

1 **Evaluation of three cycles of full-sib reciprocal recurrent selection in two maize populations**  
2 **from the Northeast of Spain**

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50 **ABSTRACT**

51 In Europe a heterotic pattern commonly used in maize breeding is “American Dent× European  
52 Flint”. Maize breeding programs generally use only a small portion of the useful genetic  
53 variability present in the local open-pollinated varieties which, otherwise, have a poor  
54 performance that hampers their use. Two maize composites, EZS33 formed by open-pollinated  
55 flint varieties from dry or Mediterranean Spain, and EZS34 from USA dent populations, were  
56 developed in Zaragoza (Spain). Both were subjected to three cycles of full-sib reciprocal  
57 recurrent selection (RRS) for yield. The objective of our study was to evaluate after three cycles  
58 of selection the effect of RRS. The selection significantly increased yield in the population  
59 crosses (3.0% per cycle) and in the crosses of the populations with testers of different origin. The  
60 correlated responses for the population crosses in other agronomic traits like flowering and  
61 early vigor were in the desired direction, but plant height was reduced. We propose that stover  
62 yield or related traits could be included in selection programs as selection criteria to prevent  
63 their decline. The adapted population increased the frequency of favorable alleles for yield, but  
64 the inbreeding depression counteracted their effect on the mean. RRS had a positive effect on  
65 other traits. According to our data, RRS can be useful to develop improved populations from  
66 which it would be possible to develop lines with improved both specific and general combining  
67 ability with different heterotic groups. We conclude that the heterotic pattern “Mediterranean  
68 Spain x US Dent” is potentially very useful for maize breeding for adaptation to Mediterranean  
69 conditions and an interesting source of cultivars for low-input agriculture.

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72 **Keywords:** Selection, maize, combining ability, breeding, reciprocal recurrent, *Zea mays* L.

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75 **Abbreviations:**

76 RRS                      Reciprocal recurrent selection

77 **Introduction**

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79 Maize (*Zea mays* L.) is the highest tonnage cereal crop worldwide with a production of about  
80 819 million Mg, followed by paddy rice with 682 million Mg, and wheat with 679 million Mg  
81 (FAO, 2012). Single-cross hybrids are the predominant type of variety grown in the developed  
82 world and other areas.

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84 Inbred lines were obtained from open pollinated varieties at the beginning of maize breeding,  
85 but after that initial period, inbreds were and are nowadays mainly obtained from populations  
86 generated by crossing elite inbred lines (Mikel, 2011). Two points are relevant regarding the  
87 genetic variability of the current elite inbred lines. First, only a small proportion of the genetic  
88 variation of the original open-pollinated varieties is used (Ho et al., 2005; Reif et al., 2005), and  
89 second, the recycling of elite inbreds is the cause that many of them have become genetically  
90 related (Mikel and Dudley, 2006; Mikel, 2011). A low genetic variability reduces the expected  
91 rate of genetic improvement (Yu and Bernardo, 2004) and increases the susceptibility to stresses  
92 (Reif et al., 2010).

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94 The term heterotic pattern refers to a specific pair of two heterotic groups, which express high  
95 heterosis in their cross (Reif et al., 2005). The concept of heterotic pattern is widely used in  
96 maize breeding because helps to elect parents of crosses for line development and to choose  
97 testers for evaluating new hybrids (Reif et al., 2005). In Europe a successful heterotic pattern is  
98 “American Dent × European Flint” (Ordás, 1991). American cultivars can be useful sources of  
99 favorable alleles for yield, while European open-pollinated cultivars contain genes for  
100 adaptation to specific environments (Malvar et al., 2004; Revilla et al., 2006). Within the pattern  
101 “American Dent × European Flint”, the crossing between Reid germplasm or derivatives from the  
102 US Corn Belt with germplasm from dry or Mediterranean Spain has resulted in a large amount  
103 of heterosis (Garay et al., 1996a). Other related heterotic combinations that have, alternatively  
104 been proposed for Europe are “Lancaster (another US Corn Belt dent variety) × Spain varieties”,  
105 “Reid × Argentinean Flint”, and “Reid × varieties from wet or Atlantic Spain” (Soengas et al.,  
106 2003). In addition, the varieties of dry Spain have been also found to produce large heterosis  
107 with the varieties of wet Spain (Ordás, 1991).

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109 In general, using a wide range of germplasm sources seems to be necessary for sustaining long-  
110 term breeding progress in maize and for avoiding an increase in the susceptibility to stresses  
111 (Reif et al., 2010). Exotic or adapted, but non improved, germplasm can be used to increase the  
112 genetic base of maize breeding germplasm (Romay et al., 2011). The adapted, non-improved  
113 germplasm, needs to be improved before it can be included in hybrid breeding programs  
114 because of its poor agronomic performance, particularly low yield and high lodging.

