

1 **Causes of agronomic differences between synthetics developed by the random and**
2 **convergent cross methods**

3

4 A Butrón, P Revilla, MC Romay, A Ordás, RA Malvar

5

6 Misión Biológica de Galicia (CSIC), Apdo. 28, 36080 Pontevedra, Spain

7 E-mail: abutron@mbg.cesga.es Telephone: 34 986 854800

8 Fax number: 34 986 841362

9

1 **Abstract.** The theoretical utility of two alternative methods, random (r) and convergent (c)
2 cross methods, of producing maize synthetics has previously been ascertained in two
3 different genetic backgrounds (EPS20 derived from eight Reid inbred lines with origin
4 from the U.S.Corn Belt population “Reid”, and EPS21 formed from eight non-Reid
5 inbreds). However, the agronomical consequences of using one or another methodology
6 have not been tested. The objectives of the present study were to determine, in two
7 genetic backgrounds, whether synthetics developed by the random and convergent cross
8 methods differed in agronomic performance and to investigate whether some allelic
9 changes previously observed by Butron et al (2003) could be directly implicated in
10 those differences. The synthetics and the diallel crosses among them, testcrosses of
11 EPS20c and EPS20r to their Reid parental inbreds and testcrosses of EPS21c and
12 EPS21r to their non-Reid parental inbreds were evaluated for grain yield in three trials
13 in 2004 and 2005. Our results suggest that directional selection for germination, which
14 occurs during the process of formation of synthetics using the random method (but
15 absent with the convergent cross method) was responsible for agronomic and genetic
16 differences between synthetics obtained by alternative methods from the same set of
17 inbreds. Although selection for germination increased the yield performance of the
18 synthetic obtained from the Reid inbreds, in a more heterogeneous genetic background,
19 natural selection against non-competitive inbred lines at germination would be responsible
20 for an important reduction of variability that would reduce yield.

1 The term synthetic variety has been used extensively to refer to those populations that
2 result after randomly mating a balanced bulk of several inbred lines or populations.
3 According to this broad definition, synthetic varieties are produced by mating several
4 parents so that all possible crosses between the parents have equal probability of being
5 represented in the synthetic. Mating randomly all possible (diallel) crosses among n
6 lines has been proposed as the most effective procedure for developing synthetic
7 varieties (Allard 1960). When the number of lines is large, however, this protocol
8 becomes too burdensome, and a labor and time saving method is desirable.

9 Maintaining the original genetic diversity in a synthetic can have practical
10 implications because, according to Busbice (1970), yield of a synthetic (Y_{Syn}) depends on
11 yield of homozygotes (Y_{Hom}) in the ancestry of the population plus the product of the yield
12 of the heterozygote (Y_{Het}) multiplied by the difference between one and the inbreeding
13 coefficient (F):

$$14 \qquad Y_{Syn} = Y_{Hom} + Y_{Het} (1 - F)$$

15 Thus, loss of diversity due to factors such as genetic drift can result in reduced yield
16 of the synthetic. Variability leaks should also be avoided in the process of producing
17 synthetics when the final goal of the population is the development of inbred lines, in order
18 to prevent losses of potentially useful alleles.

19 Márquez-Sánchez (1992; 1993) found that the inbreeding coefficients of synthetic
20 varieties obtained by different mating methods were the same regardless of whether the
21 first generation is obtained by intercrossing lines or by randomly mating the plants from a
22 seed bulk. He established that any random mating method could be useful as long as each
23 component line had the same chance of contributing to the synthetic variety. However,
24 factors leading to non-balanced contribution of inbreds to the synthetic can result in loss of
25 genetic variability.

1 The two main factors that can change allele frequencies of a synthetic developed
2 from a random sample of plants from a parental seed bulk are as follow: i) natural selection
3 triggered by heritable differences among lines in germination success, vigor, seed
4 production, etc. For example, Revilla et al. (2000) showed that reduced germination of *su1*
5 kernels could account for most of the decrease in *su1* frequency in crosses between *su1* and
6 *Su1* maize populations across generations of recombination. Under these circumstances,
7 the expected number of parents involved in any sample drawn from the bulk will be less
8 than the number of parents included in the bulk (Crossa 1989), (ii) genetic drift associated
9 with reduced effective population size that can cause random fluctuations in allele
10 frequencies (Crossa 1989). However, when synthetics are the base material for breeding
11 programs, those genetic changes could be of little importance if no impact is observed on
12 the agronomical performance of synthetics. In summary, when considering alternative
13 mating methods for the development of synthetic varieties, not only should the methods
14 be simpler and faster, but they should also prevent the unequal contribution of parents and
15 random genetic drift (if conservation of genetic variability is the goal) and maximize
16 agronomic performance (if selection is intended).

