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ORIGINAL PAPER

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Doubled haploid versus S_1 family recurrent selection for testcross performance in a maize population

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Abstract Theoretically, in a recurrent selection program, the use of doubled haploids (DH) can increase genetic advance per unit of time. To evaluate the efficiency expected from the use of DH for the improvement of grain yield in a maize (*Zea mays* L.) population, two recurrent selection programs for testcross performance were initiated using testcross progenies from DH lines and S_1 families. In 4 years one selection cycle using DH and two selection cycles using S_1 families were carried out with the same selection intensity for both methods. As expected, testcross genetic variance was twice as high among DH lines as among S_1 families. The predicted genetic gain was 8.2% for the DH selection cycle, and 10.6% for the two S_1 selection cycles, giving a per year advantage of 29% for the S_1 family method over the DH method with a cycle of 4 years. With a 3-year cycle for the DH method, both methods were expected to be equivalent. Using a tester related to the one used for selection, the genetic gains obtained were equivalent for both methods: 6.6% for the DH cycle and 7.0% for the two S_1 cycles. With a 3-year cycle for the DH method, the advantage would have been in favor of DH method. Furthermore, the DH method has the advantage of simultaneously producing lines that are directly usable as parents of a hybrid. Thus, if the genetic advance per unit of time is evaluated at the level of developed varieties even with the same or with a lower genetic advance in population improvement, the DH method appears to be the most efficient.

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Introduction

Until the 1990s, the low efficiency of the different techniques for the derivation of doubled haploids (DH) in maize prevented the use of this tool at the scale of a selection program. Production of large numbers of DH lines by induced in-situ gynogenesis (Coe 1959) is now successful in maize (Lashermes and Beckert 1988; Chalyk 1994; Deimling et al. 1997; Bordes et al. 1997). The creation of elite populations with the recessive glossy character (Hayes and Brewbaker 1928; Bianchi and Marchesi 1960) allows unambiguous differentiation of haploid plants from diploid plants and facilitates the use of DH in recurrent selection programs (Bordes et al. 1997).

Theoretically, the use of DH can increase the efficiency of recurrent selection methods aimed at improving per se value and testcross performance of lines that can be derived from a given population (Griffing 1975; Gallais 1989, 1990a, b; Bouchez and Gallais 2000). This is due to the increase in genetic variance among tested units which leads to an increase in heritability (Gallais 1990b). However, the main parameter affecting selection using DH as compared to selection using S_1 or S_2 families is cycle length (Gallais 1993). If we consider the progress per unit of time, Strahwald and Geiger (1988) showed that the use of off-season nurseries reduces the usefulness of recurrent selection using DH. In their theoretical study, Bouchez and Gallais (2000) compared the efficiency of selection using DH, S_0, S_1 and S_2 testcross progenies and concluded that for an annual plant like maize, without the use of off-season nurseries, recurrent selection using DH is the most effective, in particular at low heritability. This advantage disappears when off-season nurseries are used. However, from the point of view of applied breeding, when choosing a breeding method with the aim of developing new hybrids, the fact that recurrent selection using DH produces inbred lines that can be directly used as parents of new hybrids should be considered. Indeed, with the

other schemes using S_0, S_1 or S_2 progenies, the time needed for the derivation of new lines has to be taken into account.

To our knowledge, no experimental studies have compared the DH method with other classical recurrent selection methods. In order to evaluate whether the conclusions of Bouchez and Gallais (2000) are valid for a maize population improvement using our material and our experimental means, two recurrent selection programs for testcross performance were initiated using testcross progenies from DH lines on the one hand and S_1 families on the other. During the selection process, with one 4-year cycle for the DH method and two 2-year cycles for S_1 family selection, we estimated the different parameters of the response to selection and the expected genetic gain was derived from these parameters. Realized genetic gain in testcross performance was evaluated with a tester related to the one used for selection. It was then possible to explore the impact of the cycle length on the relative efficiency of the DH selection method.

Materials and methods

Doubled haploid process

The production of DH lines involves four main stages: (a) haploid induction, (b) haploid identification, (c) artificial chromosome doubling and, (d) plant growth after colchicine treatment (Bordes et al. 1997). The haploid lines were obtained by gynogenesis using a FIGH1 haploid inductor derived from WS 14 (Lashermes and Beckert 1988). The haploid induction rate of FIGH1 was about 1%. Haploid plants were identified by the glossy character when the first ligulate leaf appeared. Chromosome doubling to restore diploidy was accomplished by colchicine treatment at the three ligulate leaf stage. Chromosome doubling was performed only on some cells and on chimera haploid plants with some diploid sectors (Kato 2002). After colchicine treatment the plants were transferred to a greenhouse and were selfed. As plants were stressed by transplanting, the number of kernels produced per plant varied between 0 and 50. Finally, we derived approximately one DH line per 500 kernels resulting from FIGH1 crossing.

Development of genetic material

A synthetic population was developed by crossing 48 lines with a *glossy* gene from the three main North American genetic groups ('Iodent', 'Minnesota 13', 'Iowa Stiff Stalk Synthetic'). The C0 population was derived by two intercrossing generations by developing 24 independent crosses (1×2, 3×4, 5×6...47×48) for the first generation and by chain crossing between these crosses [(1×2) × (3×4), (3×4) × (5×6) ... (47×48) × (1×2)] for the second generation. Plants from the C0 population were self-fertilized to produce 150 S_1 families, and haploids were derived from these families. After chromosome doubling, 261 DH lines were obtained from 94 S_1 families with 1–8 DH lines per S_1 family and an average of 2.8. The reduction in the number of S_1 families from which the DH lines originated was due to the elimination of families with only one haploid to prevent the risk of failure of chromosome doubling. We considered that the 94 S_1 families from which the DH lines originated were a random sample of the 150, and that the number of haploids per plant was not related to agronomic traits such as grain yield.