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116 With the aim of increasing the genetic base of the germplasm used in maize breeding, two  
117 composites of populations have been developed in the Aula Dei Experimental Station, Zaragoza  
118 (Spain). These composites follow the heterotic pattern “Reid × Dry Spain”. The dry Spain  
119 varieties have a special interest for increasing genetic variability because they are very variable  
120 and a source of favorable alleles for adaptation to Mediterranean climatic conditions (Garay et  
121 al., 1996b).

122

123 The composites were subjected to three cycles of  $S_1$  progeny intrapopulation recurrent selection  
124 for grain yield (Alvarez et al., 1993). Garay et al. (1996b) confirmed that both composites  
125 showed increased grain yield and good response to selection. After that, the composites (named  
126 EZS33 and EZS34) were subjected to three cycles of full-sib reciprocal recurrent selection (RRS)  
127 for yield. RRS is a long-term breeding procedure designed to improve population crosses while

128 maintaining genetic variability for continued selection (Hallauer and Eberhart, 1970). RRS also  
129 enhances the performance of hybrids obtained from lines developed from the succeeding cycles  
130 of selection (Betrán and Hallauer, 1996). Thus, both general and specific combining abilities are  
131 exploited by this breeding method. RRS has been shown very effective to improve the cross of  
132 two divergent US (Eyherabide and Hallauer, 1991; Keeratinijakal and Lamkey, 1993) or two  
133 divergent European (Romay et al., 2011) populations, but this procedure has not been evaluated  
134 for populations that follow the “American Dent × European Flint” heterotic pattern.  
135 Keeratinijakal and Lamkey (1993) showed that RRS improves the testcrosses of the reciprocal  
136 populations using two lines, one derived from the RRS program and another from a different  
137 heterotic group. Thus, RRS could be useful for improving germplasm sources, not only for the  
138 extraction of inbred lines with specific combining ability, but also for the extraction of lines with  
139 a wide general combining ability.

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141 The objective of our study was to evaluate in populations from the “American Dent × European  
142 Flint” heterotic pattern, the effect of RRS on: (a) the direct and correlated responses in the  
143 populations and the interpopulation crosses (b) the inbreeding of the populations and the  
144 heterosis between them, and (c) on the crosses of the populations with germplasm from  
145 different origin.

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## 148 **Materials and methods**

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### 150 **Plant Materials**

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152 Starting in 1983 two composites were developed in Zaragoza (Spain). EZS1 was formed by  
153 intercrossing flint populations from dry Spain and EZS2 by intercrossing North American dent  
154 populations with a predominance of Reid germplasm (Garay et al., 1996a). Preliminary results  
155 indicated that superior hybrids might be developed from the cross between these composites  
156 (Alvarez et al., 1993). As the initial yields of both composites were low, they were separately  
157 subjected to  $S_1$  progeny recurrent selection for three cycles. Cycles 0, 1, 2, and 3 of EZS1 and  
158 EZS2 have been previously evaluated for their response (Garay et al., 1996a), as well as to  
159 determine their ability to combine (Garay et al., 1996b). Later, EZS1C3 and EZS2C3 were  
160 renamed to EZS33C0 and EZS34C0, respectively, and both populations were submitted to three  
161 cycles of full-sib reciprocal recurrent selection for grain yield (figure 1). The full-sib reciprocal  
162 recurrent selection breeding program started in 1995. EZS33C0 and EZS34C0 were self-  
163 pollinated to obtain  $S_1$  progenies (about 150 plants from each population). In 1996, 100  $S_1$   
164 families from EZS33C0 were randomly crossed to 100  $S_1$  families from EZS34C0 and the  $S_1 \times S_1$   
165 crosses were evaluated in 1997. The 100  $S_1$  families from each composite were also self-  
166 pollinated to obtain  $S_2$  progenies. In each population the 10  $S_2$  families derived from the 10  $S_1$   
167 families with the highest yield in crosses were recombined in 1998 to generate the first cycle of  
168 selection [EZS33(FR)C1 and EZS34(FR)C1]. This methodology was also used to obtain the  
169 second cycle [EZS33(FR)C2 and EZS34(FR)C2] in 2003 and the third cycle [EZS33(FR)C3 and  
170 EZS34(FR)C3] in 2007.

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172 In 2008 seeds from all populations (EZS33C0, EZS34C0, EZS33(FR)C1, EZS34(FR)C1,  
173 EZS33(FR)C2, EZS34(FR)C2, EZS33(FR)C3, and EZS34(FR)C3) were multiplied and the 28  
174 possible crosses between the 8 populations were made. Additionally, the four cycles of each  
175 population (C0, C1, C2 and C3) were testcrossed to two inbred lines to know the effect of RRS  
176 on the crosses of the populations with other germplasm. EZS33 (the flint composite from dry  
177 Spain) and its cycles of selection were crossed to B93 (a Lancaster inbred) and to EP42 (a flint  
178 inbred derived from a wet Spain’s population). EZS34 (the dent Reid composite) and its cycles

179 of selection were crossed to EZ6 (an Argentinean flint inbred) and to EP42.