17 In a previous study, the theoretical utility of two alternative methods of producing
18 maize synthetics was ascertained in two different genetic backgrounds (eight Reid inbred
19 lines originated from the U.S.Corn Belt population “Reid”, and eight non-Reid inbreds),
20 assuming an equal contribution of each parental inbred line (Butrón et al. 2003). Results
21 showed that the convergent cross method could modify gene frequencies of some SSR
22 markers if few individuals were sampled among segregating-individuals of each double
23 cross hybrid, but could not cause allele losses or significant reduction of heterozygosity
24 (Butrón et al. 2003). On the other hand, the random method caused drastic deviations of
25 allelic frequencies from expected ratios in a synthetic developed from non-related materials,

1 decreasing significantly the heterozygosity, and modifying genetic distances between the
2 synthetic and their parental inbreds. Based on those results, the convergent cross method
3 was found to be a valid method in both backgrounds. It was concluded that the random
4 method should be used with caution when the inbreds intermated are genetically diverse.
5 However, to determine the real utility and the risk associated with the use of the random
6 method, the agronomical performance of a synthetic developed by the random method
7 should be compared to that of the synthetic developed by the cross convergent method.
8 The objectives of the present study were to determine, in two genetic backgrounds,
9 whether synthetics developed by the random and convergent cross methods differed in
10 agronomic performance and to investigate, through data mining, whether some allelic
11 changes previously observed by Butron et al (2003) could be directly implicated in
12 those differences.
13

1 **Materials and methods**

2

3 The synthetic varieties EPS20 and EPS21 were developed using two mating methods,
4 referred to as convergent cross (EPS20c and EPS21c) and random (EPS20r and EPS21r)
5 methods. Eight Reid inbred lines originated from the US Corn Belt population “Reid” and
6 eight inbreds that were unrelated to “Reid” population were the base materials for synthetic
7 varieties EPS20 and EPS21, respectively (Table 1). The synthetics EPS20c and EPS20r
8 were formed from inbreds lines derived from B14 or WF9, both of which originated,
9 directly or indirectly, from the population “Reid” (Messmer et al. 1991; Gerdes et al. 1993).
10 The synthetics developed by the convergent cross method (c) were obtained from specific
11 crosses involving n parental inbreds. Specifically, the 8 parents were crossed in pairs, then
12 the 4 hybrids were crossed to make 2 double-crosses, and so on until a final cross involving
13 all n parents was completed. The random method (r) involved random intermating of a
14 sample of plants obtained by bulking equal number of seeds from each parental line.

15 In the convergent cross method, the single crosses, CM109 \times CM151, A652 \times
16 A664, W64A \times A634, and A639 \times CM139, were made in 1995 as the first step in forming
17 the balanced synthetic variety EPS20c. Crosses A509 \times CO125, PB60 \times PB130, F473 \times
18 EP53, and EP17 \times EP43 were also made the same year to form the balanced synthetic
19 variety EPS21c. The double crosses, (CM109 \times CM151) \times (A652 \times A664) and (A639 \times
20 CM139) \times (A634 \times W64A) were produced for EPS20c, and (A509 \times CO125) \times (PB60 \times
21 PB130), and (F473 \times EP53) \times (EP17 \times EP43) for EPS21c in 1996. Finally, in 1997, crosses
22 between the two double cross-hybrids within each synthetic variety were made to obtain
23 the synthetic varieties EPS20c and EPS21c. For each synthetic, equal number of seeds
24 from each ear was bulked and three hundred seeds out of the bulk were sown in ten rows

1 each with 15 hills and two seeds per hill. The seedlings were later thinned to one plant per
2 hill leaving 150 plants for plant-to plant crosses. Each plant was used only once as male or
3 female resulting in at least 50 ears. Equal numbers of seeds were bulked from each ear and
4 another generation of recombination was carried out.

5 To initiate the random method, three hundred and four seeds from the eight inbred
6 lines that constituted the base material for each synthetic variety (EPS20r and EPS21r)
7 were bulked and sown in 1998. Each inbred line contributed 38 seeds to the bulk. The 304
8 seeds were sown in ten rows, each with 15 hills and two seeds per hill. Following thinning,
9 150 plants were available to form each random synthetic variety (EPS20r and EPS21r).
10 Plant-to plant crosses were made using each plant only once as male or female. This
11 resulted in 38 and 39 ears that constituted the synthetic varieties, EPS20r and EPS21r,
12 respectively, after two generations of recombination. Recombinations were made as
13 described earlier for synthetics EPS20c and EPS21c.

