015 ± 0.001**	2.67	0.99	0.56

*Significant at .05 probability levels; **Significant at .01 probability levels; ***Significant at .001 probability levels; 50Fi, days to silk beginning; 50Ff, days to silk end; 50Mi, days to anthesis beginning; 50Mf, days to anthesis end (no. of days for a particular stage in 50% of plants); OI, Overlap Index between beginning and end of anthesis and silking; MO, moisture, %; CWEW, ratio of cob weight in the ear weight per plot (sample of four ears), %; Yld, yield, Mg/ha; (grain yield 15% moisture (Mg/ha) = total ear weight per ha × CWEW × (100%–%moisture at harvest)/(100%–15% moisture); grain moisture measured with the FARMPOINT moisture meter using mixed sample of four shelled ears grain At 15% moisture U, uniformity (1 as minimum to 9 as maximum, 1–5 to populations and 6–9 to inbreed lines.); *N*, angle (angle of the adaxial side of the leaf above the ear with the stalk (5 = 45°, <5 = <45° and >5 = >45°); T, tassel (tassel branching; 1—absent tassel [inbreeds and hybrids] and 9—a much branched tassel [frequent in populations with abnormal fasciated ears]); E, ear placement (1 as minimum to 9 as maximum; 5, indicates a ear placement in the middle of the plant); R, root lodging, % percentage of plants leaning more than 30° from vertical); S, stalk lodging, % ([percentage of plants broken at or below the primary ear node], related to the quality of the stalk and the stalk damage caused by some insect attack); H, plant height, cm (plant height, from the stalk basis to the last leaf insertion before the tassel); H1E, ear height, cm (ear height, from the stalk basis to the highest ear bearing node); *L*, ear length, cm; ED1, ear diameter 1 and 3, cm (large diameter in the 1/3 bottom and top of the ear, respectively); ED2 and ED4, cm (small diameter in the 1/3 bottom and top of the ear, respectively (90° rotation from large diameter)); R1 and R2, kernel-row number 1 and 2, no. (row number in the 1/3 bottom and top of the ear, respectively); Fa, fasciation degree (1—without fasciation and 9—a maximum of fasciation); DI, determinate versus indeterminate ears (1—indeterminated [unfilled ear tips]; 2—determinated [complete ear tip fill]); CV, ear convulsion, convulsion intensity, kernel-row arrangement in the ear (0—without convulsion, regular kernel-row arrangement, 5—maximum of convulsion, without kernel-row arrangement); E_CWEW, cob/ear weight (percentage of cob weight in the ear weight measured per ear at laboratory); EW, ear weight, g (ear weight, adjusted to 15% of grain moisture); CW, cob weight adjusted to 15% grain moisture, g; KW, kernel weight, g (measured from the difference between ear and cob weight at 15% grain moisture); Emo, ear moisture, %; KD, kernel depth, cm (kernel depth, one kernel in the middle of the ear); SW, thousand kernel weight at 15% grain moisture, g; KN, kernel number per ear, no.; KR, kernel number per row, no.; CD1 and CD3, cob diameter 1 and 3 (cd1 and 3 measure in the same way for EDs), cm; CD2 and CD4, cob diameter 2 and 4 (cd2 and 4 measure in the same way for EDs), cm; M1, medulla 1 and 2, cm (large and small length of medulla, respectively, cob is cut in the diameter 1 position; Rq1 and Rq2, rachis 1 and rachis 2, cm (large and small length of rachis; cob is cut in the diameter 1 position, respectively); trait's detailed information in Mendes Moreira et al. (2008), IBPGR (1991) and IPGRI(2000).

Amplification fragment size was determined in base pairs and visually scored at least twice independently for each entry, to ensure data accuracy. Data from Vaz Patto et al. (2008) were added (adding up to a total of 179 individuals) for the comparative analysis of all FS and BS cycles.

2.5 | Molecular data analysis

Several genetic diversity parameters, such as polymorphism information content (PIC), allele frequencies, average number of alleles (N_a), number of private alleles (N_{pa}), observed and expected heterozygosities (H_O, H_E), inbreeding coefficient (*f*) and allelic richness (N_{ar}), were calculated using the SSR data matrix, as in Vaz Patto et al. (2008).

