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ORIGINAL PAPER

Hybrid maize breeding with doubled haploids: III. Efficiency of early testing prior to doubled haploid production in two-stage selection for testcross performance

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Abstract Early testing prior to doubled haploid (DH) production is a promising approach in hybrid maize breeding. We (1) determined the optimum allocation of the number of S_1 families, DH lines, and test locations for two different breeding schemes, (2) compared the maximum selection gain achievable under both breeding schemes, and (3) investigated limitations in the current method of DH production. Selection gain was calculated by numerical integration in two-stage breeding schemes with evaluation of testcross progenies of (1) DH lines in both stages (DHTC), or (2) S_1 families in the first and DH lines within S_1 families in the second stage (S_1TC-DHTC). Different assumptions were made regarding the budget, variance components, and time of DH production within S_1 families. Maximum selection gain in S_1TC-DHTC was about 10% larger than in DHTC, indicating the large potential of early testing prior to DH production. The optimum allocation of test resources in S_1TC-DHTC involved similar numbers of test locations and test candidates in both stages resulting in a large optimum number of S_1 families in the first stage and DH lines within the best two S_1 families in the second stage. The longer cycle length of S_1TC-DHTC can be compensated by haploid induction of individual S_1 plants instead of S_1 families. However, this reduces selection gain largely due to the current limitations in the DH technique. Substantial increases in haploid induction and chromosome doubling rates as well as reduction in costs of DH production would allow early testing of S_1 lines and subsequent production and testing of DH lines in a breeding scheme that combines high selection gain with a short cycle length.

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Introduction

Inbred line development by the doubled haploid (DH) technique is currently adopted as a routine method in commercial hybrid maize breeding programs (Schmidt 2004; Seitz 2005). The use of DHs offers the possibility to evaluate potential hybrid cultivars from the very beginning of the selection process. Alternatively, an early test on testcross performance in generation S_1 or S_2 could be made before production of DHs. This elongates the breeding scheme but permits the restriction of the production and testing of DH lines to those derived from segregation in the most promising families.

Early testing is based on the assumption that the combining ability of a line is determined during the early generations of selfing (cf. Hallauer et al. 1988). Experimental results reported in literature have been proving (Sprague 1946; Lonnquist 1950; Hallauer and Lopez-Perez 1979; Jensen et al. 1983) or disproving this assumption (Richey 1945; Payne and Hayes 1949). However, the genetic correlation for testcross performance between S_1 plants and inbreds is larger than 0.7, thus supporting the determination of combining ability in the early stages of selfing (Bernardo 1991). An assessment of the potential of

early testing in hybrid maize breeding with DHs is not available in the literature.

Early testing prior to DH production requires selection among two different types of test candidates: families and DH lines within families. As plant breeders have only a fixed budget available, they must find a compromise between (1) the number of families and (2) the number of DH lines within families to be tested, as well as (3) the intensity of their testing as determined by the number of test locations, years, and replications. For self-pollinated crops, Utz (1981) and Weber (1981) investigated two consecutive selfing generations with selection among families in the first stage and selection among and within families in the second stage. Almost equal parts of the budget were used for selection among and within families. For the second stage, this approach resulted in a small optimum number of families but a large optimum number of lines within families. However, hybrid maize breeding schemes have not been taken into account. In addition, the number of test locations was not optimized.

We calculated the maximum selection gain by numerical integration to optimize the allocation of test resources in hybrid maize breeding with DHs. Two-stage selection schemes were considered with evaluation of testcross progenies of (1) DH lines in both stages, or (2) S_1 families in the first and DH lines within S_1 families in the second stage. Different assumptions were made regarding the budget, variance components, and time of DH production within S_1 families. Our objectives were to (1) determine the optimum allocation of the number of S_1 families, DH lines, and test locations for two different breeding schemes, (2) compare the maximum selection gain achievable under both breeding schemes, and (3) investigate limitations in the current method of DH production.

Materials and methods

Breeding schemes

We investigated two breeding schemes for second-cycle breeding, where new lines are developed by crossing elite inbreds within heterotic groups. Both breeding schemes comprise two-stage selection of test candidates within one cross of two homozygous lines (Fig. 1). The target variable is the genotypic value of testcross performance for yield with a given tester. In applied maize breeding, per se evaluation of DH lines for traits with high heritability but not for yield is commonly performed before testcross evaluation. Therefore, we considered per se evaluation of DH lines with regard to the time length of the breeding scheme but neglected it in the selection process.