14 In 2002, the promising diallel crosses of the four maize synthetic populations
15 (EPS20c, EPS20r, EPS21c, and EPS21r) were made, and the synthetics were multiplied to
16 obtain homogeneous seed. More than 50 ears were obtained for each cross and synthetic.
17 Furthermore, each synthetic was crossed to each of its parental inbreds in 2002 and 2003
18 using the synthetics as males. Bulk pollen from a minimum of 50 male plants of each
19 synthetic was used to pollinate more than 30 females of each inbred. The crosses A639 ×
20 EPS20r and EP43 × EPS21c failed in both years and were therefore not included in field
21 evaluations.

22 The diallel crosses and parental populations, testcrosses of EPS20c and EPS20r to
23 Reid parental inbreds and testcrosses of EPS21c and EPS21r to non-Reid parental inbreds
24 were evaluated in three adjacent trials in 2004 and 2005, at Pontevedra. For each trial, a
25 randomized complete block design with 3 replications was used. Each genotype was

1 planted in a two-row plot with 17 hills per row. The rows were spaced 0.80 m with 0.21 m
2 between hills. Two seeds were planted per hill and later thinned to one resulting in a final
3 population density of about 60 000 plants ha⁻¹.

4 Grain yield, the most important agronomic trait, has important dominance genetic
5 effects. Therefore, it is expected that crosses between genetically distinct varieties would
6 produce larger yields than genetically related varieties. Grain yield was computed as the
7 shelled grain weight at 140 g kg⁻¹ moisture per plot converted to Mg ha⁻¹.

8 Combined analyses of variance across years were performed on diallel data
9 including parental populations using the PROC GLM procedure of SAS (SAS Institute
10 2002). Genotypes were considered fixed, and years and replications random. Midparent
11 heterosis was estimated as the mean of crosses minus the mean of parental populations.
12 The standard error of heterosis was calculated as the square root of 1.5 times the variance
13 of the entry mean, according to the method of Keeratinijakal and Lamkey (1993). All
14 analyses were made using SAS, version 9.1 (SAS Institute 2002). Combined analyses of
15 variance across years were also performed, independently for each background (Reid and
16 non-Reid) synthetic, on testcrosses data of synthetics to their parental inbreds. Testcrosses
17 were assumed to be fixed effects. Mean comparisons among the genotypes of the diallel
18 design and among testcrosses of synthetics to their parental inbreds were made using the
19 Fisher's protected LSD.

20 In a previous study (Butrón et al. 2003), 40 individuals from each synthetic
21 (EPS20c, EPS20r, EPS21c, and EPS21r) and parental inbreds were genotyped with several
22 polymorphic SSRs (*phi083*, *nc132*, *phi090*, *phi036*, *bnlg197*, *phi046*, *phi021*, *phi076*, *phi113*,
23 *phi101*, *phi128*, *phi075*, *phi112*, *phi114*, *phi116*, *phi115*, *bnlg240*, *phi028*, *phi065*, *phi027*, and
24 *phi050*) randomly distributed across the maize genome, except on chromosome 1. Marker
25 locations and primer sequences could be down loaded from the Maize Genetics and

1 Genomics Database (<http://www.maizegdb.org>). In the present study, we have
2 investigated, through data mining in the ‘Maize Genetics and Genomics Database’
3 (MaizeGDB), accessible through <http://www.maizegdb.org>, whether each of the 12 loci
4 detected by Butrón et al. (2003) as showing allelic frequencies significantly different from
5 expected (under the assumption of equal contribution of each inbred) are located on
6 known genes that could be directly implicated in agronomic differences between
7 synthetics. Besides, genetic distances among synthetics varieties developed from the same
8 materials by different methods were computed according to Nei (1972) using the program
9 NTSYS-PC (Rohlf, 1997) and empirical estimates for the effective population size were
10 obtained as well as their 95 % confidence intervals using temporal method of Waples
11 (1989). Expected allele frequencies under the assumption of equal contribution of
12 parental lines to the synthetic variety were assumed as the frequencies of an ideal initial
13 sample of 10^{10} individuals (generation 1), while the variety synthetic was the generation
14 3. The standardized variance in allele frequency change (F_c) was calculated following
15 the method of Nei and Tajima (1981) for all loci and for loci that exhibited allelic
16 frequencies not significantly different from the expected ones (neutral loci). To avoid
17 the possible bias in the estimation of the effective population size caused by alleles at
18 initially high frequencies, loci with an expected frequency of the most common allele
19 larger than 0.90 were removed (Labate et al. 1999).