Selection process

With the use of off-season nurseries, the S_1 selection cycle required four generations over 2 years (Figs. 1, 2). Two S_1 family cycles were obtained in 4 years. During this time, one DH selection cycle was developed. Off-season nurseries were located in Chile, south of Santiago. The S_1 families and DH lines were crossed with the flint tester D171 to produce the testcross progenies. The elite line D171 selected at the University of Hohenheim (Germany) was chosen as tester because it combined well with our material. Testcross progenies were produced in isolated fields: S_1 progenies and DH lines sown in rows of 25 kernels were used as female parent, and the tester was used as male parent. A common

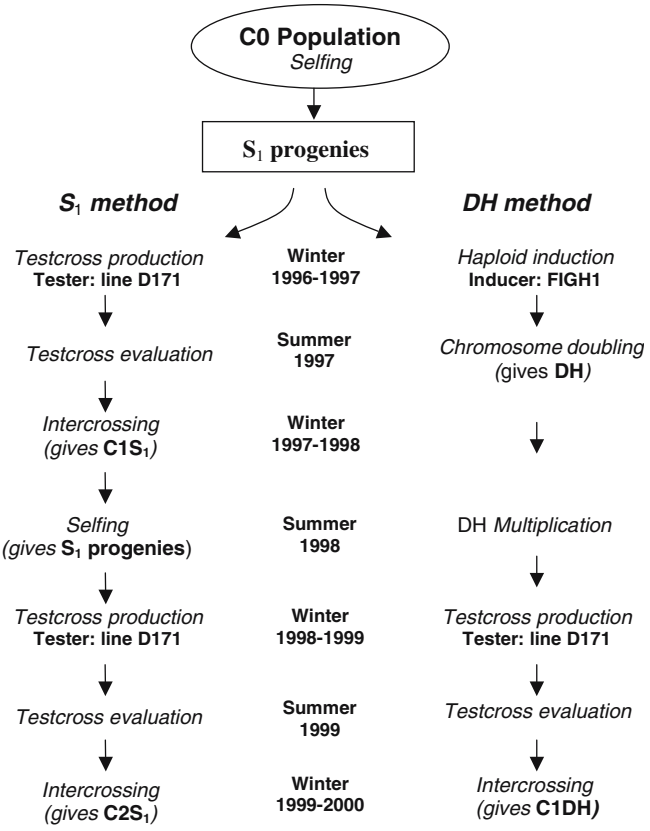

Fig. 1 Selection process with both S_1 family progenies and DH lines

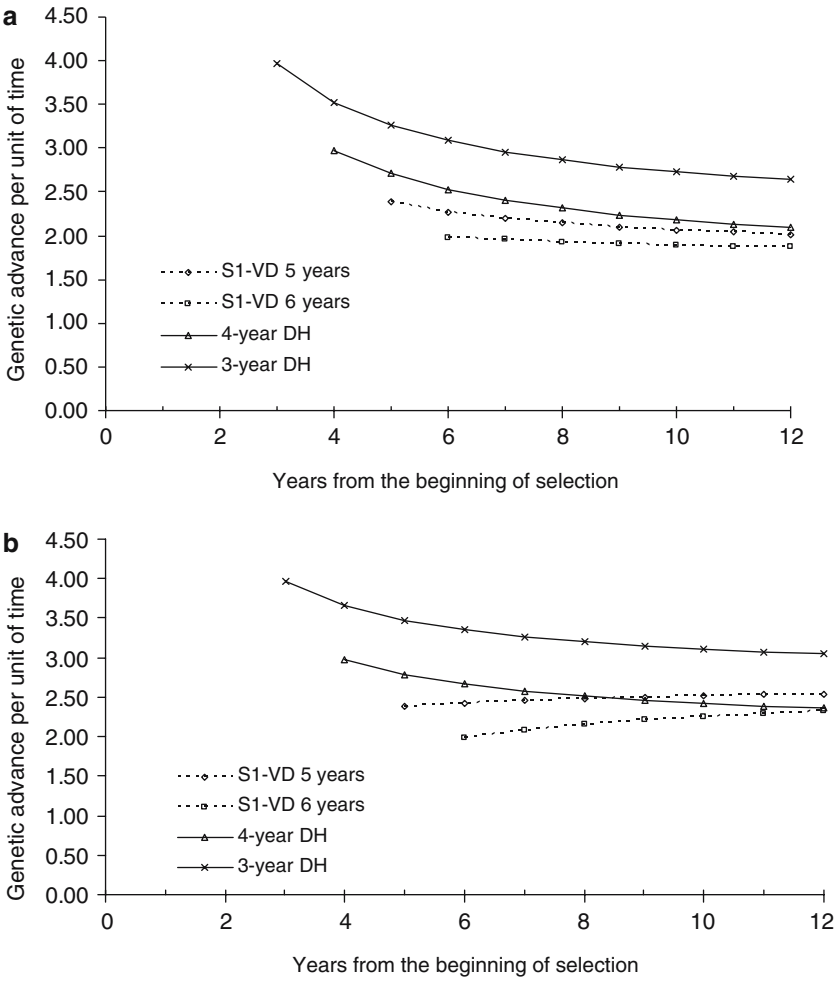

Fig. 2 Change in average genetic advance per unit of time for varietal development (*VD*), computed from the beginning of selection according to the selection method. **a** From realized gains, **b** from expected gains (see appendix for details on the computation). S_1-VD 5(6) years: S_1 method with varietal development in 5 (6) years; 3(4)-year DH: DH method with 3 (4) years for a cycle