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181 Evaluation trials

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183 The populations (8), the crosses between them (28), the testcrosses (16), and four hybrid checks  
184 were tested for two years (2009 and 2010) at three locations in Aragón (Spain) which are  
185 representative of dry areas: Montañana (41° 44'N, 0° 47' O, 243 masl), Torres de Alcanadre (42°  
186 04'N, 0° 09' O, 389 masl), and Zuera (41° 45'N, 0° 47' O, 325 masl). The total number of  
187 environments was finally five because the evaluation in Zuera was carried out only in 2010. The  
188 experimental design for all trials was a 7 × 8 triple lattice. However, for conciseness, we report  
189 here only the data of populations *per se*, interpopulation crosses (C0×C0, C1×C1, C2×C2, C3×C3),  
190 the crosses to the initial cycle (C0×C1, C0×C2, C0×C3), the testcrosses, and the hybrid checks  
191 (Table 1). The trials were irrigated throughout the growing seasons. Management, fertilization,  
192 pest, and weed control were carried out according to local practices, with the trials being  
193 machine planted. Each experimental plot consisted of two rows spaced 0.75 m apart, with 29  
194 one-plant hills spaced 0.18 m apart, for a plant density of approximately 71,000 plants ha<sup>-1</sup>. All  
195 the plots were manually harvested.

196

197 The following data were recorded on each plot: grain yield (kg ha<sup>-1</sup> adjusted at 140 g H<sub>2</sub>O kg<sup>-1</sup>),  
198 early vigor (number of leaves when plants were 31 days old), flowering (days from planting to  
199 50% of the plants showing silks), plant height (in centimeters), total lodging (%), and kernel  
200 moisture. Dates were recorded on the whole plot, except for plant height and kernel moisture,  
201 which were recorded on 10 plants and 10 ears per plot, respectively.

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203 Combined analyses of variance were made using the adjusted means obtained from the lattice  
204 analysis of each environment if the effectiveness of the lattice design was above 105%;  
205 otherwise, the agronomic traits were analyzed as randomized complete blocks. Environments  
206 were considered random and populations were considered fixed. The entry-by-environment  
207 mean squares, when significant, were used in tests of significance for differences among entries.  
208 The sums of squares due to genotypes were partitioned orthogonally for populations,  
209 population crosses, testcrosses, and hybrid checks. The populations were divided into effects  
210 due to populations from EZS33 [EZS33C0, EZS33(FR)C1, EZS33(FR)C2, and EZS33(FR)C3] and  
211 to populations from EZS34 [EZS34C0, EZS34(FR)C1, EZS34(FR)C2, and EZS34(FR)C3]. Linear  
212 and quadratic regression models were fit for entry means across environment on selection  
213 cycles for populations derived from EZS33 and EZS34, and for crosses between both  
214 populations to assess the response to selection. The mean square for each partition was tested  
215 with the mean square of the corresponding partition of the interaction (genotype ×  
216 environment) or experimental error. Means were compared by Fisher's protected LSD method  
217 (Steel et al., 1997). The sum of squares of testcrosses was divided into crosses defined as  
218 EZS33xB93, EZS33xEP42, EZS34xEZ6, and EZS34xEP42.

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220 The evolution of heterosis in the breeding program was compared respect to difference between  
221 the mean of a cross and the average of the two parents (mid-parent heterosis) (Eyherabide and  
222 Hallauer, 1991). Genetic drift was measured for each population and cycle of selection, which let  
223 us know the genetic loss and the favorable alleles accumulated in the populations  
224 (Keeratinijakal and Lamkey, 1993). Data analyses were made using the SAS software package  
225 (SAS Institute, 2008).

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## 227 **Results and discussion**

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229 The combined analysis of variance across environments showed highly significant differences

230 between genotypes for all agronomic traits, although the interactions between environment and  
231 genotypes were also significant (online source 1).

232

233 Populations and crosses between populations

234

235 For yield, the linear rate of response was significant ( $0.25 \text{ Mg ha}^{-1} \text{ cycle}^{-1}$ ,  $3.0\% \text{ cycle}^{-1}$ ) in the  
236 interpopulation crosses (Table 2). The linear response did not interact with environments (online  
237 source 1) indicating that the response was consistent; moreover, the lack of quadratic response  
238 suggests future possibilities of improvement (data not shown). The linear response obtained in  
239 our program was similar to the response obtained in several full sib RRS programs that  
240 reported a gain between 3 and 5% per cycle (Menkir and Kling, 1999; Butruille et al., 2004;  
241 Moreno-Pérez et al., 2004; Ordás et al. 2012), although Eyherabide and Hallauer (1991) obtained  
242 a higher response ( $7.5\% \text{ cycle}^{-1}$ ).