The estimates of N_{ar}, H_O, H_E and *f* in selection cycles were compared using the Kruskal–Wallis test. Average values of N_{ar}, H_O, H_E and *f* were tested for significant differences between BS and FS. Genotypic frequencies were tested for conformance to Hardy–Weinberg (HW) expectations, as well as to estimate the significance of genic differentiation between selection cycle pairs. Analysis of molecular variance (AMOVA) (Excoffier, Smouse, & Quattro, 1992) was used to partition the total microsatellite diversity among and within groups defined by taking into account different selection methods and cycles. All these analyses were performed as in Vaz Patto et al. (2008).

In order to graphically represent genetic relationships among individual genotypes, a factorial correspondence analysis (FCA) was carried out using GENETIX 4.05 (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 2004). FCA is a multidimensional statistical method suitable for categorical data allowing the assessment of correspondence between rows (e.g., individuals) and columns (e.g., alleles) in a two-way table. The aim of the analysis was to find composite axes generated from combinations of alleles that explain portions of the total observed inertia of the table (She, Autern, Kotoulas, Pasteur, & Bonhomme, 1987). In this way, the individuals are plotted on two composite axes that optimize the differences between the analysed individuals using the average inertia of predefined groups (i.e., selection cycles).

3 | RESULTS

At the phenotypic level, although a few traits have evolved in the same direction in both selection methods, FS was more effective in increasing fasciation-related traits and cob weight, with an overall significant contribution for yield increase (Table 2, Appendix S1). In comparison, BS was more effective in achieving crop uniformity, plant and ear height reduction and greater resistance to stalk lodging (Table 2, Appendix S1). In our study, we detected only an increase in yield as a result of FS selection. An ear fasciation increase by FS was also confirmed and this contrasted with the BS output (0.21% and −0.39% for yield selection gain, respectively, for FS and BS cycles)

TABLE 3 AMOVA for partitioning of SSR variation between selection methods (breeder's vs. farmer's), among cycles within selection methods and within selection cycles

Source of variation	% Total variance		Φ-statistics	
	Between	Within	Φ	*p* (Φ)
Breeder versus farmer selection methods	2.43		Φ_{CT} = 0.024	<.001
Among cycles within selection methods		5.16	Φ_{SC} = 0.053	<.001
Within cycles		92.40	Φ_{ST} = 0.076	<.001
All cycles	6.40	93.60	0.064	<.001
Breeders' cycles[a]	6.77	93.23	0.068	<.001
C0-84 versus BSC1-89	8.75	91.25	0.087	<.001
BSC1-89 versus BSC2-94	6.04	93.96	0.060	<.001
BSC2-94 versus BSC3-98	4.53	95.47	0.045	<.001
C0-84 versus BSC3-98	5.52	94.48	0.055	<.001
Farmers' cycles[a]	3.24	96.76	0.032	<.001
C0-84 versus FSC9-93	2.62	97.38	0.026	<.001
FSC9-93 versus FSC20-04	4.07	95.93	0.041	<.001
C0-84 versus FSC20-04	3.03	96.97	0.030	<.001

[a]Comparisons of both breeder's and farmers' cycles include the initial population (C0-84); *p*(ϕ), ϕ-statistics probability level after 10,000 permutations.

(Table 2). In addition, during BS, and contrary to the FS outputs, kernels became heavier. Finally ears became heavier for both FS (between C0-84 vs. FSC20-04) and BS (between BSC2-94 vs. BSC3-98) selection, especially due to cob weight increase (R^2 = 0.81 and gain cycle/year = 1.48% in FS, R^2 = 0.87 and gain cycle/year = 2.17% in BS for cob weight increase according to breeding approach) (Table 2).

Molecular results confirm that both breeding approaches seem to have achieved phenotypic modifications though preserving genetic diversity. The lack of significant differences among FS and/or BS cycles in any of the diversity parameters analysed (N_{ar}, H_O, H_E, *f*) (Table 4) indicates no effective loss of genetic diversity occurring during the two selection methods. Of the 81 different originally detected alleles, using 15 SSR markers, 61 alleles were maintained in FSC20-04 and 59 alleles were maintained in BSC3-98, reinforcing the idea of that genetic variability was maintained (Figure 2, Table 3). In addition, the number of common/shared alleles among selection cycles was 75.31% and 72.84% for FS and BS, respectively.

AMOVA among selection cycles also indicated a greater proportion of genetic diversity maintained within each selection cycle; 94.48% and 96.97% of the variation were attributable to within-selection cycles diversity for BS and FS, respectively (Table 3). In addition, this analysis also showed that the percentage of total variance among cycles within selection methods *per se* (5.16%) was two times greater than between selection methods (2.43%).