selection rate of 20% was applied for each selection method. Consequently, for S_1 family selection, 30 were selected from the 150 S_1 derived from the C0 population on the basis of their testcross performance and intercrossed by chain crossing to obtain the $C1S_1$ population as follows: (1×2, 2×3, 3×4, ... 30×1). To initiate the second selection cycle, again 150 S_1 progenies were derived at random from the $C1S_1$ population, and after their evaluation, 30 were selected and intercrossed by chain crossing to obtain the $C2S_1$ population. For DH selection, after evaluation of the testcross progenies from the 261 DH lines derived from the C0 population, 52 DH lines were selected and also intercrossed by chain crossing to obtain the C1DH population. To obtain a similar genetic base at the DH population level after intercrossing, it would have been necessary to intercross 60 independent DH lines, which would have implied studying 300 DH lines in order to obtain the same selection intensity. Assuming independence among DH lines, the expected inbreeding coefficient after intercrossing the 52 selected DH lines was $1/52 = 0.019$, whereas with intercrossing 30 S_1 families it was $1/60 = 0.017$. The two expected coefficients were thus similar. However, selected DH lines were not completely independent: 28 S_1 families produced one DH line each, 9 S_1 families produced two DH lines each and 2 S_1 families produced three DH lines each. This parentage increased the coefficient of inbreeding to 0.025 (see Appendix 1). This means that the expected size of the C1DH population was less than that of the $C1S_1$ population. However, we had to compare it to the $C2S_1$ population which had an expected inbreeding coefficient of 0.033 (Gallais 1989). The situation was then reversed; the expected population size for C1DH was higher than for $C2S_1$.

The experimental testing design used for the whole selection program consisted of 150 testcross progenies from S_1 families in 1997 and 1999, and 261 testcross progenies from DH lines in 1999. Progeny evaluation trials were conducted in only 1 year at three locations in the INRA experimental network: Le Moulon (Yvelines, France), Clermont-Ferrand (Puy de Dôme, France) and Lusignan (Vienne, France). In each location, the progenies and hybrid checks were arranged in sets of 56 entries and evaluated using a randomized complete block design with two replications. Each experimental plot comprised two rows, 5.5 m in length with 0.80 m between rows. Plots were machine planted at approximately 90,000 plants ha^{-1}

and thinned to approximately 82,000 plants ha^{-1}. All plots were machine harvested. Grain yield (adjusted to 15.5% moisture), root lodging and kernel moisture were determined for all plots at harvest. As the initial population was composed of elite lines belonging to equivalent maturity groups, neither root lodging nor significant differences in kernel moisture were observed between genotypes. Consequently, the only selection criterion taken into account was grain yield.

Expected genetic gain for direct selection

Expected genetic gain in one cycle of recurrent selection can be written as (Gallais 1991):

$$\Delta G = \frac{i\theta \operatorname{cov} P_T O_T}{\sigma_{P_T}}, \quad (1)$$

where i is the selection intensity, θ is the degree of selection control on both sexes, cov $P_T O_T$ is the parent–offspring covariance for testcross value and $\sigma^2_{P_T}$ is the phenotypic variance among selection units. In our experiment, with a selection rate of 20%, the i value was 1.40 (Falconer 1981) and the degree of selection control on both sexes was $\theta = 2$ for both methods. As we considered the combining ability of diploid genotypes with a tester, there is no dominance effect. In a noninbred population evaluated for its testcross performance, with the assumption of absence of epistasis, the genotypic variance is equal to the additive variance (Gallais 1990b). With an inbred population cov $P_T O_T$ can then be written (Bouchez and Gallais 2000):

$$\operatorname{cov} P_T O_T = \frac{(1+F)}{2\sigma^2_{A_T}},$$

where $\sigma^2_{A_T}$ is the additive variance for testcross performance and F is the inbreeding coefficient of the selected units ($F = 0$ for S_1 progenies and $F = 1$ for DH lines). Expression 1 can be written as:

$$\Delta G = i\sqrt{1+F}\, h_F\, \sigma_{A_T}, \quad (2)$$

with $h^2_F = (1+F)\sigma^2_{A_T}/\sigma^2_{P_T}$ and $\sigma^2_{P_T} = (1+F)\sigma^2_{A_T} + \sigma^2_{GL}/l + \sigma^2_E/bl$, where l is the number of locations, b the number of replications in one location, σ^2_{GL} the genotype × location interaction and σ^2_E the residual variance.

Finally, the relative efficiency of each method depends on three parameters: the inbreeding coefficient F, the heritability h^2_F and the cycle length (the additive variance being unchanged). When expressed per unit of time t, genetic advance becomes:

$$\Delta G = \frac{(i\sqrt{1+F}\, h_F\, \sigma_{A_T})}{t}. \quad (3)$$

The predicted genetic gain per cycle and per year was calculated with Eqs. 2 and 3, respectively. To estimate variance components, analysis of variance of experimental designs was carried out according to the following model:

$$Y_{ijk} = \mu + L_i + (b/L)_{ij} + G_K + (GL)_{ik} + E_{ijk},$$

where L is the location effect, (b/L) is the block/location nested effect, G the genotype effect, (GL) is the genotype × location interaction effect, E the residual variance. The last three effects were random, whereas the others were fixed. Variance components were estimated with the Restricted Maximum Likelihood procedure (VARCOMP REML option of SAS software; SAS Institute 2000). The confidence interval of heritability was calculated according to the method described by Knapp et al. (1985). The same model was used for DH testcross progenies as for S_1 testcross progenies despite the presence of several DH lines per S_1. Indeed, with the number of S_1 from which the DHs were derived (94) and with an average of 2.8 DH lines per S_1, the expected component for the genetic variance among DH lines estimated by ignoring this nested structure is very close to that estimated with the nested design. Assuming the same number n of DH lines per S_1, the expected mean square would be $\sigma^2 + \sigma^2_W + [n(g-1)/gn-1]\sigma^2_B$ (g being the number of parental S_1 lines and $\sigma^2 + \sigma^2_W + \sigma^2_B$, respectively, the residual variance on family means and the within and between S_1 variance) instead of $\sigma^2 + \sigma^2_W + \sigma^2_B$. It can be seen that, with $g = 94$ and $n = 2$ or 3, the coefficient of σ^2_B is close to 1.

Observed genetic gains

For each method, observed gains were studied at the level of testcross progenies from the improved population. This study also allowed the evaluation of genetic variance after selection. The tester used, the elite UH002 line, was different from that used for selection. This line, like D171, was selected at the University of Hohenheim, and belongs to the same heterotic group but represents an improved inbred. As the tester used to evaluate initial and improved populations was different from the tester used in the selection process, the realized genetic advance was the result of an indirect response to selection. Testcross performance was evaluated for each population selected (C1S_1, C2S_1, C1DH) and for the initial population (C0). To produce the testcross progenies for each population, 86 S0 plants were selfed and S_1 families were crossed to the tester UH002. Progenies were evaluated in the INRA experimental network over a period of 2 years, in 2002 in five locations: Le Moulon, Clermont-Ferrand, Lusignan, Dijon (Côte d'Or, France) and Saint Martin de Hinx (Landes, France), and three locations in 2003: Le Moulon, Clermont-Ferrand and Lusignan. However, in 2003, due to a limited quantity of seeds available, only 59 progenies were evaluated. In each location, the progenies and hybrid checks were arranged in sets of 56 entries and evaluated using a randomized complete block design with one replication in 2002 and two replications in 2003. Each experimental plot was made up of two rows 5.5 m in length with 0.80 m between rows. Plots were machine planted at approximately

90,000 plants ha^{-1} and thinned to approximately 82,000 plants ha^{-1}. All plots were machine harvested. Grain weight and grain moisture were measured on all replications at harvest. Because of the differences between experimental designs (no replications within a given location in 2002), the data of each year were first analyzed separately according to the following model:

$$\text{for } 2002: Y_{ik} = \mu + L_i + G_k + (GL)_{ik} + E_{ik},$$

where l is the location effect, G is the genotype random effect, $(G\times\text{L})$ is the genotype × location interaction effect and E is the residual variance.

$$\text{For } 2003: Y_{ijk} = \mu + L_i + (b/L)_{ij} + G_k + (GL)_{ik} + E_{ijk},$$

where b/L is the hierarchical block/location effect.

For each year of the experiment, variance components of random effects $\left(\text{i.e. } \sigma^2_{\text{G}}, \sigma^2_{\text{GL}} \text{ and } \sigma^2_{\text{E}}\right)$ were estimated. In 2002, because each location had only one replication, the genotype × location × year interaction variance was confounded with the residual variance. By pooling both years, we also estimated σ^2_{G}, σ^2_{GL} and σ^2_{GY} the genotype × year interaction variance.

Results

Variance components and predicted genetic gain

Genetic variance among DH lines in the C0 population was close to twice the genetic variance among S_1 families in the C0 or the $C1S_1$ population (Table 1). Genetic × location interaction variance for S_1 families from the C0 population was about twice that for S_1 progenies from the C1 population and that for DH progenies, which were equivalent. The residual variance for S_1 progenies from the C0 population was also significantly higher than residual variance for S_1 progenies from the $C1S_1$ population and residual variance for DH progenies. As a consequence, heritability $\left(h^2_{\text{F}}\right)$ for S_1 progenies from the C0 population was significantly lower than for S_1 progenies from the C1 population and for DH progenies from the C0 population.

The predicted gain per cycle with the DH method (8.2%) was 1.7 times higher than the gain predicted with the S_1 method in the first cycle (4.8%) and 1.4 times the gain predicted in the second cycle (Table 2). The sum of the two S_1 selection cycles led to a total expected genetic advance of 10.6% which was 1.3 times as high as the expected advance with DH selection. Thus, per year, with a 4-year cycle for the DH method, the predicted gain for this method (2.0%) was lower than the predicted gain for the S_1 selection method: 2.4% in the first cycle and 2.9% in the second cycle.

Realized genetic gain

Realized gain in the DH cycle (6.6%) was about the same as the sum of gain in the two S_1 cycles (7.0%) (Table 2). Consequently, with a 4-year cycle for the DH method, the per year gain for this method (1.6%) was almost the same as the gain per S_1 cycle (1.8%). However, the per year gain obtained in the first S_1 cycle (1.1%) was lower than that obtained in the second S_1 cycle 4.8% (2.3%). Therefore, per year, the DH method was more efficient than the first cycle of the S_1 method, whereas it was less efficient than the second cycle.