243

244 On the other hand, the linear response for yield was not significant in the populations *per se*  
245 (Table 2). Contrary to the positive and consistent response across experiment found in the  
246 interpopulation crosses, some authors reported a lack of response in both populations *per se*  
247 (Butruille et al., 2004; Moreno-Pérez et al., 2004), while other authors reported a positive  
248 response in one population (Romay et al. 2011; Ordas et al. 2012) or both (Eyherabide and  
249 Hallauer (1991). Romay et al. (2011) and Ordas et al. (2012) carried out a RRS program with one  
250 population adapted and other non-adapted to the environment where the selection was  
251 performed. In both RRS programs the positive response was achieved in the adapted  
252 population. In the RRS program of BS10 and BS11, carried out in the Corn Belt, BS10 can be  
253 considered more adapted than BS11, because BS10 was made primarily from Reid Yellow Dent  
254 germplasm, while BS11 included southern, non-adapted materials to the Corn Belt environment  
255 (Eyherabide and Hallauer, 1991). In this program, although both populations had a positive  
256 response, the response in the adapted population almost doubled the response in the non-  
257 adapted population (Eyherabide and Hallauer, 1991). In our program, although the response  
258 was not significant in the populations *per se*, a positive tendency was observed in the adapted  
259 population ( $b=0.08$ ), with a negative tendency in the non-adapted population ( $b=-0.08$ ). From  
260 ours and previous results, it seems that, when selection is performed in one adapted and one  
261 non-adapted population, full-sib RRS effectively improves the inter-population cross and tends  
262 to improve the adapted population.

263

264 Regarding the changes correlated with selection in the interpopulation cross, there was a  
265 significant linear reduction in flowering and plant height, while the kernel moisture and the  
266 lodging remained unchanged. Therefore, the increase of yield was not at expense of an increase  
267 in the maturity of the crop (Table 2). In most of the cases, the reduction in flowering days can be  
268 an advantage to escape from high temperatures and/or drought in the period more critical to  
269 the plants. However, the decreasing in plant height could reduce the interest of the  
270 interpopulation crosses for alternatively used as forage or biomass production. The selection for  
271 grain has not affected the stover yield in the US Corn Belt varieties; however the opposite  
272 situation occurred in tropical germplasm (Lorenz et al., 2010). Our results suggest that stover  
273 yield, or related traits, should be included in selection programs as selection criteria to prevent  
274 their decline. The early vigor of each cycle of selection was higher than the early vigor of the  
275 previous cycle in the interpopulation cross, although the linear rate of increase did not reach the  
276 significant level. The selection for yield probably had a weak effect on early vigor, but more  
277 cycles of selection are needed to detect a significant effect.

278

279 Regarding the correlated responses in the populations *per se*, EZS34 reduced flowering  
280 significantly and kernel moisture, while EZS33 did not. The changes in flowering time approach

281 the values of EZS34 to the values of EZS33. This could be indicative of selection for adaptation  
282 in the non-adapted composite or, alternatively, the methodology of RRS, where crossing  
283 families of two composites could tend to approach the flowering dates of the populations.  
284 Similar results have been found in others experiments (Ruiz de Galarreta and Álvarez, 2007;  
285 Romay et al., 2011). The linear regression of lodging on cycles of selection was negative in both  
286 populations, EZS33 and EZS34. The linear coefficient was significant in EZS33, but not in EZS34,  
287 although a linear tendency was also observed. The lodging of EZS33 (21%) was higher than the  
288 lodging of EZS34 (11%) in the original populations. This result was expected because EZS34 was  
289 formed with four maize populations with a good resistance to borers, while EZS33 was formed  
290 with four adapted varieties, but never improved for lodging. One of the main agronomic  
291 disadvantages found in several local open-pollinated varieties is high lodging (Malvar et al.,  
292 1996; Carena and Wicks, 2006). The greatest reduction of lodging was reached in the first cycle,  
293 which is in agreement with Keeratinijakal and Lamkey (1993). On the other hand, Romay et al.  
294 (2011) observed this reduction in the third cycle. In general, we can resume that RRS has a good  
295 effect for decreasing lodging (Martin and Hallauer, 1980; Popi and Kannenberg, 2001).

296

297 Heterosis and inbreeding

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299 Mid-parent heterosis for yield in the interpopulation crosses increased  $0.26 \text{ Mg ha}^{-1} \text{ cycle}^{-1}$ , but  
300 the linear tendency was not significant (Table 3). Others studies showed a significant linear  
301 coefficient of about  $0.22 \text{ Mg ha}^{-1} \text{ cycle}^{-1}$  after 11 cycles of selection (Keeratinijakal and Lamkey,  
302 1993).

