

Comparison of two methods of reciprocal recurrent selection in maize (*Zea mays* L.)

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Abstract Reciprocal recurrent selection (RRS) was proposed for simultaneously improving two populations and their cross. A modification of the classical full-sib RRS (FS-RRS) was proposed in which the performance of full-sibs and S_2 families is combined in a selection index (FS- S_2 -RRS). The Mediterranean corn borer (MCB) is the main corn borer species in the Mediterranean and adjacent areas and produces important yield losses. We started two RRS programs (FS-RRS and FS- S_2 -RRS) from the same maize population in which the selection criterion was grain yield under artificial infestation with MCB eggs. Original populations, two cycles of selection derived from them by both RRS methods, and population crosses were evaluated under MCB attack and under insecticide treatment in three different environments. The objective was to compare the efficiency of the FS-RRS and the FS- S_2 -RRS methods for improving grain yield. We found that the FS- S_2 -RRS method was successful for improving the yield of the population cross under optimum conditions (the regression coefficient over cycles was $b = 0.87^{**}$ Mg ha⁻¹ cycle⁻¹) without losing yield under high pressure of MCB attack ($b = 0.07$). On the contrary, FS-RRS failed to improve the yield of the population cross under optimum conditions ($b = 0.65$) and tended to decrease the yield under high levels of MCB attack ($b = -0.26$). We conclude that for

developing high yielding and stable varieties, FS- S_2 -RRS is more efficient than the classical FS-RRS method.

Introduction

In crops in which the commercial varieties are mainly hybrids, interpopulation are preferred to intrapopulation selection methods to capitalize heterosis. Reciprocal recurrent selection (RRS) was first proposed by Comstock et al. (1949) to simultaneously improve two populations and the cross between them. RRS is being widely applied in multiple species, for example, in *Coffea canephora* Pierre ex A. Froehn (Montagnon et al. 2008), cocoa, *Theobroma cacao* L. (Pokou et al. 2009), oil palm, *Elaeis guineensis* Jacq (Bakoume et al. 2010) and particularly in maize (*Zea mays* L.) (Butruille et al. 2004; Hinze et al. 2005; Pinto et al. 2003; Romay et al. 2011). Half-sib RRS (HS-RRS) uses interpopulation half-sib progenies as evaluation units (Comstock et al. 1949), while full-sib RRS (FS-RRS) uses interpopulation full-sib progenies (Hallauer and Eberhart 1970). Peiris and Hallauer (2005) compared both methods, by means of computer simulations and found that the genetic responses, under similar conditions, for HS-RRS and FS-RRS were similar. However, FS-RRS samples twice as many plants as HS-RRS for the same amount of testing resources and, therefore, the number of evaluation plots could be halved while maintaining the selection intensity. Jones et al. (1971) compared both methods by simulation approaches, but applying higher selection intensity to FS-RRS, and concluded that the response rate was higher for FS-RRS. A modification of FS-RRS was proposed by Moreno-González and Hallauer (1982), in which additional evaluation of S_2 families of each one of the members that generate the full-sib families

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was carried out and the performance of full-sibs and S_2 families was combined in a selection index (FS- S_2 -RRS). The selection index was constructed as a lineal combination of the performance of full-sib families and S_2 families. Each measurement was weighted by a factor, which was calculated by standard selection index theory, to maximize the genetic gain of the breeding value of the populations (Moreno-González and Hallauer 1982). The authors made a theoretical comparison of FS-RRS and FS- S_2 -RRS and concluded that FS- S_2 -RRS has some advantage for traits with low heritability, such as maize yield, when one or two locations with two replications were involved in selection experiments. However, theoretical expectations or computer simulations have two limitations: simplification of genetic situations and general conclusions are limited to a specific set of genetic conditions. To determine the relative effectiveness of different selection methods, more reliable information is provided by field evaluations (Peiris and Hallauer 2005).

We started two parallel RRS programs (FS-RRS and FS- S_2 -RRS) from the same original population using grain yield as selection criteria. In the evaluation of the interpopulation full-sib progenies and the S_2 families during the selection process, the genotypes were artificially infested with eggs of Mediterranean corn borer (MCB, *Sesamia nonagrioides* Lefebvre), which is one of the main corn borer pests in the Mediterranean and adjacent areas. The infestation was made to avoid an unexpected increment in the susceptibility to MCB of the improved varieties. After two cycles of selection, we evaluated the efficiency of the FS-RRS and the FS- S_2 -RRS for improving grain yield of the populations and the population cross.