TABLE 4 Genetic variability estimates for the initial population (C0-84), three breeder's selection cycles (BSC1-89, BSC2-94, BSC3-98) and two farmer's selection cycles (FSC9-93, FSC20-04)

Selection cycle	*n*	N_a	N_{ar}	N_{pa}	H_O	H_E	*f*
C0-84	30	5.400	3.718	8	0.483	0.584	0.176
BSC1-89	30	4.800	3.348	2	0.442	0.547	0.195
BSC2-94	30	4.933	3.522	3	0.469	0.592	0.212
BSC3-98	30	4.800	3.760	2	0.552	0.652	0.156
FSC9-93	29	4.667	3.409	1	0.570	0.588	0.032
FSC20-04	30	4.733	3.503	3	0.509	0.597	0.153
Average		4.889	3.543		0.504	0.593	0.154
P(KW)[a]			0.729		0.219	0.654	0.682
P(BSC vs. FSC)[b]			0.598		0.317	0.917	0.065

N, number of individuals; N_a, average number of alleles; N_{ar}, allelic richness; N_{pa}, number of private alleles; H_O, observed heterozygosity; H_E, gene diversity or expected heterozygosity; *f*, inbreeding coefficient.
[a]Probability of Kruskal–Wallis test among all selection cycles.
[b]*p*-Value of the permutation tests for difference between selecting methods (BSC vs. FSC).

Factorial correspondence analysis indicated, along its first axis, two different genetic directions for the two selection methods (Figure 3). The first farmer selection cycle analysed, FSC9-93, was however closer to the BS. This corresponded with a more stratified mass selection applied since 1986 until 1999. More recent FS was much more differentiated from the BS and more differentiated among them. Along the second axis, a major distance between the final farmer's cycle analysed, FSC20-04, and the original population was observed. BS gave rise to much more uniform populations than FS (Figure 3).

Allele frequency distributions have changed significantly between selection cycles for a few of the loci under evaluation (data not shown). The number of private alleles, however, varied among selection cycles, being, as expected, the highest in the original population (Table 4). We observed that locus umc1907 significantly deviated from Hardy–Weinberg equilibrium ($p < .05$) in all selection cycles (farmer's and breeder's), umc1823 only for the BS and umc1229 only for the FS cycles.

4 | DISCUSSION

The maize "Pigarro" population was under selection since 1985, within the PPB VASO Project, using a farmer's and a breeder's approach. To identify the most useful selection approach to support participatory maize breeding in sustainable farming systems, we compared "Pigarro" molecular diversity evolution and agronomic selection response between the two applied selection approaches. We confirmed that during both selection approaches, the Pigarro's genetic diversity changed and the population responded phenotypically. Nevertheless, genetic diversity was not reduced even with the more intensive BS, suggesting further response to selection can be expected.