Table 1 Estimates of variance components among maize S_1 families and DH lines for each selection cycle

Method	Population	F	σ^2_{G}	σ^2_{GL}	σ^2_{E}	h^2_{F}
S_1	C0	0	22.1 (5.3)[a]	41.8 (2.7)	45.4 (3.0)	0.53 (0.39–0.62)[b]
	$C1S_1$	0	25.9 (4.3)	21.3 (2.8)	22.8 (1.5)	0.74 (0.67–0.80)
DH	C0	1	44.3 (4.8)	22.0 (1.9)	16.5 (0.8)	0.83 (0.79–0.88)

F Coefficient of inbreeding of selection units; σ^2_{G} genetic variance; σ^2_{GL} genotype × location interaction variance; σ^2_{E} residual variance; $h_{\text{F}}{}^2$ heritability
[a]Standard error
[b]Confidence interval

Table 2 Predicted and realized genetic gain per cycle and per year from recurrent selection among maize S_1 families and DH lines

Method	Cycle	Cycle length years	Predicted genetic gain		Realized genetic gain (%)[a]		Ratio realized/ expected[b]	Realized h^2
			Per cycle	Per year	Per cycle	Per year		
S_1	C1	2	4.8	2.4	2.2 ± 1.4	1.1 ± 0.7	0.47	0.26
	C2	2	5.8	2.9	4.8 ± 1.4	2.3 ± 0.7	0.81	0.60
	Total	4	10.6	2.7	7.0 ± 2.0	1.8 ± 0.5	0.66	
DH	C1	4	8.2	2.0	6.6 ± 1.4	1.6 ± 0.3	0.80	0.64
		3	8.2	2.7	6.6 ± 1.4	2.2 ± 0.5	0.80	

[a] ± twice the standard error
[b]Ratio realized gain/predicted gain

The realized genetic gain was always less than the expected gains. The ratio of realized-to-expected gain was 0.47 for $C1S_1$, 0.81 for $C2S_1$ and 0.80 for C1DH. Thus, there was about the same overestimation of genetic advance by expected gain for $C2S_1$ as for C1DH (Table 2). However, the overestimation for $C1S_1$ was much higher. Similarly, the realized heritability computed from the ratio of observed genetic advance to the differential selection, appeared to be lower for $C1S_1$. It should be noted that although selection was only on grain yield, kernel moisture was not significantly modified by such a selection.

Change in genetic variances

In 2002, the estimates of genetic variances in initial and improved populations showed no significant change due to the breeding methods (Table 3). In 2003 and in the pooled analysis, genetic variance in the first S_1 selection cycle appeared to be higher than in the initial population and than in the second cycle of selection. However, taking into account the large confidence interval for variance components, these differences were not significant. On average, we can conclude that there was no significant change in the genetic variance. In 2002 and 2003 the genotype × location variance was about equal to the genetic variance. This was confirmed in the pooled analysis. Furthermore, this analysis showed that the genotype × year interaction variance was, on average, lower than the genotype × location variance.

Discussion

Variance component estimates

The fact that the estimates of genetic variance among testcross progenies from DH were about twice the estimates of genetic variance among testcross progenies from S_1 families is quite consistent with what was expected and shows that there was no significant epistatic variance component (Gallais 1990a). The higher heritability for DH progenies was also expected. However, we did not expect a significant difference in heritability between $C0S_1$ and $C1S_1$. The lower heritability for $C0S_1$ was due to higher genotype × location interaction and environmental variances. In comparison with DH progenies, the higher residual variance for S_1 progenies was expected because of their heterogeneity leading to competition among plants of different genotypes thereby increasing random variation. Concerning the higher genotype × location interaction, the test of S_1 progenies from C0 and $C1S_1$ in the same year at the end of the experiment did not show a greater genotype × location variance. Therefore, this leads to the conclusion that in the first cycle of S_1 selection the higher genotype × location interaction could have been due to a complex interaction genotype × location × year. Furthermore, heritability for DH progenies could have been overestimated because, in comparison with results for S_1 progenies, a higher genotype × location interaction was expected with DH progenies as a consequence of higher genetic variation. This again could be the result of interaction with the year of the test. It is known that genotype × year interactions are often more important than genotype × location (Sprague and Federer 1951). In our experiment they were about the same.

Predicted gain versus realized gain

For the predicted genetic gain, the lower efficiency of the first S_1 cycle as compared to the second S_1 cycle is due to the lower heritability (0.53 compared 0.74). On a per cycle basis, the greater efficiency of DH method is due to

Table 3 Variance components and heritabilities in initial and improved populations of maize S_1 families and DH lines

Method	Population	Year	σ^2_{GT}	σ^2_{GL}	σ^2_{GY}	h^2_Y
	C0	2002	25.7 (6.2)[a]	24.0 (5.7)[a]		0.64 (0.55–0.71)[b]
		2003	18.4 (6.1)	20.0 (5.4)		0.58 (0.47–0.66)
		Pool	14.6 (5.4)	16.5 (4.4)	10.6 (3.8)	(–)[c]
S_1	$C1S_1$	2002	32.5 (7.0)	21.8 (5.2)		0.71 (0.64–0.77)
		2003	31.9 (9.6)	34.2 (7.3)		0.63 (0.54–0.70)
		Pool	26.5 (6.7)	23.2 (4.2)	7.6 (3.0)	(–)
	$C2S_1$	2002	25.9 (6.6)	27.9 (6.6)		0.61 (0.51–0.69)
		2003	19.1 (6.8)	17.2 (6.8)		0.54 (0.43–0.63)
		Pool	14.2 (5.3)	8.0 (4.5)	10.2 (4.3)	(–)
DH	C1DH	2002	32.2 (7.4)	26.9 (6.3)		0.67 (0.58–0.73)
		2003	18.2 (6.6)	23.9 (6.5)		0.53 (0.41–0.62)
		Pool	17.6 (5.7)	17.1 (4.8)	10.6 (3.9)	(–)

σ^2_G genetic variance; σ^2_{GL} genotype × location interaction variance; σ^2_{GY} genotype × year interaction variance; h^2_F heritability for the type of families considered

[a] Standard error of the variance component

[b] Confidence interval

[c] h^2 not computed because of the absence of replications in each location in 2002

the higher variance among DH lines than among S_1 families. This is mostly a consequence of inbreeding which resulted in higher heritability. In comparison with the S_1 second cycle, the lowest residual variance of the DH cycle, due to the plant homogeneity within plots, increased heritability. Indeed, when selection units are DH lines and the tester is a homozygous line, the within-progeny genetic variance is equal to zero.