Materials and methods

Selection programs

The selection program started with the development of two eight-line synthetic varieties, EPS20 and EPS21 (Table 1). The eight inbred lines for each synthetic variety were chosen because they had partial resistance to MCB according to a previous evaluation of 121 inbred lines (Butron et al. 1999). The inbreds involved in EPS20 belong to the Reid group, while the inbreds for EPS21 do not belong to that heterotic group. Therefore, we should expect good hybrid performance when crossing improved cycles of these synthetic varieties or inbreds derived from them. Prior to generating the synthetic varieties, the single crosses between inbreds and double crosses were made in 1995 and 1996, respectively. For each synthetic variety, about 20 plants from each double-cross hybrid were mated only once (as male or female) in plant-to-plant crosses

Table 1 Inbred lines of the synthetic varieties EPS20 and EPS21

Inbred line	Pedigree
EPS20 synthetic variety	
CM109	(V3 × B14)B14
CM139	(V3 × B14)B14
CM151	(Mt42 × WF9)WF9
A634	(Mt42 × B14)B14 ³
A639	A158 × B14
A652	A90 × WF9
A664	(ND203 × A636)A636 ²
W64A	WF9 × C.I. 187-2
EPS21 synthetic variety	
EP17	A1267
EP43	Parderrubias (O.P. variety from northwestern Spain)
EP53	Laro (O.P. variety from northwestern Spain)
PB60	Nostrato dell'Isola (O.P. variety from Italy)
PB130	Rojo Vinoso de Aragón (O.P. variety from northeastern Spain)
F473	Doré de Gomer (O.P. variety from France)
CO125	Wisc. Exp. Single cross
A509	A78 × A109

O.P. open pollinated variety

between double-cross hybrids in 1997, to generate about 20 ears that provided the base material. Two parallel programs of RRS, modified for single-eared populations, were started in 1998. The FS-RRS involves only crossbred family selection (Hallauer 1973), whereas the FS- S_2 -RRS combines simultaneously S_2 and crossbred family selection (Moreno-González and Hallauer 1982). The RRS programs started with the development of the S_1 progenies in 1998. In 1999, 100 S_1 families from EPS20 were randomly crossed to 100 S_1 families from EPS21. In addition, the S_1 families were self-pollinated to obtain S_2 families. The crosses between S_1 families and the S_2 families from EPS20 and EPS21 were evaluated in three adjacent experiments under artificial infestation with eggs of MCB using simple lattice designs in 2000 in Pontevedra (42° 24' N, 8° 38' W, 20 masl). Pontevedra is located in northwestern Spain with cold and wet springs and short summer, conditions similar to those found in many areas of Atlantic Europe. In each population, ten families that were the parents of the S_1 crosses with the highest yields were chosen and recombined in 2001 to produce the first cycle of FR-RRS selection. Simultaneously, the ten best families were selected based on performance of S_2 and full-sib families by applying a selection index (Moreno-González and Hallauer 1982) and recombined in 2001 to develop the first cycle of FS- S_2 -RRS. From 2002 to 2005, the process was repeated to obtain the second cycles of selection.

Estimation of genetic parameters

Since S_2 families were obtained by bulking seed from selfing four plants within each S_1 family, the additive variance (V_a) for each population was estimated as 8/9 times the variance among the S_2 families. This estimate will be biased down to 1/16 the dominance variance. The FS families evaluated during selection were used to estimate, assuming that the dominance variance is negligible, the additive variance for the population cross (V_{ap}) as 1/2 of the variance between families. For both selection methods, the heritability (h^2) and the expected genetic gain (Δg) for the population cross were estimated following Moreno-González and Hallauer (1982).

Evaluation of the selection programs

In 2006, seeds from the original (C0) and improved cycles (C1 and C2) were multiplied and the three interpopulation crosses (C0 × C0, C1 × C1 and C2 × C2) were made. In all intrapopulations and interpopulation crosses, at least 70 plants from each population were used. The genotypes were evaluated at Pontevedra in 2007 (we named this environment 1) and 2008 (environment 2) and Zaragoza (41° 44' N, 0° 47' W, 230 masl) in 2007 (environment 3). Zaragoza, located in northeastern Spain, has a dry and hot summer, typical of the Mediterranean area. In each environment, there were two adjacent trials: one trial in which successive granular insecticide treatments were applied to guarantee protection against MCB and another trial in which ten plants per plot were artificially infested at silking with a mass of 40 MCB eggs. The experimental design for each trial was a 7 × 7 lattice design with three replications that included entries which were not analyzed in this paper. Each experimental plot consisted of two rows spaced 0.8 m apart, with 25 two-plant hills spaced 0.21 m apart. The hills were thinned after emergence to obtain a final density of 60,000 plants ha⁻¹. Appropriate techniques for the cultivation of maize were performed in each location. The following data were taken on each plot: grain yield (Mg ha⁻¹), days to pollen shedding (days from planting until 50% of plants shed pollen), days to silking (days from planting until 50% of plants showed silks), grain moisture (g H₂O kg⁻¹), lodging (percentage of plants broken below the main ear or leaning more than 45° from the vertical), stalk tunnel length (the length in centimeters of tunnels made by MCB into the stalk) and kernel damage recorded on a 9-point rating scale (9 = non-damaged kernels; 1 = more than 80% of kernels tunneled by MCB).