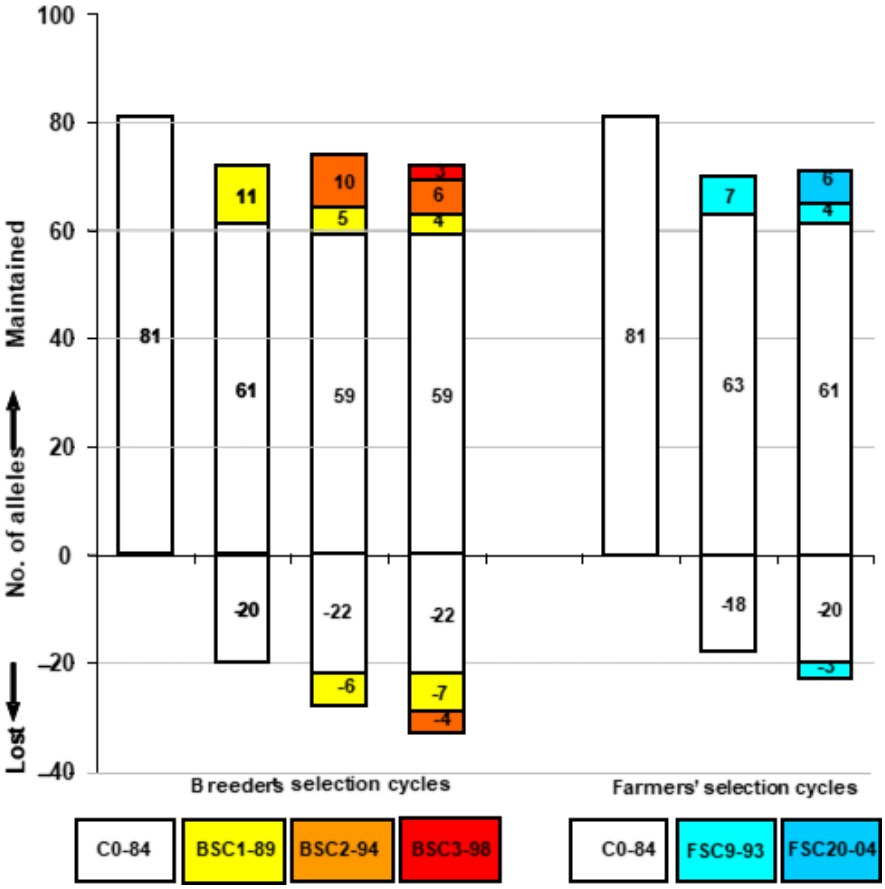

FIGURE 2 Number of alleles in each selection cycle (represented by different colour) lost or maintained from previous cycles detected using 15 SSR markers. Negative numbers refer to alleles lost comparing with previous analysed cycle. Positive numbers refer to new alleles or alleles maintained comparing with previous analysed cycle [Colour figure can be viewed at wileyonlinelibrary.com]

The evaluation of both selection methods indicates that both selection approaches were effective for achieving the main breeding objectives. As an example, crop uniformity was significantly improved by breeder selection ($R^2 = 0.93$ and gain per year −1.10%), but not by FS. Uniformity is important for hybrid development and to comply with seed commercialization requirements. In our study, we only detected yield increase during FS, which is of great value considering PPB applications. Increased ear fasciation might be partially responsible for this observed yield improvement. The ear fasciation increase by farmer selection was reported previously by Mendes Moreira et al. (2008). Ear fasciation is a particularly important trait for farmers during their seed selection, where they balance the choice of fasciated ears with other ear types to maintain a certain level of diversity, towards a long-term gain in ear diameters, kernel-row numbers, medulla and rachis dimensions (Vaz Patto et al., 2007). This positive selection of fasciation by farmers, contrast with BS, suggests an important role of fasciation for yield improvement. In the case of BS, the yield improvement strategy can be probably associated with adaptation to increased plant densities, considering that during BS, it was observed a reduction in plant height and yield.

During FS, an increasing level of kernel convulsion and the number of kernels per ear were associated with a decrease in thousand kernel weight, indicating a reduction in kernel size. In parallel, during this selection, ear length decreased significantly, and kernel-row number as well as ear diameter increased, in agreement with Emerson and East (1913) and Hallauer et al. (2010) for long-term divergent selection of ear length in maize. Nevertheless, contrary to Hallauer et al. (2010), yield slightly increased even though ear length was reduced. During breeders' selection, in contrast to the FS outputs, kernels became heavier, indicating a tendency for larger kernels, considering that the kernel type did not change.

Ear weight increase can be highly demanding for both stalk lodging resistance and root anchorage. These reasons are potentially associated with the lower values of stalk and root lodging, respectively. However, this association was only observed in the FS, with a moderated correlation of root or stalk lodging with cob weight ($r = 0.529$; 0.234) and with cob and ear weight ratio ($r = 0.573$; 0.266). The observed higher correlations between cob/ear weight ratio at harvest and per ear, with medulla and rachis 1 and rachis 2 (data not shown), suggested a higher lignification of the rachis, which may be important for ear architecture regarding kernel support.

Mendes Moreira et al. (2008) stated that differences in yield response between both selection methods could be related to a reduction in diversity along BS. Concerns have also been expressed that genetic diversity may be reduced by natural and artificial (human) selection (Vaz Patto et al., 2008).

Genetic differentiation for BS cycles decreased progressively, while during FS, genetic differentiation changed more erratically, being higher between FSC9-93 and FSC20-04 (4.07%) than between C0-84 and FSC9-93 (2.62%) (Appendix S1, Table 3). This difference can be associated with changes reported on the FS objective since