The overestimation of genetic advance by expected genetic gain could be due to two factors: the change in the tester and the change in environmental conditions with the effect of genotype environment interactions. Let us first consider the effect of the change in the tester. By selection with the first tester (T1 = D171), but evaluation with the second tester (T2 = UH002), the expected genetic advance is

$$\Delta G_{\mathrm{T2/T1}} = i\sqrt{1+F}\rho h_{\mathrm{F}}\sigma_{A_{\mathrm{T2}}},$$

where ρ is the genetic correlation between the two testers and $\sigma_{A_{\mathrm{T2}}}$ the additive variance in the test with tester T2. The ratio of this expected genetic advance to the expected genetic advance by direct selection is thus:

$$R_{\mathrm{T}} = \frac{\Delta G_{\mathrm{T2/T1}}}{\Delta G_{\mathrm{T1/T1}}} = \frac{\rho\,\sigma_{A_{\mathrm{T2}}}}{\sigma_{A_{\mathrm{T1}}}}, \qquad (4)$$

ρ is necessarily less than 1. Assuming a correlation of 0.70 between the two testers (which could be expected as the two testers are related), if both additive variances are equivalent, this could be sufficient to explain the observed results (at least for the DH method). Now, in comparison with the first tester, if the second tester had a greater proportion of loci with dominant alleles, the additive variance would be expected to be lower. This could be a result of the improvement of the tester and would then also contribute to decreasing the observed genetic advance.

If overestimation of the genetic advance were only due to the change in the tester, the R_{T} ratio would be the same whatever the selection method and the cycle. As this was not the case, the change in the tester is not sufficient to explain the results. Overestimation could also be due to the variation in environmental conditions, depending on the year of testing. The evaluation of testcross progenies during the selection process was performed at three sites but only in 1 year, whereas the evaluation of genetic advance was performed in 2 years, representing eight site × year combinations. Consider, then the genetic advance in one cycle, with selection in environment *E1* for response in environment *E2*. It can be written

$$\Delta G = i\sqrt{1+F}\,\rho_{12}\,h_{\mathrm{F}}\,\sigma_{\mathrm{A}_2}, \qquad (5)$$

where ρ_{12} is the genetic correlation between the two types of environments and $\sigma^2_{\mathrm{A}_2}$ the additive variance in the test in environment 2. Then again the ratio of this expected genetic advance to the expected genetic advance by direct selection will be

$$R_{\mathrm{E}} = \frac{\rho_{12}\,\sigma_{\mathrm{A}_2}}{\sigma_{\mathrm{A}_1}}.$$

Again assuming the same amount of genetic variance in both types of environments, R_{E} would be less than 1, all the more as the genotype × environment (genotype × year) interaction would be high. It is then possible that the genetic correlation between the testing environments of S_1 families of C0 and the environments for final evaluation was lower than the other correlations. The higher genotype × environment interaction variance observed for S_1 progenies in the first cycle of selection is quite consistent with such an assumption (Table 1). The combination of both factors, i.e., the change in tester and the change in environments, then seems sufficient to explain the results observed.

Change in variance due to selection and genetic advance

In spite of the change in tester, the variance components estimated for each cycle of S_1 selection (Table 1) were comparable with the variance components of S_1 progenies used for the evaluation of observed genetic gain (Table 3). Estimated heritabilities in C0 and $C1S_1$ populations were consistent in both evaluations. Finally, the absence of significant change in genetic variances in the first cycles of selection was consistent with what has been already observed in many selection experiments in maize with relatively low selection intensity (Hallauer and Miranda 1981). However, a decrease in genetic variance is to be expected with further cycles. The absence of differences between the two methods after selection was expected because we tried to obtain the same effective population size after both types of selection. Indeed the effective population size can be computed as $1/(2F)$, F being the coefficient of inbreeding (Hallauer and Miranda 1981; Crow and Kimura 1970). According to F values given in the Materials and methods section, the effective population size was 30 for population $C1S_1$, 15 for $C2S_1$ and 20 for C1DH. We expected the genetic variance for C1DH to be closer to variance for $C2S_1$ than to variance for $C1S_1$, which was observed, although the differences were not significant. However, this means that on the whole, the selection intensity was higher with the two S_1 selection cycles than with the DH selection cycle. To obtain the same inbreeding coefficient in C1HD as in $C2S_1$, it would have been necessary to select only 42 DH. Through an increase in the selection intensity for DH method, this means multiplying the expected genetic advance by 1.1 (8.9% instead of 8.2%). This does not change the conclusion: in 4 years, the DH method is expected to be less efficient than the S_1 method. We could also expect the same increase for realized gain. Again, this would not change the conclusion: with a 4-year cycle, the DH method is equivalent to the S_1 method and with a 3-year cycle, it tends to be better. This shows that it is difficult to compare the efficiency of breeding methods that do not maintain the

same effective genetic size. Methods can be compared for the same effective genetic size only at given cycles. Without this problem, the DH method could be better because it narrows the genetic base faster than the S_1 method. In our experiment, we increased the number of selection units (DH lines) in order to apply the same selection intensity, but with the same number of selection units as in the S_1 method it would also be possible to reduce the selection intensity.