20

21 **Results**

22

23 Nei’s genetic distance between EPS20c and EPS20r was 4.2, while the distance between
24 EPS21c and EPS21r was almost double, 7.9. EPS20c and EPS21c did not differ in grain
25 yield, but EPS20r yielded significantly more than EPS21r (Table 2). In the diallel analysis,

1 the genotypes that showed the highest grain yield were EPS20r × EPS21c and EPS20c ×
2 EPS21c. On the other hand, EPS20c × EPS21r and EPS20r × EPS21r were similar to the
3 synthetics *per se* in grain yield, except EPS21r, and the crosses between synthetics
4 developed by alternative methods from the same set of inbred lines (EPS20c × EPS20r and
5 EPS21c × EPS21r). Crosses EPS20c × EPS21c and EPS20r × EPS21c showed significant
6 heterosis for grain yield.

7 The analysis of variance of testcrosses of EPS20c or EPS21c to their parental
8 inbreds did not show any significant differences for grain yield. However, significant
9 differences were detected in grain yield of crosses of EPS20r or EPS21r with their parental
10 inbreds (Table 3). The crosses CM151 × EPS20r, A634 × EPS20r, A652 × EPS20r, and
11 W64A × EPS20r were among the highest yielding genotypes while CM139 × EPS20r was
12 the least productive. EPS21r testcrossed to EP17, EP53, CO125, and A509 yielded
13 significantly less than the best testcross, F473 × EPS21r (Table 3).

14 The estimated effective numbers computed from all loci were similar to the
15 expected ones for the variety synthetics developed by the convergent cross method, but the
16 estimated effective numbers were significantly lower than expected for synthetics obtained
17 by the random method (Table 4). The estimated effective numbers computed with neutral
18 loci approximated the expected effective numbers in all cases; while when computed with
19 non neutral loci only they approximated the expected ratios in EPS21c.

20 The SSR markers for which Butrón et al. (2003) detected allelic frequencies
21 significantly different from expected are shown in Table 5, along with information on the
22 inbred lines that reduced or increased their contributions to the synthetic, and the locus
23 where the SSR marker is located. Quantitative information on the significance, size and
24 direction of allele frequency changes have previously been reported by Butrón et al. (2003).
25 Some markers for which allelic frequencies significantly changed from expected under the

1 assumption of equal contribution of each inbred could be non neutral because they are
2 located in locus involved in the responses of seeds or seedlings to *Fusarium* infection
3 (*pathogenesis-related protein homolog2*), anoxia (*alcohol deshidrogenase2*), oxidative (*catalase3*), heat
4 and cold (*oxygen-evolving complex17*), and drought (*oleosin2*) stresses
5 (<http://www.maizegdb.org>). The SSR markers located in the *pathogenesis-related protein*
6 *homolog2* showed modified allelic frequencies compared to the expected frequencies under
7 the assumption of equal contribution of each inbred line in EPS20r and EPS21r (Table 5).
8 The frequencies of alleles at the marker *phi114*, located in the locus *oxygen-evolving complex17*,
9 changed from the expected ones in the four synthetics.
10

1 **Discussion**

2

3 The lack of heterosis in crosses of EPS21r with the two Reid synthetics (EPS20c and
4 EPS20r) could be attributed to the significant loss of diversity that was reported by Butrón
5 et al. (2003) when the random method was used to develop the synthetic EPS21r. The
6 superior agronomic performance of synthetics EPS20r and EPS21c along with their
7 significant specific heterosis for yield might have caused EPS20r × EPS21c to yield more
8 than the other crosses, except EPS20c × EPS21c. Therefore, from an agronomical point of
9 view, the random method which is simpler and faster was beneficial when forming the
10 synthetic with Reid-related inbreds, and was totally inappropriate when the base materials
11 were unrelated inbreds. This finding is in partial agreement with predictions made by
12 Butrón et al. (2003), based on the loss of genetic variability resulting from the use of the
13 random method. Butron et al. (2003) hypothesized the superior performance of EPS21c
14 compared to EPS21r, but no variability difference between EPS20c and EPS20r was found
15 in that previous study.

16 Results of yield evaluations involving crosses between synthetics EPS20c and
17 EPS20r and their parents were generally similar to the genetic relative distances between
18 synthetics and their parental inbreds based on molecular data (Butrón et al. 2003). The
19 close correspondence between genetic distances and yield performance supports the
20 findings of previous studies that showed that among genotypes with similar pedigree
21 background, there were high correlation coefficients between genetic diversity values
22 estimated from field data and the genetic distances based on molecular markers (Williams
23 and Hallauer 2000, Reif et al. 2003, García et al. 2004).