303

304 For the cycles C2 and C3 the yield of the populations *per se* was lower than the yield of the  
305 populations crossed to the original populations (Table 4). Furthermore, all the differences were  
306 significant, except for the cycle C3 of EZS33. This is most likely the result of inbreeding  
307 depression, which is present in the selected populations reducing their mean, but disappears  
308 when the improved populations are crossed with the original population (C0). This response is  
309 similar to the response reported by Rodriguez and Hallauer (1988), and Keeratinijakal and  
310 Lamkey (1993). Our data suggest that with 10 families selected per generation, a usual number  
311 in selection programs, fixation of deleterious alleles is probably happening as a consequence of  
312 random genetic drift. For that reason, to increase the frequency of the maximum number of  
313 favorable alleles in the long term, it would be worthwhile to increase the number of selected  
314 families at the expense of the response in the short term. This was made in the RRS of the BSSS  
315 and BSCB1 populations conducted by the Cooperative Federal-State maize breeding program in  
316 Iowa, in which the number of  $S_i$  selected progenies was increased from 10 to 20 in order to  
317 reduce the cumulative effects of genetic drift (Keeratinijakal and Lamkey, 1993). The testcrosses  
318 of the cycles of selection of EZS33 and EZS34 to their original parents (cycle C0) linearly  
319 improved and worsened, respectively, their grain yield with selection (Table 4). This confirms  
320 the previous hypothesis that RRS effectively increases the frequency of favorable alleles for  
321 yield in the adapted population, although the inbreeding depression could counteract the effect  
322 of selection on the mean.

323

324 The early vigor (one trait related to adaptation) of the testcrosses of EZS34 (the non-adapted  
325 population) to cycle C0 changed linearly with selection, indicating that selection was effective  
326 increasing the frequency of favorable alleles. Thus, our results suggest that RRS for yield could  
327 be effective increasing the favorable alleles for adaptation of non-adapted germplasm.

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329 Crosses of cycles with testers

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331 For yield we found a significant ( $P \leq 0.05$ ) linear response in the testcrosses of EZS33 and EZS34

332 to all testers except B93, in which the linear response was significant at  $P=0.10$  (Table 2). These  
333 results suggest that RRS is able to improve not only the crosses between the reciprocal  
334 populations, targets of the selection program, but also the crosses with germplasm from  
335 different heterotic groups. Thus, RRS can be useful to develop improved populations from  
336 which in turn it would be possible to develop lines with general and specific combining ability  
337 with other alternative heterotic groups. Therefore, RRS seems to be an appropriate  
338 methodology to make new germplasm available for elite breeding. The flowering time of most  
339 of the testcrosses was linearly reduced by RRS, which could be an advantage in order to escape  
340 from elevated temperatures in the more susceptible stage of the plant development.

341

342 On the other hand, the interpopulation crosses can be directly cultivated, mainly in agriculture  
343 of low inputs. We compared the yield of the improved population crosses with hybrid checks to  
344 assess the possibility of cultivation of interpopulation crosses (Table 2). We found that the yield  
345 of the population crosses in the third cycle of improvement did not differ significantly from the  
346 mean yield of the hybrid checks. These results confirm the hypothesis that some  
347 interpopulation crosses could produce similar yields than some hybrids in determinate  
348 conditions (Carena, 2005). We conclude that RRS could be an appropriate tool to develop  
349 improved interpopulation crosses for direct use by farmers.

350

### 351 **Conclusions**

352

353 Some useful genetic variability present in local varieties or synthetic populations is hampered  
354 by the poor agronomic performance of the populations, which prevents their use in elite  
355 breeding. Our results suggest that the RRS methodology is useful for improving non-elite  
356 varieties because efficiently improves grain yield, lodging, and adaptation of the populations.  
357 The use of heterotic patterns is widely spread in maize breeding and the RRS methodology  
358 allows the improvement of the specific combining ability of populations within a concrete  
359 heterotic pattern scheme. RRS also improves the general combining ability of populations,  
360 which gives more flexibility to the exploitation of the improved populations.