Individual analyses of variance were made for each trial. If the effectiveness of the lattice design was above 105%, the adjusted means and the intrablock errors were used; otherwise, the unadjusted means and the errors considering

a randomized complete block design were used (Cochran and Cox 1957). The combined analyses of variance were made using the errors and the means of the individual analyses. These analyses have the following sources of variation: environments, treatments, genotypes and their interactions. The treatment factor has two levels: insecticide and artificial infestation with MCB larvae. Environments and their interactions were considered random effects and genotypes and treatments were considered fixed effects. The means of the different cycles of selection were compared by means of the Fisher's protected least significant difference (LSD). The mean squares of the linear component of the main effects and interactions corresponding to the linear regression on cycle of selection were calculated and the significance of the linear components tested by an F test with the appropriate error denominator (Cochran and Cox 1957). The linear regression coefficients of each character on cycles of selection were estimated by least squares to quantify the response per cycle of selection. The analyses were carried out with the GLM procedure of SAS (SAS Institute 2009).

Results

According to the evaluation of S_2 families, EPS20 has a lower additive variance than EPS21, although both populations had a similar reduction in V_a after one cycle of FS- S_2 -RRS (Table 2). V_{ap} and h^2 were reduced after one cycle of FS-RRS, but they were not reduced after one cycle of FS- S_2 -RRS. For cycle 1, the expected Δg was similar for both selection methods, while for cycle 2 the expected Δg was higher for FS- S_2 -RRS than for FS-RRS.

Cycles 0 and 1 were not significantly different in yield for both populations per se and their respective crosses for both selection methods (Table 3). For EPS20 × EPS21, cycle 2 yielded significantly ($P < 0.05$) higher than cycle 0 with the FS- S_2 -RRS method, but not with the FS-RRS method. Regarding the populations per se, cycle 2 of EPS21 had higher yield than cycle 1 with FS-RRS, while cycle 2 of EPS20 had higher yield than cycle 0 with FS- S_2 -RRS.

The linear response of EPS20 and EPS21 to FS-RRS, averaged over environments and treatments, was significant for yield, at the 10 and 1% level of significance, respectively, but the response of the cross EPS20 × EPS21 was not significant (Table 4). The average linear response of EPS21 to FS-RRS was positive, but the response of EPS20 was negative (Fig. 1). The interaction of the linear response of population per se with environments and treatments was not significant, but the interaction of the response of population cross with environments was highly significant. For the populations cross, the sign of the linear

Table 2 Additive variances, heritabilities and expected genetic gains for grain yield (Mg ha^{-1}) estimated from FS and S_2 families which were evaluated during the selection process

	V_a^a (EPS20)		V_a^a (EPS21)		V_{ap}^b (EPS20 and EPS21)		h^{2c}		Δg^d	
	FS-RRS	FS- S_2 -RRS	FS-RRS	FS- S_2 -RRS	FS-RRS	FS- S_2 -RRS	FS-RRS	FS- S_2 -RRS	FS-RRS	FS- S_2 -RRS
Cycle 1		0.3650		0.5444	0.1612	0.1612	0.21	0.21	0.65	0.61
Cycle 2		0.2292		0.4343	0.0941	0.2422	0.12	0.35	0.36	1.01

^a Additive variance estimated from the variance between S_2 families

^b Additive variance for the population cross estimated from the variance between FS families

^c Heritability for the population cross

^d Expected genetic gain for the population cross

Table 3 Means for grain yield (Mg ha^{-1}) of the original and improved by FS-RRS and FS- S_2 -RRS populations and interpopulation crosses evaluated at three environments and two levels of infestation

	FS-RRS			FS- S_2 -RRS		
	EPS20	EPS21	× EPS21	EPS20	EPS21	× EPS21
C0	7.54	7.00	8.46	7.19	6.90	8.42
C1	7.82	6.78	8.82	7.75	7.40	8.93
C2	7.15	7.62	9.09	8.20	7.05	9.35

LSD (0.05) = 0.65 Mg ha^{-1}

Table 4 Mean squares of the linear component of the main effects and interactions corresponding to the regression on cycle of selection from the analysis of variance of the original and improved by FS-RRS populations and interpopulation crosses evaluated at three environments and two treatments (artificial infestation with MCB eggs and insecticide)

Source	df	MS					
		Grain yield (Mg ha^{-1})	Shedding (days)	Silking (days)	Grain moisture ($\text{g H}_2\text{O kg}^{-1}$)	Lodging (%)	Kernel damage (1–9) ^a
Genotypes							
EPS20 linear	1	0.4615†	8.5103**	6.7243**	0.3089	3.8957	0.0363
EPS21 linear	1	1.1307**	0.0667	0.2726	2.8320**	505.5044	0.1284
EPS20 × EPS21 linear	1	0.4474	0.6401	1.3054	0.0027	7.7054	0.9017*
Environments (E) × genotypes							
E × EPS20 linear	2 ^b	0.2965	0.6195	0.5006	0.9606†	0.5807	0.4169
E × EPS21 linear	2 ^b	0.1648	0.2217	1.5109†	0.2519	197.7046**	0.0662
E × EPS20 × EPS21 linear	2 ^b	1.1441**	0.0496	0.0013	0.5185	8.4472	0.4078
Treatments (T) × genotypes							
T × EPS20 linear	1	0.0031	3.2983**	0.2378	0.04343	3.8447	
T × EPS21 linear	1	0.0585	0.0277	0.8514	0.0062	53.0070†	
T × EPS20 × EPS21 linear	1	2.4520**	0.4295	2.0397*	0.0006	3.7745	
E × T × genotypes							
E × T × EPS20 linear	2 ^b	0.4835*	0.4863	0.0003	0.1778	3.0793	
E × T × EPS21 linear	2 ^b	0.2288	0.2447	0.0465	3.1485**	31.3469	
E × T × EPS20 × EPS21 linear	2 ^b	0.0194	0.3845	0.0623	0.0982	11.4902	
Combined error		0.1291	0.3463	0.4811	0.3432	18.5458	0.1830
df of combined error		540	540	348	522	348	288