In breeding scheme DHTC, test candidates are DH lines produced by in vivo haploid induction from S_0 plants and evaluated for their testcross performance (Fig. 1). With S_0 we refer to the F_1 of a biparental cross (cf. Bauman 1981). In the first stage, N_1 DH lines are evaluated at L_1 test locations and N_2 of the most superior DH lines are selected for evaluation at L_2 test locations in the second stage. Without restrictions on L_j in stage j (j = 1, 2), selection gain is maximum for one replication per location (Sprague and Federer 1951; Utz 1969; Melchinger et al. 2005). Thus, we set the number of replications to one for all calculations. The four best DH lines are selected after two test stages.

In breeding scheme S_1TC-DHTC, an early test for testcross performance of the S_1 families is made and remnant seed is used for a simultaneous in vivo haploid induction of these S_1 families. However, chromosome doubling was only performed with haploid kernels produced in selected S_1 families. Therefore, test candidates are either S_1 families or DH lines within S_1 families evaluated for their testcross performance. Testcross progenies of N_1 S_1 families are evaluated at L_1 test locations in the first stage and N_{2_F} of the most superior S_1 families are selected. Within each of the selected S_1 families, a constant number of $N_{2_{DH/F}}$ DH lines are produced and evaluated at L_2 test locations in the second stage. Selection in the second stage is made first among S_1 families and then among DH lines within S_1 families. A final number of one S_1 family and four DH lines within this S_1 family is selected.

Calculation of selection gain

In the first stage, selection among N_1 test candidates was based on the phenotypic mean of testcross performance (x_1) at this stage with the given tester evaluated at L_1 test locations. In the second stage, the selection criterion was an optimum index of the phenotypic means of the test candidates evaluated in both stages with $I = b_1x_1 + b_2x_2$, where b_1 and b_2 refer to the weight of the phenotypic mean in stage one or two (Supplementary Table S1). Calculation of selection gain was based on the well-known formula of Cochran (1951). For DHTC, the selection gain (ΔG) was calculated as

$$\Delta G = \sigma\left(\frac{\rho_{x_1} o_1 J_2 + \rho_{x_2} o_2 J_1}{\alpha_1 \alpha_2}\right), \quad (1)$$

where σ is the standard deviation of the target variable, α_j the selected fraction in stage j (i.e., the ratio of selected by tested candidates), ρ_{x_j} the coefficient of correlation between the phenotypic mean of testcross performance x_j in stage j and the target variable, o_j the ordinate of the univariate normal distribution at the truncation point of