361

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Table 1: Populations of maize used in this study, all crosses between them, the testcrosses of the cycles of selection to two inbred lines, and the hybrid checks, grown at five environments in 2009 and 2010 in Spain.

|                                     |                              |
|-------------------------------------|------------------------------|
| <b><u>per se populations</u></b>    | EZS33C1 × EP42               |
| EZS33C0                             | EZS33C2 × B93                |
| EZS33C1                             | EZS33C2 × EP42               |
| EZS33C2                             | EZS33C3 × B93                |
| EZS33C3                             | EZS33C3 × EP42               |
| EZS34C0                             | EZS34C0 × EZ6                |
| EZS34C1                             | EZS34C0 × EP42               |
| EZS34C2                             | EZS34C1 × EZ6                |
| EZS34C3                             | EZS34C1 × EP42               |
| <b><u>Intervarietal crosses</u></b> | EZS34C2 × EZ6                |
| EZS34C0 × EZS33C0                   | EZS34C2 × EP42               |
| EZS34C1 × EZS33C1                   | EZS34C3 × EZ6                |
| EZS34C2 × EZS33C2                   | EZS34C3 × EP42               |
| EZS34C3 × EZS33C3                   | <b><u>Hybrids checks</u></b> |
| <b><u>Testcrosses</u></b>           | B73 × Mo17                   |
| EZS33C0 × B93                       | COSTANZA                     |
| EZS33C0 × EP42                      | DRACMA                       |
| EZS33C1 × B93                       | HELEN                        |

Table 2: Means for yield and some agronomic traits for the cycles (C0-C3) of the EZS33 and EZS34 population of maize, all crosses between them, the testcrosses of the cycles of selection to two inbred lines, and the hybrid checks, grown at five environments in 2009 and 2010 in Spain. The regression coefficients of each character on cycles of selection are also shown

| <i>Material</i>                               | <i>Grain yield</i>        | <i>Total lodging</i> | <i>Early vigor</i>    | <i>Flowering</i> | <i>Kernel moisture</i> | <i>Plant height</i> |
|---|---------------------------|----------------------|-----------------------|------------------|------------------------|---------------------|
|   | <i>Mg ha<sup>-1</sup></i> | <i>%</i>             | <i>leaves</i>         | <i>Days</i>      | <i>%</i>               | <i>cm</i>           |
| <b>Populations</b>                            |                           |                      |                       |                  |                        |                     |
| EZS33C0                                       | 6.17                      | 20.8                 | 7.5                   | 72.6             | 20.0                   | 176                 |
| EZS33C1                                       | 6.25                      | 19.0                 | 7.3                   | 72.8             | 21.1                   | 174                 |
| EZS33C2                                       | 6.05                      | 17.5                 | 7.4                   | 72.3             | 19.9                   | 166                 |
| EZS33C3                                       | 6.51                      | 16.2                 | 7.3                   | 71.9             | 18.6                   | 166                 |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>0.08</i>               | <i>-1.52**</i>       | <i>-0.03</i>          | <i>-0.26</i>     | <i>-0.48</i>           | <i>-3.7**</i>       |
| <b>EZS34C0</b>                                |                           |                      |                       |                  |                        |                     |
| EZS34C0                                       | 6.01                      | 10.8                 | 7.3                   | 77.8             | 20.3                   | 198                 |
| EZS34C1                                       | 7.06                      | 8.7                  | 7.3                   | 76.4             | 19.6                   | 182                 |
| EZS34C2                                       | 6.72                      | 7.6                  | 7.5                   | 75.3             | 18.7                   | 169                 |
| EZS34C3                                       | 5.85                      | 7.7                  | 7.3                   | 74.8             | 18.9                   | 167                 |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>-0.08</i>              | <i>-1.04</i>         | <i>0.03</i>           | <i>-1.01**</i>   | <i>-0.50**</i>         | <i>-10.6**</i>      |
| <i>LSD (5%)</i>                               | <i>0.45</i>               | <i>10.5</i>          | <i>ns<sup>b</sup></i> | <i>1.8</i>       | <i>1.64</i>            | <i>62</i>           |
| <b>Crosses</b>                                |                           |                      |                       |                  |                        |                     |
| EZS34C0×EZS33C0                               | 8.25                      | 13.4                 | 7.4                   | 74.7             | 19.9                   | 208                 |
| EZS34C1×EZS33C1                               | 8.73                      | 18.8                 | 7.6                   | 73.0             | 19.7                   | 200                 |
| EZS34C2×EZS33C2                               | 8.60                      | 12.6                 | 7.7                   | 73.3             | 19.8                   | 187                 |