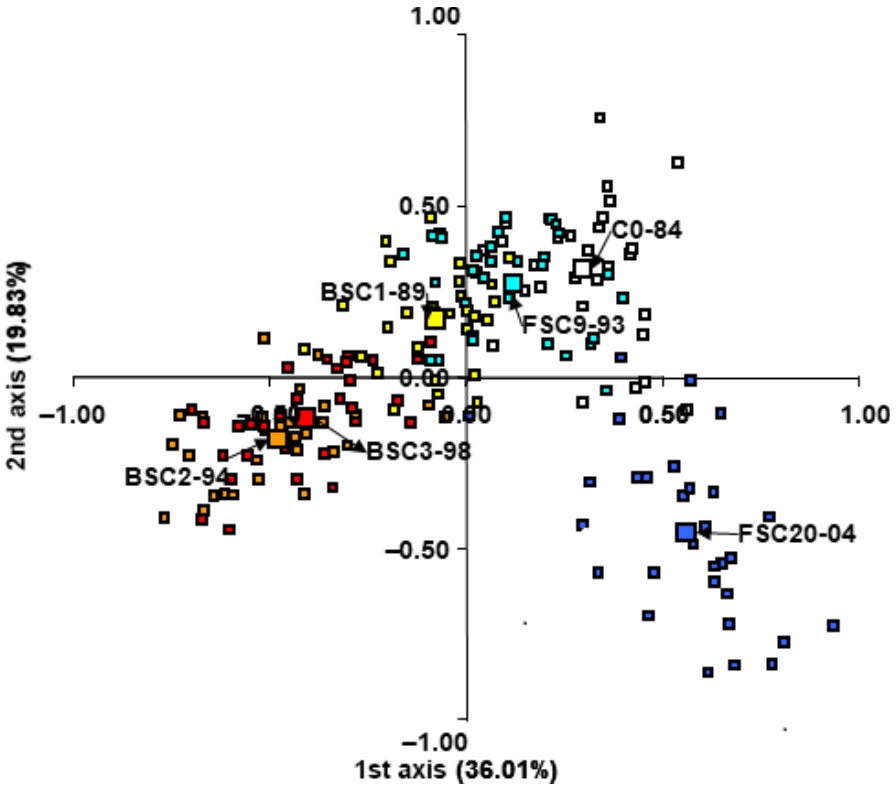

FIGURE 3 The factorial correspondence analysis (FCA) of 179 maize genotypes belonging to the initial population (C0-84, white), three breeder's selection cycles (BSC1-89, yellow; BSC2-94, orange; BSC3-98, red) and two farmer's selection cycles (FSC9-93, light blue; FSC20-04, dark blue). Each individual genotype is indicated by a small symbol, while the population barycentres are represented by larger symbols [Colour figure can be viewed at wileyonlinelibrary.com]

1993 (beginning of "Sousa Valley Best Ear" competition) towards increased ear sizes.

Selection increases the frequency of favoured alleles in a population, and due to genetic hitchhiking, the neighbouring closely linked neutral allele's variation may diminish (Pfaffelhuber, Lehnert, & Stephan, 2008). Changes observed in allelic frequency distribution and number of private alleles suggested that genetic diversity has not been reduced from "Pigarro" population in 1984 to those improved by FS or BS, but the genetic diversity maintained was not exactly the same. These molecular changes, depending on the selection approach, also had a phenotypic expression according to the previously discussed phenotypic data evolution. The seed maintenance procedure used during this PPB selection was by isolation plantings and a FS or BS pressure of 1%–5% or 15%–20%, respectively (Mendes Moreira et al., 2008); it is expected that assortative mating and selection were the most likely reasons for explaining deviations from the Hardy–Weinberg equilibrium. In the case of BS, possible inbreeding effects could have also contributed to the observed deviations from the Hardy–Weinberg equilibrium.