Cycle length

In our experiment, the cycle length for the DH selection method was 4 years. It would have been possible to reduce it to 3 years by two ways: first, by deriving haploids from S_0 plants; second, by eliminating the DH multiplication stage due to the low number of kernels produced by DH. The number of kernels on haploid plants varied from almost 0 to 50. With DH lines that produce enough kernels (about 20), it would be possible to avoid the DH multiplication stage. To use DH lines with only a few kernels, the simultaneous selfing and crossing to the tester as female parent would have been possible.

The improvement of the DH process (haploid induction, haploid identification and artificial chromosome doubling) would also make it shorter. Indeed, the increase in the efficiency of haploid induction, which is currently from 5 to 10% (Deimling et al. 1997; M. Pollacsek, unpublished), allows the production of at least one line per plant. The haploid identification at the embryonic stage (Nanda and Chase 1966) would allow colchicine treatment of the embryo or at an early stage (Demling et al. 1997; Kato 2002). This would avoid plant stress caused by the colchicine treatment at the plantlet stage and thus improve the vigor of haploid plants. Grain production per DH line at the first selfing would then be increased. Finally, DH selection cycle could be developed in five generations instead of seven, and with the use of off-season nurseries the cycle length could be reduced to 3 years without much difficulty. It is unlikely that it could be reduced to 2 years.

As far as the cycle length of the S_1 method is concerned, it is unlikely that it could be accomplished in less than 2 years, although it is theoretically possible in 1 year with: (1) simultaneously selfing and crossing to the tester used as female parent in off-season nurseries, (2) evaluating testcross progenies in a normal growing season, and (3) intercrossing in off-season nurseries. However, this type of organization would be difficult to manage due to the short time between each phase.

Another point to consider with respect to the DH method is the intercrossing procedure which can affect cycle length. We considered intercrossing in only one generation. Thus, the resulting population is a mixture of F1s. For the next cycle, DH would be derived from from F1s. To avoid the risk of selecting sister DH lines from the best F1s, which would lead to a rapid reduction of the genetic base and would severely limit recombination between selected lines, the F1s would then have to be identified. Another way would be to develop a second intercrossing generation in order to increase the recombination between selected parents. If this is possible without increasing the cycle length in years, it is to be recommended. Otherwise, it will reduce the genetic advance per unit of time. Note that in both situations it would be better to derive only one DH line per plant (i.e., per F1 in the first scheme) (Gallais 1988).

Genotype × environment interaction

From the point of view of the genotype × environment interaction, 2-year testing is possible; this would reduce the genetic advance per unit of time more than the method with the shorter cycle, i.e., the S_1 method. However, due to its shorter cycle, the S_1 family method has the advantage of comprising twice as many years of evaluation as one cycle of the DH method, with the same number of locations for the progeny test for both methods, at each cycle. We could then expect the S_1 method to produce material with a wider adaptation than the DH method. However, it would be possible to use additional diverse environments for the DH method to try to compensate for its lower sampling of genotype × year interactions. The cost per unit of time thus depends on this choice. With the same number of progenies tested, without supplementary locations for the DH method, S_1 would be the most expensive, whereas with twice as many locations for the DH method, the cost would be the same. However, if the objective was to maintain the same effective population size, with the DH method, the number of progenies tested would need to be doubled. In this case, without supplementary locations for the DH method, both methods would have the same annual cost. With supplementary locations, the DH method would then be more costly. However, in practice, with elite material, it would be better to find a compromise between keeping the same population size and doubling it (Bouchez and Gallais 2000) and also between keeping the same number of locations and doubling them. In this case the cost per unit of time would be about the same for both methods.

Genetic advance per unit of time

Consider the consequence of a 3-year cycle for DH selection and a 2-year cycle for S_1 selection which could be considered as the schemes that make the best use of time for both methods. In terms of gain per year, this scheme would increase the efficiency of DH method by 33%. With a 4-year cycle for DH selection, the genetic advance per year with this method was 23% less than for S_1 selection (Table 2), whereas with a 3-year cycle, both methods were equivalent with 2.7% for both methods. In terms of realized gains with a 4-year cycle, the DH method was equivalent to the S_1 method and would thus

be expected to be more efficient with a 3-year cycle. In conclusion, from the study of both expected and realized genetic advance in population improvement, it appears that to make the DH method competitive per unit of time it needs to have a 3-year cycle.