| EZS34C3×EZS33C3                               | 9.11                      | 12.3                 | 7.8                   | 72.5             | 20.2                   | 180                 |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>0.25**</i>             | <i>-0.94</i>         | <i>0.13</i>           | <i>-0.59**</i>   | <i>0.08</i>            | <i>-9.5**</i>       |
| <i>LSD (5%)</i>                               | <i>0.79</i>               | <i>10.5</i>          | <i>0.07</i>           | <i>1.43</i>      | <i>1.09</i>            | <i>40</i>           |
| <b>TestCrosses</b>                            |                           |                      |                       |                  |                        |                     |
| EZS33C0×B93                                   | 8.83                      | 12.9                 | 7.8                   | 72.1             | 19.4                   | 183                 |
| EZS33C1×B93                                   | 9.95                      | 16.5                 | 7.6                   | 71.5             | 21.4                   | 185                 |
| EZS33C2×B93                                   | 9.29                      | 14.7                 | 7.7                   | 72.7             | 21.6                   | 175                 |
| EZS33C3×B93                                   | 9.79                      | 14.9                 | 7.7                   | 71.5             | 20.1                   | 178                 |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>0.22</i>               | <i>0.42</i>          | <i>0.00</i>           | <i>-0.07</i>     | <i>0.22</i>            | <i>-2.55</i>        |
| <b>EZS33C0×EP42</b>                           |                           |                      |                       |                  |                        |                     |
| EZS33C0×EP42                                  | 7.33                      | 17.0                 | 7.4                   | 68.8             | 16.3                   | 179                 |
| EZS33C1×EP42                                  | 7.33                      | 18.6                 | 7.6                   | 67.1             | 16.4                   | 168                 |
| EZS33C2×EP42                                  | 8.53                      | 13.5                 | 7.8                   | 67.3             | 15.8                   | 166                 |
| EZS33C3×EP42                                  | 8.07                      | 10.4                 | 7.9                   | 67.6             | 16.8                   | 174                 |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>0.34**</i>             | <i>-2.48**</i>       | <i>0.16**</i>         | <i>-0.33*</i>    | <i>0.09</i>            | <i>-1.57</i>        |
| <b>EZS34C0×EZ6</b>                            |                           |                      |                       |                  |                        |                     |
| EZS34C0×EZ6                                   | 8.57                      | 10.2                 | 8.0                   | 74.3             | 18.2                   | 200                 |
| EZS34C1×EZ6                                   | 9.52                      | 11.0                 | 8.1                   | 73.2             | 17.9                   | 198                 |
| EZS34C2×EZ6                                   | 9.60                      | 9.5                  | 8.0                   | 73.5             | 18.3                   | 192                 |
| EZS34C3×EZ6                                   | 9.63                      | 11.1                 | 8.2                   | 72.2             | 17.1                   | 196                 |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>0.32**</i>             | <i>0.11</i>          | <i>0.05</i>           | <i>-0.61**</i>   | <i>-0.28</i>           | <i>-1.74</i>        |
| <b>EZS34C0×EP42</b>                           |                           |                      |                       |                  |                        |                     |
| EZS34C0×EP42                                  | 8.45                      | 10.4                 | 7.6                   | 71.7             | 16.1                   | 187                 |
| EZS34C1×EP42                                  | 8.38                      | 10.8                 | 7.8                   | 68.7             | 15.9                   | 185                 |
| EZS34C2×EP42                                  | 8.25                      | 9.5                  | 7.6                   | 67.9             | 15.3                   | 178                 |
| EZS34C3×EP42                                  | 9.25                      | 11.5                 | 7.9                   | 69.4             | 15.6                   | 180                 |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>0.28*</i>              | <i>0.21</i>          | <i>0.07</i>           | <i>-0.76**</i>   | <i>-0.21</i>           | <i>-2.9</i>         |
| <i>LSD (5%)</i>                               | <i>1.22</i>               | <i>10.5</i>          | <i>0.07</i>           | <i>1.43</i>      | <i>1.24</i>            | <i>42</i>           |
| <b>Hybrids checks</b>                         |                           |                      |                       |                  |                        |                     |
| B73×Mo18                                      | 7.48                      | 12.9                 | 6.9                   | 79.3             | 23.2                   | 198                 |