24 The different contributions of inbreds to each synthetic (EPS21c and EPS21r),
25 detected at the molecular level (Butrón et al. 2003), was not always reflected on the yield

1 performance of crosses between synthetics and inbreds. The differences in the results of
2 the two studies could be due to the fact that most markers were unrelated to genomic
3 regions relevant to grain yield in this genetic background (Boppenmaier et al. 1992).

4 Nei's genetic distance between EPS20c and EPS20r was low and the yield of the
5 cross EPS20c \times EPS20r was about the same as the mid parental value while the cross
6 between more genetically distinct synthetics, EPS21c \times EPS21r, yielded more than the
7 mean of their parents. This remarkable correspondence between genetic distances and
8 midparent heterosis suggests that some markers could be indirectly related in yield
9 performance. Therefore, the differences in allelic frequencies observed by Butrón et al.
10 (2003) between synthetics obtained from the same materials by different methods could
11 have been responsible for the differences in yield observed in the present study. This
12 finding supported the idea of investigating, through data mining in the 'Maize Genetics and
13 Genomics Database' (MaizeGDB), whether loci detected by Butrón et al. (2003) as
14 showing allelic frequencies significantly different from expected (under the assumption of
15 equal contribution of each inbred) are located on known genes that could be directly
16 implicated in agronomic differences between synthetics.

17 The causes of the departure from a model with equal contribution from each
18 inbred to the synthetic could be two: (1) random allelic changes due to genetic drift and (2)
19 directional selection. When using the random method, the contribution of directional
20 selection to changes in allele frequencies from expected ones under equal contribution of
21 each inbred could be important because the effective population sizes obtained with all loci
22 were underestimated. On the contrary, selection did not have a big contribution to allelic
23 changes when using the convergent cross method because the estimates of the effective
24 population sizes were closed to the expected ones assuming that random drift was acting
25 alone. However, the estimated population size computed with non neutral loci in EPS20c

1 differed from the expected one suggesting that selection could have some minor impact on
2 frequency changes when using the convergent cross method in the Reid background, while
3 no effect of selection was detected when using the same method in a more genetically
4 diverse background.

5 The marker *phi114* exhibited allelic frequencies significantly different from expected
6 in all synthetics with the allelic changes in the same direction when both methods were
7 employed suggesting that natural selection for the locus *oxygen-evolving complex17*, where the
8 maker is located, could have been acting when random and convergent methods were used.
9 The locus *oxygen-evolving complex17* is involved in the response to cold stress. An important
10 role in adaptation to abiotic stresses affecting water status, drought and cold, has also been
11 suggested for the enzyme encoded by the locus *phosphoenolpyruvate carboxylase* (González et
12 al. 2003) and the marker *phi065*, located on this locus, exhibited different allelic frequencies
13 from expected in EPS20c. Natural selection for these loci was expected because the
14 synthetics were developed at the Atlantic European area where cold is the most important
15 stress (Malvar et al. 2005), but natural selection for cold tolerance did not seem to have
16 contributed to agronomic differences between synthetics obtained from the same set of
17 inbreds.

18 When using the convergent cross method, recombination of alleles from different
19 inbreds occurs before random drift and/or selection could act. However, when using the
20 random method, these factors could affect all allele frequencies of an inbred because they
21 begin to act before any recombination of alleles occurs. Therefore, alleles whose
22 frequencies had been significantly increased by the random method could correspond to
23 markers linked to traits under selection, but could also be from inbreds that were favored
24 by selection in the initial year (before alleles from different inbreds were recombined).
25 Several SSRs that exhibited allelic frequencies different from expected in EPS20r and/or

1 EPS21r are not neutral because they are located in genes involved in responses to stresses.
2 However, *phi083*, a marker located in the *pathogenesis-related protein homolog2*, was the only
3 SSRs for which allelic frequencies were different from expected in both genetic
4 backgrounds. All lines that increased their contributions to EPS20r and EPS21r, based on
5 agronomic and previous molecular evaluations, supplied a fragment similar in size for the
6 marker *phi083*; while CM151, A634, F473, and EP17, that showed reduced contribution to
7 EPS20r or EPS21r, supplied SSR fragments for *phi083* with significant decrease in
8 frequencies in those synthetics. Previously, *phi083* or a QTL linked to it was found to be
9 involved in germination of aged seeds of the inbred P39 (Revilla personal communication).
10 The inbred seed used to generate the synthetics was partially aged because it was not
11 multiplied the year before inbred recombination. Therefore, we hypothesize that inbreds
12 carrying unfavorable variation for the marker *phi083* could decrease their contribution to
13 synthetics when using the random method because of reduced germination rate. Reedy et
14 al. (1995) reported that differential survival in storage may result in changes in the genetic
15 makeup of an accession by selection. Therefore, the differences in germination could favor
16 the contribution of the B14-related inbreds to the synthetic EPS20r compared to WF9-
17 related inbreds and, indirectly, could contribute to the increase of yield because B14 and
18 their relatives are among the most promising elite inbreds (Lu and Bernardo 2001).
19 However, natural selection in EPS21r against non-competitive inbred lines at germination
20 would be responsible for the important reduction of variability that would affect yield
21 performance because performance at germination is not always correlated to performance
22 at later stages (Soldati et al. 1999).