^a Kernel damage was taken on a 9-point rating scale (9 = non-damaged kernels; 1 = more than 80% of kernels with injuries due to MCB larvae activity)

^b The degrees of freedom for silking and lodging were 1

** , * , †, Significant at 1, 5 and 10% of probability

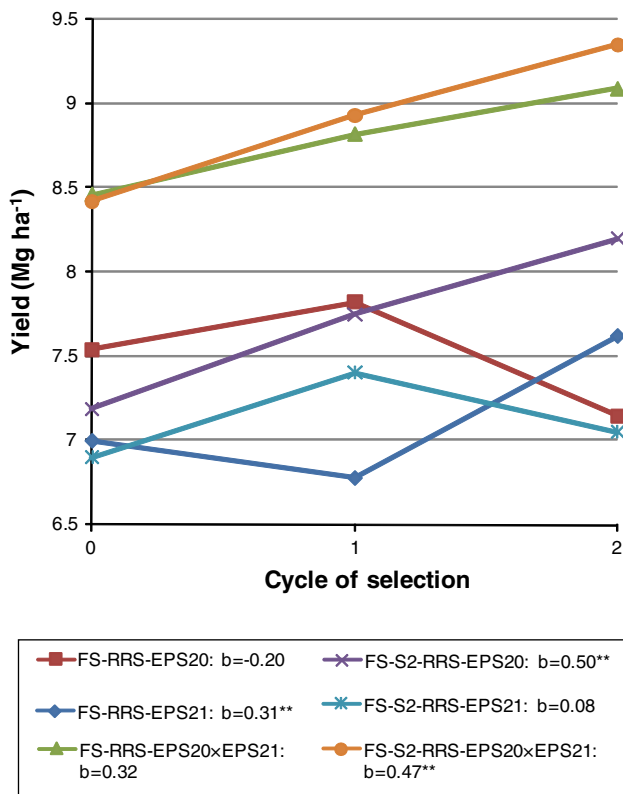


Fig. 1 Average response of the populations EPS20 and EPS21 and the crosses between them to FS-RRS and FS-S₂-RRS evaluated in six conditions: three environments and presence and absence of MCB attack within each environment **, *, †, significant at 1, 5 and 10% of probability

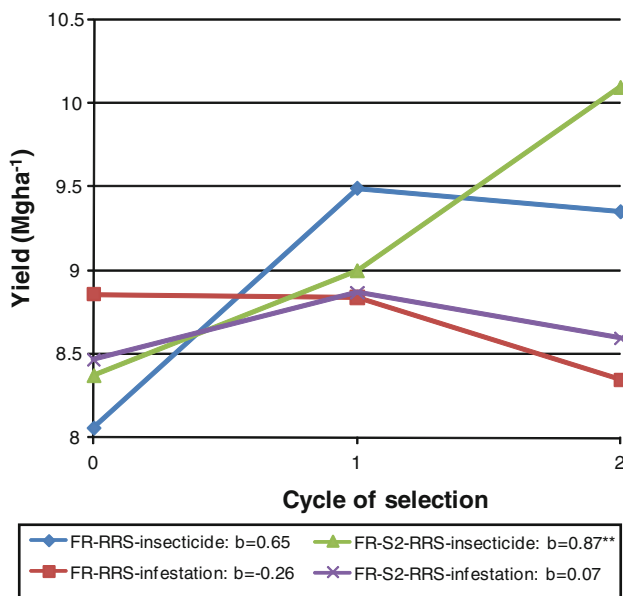


Fig. 2 Response of the population cross EPS20 × EPS21 to FS-RRS and FS-S₂-RRS evaluated in the presence and absence of MCB attack and averaged over three environments **, *, †, significant at 1, 5 and 10% of probability

regression coefficient was positive when the progress of selection was evaluated in the environment where insecticide was applied, but negative when the progress of selection was evaluated under artificial infestation, although the regression coefficients were not significant (Fig. 2). For the populations cross, the sign of the linear coefficient also varied between environments ($b = 0.72$, $b = 0.20$ and $b = -0.35$ in environments 1, 2 and 3, respectively).