However, from an applied plant breeding point of view, we have to consider the whole process of population improvement and varietal development. If we consider that one cycle of DH selection is about equivalent to two cycles of S_1 selection for population improvement, the DH method would be expected to be more efficient in terms of genetic advance per unit of time from the point of view of varietal development. Indeed with the DH method, if the tester is an elite line, then recurrent selection for testcross performance leads directly to new hybrids: after testing the DH testcross progenies it is only necessary to identify the best one or two, whereas with the S_1 method it is necessary to add the whole process of varietal development from the best S_1 families. Figure 2 illustrates what can be expected in terms of genetic advance per unit of time with the 3- and 4-year cycle for the DH method, and considering that lines are developed from selected S_1 by single seed descent in 2 years, which is optimistic for such a scheme (see Appendix 2 from more details on the assumptions and computations). Based on realized gains, 4-year DH selection is better than the S_1 method for the first cycle (2.97 q ha^{-1} $year^{-1}$in 4 years versus 2.4 q ha^{-1} $year^{-1}$) and then after two cycles both methods would be expected to be equivalent. The same conclusion can be drawn on the basis of expected gains, although after two cycles of 4-year DH selection, the S_1 method tends to be better than the DH method. Therefore, with our material and considering varietal development, with a 4-year cycle for the DH method, such a method would be expected to be significantly more efficient than the S_1 method only in the first selection cycle. In both situations the use of a 3-year cycle for the DH method gives a clear advantage to the DH method: 3.08 q ha^{-1} $year^{-1}$ after 8 years for the DH method versus 2.3 q ha^{-1} $year^{-1}$ for the S_1 method based on realized gains. After 12 years its advantage is still significant: 2.64 q ha^{-1} $year^{-1}$ versus 2.09 q ha^{-1} $year^{-1}$ based on realized gains and 3.05 q ha^{-1} $year^{-1}$ versus 2.54 q ha^{-1} $year^{-1}$ based on expected gains. Such derivations were made with assumption of no change in genetic variance in the first selection cycles. If the variance decreases, as we have assumed a greater population size for the DH method, we do not expect a greater decrease with this method and thus the previous conclusions would remain.

The total cost of both methods could also be taken into consideration, although for a plant breeder what is important is to develop the best variety as quickly as possible in order to put it on the market as early as possible which will make the investment profitable (Bouchez and Gallais 2000). In our conditions, per unit evaluated (including the cost of production), the cost of DH was only about 1.2 times more than the cost of S_1 (data not shown). Thus, from the point of view of population improvement, considering the number of progenies that were studied for each method in 4 years, the total cost of the DH cycle was slightly higher than the cost of the two S_1 cycles. From the point of view of varietal development, taking the cost of pedigree selection required for S_1 method into consideration would give a clear advantage to DH method.

Conclusion

Considering only population improvement, on a per year basis, with one cycle in 4 years, the DH method was approximately equivalent to the S_1 family method. For the DH method to be better, it would be necessary to develop one cycle in 3 years. This is quite consistent with the theoretical conclusion of Bouchez and Gallais (2000). Considering population improvement and varietal development simultaneously, the DH system has the advantage of producing potential hybrid cultivars at each cycle. Thus, if the genetic advance per unit of time is evaluated from the point of view of varietal development, there is a clear advantage to using the DH method. In this case, varietal development can be considered as integrated in the process of recurrent selection which becomes a recurrent varietal development.

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Appendix 1: derivation of the coefficient of inbreeding of the C1DH population

Among the 52 intercrossed DH lines used to develop the C1DH population, 28 derived from independent S_1 with one DH line per S_1, 18 derived from nine independent S_1 with two DH lines per S_1 and 6 derived from two independent S_1 with three DH lines per S_1.

The coefficient of inbreeding is defined as the probability of drawing at a locus in a zygote two genes identical by descent, i.e., deriving from the same ancestor gene. Assuming random mating, identity by descent in one zygote can result either from selfing with a probability of 1/52, or by crossing between sister lines, i.e., from the same S_1. In the case of crossing between two sister lines, the expected inbreeding coefficient of progeny is1/2. One S_1 with two sister lines generates two crosses between sister lines (including reciprocal); one S_1 with three sister lines generates six crosses between sister lines. The total inbreeding coefficient will thus be:

$$1/52 + 9\ (2\ 1/2)1/522 + 2\ (6\ 1/2)1/522 = 0.0247.$$

Appendix 2: genetic advance in varietal development

Total genetic advance with varietal development (ΔG_{TV}) is derived by adding genetic advance due to varietal development from one selection cycle of population improvement (ΔG_V) and the cumulated genetic advance due to population improvement (ΔG_P). Assuming linear response over the first cycles of selection, the expression of total genetic advance after n cycles of recurrent selection followed varietal development is

$$\Delta G_{TV} = n\Delta G_{P1} + \Delta G_V,$$

where ΔG_{P1} is the genetic advance in one cycle of population improvement. Such a genetic advance needs a time $t = n\ c_P + c_V$, where c_P is the cycle length in population improvement (3 or 4 years for the DH method) and c_V is the duration of varietal development. Therefore, genetic advance per unit of time can be derived.

To consider varietal development with the S_1 method, several schemes are possible. As this is not the place to discuss all the schemes that are possible, we simplified by considering SSD from the best S_1 and assumed that the same potential can be achieved as with DH. Using off-season nurseries, S_5 or even S_7 lines can be derived in 2 years with 1 year more for the evaluation of testcross progenies (which can begin at the S_4 level). This gives a minimum duration of 5 years after the population resulting from intercrossing. The same formula as previously can then be used with appropriate parameters. Obviously, it is not justified to study more than 3–4 cycles without considering the possible decrease in genetic variance. It should be noted that 5 years is the minimum time for the derivation of lines without the use of DH; such a value favors S_1 method.

Figure 1 shows the comparison of genetic advance per year for both methods with the following conditions:

- the potential of varietal development for DH selection was computed by selecting the best 3% lines;
- genetic variance among DH lines was taken to be equal to 44 which is the value estimated at the first DH selection cycle and which is quite consistent with variance among S_1 estimated at the end of the experiment;
- heritability at the level DH lines was taken to be equal to 0.80: it was estimated to be equal to 0.83 in the first selection cycle. Genetic advance due to varietal development ΔG_V from any selection cycle was then computed as $i\ h^2\ \sigma_G = 11.9$ q ha^{-1}; it should be noted that, with the assumption of the same potential for both methods in varietal development, such a quantity does not affect the difference between the two methods;
- for the genetic advance due to population improvement, expected and realized values were taken into consideration.

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