23 In conclusion, results suggest that directional selection for germination was
24 responsible for agronomic and genetic differences between synthetics obtained by
25 alternative methods from the same set of inbreds. Selection for germination increased

1 the yield performance of the synthetic obtained from the Reid inbreds, but, in a more
2 heterogeneous genetic background, natural selection against non-competitive inbred lines
3 at germination would be responsible for an important reduction of variability that would
4 reduce yield.

5

6 **Acknowledgment**

7

8 Research supported by the Ministry of Science and Education of Spain (AGL2004-06776),
9 the Autonomous Government of Galicia (Xunta-PGDIT05PXIC40301PN), and the
10 Excma. Deputación Provincial of Pontevedra, Spain. MC Romay acknowledges a
11 fellowship from the Ministry of Science and Education of Spain.

12

13 **References**

14

15 Allard, R.W., 1960. Principles of plant breeding. John Wiley and Sons. Inc., New York.

16 Boppenmaier, J., Melchinger, A.E., Brunklaus-Jung, E., Geiger, H.H., Herrmann, R.G.,

17 1992 Genetic diversity for RFLPs in European maize inbreds: I. Relation to
18 performance of flint \times dent crosses for forage traits. *Crop Sci* 32, 895-902.

19 Busbice, T.H., 1970. Predicting yield of synthetic varieties. *Crop Sci* 10, 265-269.

20 Butrón, A., Tarrío, R., Revilla, P., Malvar, R.A., Ordás, A., 2003. Molecular evaluation of
21 two methods for developing maize synthetic varieties. *Mol Breed* 12, 329-333.

22 Crossa, J., 1989. Methodologies for estimating the sample size required for genetic
23 conservation of outbreeding crops. *Theor Appl Genet* 77, 153-161.

- 1 García, A.A.F., Benchimol, L.L., Barbosa, A.M.M., Geraldi, I.O., Souza, C.L., de Souza,
2 A.P., 2004. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity
3 studies in tropical maize inbred lines. *Genetics and Molecular Biology* 27, 579-588.
- 4 Gerdes, J.T., Behr, C.F., Coors, J.G., Tracy, W.F., 1993. Compilation of North American
5 Maize Breeding Germplasm. Tracy WW, Coors JG and Geadelmann (eds.), Crop
6 Science Society of America Inc., Madison, USA.
- 7 González, M.C., Sánchez, R., Cejudo, F.J., 2003. Abiotic stresses affecting water balance
8 induce phosphoenolpyruvate carboxylase expression in roots of wheat seedlings.
9 *Planta* 216, 985-992.
- 10 Keerantinijakal, V., Lamkey, K., 1993. Responses to reciprocal selection in BSSS and BSB1
11 maize populations. *Crop Sci* 33, 73-77.
- 12 Labate, J.A., Lamkey, K.R., Lee, M., Woodman, W., 1999. Temporal changes in allele
13 frequencies in two reciprocally selected maize populations. *Theor Appl Genet* 99,
14 1166-1178.
- 15 Lu, H., Bernardo, R., 2001. Molecular marker diversity among current and historical maize
16 inbreds. *Theor Appl Genet* 103, 613-617.
- 17 Malvar, R.A., Revilla, P., Butrón, A., Gouesnard, B., Boyat, A., Soengas, P., Alvarez, A.,
18 Ordás, A., 2005. Performance of crosses among French and Spanish maize
19 populations across environments. *Crop Sci* 45, 1052-1057.
- 20 Márquez-Sánchez, F., 1992. Inbreeding and yield prediction in synthetic variety maize
21 cultivars made with parental lines: I. Basic methods. *Crop Sci* 32, 345-349.
- 22 Márquez-Sánchez, F., 1993. Inbreeding and yield prediction in synthetic variety cultivars of
23 maize: II. Random methods. *Crop Sci* 33, 1153-1157.
- 24 Messmer, M.M., Melchinger, A.E., Lee, M., Woodman, W.L., Lee, E.A., Lamkey, K.R.,
25 1991. Genetic diversity among progenitors and elite lines from the Iowa stiff stalk

1 synthetic (BSSS) maize population: comparison of allozyme and RFLP data. Theor
2 Appl Genet 83, 97-107.