For yield, the linear responses of EPS20 and EPS20 × EPS21 to FS-S₂-RRS averaged over environments and treatments were significant (Table 5). The average response of EPS20 and EPS20 × EPS21 was 0.50 and 0.47 Mg ha⁻¹ per cycle, respectively (Fig. 1). The interactions of the linear response of population cross with environments and treatments were significant at the 10 and the 1% level of significance, respectively. The average response of the populations cross was high (0.87) and significant ($P < 0.01$) in the treatment with insecticide, but low (0.07) and not significant under artificial infestation (Fig. 2). The linear regression coefficients of the population cross on cycles of selection were positive in all environments ($b = 0.76$, $b = 0.48$ and $b = 0.16$ in environments 1, 2 and 3, respectively).

FS-RRS produced a highly significant increase in grain moisture of EPS21 ($b = 0.48$) and in days to pollen shedding and silking of EPS20 ($b = 0.84$ and $b = 0.92$, respectively) (Table 4). FS-S₂-RRS produced a highly significant increase in days to pollen shedding of the populations and the populations cross ($b = 0.74$, $b = 0.57$, and $b = 0.63$ for EPS20, EPS21 and EPS20 × EPS21, respectively), while grain moisture did not change during the selection process (Table 5). For lodging, the linear response with both methods was not significant for the populations per se and for the population crosses, although the cycle 2 of EPS21 lodged significantly ($P < 0.05$) less than cycle 0 with FS-RRS.

Regarding traits related to corn borer damage, the linear responses of the population cross for kernel damage were significant at the 5 and 10% for FS-RRS and FS-S₂-RRS, respectively (Tables 4, 5). The responses of the populations cross to FS-RRS ($b = -0.39$) and FS-S₂-RRS ($b = -0.33$) for kernel damage were negative. On the other hand, none of the selections produced significant changes in stalk tunnel length.

Discussion

In maize and other crops, most of the commercial varieties are hybrids resulting from the cross between two inbred lines. By improving the cross of two populations by RRS, we expected to improve the cross of inbred lines derived

Table 5 Mean squares of the linear component of the main effects and interactions corresponding to the regression on cycle of selection from the analysis of variance of the original and improved by FS-S₂-RRS population and interpopulation crosses evaluated at three environments and two treatments (artificial infestation with MCB eggs and insecticide)

Source	df	MS					
		Grain yield (Mg ha ⁻¹)	Shedding (days)	Silking (days)	Grain moisture (g H ₂ O kg ⁻¹)	Lodging (%)	Kernel damage (1–9) ^a
Genotypes							
EPS20 linear	1	3.0348**	6.6752**	0.5882	0.2032	9.1562	0.0091
EPS21 linear	1	0.0743	3.9416**	0.2839	3.7310	5.7602	1.1631*
EPS20 × EPS21 linear	1	2.6163**	4.7108**	0.1417	0.0222	1.4844	0.6667†
Environments (E) × genotypes							
E × EPS20 linear	2 ^b	0.3544†	0.6044	2.5284*	0.1343	1.4209	0.0557
E × EPS21 linear	2 ^b	0.1128	0.3142	0.7769	1.1448*	5.7214	0.0597
E × EPS20 × EPS21 linear	2 ^b	0.3681†	0.0070	0.3747	0.4410	2.5727	0.2467
Treatments (T) × genotypes							
T × EPS20 linear	1	0.0413	0.0386	0.0873	0.0617	0.1424	
T × EPS21 linear	1	0.0659	1.6330*	0.1109	0.0992	27.0348	
T × EPS20 × EPS21 linear	1	1.9025**	3.1649**	0.5828	1.5853*	0.3089	
E × T × genotypes							
E × T × EPS20 linear	2 ^b	0.1291	0.3382	0.5882	0.6304	1.3083	
E × T × EPS21 linear	2 ^b	0.0095	0.0427	0.0191	0.1096	38.4051	
E × T × EPS20 × EPS21 linear	2 ^b	0.0607	0.0991	0.0032	0.3706	30.2294	
Combined error		0.1291	0.3463	0.4811	0.3432	18.5458	0.1830
df of combined error		540	540	348	522	348	288

^a Kernel damage was taken on a 9-point rating scale (9 = non-damaged kernels; 1 = more than 80% of kernels with injuries due to MCB larvae activity)

^b The degrees of freedom for silking and lodging were 1

**, *, †, Significant at 1, 5 and 10% of probability

from the two populations. In addition, an RRS program improves the performance of the populations per se, which is expected to improve the performance of the inbred lines derived from the populations, making the production of the seed cheaper for the seed companies. Improved open pollinated varieties or crosses between them could be also of interest in sustainable or low-input agriculture.

We evaluated the progress of two cycles of selection in six different conditions (3 environments and two treatments) and found that the standard method of FS-RRS did not significantly improve the yield of the population cross when we consider the average response over the six conditions. However, the difference between cycle 2 and cycle 0 was near the 5% level of significance and some linear trend, although not significant, was observed which preclude a significant progress after more cycles of selection. On the contrary, a significant average linear response of the populations cross over the six conditions was obtained with FS-S₂-RRS after two cycles of selection. The expected genetic gains estimated from FS and S₂ families were higher than the realized values. Inherent to theoretical expectations are simplifications that make genetic models

approachable, but different to reality. In addition, the evaluation of the families in one environment could cause a bias upward in the estimation of genetic gains, because the genotype × environment interaction was not taken into account. In spite of that, we found an acceptable agreement between the expected efficiency (1.6) and the realized efficiency (1.5) of the FS-S₂-RRS method relative to the FS-RRS method after two cycles of selection.