|          |       |      |                 |      |      |     |
|----------|-------|------|-----------------|------|------|-----|
| COSTANZA | 10.83 | 4.0  | 6.7             | 78.4 | 19.0 | 197 |
| DRACMA   | 9.41  | 4.3  | 6.9             | 78.3 | 20.2 | 189 |
| HELEN    | 10.80 | 3.5  | 6.4             | 77.3 | 19.7 | 213 |
| LSD (5%) | 1.96  | 10.5 | ns <sup>b</sup> | 0.90 | 1.94 | 27  |

<sup>a</sup>the significance of the regression coefficients were tested with an analysis of variance (online source 1)

<sup>b</sup>ns: the differences between the means were not significant according to the analysis of variance (online source 1)

Table 3: Mid-parent heterosis for yield and some agronomic traits exhibited by the crosses of the EZS33 and EZS34 populations of maize evaluated in five environments in 2009 and 2010 in Spain. The regression coefficients of mid-parent heterosis on cycles of selection are also shown

| <i>Material</i>                               | <i>Grain yield</i>                      | <i>Total lodging</i> | <i>Early vigor</i> | <i>Flowering</i> | <i>Kernel moisture</i> | <i>Plant height</i> |
|---|---|----------------------|--------------------|------------------|------------------------|---------------------|
|   | <i>Mg ha<sup>-1</sup></i>               | <i>%</i>             | <i>leaves</i>      | <i>Days</i>      | <i>%</i>               | <i>cm</i>           |
| <i>Cycle of selection</i>                     | <u>Mid-parent heterosis<sup>b</sup></u> |                      |                    |                  |                        |                     |
| EZS34C0 x EZS33C0                             | 2.15**                                  | -2.4                 | -0.0               | -0.6             | -0.2                   | 16.4**              |
| EZS34C1 x EZS33C1                             | 2.07**                                  | 4.9**                | 0.3*               | -1.7**           | -0.7                   | 17.9**              |
| EZS34C2 x EZS33C2                             | 2.22**                                  | 0.0                  | 0.2*               | -0.5             | 0.4                    | 16.1**              |
| EZS34C3 x EZS33C3                             | 2.93*                                   | 0.3                  | 0.4                | -0.8             | 1.4**                  | 10.7**              |
| <i>Regression coefficient (b)<sup>a</sup></i> | 0.25                                    | 0.33                 | 0.13*              | 0.04             | 0.58**                 | -1.89               |

\* \*\* significant at the 0.05 and 0.01 probability level respectively

<sup>a</sup>the significance of the regression coefficients were tested with an analysis of variance (online source 2)

<sup>b</sup> Evaluation of the EZS34xEZS33 cross against the best parent

Table 4: Means of yield and some agronomic traits for the improved cycles (C1-C3) of the EZS33 and EZS34 population of maize crossed to the original cycles (C0) and differences between the cycles *per se* and crossed to C0. The regression coefficients of each character on the cycles of selection are also shown for the improved cycles crossed to C0. All populations were evaluated in five environments in 2009 and 2010 in Spain.

| <i>Material</i>                               | <i>Grain yield</i>        | <i>Total lodging</i> | <i>Early vigor</i> | <i>Flowering</i> | <i>Kernel moisture</i> | <i>Plant height</i> |
|---|---------------------------|----------------------|--------------------|------------------|------------------------|---------------------|
| <i>Cycle of selection</i>                     | <i>Mg ha<sup>-1</sup></i> | <i>%</i>             | <i>leaves</i>      | <i>Days</i>      | <i>%</i>               | <i>cm</i>           |
| <b>EZS33C1 x EZS33C0</b>                      | 6.10                      | 23.2                 | 7.6                | 71.7             | 20.6                   | 182                 |
| EZS33C1 <i>vs</i> (EZS33C1xEZS33C0)           | 0.1                       | -4.2                 | -0.3               | 1.2              | 0.5                    | -6*                 |
| <b>EZS33C2 x EZS33C0</b>                      | 7.14                      | 27.7                 | 7.5                | 72.5             | 19.6                   | 176                 |
| EZS33C2 <i>vs</i> (EZS33C2xEZS33C0)           | -1.1**                    | -10.2**              | -0.1               | -0.1             | 0.3                    | -8**                |
| <b>EZS33C3 x EZS33C0</b>                      | 6.72                      | 16.8                 | 7.7                | 73.2             | 20.9                   | 176                 |
| EZS33C3 <i>vs</i> (EZS33C3xEZS33C0)           | -0.2                      | -0.5                 | -0.3*              | -1.3*            | -2.2**                 | -7**                |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>0.31**</i>             | <i>-3.20</i>         | <i>0.08</i>        | <i>0.76**</i>    | <i>0.14</i>            | <i>-3.00</i>        |
| <b>EZS34C1 x EZS34C0</b>                      | 7.35                      | 14.7                 | 7.5                | 75.4             | 20.4                   | 191                 |
| EZS34C1 <i>vs</i> (EZS34C1xEZS34C0)           | -0.3                      | -6.0**               | -0.1               | 1.0              | -0.7                   | -7**                |
| <b>EZS34C2 x EZS34C0</b>                      | 8.00                      | 9.9                  | 7.6                | 74.7             | 18.4                   | 193                 |
| EZS34C2 <i>vs</i> (EZS34C2xEZS34C0)           | -1.3**                    | -2.3                 | -0.0               | 0.6              | 0.4                    | -20**               |
| <b>EZS34C3 x EZS34C0</b>                      | 6.86                      | 8.7                  | 7.6                | 75.4             | 19.6                   | 185                 |
| EZS34C3 <i>vs</i> (EZS34C3xEZS34C0)           | -1.0**                    | -1.0                 | -0.2               | -0.6             | -0.6                   | -14*                |
| <i>Regression coefficient (b)<sup>a</sup></i> | <i>-0.24**</i>            | <i>-3.00</i>         | <i>0.06**</i>      | <i>-0.00</i>     | <i>-0.37</i>           | <i>-0.24</i>        |

\*\*significant at the 0.05 and 0.01 probability level respectively

<sup>a</sup>the significance of the regression coefficients were tested with an analysis of variance (online source 3)