3 Nei, M., 1972. Genetic distances between populations. American Naturalist 106, 283-292.

4 Nei, M., Tajima, F., 1981. Genetic drift and estimation of effective population size.
5 Genetics 98, 625-640.

6 Reedy, M.E., Knapp, A.D., Lamkey, K.R., 1995. Isozyme allelic frequency changes
7 following maize (*Zea mays* L.) germplasm regeneration. Maydica 40, 269-273.

8 Reif, J.C., Melchinger, A.E., Xia, X.C., Warburton, M.L., Hoisington, D.A., Vasal, S.K.,
9 Beck, D., Bohn, M., Frisch, M., 2003. Use of SSRs for establishing heterotic groups
10 in subtropical maize. Theor Appl Genet 107, 947-957.

11 Revilla, P., Malvar, R.A., Abuín, M.C., Ordás, B., Soengas, P., Ordás, A., 2000. Genetic
12 background effect on the germination of *su1* maize and viability of the *su1* allele.
13 Maydica 45, 109-111.

14 Rohlf, F.J., 1997. NTSYS-PC Numerical taxonomy system. Version 2.02e. EXETER
15 software, Setauket, New York, USA

16 SAS Institute, Inc., 2002. SAS OnlineDoc, version 9. SAS Institute, Inc., Cary, NC, USA.

17 Soldati, A., Stehli, A., Stamp, P., 1999. Temperature adaptation of tropical highland maize
18 (*Zea mays* L.) during early growth and in controlled conditions. Eur J Agron 10,
19 111-1117.

20 Waples, R.S., 1989. A generalized approach for estimating effective population size from
21 temporal changes in allele frequency. Genetics 121, 379-391.

22 Williams, T.R., Hallauer, A.R., 2000. Genetic diversity among maize hybrids. Maydica 45,
23 163-171.

24

1 Table 1. Maize inbred lines that were the base material for synthetic varieties EPS20 and
 2 EPS21, and their pedigrees

3	<hr/>				
4	Synthetic variety	Inbred lines	Pedigree ^a	Group of germplasm ^b	
5	<hr/>				
6	EPS20	CM109	(V3 × B14) B14	Reid-B14	
7		CM139	(V3 × B14) B14	Reid-B14	
8		CM151	(Mt42 × WF9) WF9	Reid-WF9	
9		A634	(Mt42 × B14) B14 ³	Reid-B14	
10		A639	A158 × B14	Reid-B14	
11		A652	A90 × WF9	Reid-WF9	
12		A664	(ND203 × A636) A636 ²	Reid-B14	
13		W64A	WF9 × C.I. 187-2	Reid-WF9	
14					
15		EPS21	EP17	A1267	Spanish flint
16			EP43	Parderrubias ^c	Spanish flint
17			EP53	Laro ^c	Spanish flint
18	PB60		Nostrano dell'Isola ^c	Italian flint	
19	PB130		Rojo Vinoso de Aragón ^c	Spanish flint	
20	F473		Doré de Gomer ^c	French flint	
21	CO125		Wisc. Exp. single cross	Corn Belt (USA)	
22	A509		A78 × A109	Corn Belt (USA)	
23	<hr/>				

24 ^a Pedigrees for the US inbreds are reported following Gerdes et al. (2003).

1 ^bB14 and WF9 are two inbred lines originated from the population “Reid” and were the
2 origin of two groups of germplasm within the Reid material.

3 ^c Local European maize varieties.

4

1 Table 2. Mean grain yield (Mg ha⁻¹) of parental populations (on the diagonal) and diallel
 2 crosses (above the diagonal) among maize synthetics developed by two methods and
 3 midparent heterosis (below the diagonal).

	EPS20c	EPS20r	EPS21c	EPS21r
7 EPS20c ^a	5.4	6.0	7.1	6.2
8 EPS20r	-0.1	6.5	7.9	6.0
9 EPS21c	1.6*	1.9*	5.6	5.9
10 EPS21r	1.2	0.5	0.8	4.6

12 *Significantly different from zero at 0.05 probability level

13 ^a c was assigned to synthetics obtained by the convergent cross method and r to those
 14 obtained by the random method.

15 ^b The LSD was 1.3 for grain yield and 2.0 for midparent heterosis for yield.