The higher additive variance in S₂ values compared with the additive variance in crosses suggests that additive effects are more important than non-additive ones in our populations. In addition, the change of heterosis was not important with both methods. Some RRS programs failed to increase heterosis and this may be attributable to important additive effects in the original populations (Hallauer and Miranda 1988). The increments that showed the population cross with FS-S₂-RRS in the first and second cycle of selection (0.51 and 0.42, respectively) were similar to the increments shown by EPS20 (0.56 and 0.45, respectively). Therefore, FS-S₂-RRS was probably more successful than FS-RRS, because it was able to manipulate the additive effects, particularly those present in EPS20.

Under this scenario, another adequate selection scheme could be an intrapopulation selection method in the beginning, followed by an interpopulation method after some cycles of selection (Romay et al. 2011). Weyhrich et al. (1998) found that interpopulation methods were not superior to intrapopulation methods for improving testcross performance in BS11 \times BS10, because additive effects were probably more important than non-additive ones.

Four seasons are required for completing one cycle of selection with both methods, which would include 4 years without a winter nursery and 2 years with a winter nursery. However, for the same number of full-sib families evaluated and for the same selection intensity, the development of an FS-S₂-RRS program needs more effort than an FS-RRS program because S₂ families are developed and evaluated. If each S₂ family is developed by bulking seeds of four plants and 100 S₂ families per population are developed, then 800 self-pollinations are needed. In addition, if the 200 S₂ families are evaluated with two replications, then 400 experimental plots are needed. Moreno-González and Hallauer (1982) theoretically compared FS-S₂-RRS and FS-RRS on the basis of equivalent amount of effort for different values of heritability for the population cross and for different values of the correlation between S₂ values and values in crosses. They found that for low values of the heritability, as those found in our populations, FS-S₂-RRS was more efficient than FS-RRS when the same resources were applied to both methods. This is so even for values of the correlation as low as those found in our populations (about 0.2).

Although more cycles of selection would give a more precise estimation of the response to selection, given the clear difference between the two methods obtained in our experiment, it is reasonable to conclude that FS-S₂-RRS is more efficient than the classical FS-RRS method. Additionally, we found a reduction of the additive variance and, consequently, of the heritability with the FS-RRS method, but we did not find that effect with the FS-S₂-RRS method.

Although in several experiments the average response across environments has been reported, as far as we know there is little information about the interaction of the response to selection with environments. In our experiment, this was the case for the response of the population cross to FS-RRS, which significantly interacted with environments. With both methods, the response to selection was higher when it was evaluated in Pontevedra (environments 1 and 2), where the selection process had been carried out, than in Zaragoza (environment 3), which has climatic conditions very different from Pontevedra. Thus, our data indicate that alleles selected in one particular environment may not work well in other environments and highlight the importance of both environments: the environment where the selection is carried out and the

environment where the improved varieties will be grown. The interaction of response with environments was higher for FS-RSS, with even a negative response in environment 3 ($b = -0.35$) than for FS-S₂-RSS. Therefore, our experimental results indicate that FS-S₂-RSS gives not only a higher average response, but also a more stable response over environments.

With both methods of selection, the response of the population cross significantly interacted with MCB treatments. This is so because the magnitude of the regression coefficient was higher for plants protected with insecticide ($b = 0.65$ and $b = 0.87$, for the FS-RRS and the FS-S₂ methods, respectively) than for those artificially infested with MCB ($b = -0.26$ and $b = 0.07$, for the FS-RRS and the FS-S₂ methods, respectively). Therefore, the positive response of the FS-S₂-RRS method averaged over the six different conditions is due to a significant increase of the yield of the improved cycles under conditions of low corn borer attack and the maintenance of the yield of the improved cycles under conditions of high levels of infestation. The high level of infestation in our experiment was achieved by artificial infestation, which was superimposed to the natural infestation and, therefore, the level of infestation in our experiment was higher than expected in the usual years. Thus, the FS-S₂-RRS method has been successful in improving yield under optimum conditions without losing yield under conditions of high pressure of corn borer attack. On the contrary, the negative value, although not significant, of the response of EPS20 \times EPS21 to FS-RRS under high level of infestation suggests that if the FS-RRS would continue for some more cycles, a considerable decrease of yield under high level infestation could occur.

