Molecular marker evaluation of two methods for developing maize synthetic varieties

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Abstract . A generalized way to obtain a maize (*Zea mays* L.) synthetic variety is to randomly mate all possible crosses among inbred lines, but, when the number of lines is large, it would save time and effort to use other mating methods. This work is aimed to determine the utility of two alternative methods, assuming that an equal contribution of each inbred line to the synthetic is the goal (Márquez-Sánchez, 1992; 1993). Eight Reid inbred lines originated from the U.S.Corn Belt population “Reid”, and eight non-Reid unrelated inbreds were the base material for synthetic varieties EPS20 and EPS21, respectively (Table 1). Each variety was built using both alternative methods: i) controlling the balanced contribution of each parental inbred (convergent cross method), and ii) randomly mating a seed mixture from the eight inbreds (random method). In the convergent cross method, single crosses between inbreds, and double crosses were performed in 1995, and 1996, respectively, as the first step to create EPS20c and EPS21c. Finally, in 1997, about 40 plants from each double-cross hybrid were mated only once (as male or female) in plant-to-plant crosses between double cross-hybrids, to generate about 20 ears that provided the base material for synthetics EPS20c and EPS21c. In the random method, a total of 304 seeds from the eight inbred lines, 38 seeds from each one, were bulked and sown in ten rows of 15 two-kernel hills per row in 1998 to form EPS20r and EPS21r. After thinning, 150 plants were left, and random plant-to plant crosses were made (where each plant was used only once as male or female), that resulted in 38 and 39, respectively. The two crossing schemes were followed by two generations of recombination. A sample of 40 individuals from each synthetic variety was genotyped with 17 SSR (simple sequence repeat) markers loci (see supporting data on the attached disk, Tables 3 and 4). Primer sequences and chromosomal locations are available at the MaizeDB (www.agron.missouri.edu/ssr.html). Genetic drift due to the relatively small samples used to constitute balanced synthetic varieties, and/or natural selection could modify the original frequencies, but did not cause allele or heterozygosity losses. Therefore, the convergent cross method appeared to be appropriate for

developing synthetic varieties either from related or non related inbreds. The random method should be used with caution, especially when inbred parents are genetically different, since relative adaptive advantages of some inbred lines could cause loss of genetic variability.

Results and Discussion

Allelic frequencies were estimated for each inbred line and synthetic variety. For each SSR locus and synthetic variety, observed allelic frequencies were compared to expected frequencies if parental lines contributed equally to the synthetic variety by means of a chi-square test. To check whether the two synthetic varieties obtained from the same set of lines could be considered independent samples from the same population, a chi-square test of homogeneity was computed. Standard Bonferroni corrections for multiple comparisons were applied, considering 34 independent tests for testing the goodness of fit and 17 for testing homogeneity. In order to quantify the genetic similarities among each synthetic variety and the parental inbreds, genetic distances were computed according to Nei (1972). Coefficients of correlation were calculated between genetic distances computed for the two synthetic varieties developed by different mating methods from the same set of inbred lines. The genetic structure of each synthetic variety was assessed by computing unbiased average heterozygosities (Nei 1978) over loci.

In the convergent cross method, tests of goodness of fit showed significant chi-square values for only two and three markers out of seventeen in EPS20c and EPS21c, respectively (observed and expected allelic frequencies for the SSR loci, allelic inbred lines composition, and chi-square values are available [as Supporting Online Material, Table 3](#)). Crossa (1989) established that 40 individuals are sufficient to preserve alleles with frequencies higher than 0.1, but random frequency fluctuations are expected. Since about 40 plants were used in plant-to-plant crosses between both double cross-

hybrids, random genetic drift could introduce unexpected changes in allelic frequency. These changes did not significantly deviated observed heterozygosities in EPS20c and EPS21c (0.4670 and 0.5541) with respect to the expectation (0.5132 ± 0.0233 and 0.5715 ± 0.0226).

The convergent cross method seems to be a valid mating method since allele losses did not occur, as was expected considering the sample size used to develop the synthetic varieties, and significant deviations from the expected allelic frequencies were rare, especially when parental lines were related.

The random method produced different results to those obtained by the convergent cross method. In EPS20r, the observed allelic frequencies were significantly different from the expected values at four loci (supporting Online data, Table 4). These frequency changes did not significantly modified the observed heterozygosity (0.47) with respect to the expected (0.51 ± 0.03). However, in EPS21r, significant differences were detected for more than a half of the markers, and the expected heterozygosity (0.57 ± 0.03) was significantly higher than the observed one (0.51). In addition, one and four alleles were lost from the Reid and non-Reid populations, respectively.

To make random synthetic varieties, equal numbers of seeds from each parental inbred were bulked and sown, but crosses were conducted among a sub-group of about 75 plants that originated from those seeds. Natural selection could have reduced the contribution of those inbred lines less adapted to the crosses that were the base materials for synthetic varieties EPS20r and EPS21r. Thus, differences in rate of germination or early vigor (helping seedlings to resist unfavorable weather conditions) among parental lines can result in unequal contribution of inbreds to the synthetic varieties. Furthermore, the different seed production ability of inbred lines can cause a higher contribution by the most productive inbreds to the synthetics. Gutierrez and Sprague (1959) stated that, when it is desirable to perpetuate the average gene frequency, as in the production of a synthetic variety, bulking crosses should be preferred to bulking equal quantities of the original materials.