The majority of the screened SSR loci represented non-coding DNA regions (nine of the 15 SSR markers used were genomic SSRs) apparently not subject to strong selection pressures (Heath, Lwama, & Devlin, 1993). However, they could be linked to selected loci and therefore subjected to selection by genetic hitchhiking (Pinto, Vieira, de Souza, & de Souza, 2003). This suggests that directional selection observed on these SSR markers might indicate loci controlling the selected trait or traits linked to these markers (Butrón, Tarrío, Revilla, Ordás, & Malvar, 2005). Indeed, after accounting for multiple comparisons, several SSR loci were out of Hardy–Weinberg equilibrium in a few of the selection cycles. Due to space constraints, we will only refer to the ones consistently selected across improvement cycles. These were all genomic SSRs and all with an excess of homozygotes. In particular, umc1907, significantly out of Hardy–Weinberg equilibrium ($p < .05$) in all selection cycles (farmer's and breeder's), is located at maize genome bin 3.05, where several genes and QTLs have been identified in previous studies (Mendes-Moreira et al., 2015) that might be associated with, or indicating loci controlling, traits consistently changing in both selection approaches. The *candidate gene terminal ear1* (*te1*) (Jackson, 2009; Schnable & Freeling, 2011; Vollbrecht & Schmidt, 2009) and several QTLs controlling days to pollen 2, 7, 12 (*qdpoll2*, *7*, *12*) were detected in this region (Lawrence, Seigfried, & Brendel, 2005). In the present study, days to pollen or anthesis were inversely correlated with the average of determinate versus indeterminate ears (<−0.72 for FS and <−0.89 for BS) and are mainly associated with cycle duration. Indeed, in both selection methods, plant cycles tended to increase and ears became more indeterminate. In addition, the QTL for ear diameter 7 (*qeard7*) that can be associated with the ear diameters genetic control, was also located in this region (Lawrence et al., 2005). In the present study, the majority of the detected correlations between ear and cob diameters were higher than 90%, although Hallauer et al. (2010) reported 67% and Mendes-Moreira et al. (2015) reported 80.7%. These high correlations may be associated with loci controlling the cob diameters increase with both selection methods and cycles.

Locus umc1823, significantly deviated from Hardy–Weinberg equilibrium only for the BS cycles, is located at bin 2.02, where several genes potentially associated with traits consistently changing

with the BS have been identified in previous studies (Mendes-Moreira et al., 2015). This is the case of the QTLs for cob diameter 14 (*qcobd14*) and kernel-row number 6, 26 (*qkrow6, 26*) (Lawrence et al., 2005). Indeed, very high correlations among cob diameter 3 and row number 1, with ear diameter 3, have been described in the present study (>0.90) and in Mendes-Moreira et al. (2015) (>0.80) in comparison with Hallauer et al. (2010) (>0.67). These traits can be associated with loci controlling ear length increase, and the reduction in ear fasciation and kernel depth observed with the BS. In addition, fasciation was in this study correlated with cob diameter 3 (0.78). Mendes-Moreira et al. (2015) had also indicated a correlation of 0.59 to 0.79 among the same traits at two different locations.

Finally, umc1229, significantly deviated from Hardy–Weinberg equilibrium only for the FS cycles, is located at bin 6.01, where several genes, potentially associated with phenotypic traits consistently changing along the farmer's selection, have been identified in previous studies (Mendes-Moreira et al., 2015). With farmer selection, ears become shorter and wider, with a greater number of rows, hence with more convulsion, higher fasciation and smaller kernels that increased in number. Among bin 6.01 potentially associated genes, we may find the defective kernel 19, 28 (*dek19*, *28*), and miniature seed 3 (*mn3*) genes (Lawrence et al., 2005), associated with the observed decrease in thousand kernel weight. On this same region, the ear length 25 (*qearl25*) and days to pollen 4 (*qdpoll4*) QTLs were also detected (Lawrence et al., 2005). The *qearl25* might be associated with the observed ear length decrease, while the *qdpoll4* might be associated with the observed ear and cob diameter increases, due to the high correlation detected among these traits and the beginning of anthesis, that is, days to pollen (>0.85, in Mendes-Moreira et al. [2015]).

We confirmed that during both selection approaches, genetic diversity changed, to allow the population to phenotypically respond to selection, but was not reduced even with the most intensive BS. Although there were no significant differences detected on the studied genetic diversity parameters along selection cycles, during both selection methods, an increase in plant maturing and in the ears indeterminacy was observed. Also in both selection methods, cobs have become wider and heavier. The last cycle of both selection methods maintained the ability for polycropping systems and quality for bread production according to Vaz Patto et al. (2009, 2013). Nevertheless, particular phenotypic traits evolved in opposite directions between the two selection methods. With BS, ears became longer and less fasciated with an overall increase in crop uniformity, whereas farmers selected for shorter and wider ears, with increased levels of fasciation and smaller kernels. Our molecular diversity evolution analysis highlighted potential associations between particular neutral molecular markers and loci controlling some of the phenotypic traits under selection (e.g., ear length, fasciation and related ear traits such as ear diameter and kernel-row number). These associations need however to be better explored and validated by future linkage or association mapping approaches previous to their use for supporting trait selection in sustainable farming systems. Contrarily to breeder selection, a positive response on yield was observed under farmer's selection. This indicates a need for a further development in PPB methodologies from seed to plate, for a holistic approach of dynamic management of genetic resources.

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SUPPORTING INFORMATION

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