16
 17
 18
 19

Table 3. Mean comparisons among testcrosses of the maize synthetics EPS20c, EPS20r, EPS21c, and EPS21r to their parental inbred lines for yield (Mg ha^{-1}) evaluated for two years in Pontevedra (Spain).

Inbreds	EPS20c ^a	EPS20r	Inbreds	EPS21c	EPS21r
CM109	4.7	4.5	EP17	5.1	4.4
CM139	4.6	3.4	EP43	-	5.3
CM151	5.3	5.4	EP53	4.8	4.0
A634	4.8	5.8	PB60	5.2	5.2
A639	5.3	-	PB130	4.3	4.9
A652	5.4	6.3	F473	5.7	5.8
A664	5.8	4.6	CO125	4.8	4.0
W64A	5.1	6.0	A509	4.4	4.3
LSD	-	1.3	LSD	-	1.2

^a c indicates synthetics obtained by the convergent cross method and r to those obtained by the random method.

Table 4. Estimates of the effective population size (N_e) and 95% confidence intervals¹ by the method of Waples (1989). The standardized variance in allele frequency change (F) was calculated following the method of Nei and Tajima (1981).

N_e	Loci	EPS20c	EPS20r	EPS21c	EPS21r
Estimated	All	77.18 (31.59, 205.32)	25.35 (12.29, 46.96)	69.25 (33.23, 148.66)	15.37 (8.66, 24.87)
	Neutral ²	188.75 (54.08, ∞)	57.73 (22.69, 145.92)	162.09 (52.90, 1512.95)	59.75 (15.64, 235.43)
	Non neutral ³	15.02 (0.97, 63.68)	7.82 (1.23, 22.34)	25.48 (7.23, 67.12)	10.76 (5.42, 18.54)
Expected ⁴		68.65	95.18	65.05	99.09

¹ 95% confidence intervals are within brackets.

² Loci that exhibited allelic frequencies non significantly different from the expected ratios.

³ Loci that exhibited allelic frequencies significantly different from the expected ratios.

⁴ The expected N_e was the harmonic mean of the individuals crossed in each segregating generation: 40, 104, and 110 for EPS20c; 76, 106, and 112 for EPS20r; 36, 109, and 109 for EPS21c; and 78, 122, and 108 for EPS21r.

Table 5. SSR markers for which allelic frequency were significantly different from expected ratios if an equal contribution of each parental inbred line is assumed, indicating the maize inbred lines that reduced and increased their contributions to the synthetic (original data in Butrón et al. 2003) and the gene where the SSR marker is located.

Synthetic	SSR marker	Inbred lines (reduced)	Inbred lines (increased)	Gene
EPS20r	<i>phi083</i>	CM151, A634	CM109, CM139, A639, A664	<i>pathogenesis-related protein homolog2</i>
EPS20c and EPS20r	<i>phi114</i>	CM109, CM139 CM151, A652, W64A	A634, A639, A664	<i>oxygen-evolving complex17</i>
EPS20r	<i>bnlg240</i>	CM151, A652	W64A	unknown
EPS20c	<i>phi065</i>	CM109, CM139, W64A	A634, A639, A652 A664	<i>phosphoenolpyruvate carboxylase1</i>
EPS20r	<i>phi050</i>	CM151	CM109, CM139, A634, A639, A652, A664, W64A	unknown
EPS21r	<i>phi083</i>	EP17, F473	CO125, A509	<i>pathogenesis-related protein homolog2</i>

EPS21c	<i>pbi036</i>	PB60, CO125, EP53	EP43	unknown
EPS21r		EP17, F473, EP53 PB130, A509	EP43, PB60, CO125	
EPS21c	<i>bng197</i>	EP17, PB60, A509, PB130	EP53, CO125, EP43, F473	unknown
EPS21r		EP17, PB60, EP43, F473	EP53, CO125, A509, PB130	
EPS21r	<i>pbi021</i>	PB60	CO125	<i>alcohol dehidrogenase2</i>
EPS21r	<i>pbi076</i>	EP17, EP43, F473, A509	EP53, PB60, PB130, CO125	<i>catalase3</i>
EPS21r	<i>pbi113</i>	EP17, EP43, EP53, PB60 PB130, F473, CO125	A509	<i>oleosin2</i>
EPS21r	<i>pbi075</i>	EP17, F473	EP43, PB130, CO125	<i>ferrodixin1</i>
EPS21c	<i>pbi114</i>	EP17, EP43, PB60, A509	PB130, F473	<i>oxygen-evolving complex17</i>
EPS21r		EP17, EP43, PB60, A509	EP53, PB130, F473, CO125	
EPS21r	<i>pbi101</i>	EP17, PB60	CO125	unknown