Regarding the yield improvement of the populations per se, with both methods one of the two reciprocal populations improved its yield, although the response was clearer with the FS-S₂-RRS method. The reciprocal population, i.e., that which was not improved, decreased its yield with FS-RRS, but maintained it with FS-S₂-RRS. The advantage of FS-S₂-RRS compared to FS-RRS was expected because selection of S₂ families with the FS-S₂-RRS method should have increased the frequency of favorable alleles, which contribute to the population cross as originally designed, but also to the populations per se as an indirect contribution. Most results from reciprocal recurrent selection for grain yield in maize have shown that one of the source populations was improved, but the response was negligible or negative for the reciprocal population (Romay et al. 2011; Souza 1999). Two main processes usually operate during the improvement of populations in reciprocal recurrent selection programs: selection that increases the yield, and inbreeding depression, with the opposite effect. The final result depends on the balance between these two

processes. The original populations from our RRS program are very different: EPS21 is mainly composed of inbreds derived from populations adapted to Atlantic conditions, while EPS20 is composed of inbreds derived from WF9 and B14, which are very stable lines as evidenced by its genetic contribution to commercial hybrids during decades (Mikel 2011; Troyer 1999). The performance of the original populations in our experiment reflects the differences in their progenitor lines. Thus, EPS21, made from lines adapted to Atlantic conditions, had a good performance in Pontevedra, which has Atlantic weather, but poor performance in Zaragoza, which has Mediterranean weather. The EPS20 variety, made from stable lines, had an acceptable performance in both environments. We found, in agreement with the FS-RRS experiment reported by Romay et al. (2011), that the population made from material adapted to the environment where selection was performed (EPS21) was improved by FS-RRS. On the contrary, the population made from stable lines (EPS20) was improved by FS-S₂-RRS. These results suggest that FS-RRS increased the frequency of favorable alleles specific to the environment of selection, while FS-S₂-RRS increased the frequency of favorable alleles which are stable over environments. This is in congruence with the previously discussed fact that the population cross had a more stable response over environments when FS-S₂-RRS was used than with FS-RRS. If the only goal of the RRS is the improvement of the yield of the population cross in the short term, the FS-S₂-RRS seems the best choice; however, if we want to improve simultaneously the yield of the intervarietal cross and the yield of the two populations, a cycle of FS-RRS could be alternated with some cycles of FS-S₂-RRS.

Regarding other agronomic traits, lodging was high (about 15%) in EPS21 and was reduced to 5%, approximately, as a correlated response to yield improvement in this synthetic when FS-RRS was carried out. FS-S₂-RRS increased yield of EPS20, but did not change its lodging that was initially low (5%, approximately). Romay et al. (2011) also found a favorable correlated response for lodging in an FS-RRS for yield, although the final lodging after three cycles was still high. Another correlated change due to selection that we found with both methods was an increase in the number of days to flowering, which had to be controlled to avoid the period of flowering coinciding with the period of highest temperature and less precipitation. However, the grain moisture of the populations per se and the cross between them did not change with the selection, except that of EPS21 with the FS-RRS method. Both the increasing of the number of days to flowering and the maintenance of the grain moisture are usual effects in the RRS programs (Eyherabide and Hallauer 1991; Popi and Kannenberg 2001; Romay et al. 2011).

Kernel damage of the population cross increased with both methods of selection, suggesting a negative genetic correlation between ear resistance and yield. The increment of damage without losing yield, as with the FS-S₂-RRS method, indicates some increase in tolerance. These results disagree with those of Sandoya et al. (2010), who found a positive phenotypic correlation between yield under infestation conditions and grain appearance, although in that case the main criterion for selection was the length of the tunnels made by the corn borer instead of yield. The physical injuries to ears could promote infections by *Fusarium* spp. that prejudice grain quality, producing toxic compounds (Avantaggiato et al. 2002; Butron et al. 2006). For that reason, it could be necessary to control the effect of the selection in the susceptibility to infections by *Fusarium* spp.

In our selection experiment, breeding for yield did not involve any significant change in stalk resistance. However when traits related to insect resistance, such as tunnel length or damage ratings, were the selection criteria, other authors have reported a decrease in grain yield (Klenke et al. 1986; Nyhus et al. 1989; Russell et al. 1979; Sandoya et al. 2008). The relationship between yield and resistance is poorly understood and our data indicate that both selection programs have managed yield genes independent of stalk resistance genes. On the other hand, it is not surprising that there were changes in the ear and not in the stalk resistance because both resistances are independent (Butron et al. 1998; Cartea et al. 2001).

As a practical point of view, we conclude, based on the evaluation of two cycles of selection, that for developing high yielding and stable crosses between two varieties, FS-S₂-RRS is more efficient than the classical FS-RRS method. If we are also interested in the improvement of the populations per se, our data show that both methods are efficient, although for improving only one of the two populations.

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References

- Avantaggiato G, Quaranta F, Desiderio E, Visconti V (2002) Fumonisin contamination of maize hybrids visibly damaged by *Sesamia*. *J Sci Food Agric* 83:13–18
- Bakoume C, Galdima M, Tengoua FF (2010) Experimental modification of reciprocal recurrent selection in oil palm in Cameroon. *Euphytica* 171:235–240
- Butron A, Malvar RA, Velasco P, Cartea ME, Ordas A (1998) Combining abilities and reciprocal effects for maize ear resistance to pink stem borer. *Maydica* 43:117–122