When crosses are bulked, differences of contribution to the synthetic variety would be due to variation in probability of survival to reproductive stage. However, when inbreds are bulked, fecundity could also greatly influence each inbred's contribution to the synthetic variety.

Differences among inbred lines are expected to be less important when they are related. Therefore, bulking of the same amount of seeds from each parental inbred would not have the same effect on synthetic varieties developed from related and non-related materials. Our results are in agreement with those expectations since allelic changes obtained by using the random mating system among related inbreds (EPS20r) were not as dramatic as those observed in a synthetic variety obtained from non-related materials (EPS21r). Similarly, the small allelic changes produced by the random method in EPS20 did not significantly modify heterozygosity or the distances between EPS20r and their parental lines compared to the distances between EPS20c and their parental inbreds (Table 2). On the contrary, there were drastic deviations of allelic frequencies in EPS21r from the expected frequencies, heterozygosity in EPS21 significantly decreased by using the random method, and genetic distances between EPS21 and their parental inbreds were very different when considering EPS21c or EPS21r (Table 2).

Comparison between mating methods

Allelic frequencies obtained by using the convergent cross and the random method systems with the same set of lines were not homogeneous for some markers (data not shown), illustrating that different procedures resulted in different genetic composition of synthetics. Genetic distances between each synthetic variety and its parental lines were computed (Table 2), showing that relative distances between EPS20c and their parental inbreds were, in general, similar to the ones between EPS20r and their parental inbreds. A different pattern was observed in EPS21 where, for example,

one of the closest inbred lines to EPS21c, the line EP17, was the most distant to EPS21r. The coefficient of correlation between genetic distances computed for the synthetic varieties EPS20c and EPS20r was high and significant (0.84, $p < 0.01$), while the one computed for synthetic varieties EPS21c and EPS21r was not significant (0.13, $p = 0.76$).

The final goal of a synthetic like EPS20 should be the development of new inbred lines because the high genetic relationship among their progenitor inbreds reduces the usefulness of the synthetic as a cultivar. The only possible negative effect of using the random method in this case would be the loss of useful genes that could be linked in repulsion to adaptive factors (Rubino and Davis, 1991). In contrast, EPS21 displays higher genetic variability and could be used directly by growers. Therefore, decreased productivity associated with increased inbreeding should be prevented.

References

- Crossa, J (1989) Methodologies for estimating the sample size required for genetic conservation of outbreeding crops. *Theor. Appl. Genet.* 77: 153-161
- Gutierrez MG, Sprague GF (1959) Randomness of mating in isolated polycross plantings of maize. *Genetics* 44: 1075-1082
- Márquez-Sánchez, F (1992) Inbreeding and yield prediction in synthetic variety maize cultivars made with parental lines: I. Basic methods. *Crop Sci.* 32: 345-349
- Márquez-Sánchez, F (1993) Inbreeding and yield prediction in synthetic variety cultivars of maize: II. Random methods. *Crop Sci.* 33: 1153-1157
- Nei, M (1972) Genetic distance between populations. *American Naturalist* 106: 283-292

Nei, M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590

Rubino, DB, Davis DW (1991) Maintenance of tropical isozyme alleles during random mating in a semiexotic maize composite. *J. Hered.* 82: 423-425

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

Synthetic	Inbred line	Pedigree	Synthetic	Inbred line	Pedigree
EPS20	CM109	(V3 × B14) B14	EPS21	EP17	A1267
	CM139	(V3 × B14) B14		EP43	Parderrubias
	CM151	(Mt42 × WF9) WF9		EP53	Laro
	A634	(Mt42 × B14) B14 ³		PB60	Nostrano dell'Isola
	A639	A158 × B14		PB130	Rojo Vinoso de Aragón
	A652	A90 × WF9		F473	Doré de Gomer
	A664	(ND203 × A636) A636 ²		CO125	Wisc. Exp. single cross
	W64A	WF9 × C.I. 187-2		A509	A78 × A109

Table 2. Nei's genetic distances between each synthetic variety and their parental inbred lines.

Inbreds	EPS20c	EPS20r	Inbreds	EPS21c	EPS21r
CM109	0.2565	0.1934	EP17	0.3936	0.6706
CM139	0.2565	0.1934	EP43	0.3827	0.4877
CM151	0.4548	0.6148	EP53	0.4249	0.2926
A634	0.2674	0.3442	PB60	0.4085	0.5220
A639	0.4059	0.3709	PB130	0.4566	0.4422
A652	0.4784	0.7264	F473	0.4414	0.6570
A664	0.3421	0.3004	CO125	0.4306	0.2805
W64A	0.4768	0.4300	A509	0.5907	0.5811