- Butron A, Malvar RA, Cartea ME, Ordas A, Velasco P (1999) Resistance of maize inbreds to pink stem borer. *Crop Sci* 39:102–107
- Butron A, Santiago R, Mansilla P, Pintos-Varela C, Ordas A, Malvar RA (2006) Maize (*Zea mays* L.) genetic factors for preventing fumonisin contamination. *J Agric Food Chem* 54:6113–6117
- Butruille DV, Silva HD, Kaeppeler SM, Coors JG (2004) Response to selection and genetic drift in the three populations derived from golden glow maize population. *Crop Sci* 44:1527–1534
- Cartea ME, Malvar RA, Vales MI, Butron A, Ordas A (2001) Inheritance of resistance to ear damage caused by *Sesamia nonagrioides* (Lepidoptera: Noctuidae) in maize. *J Econ Entomol* 94:277–283
- Cochran WC, Cox GM (1957) *Experimental design*, 2nd edn. Wiley, New York
- Comstock RE, Robinson HF, Harvey PH (1949) A breeding procedure designed to make maximum use of both general and specific combining ability. *Agron J* 41:360–367
- Eyherabide GH, Hallauer AR (1991) Reciprocal full-sib recurrent selection in maize. I. Direct and indirect responses. *Crop Sci* 31:952–959
- Hallauer AR (1973) Hybrid development and population improvement in maize by reciprocal full-sib selection. *Egypt J Genet Cytol* 2:84–101
- Hallauer AR, Eberhart SA (1970) Reciprocal full-sib selection. *Crop Sci* 10:315–316
- Hallauer AR, Miranda JB (1988) *Quantitative genetics in maize breeding*. Iowa State University Press, Ames
- Hinze LL, Kresovich S, Nason JD, Lamkey KR (2005) Population genetic diversity in a maize reciprocal recurrent selection program. *Crop Sci* 45:2435–2442
- Institute SAS (2009) SAS OnlineDoc, version 9.1. SAS Institute, Inc, Cary, North Carolina
- Jones LP, Compton WA, Gardner CO (1971) Comparison of full and half-sib reciprocal recurrent selection. *Theor Appl Genet* 41:36–39
- Klenke JR, Russell WA, Guthrie WD (1986) Recurrent selection for resistance to European corn borer in a corn synthetic and correlated effects on agronomics traits. *Crop Sci* 26:864–868
- Mikel MA (2011) Genetic composition of contemporary US commercial dent corn germplasm. *Crop Sci* 51:592–599
- Montagnon C, Leroy T, Cilas C, Legnate H, Charrier A (2008) Heterozygous genotypes are efficient testers for assessing between-population combining ability in the reciprocal recurrent selection of *Coffea canephora*. *Euphytica* 160:101–110
- Moreno-González J, Hallauer AR (1982) Combined S_2 and crossbred family selection in full-sib reciprocal recurrent selection. *Theor Appl Genet* 61:353–358
- Nyhus KA, Russell WA, Guthrie WD (1989) Changes in agronomic traits associated with recurrent selection in two corn synthetics. *Crop Sci* 29:269–275
- Peiris BL, Hallauer AR (2005) Comparison of half-sib and full-sib reciprocal recurrent selection and their modifications in simulated populations. *Maydica* 50:25–37
- Pinto LR, Vieira MLC, Souza CL Jr, Souza AP (2003) Genetic diversity by microsatellites in tropical maize populations submitted to a high-intensity reciprocal recurrent selection. *Euphytica* 134:277–286
- Pokou ND, N’Goran JAK, Lachenaud P, Eskes Ab, Montamayor JC, Schnell R, Kolesnikova-Allen M, Clement D, Sangare A (2009) Recurrent selection of cocoa populations in Cote d’Ivoire: comparative genetic diversity between the first and second cycles. *Plant Breed* 128:514–520
- Popi J, Kannenberg LW (2001) Response to selection over 15 years in the HOPE maize breeding system. *Maydica* 46:93–103
- Romay MC, Ordas B, Revilla P, Ordas A (2011) Three cycles of full-sib reciprocal recurrent selection in two Spanish maize populations. *Crop Sci* 51:1016–1022
- Russell WA, Lawrence GD, Guthrie WD (1979) Effects of recurrent selection for European corn borer resistance on other agronomic characters in synthetic cultivars of corn. *Maydica* 24:33–47
- Sandoya G, Butron A, Alvarez A, Ordas A, Malvar RA (2008) Direct response of a maize synthetic to recurrent selection for resistance to corn borers. *Crop Sci* 48:113–118
- Sandoya G, Butron A, Santiago R, Alvarez A, Malvar RA (2010) Indirect response to selection for improving resistance to the Mediterranean corn borer (*Sesamia nonagrioides* Lef) in maize. *Euphytica* 176:231–237
- Souza CL Jr (1999) Recurrent selection and heterosis. In: Coors JG, Pandey S (eds) *The genetics and exploitation of heterosis in crops*. ASA, CSSA, SSSA, Madison, pp 247–255
- Troyer AF (1999) Background of US hybrid corn. *Crop Sci* 39:601–626
- Weyhrich RA, Lamkey KR, Hallauer AR (1998) Responses to seven method of recurrent selection in the BS11 maize population. *Crop Sci* 38:308–321