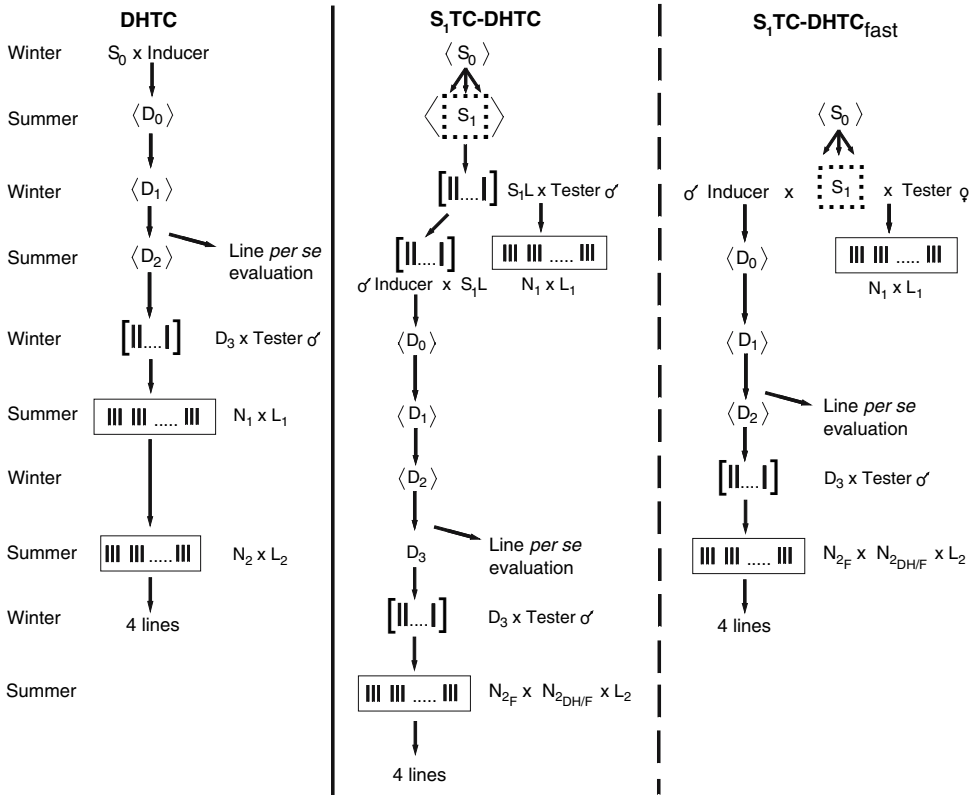

Fig. 1 Hybrid maize breeding schemes using DH lines under two-stage selection with test candidates generated within one cross of two homozygous lines. In breeding scheme DHTC, testcross progenies of N_1 doubled haploid lines produced from S_0 plants by in vivo haploid induction are evaluated in the first stage and the top N_2 DH lines again in the second stage, where four DH lines are finally selected. In breeding schemes S_1TC-DHTC and S_1TC-DHTC$_{fast}$, testcross progenies of N_1 S_1 families are evaluated in the first stage and N_{2_F} of the top S_1 families are selected. Within each of these selected S_1 families, $N_{2_{DH/F}}$ DH lines are produced by in vivo haploid induction and evaluated in the second stage. Four DH lines within one S_1 family are finally selected. (D_i = ith generation of DH multiplication, $\langle\ \rangle$ = selfing, [ ] = isolation plot, □ = performance trials of N_j test candidates at L_j locations in stage j)

selection stage j, and J_1, J_2 the convergent improper integral of the standardized bivariate normal distribution. A detailed description of the calculation of selection gain is given by Wricke and Weber (1986).

For S_1TC-DHTC, we assumed that selection among DH lines within S_1 families was independent from selection among S_1 families (cf. Falconer and Mackay 1996). Selection among S_1 families in the first stage and selection among DH lines within S_1 families in the second stage were based on their phenotypic mean of testcross performance at the corresponding stage evaluated at L_j test locations. Selection among S_1 families in the second stage was based on the optimum index I combining the phenotypic mean of S_1 families of the first stage with the phenotypic mean of all DH lines from the corresponding S_1 family in the second stage. Selection gain (ΔG) was calculated according to Utz (1981) as

$$\Delta G = \sigma\left(\frac{\rho_{x_1} o_1 J_2 + \rho_{x_{2_F}} o_{2_F} J_1}{\alpha_1 \alpha_{2_F}} + \frac{\rho_{x_{2_{DH/F}}} o_{2_{DH/F}}}{\alpha_{2_{DH/F}}}\right). \quad (2)$$

Optimum allocation of test resources

The allocation of test resources refers for DHTC to (L_1, N_1, L_2, N_2) and for S_1TC-DHTC to $(L_1, N_1, L_2, N_{2_F}, N_{2_{DH/F}})$. The allocation of test resources was considered optimum if it maximized the selection gain in the set of all integer allocation combinations feasible for a given scenario, i.e., budget, variance components, and production costs of DH lines. The optimum allocation as well as the corresponding selection gain are denoted by an asterisk, e.g., ΔG^*.

Economic frame and quantitative-genetic parameters

A fixed total budget for the production of test candidates and evaluation of their testcross progenies in two selection stages was defined in terms of testcross plot equivalents assuming equal plot sizes in both selection stages. In DHTC, the budget equals $N_1 C_{DH} + N_1 L_1 (1 + C_T) + N_2 L_2 (1 + C_T)$, where C_{DH} refers to the production costs of one DH line and C_T to the production costs of testcross seed for

one plot. In S_1TC-DHTC, the budget equals $N_1C_F + N_1L_1(1 + C_T) + N_{2_F}N_{2_{DH/F}}C_{DH} + N_{2_F}N_{2_{DH/F}}L_2(1 + C_T)$, where C_F refers to the production costs of each S_1 family. All costs are based on actual costs in the maize breeding program of the University of Hohenheim. We assumed $C_{DH} = 1/2$, $C_T = 1/25$, and $C_F = 1/12$. Three budgets were compared with a total of 200, 1,000, and 5,000 testcross plot equivalents per cross.

For DHTC, we assumed the proportions among variance components as $\sigma^2_{DH}:\sigma^2_{DH\times y}:\sigma^2_{DH\times l}:\sigma^2_{DH\times l\times y}:\sigma^2_e = 1 : 0.5 : 0.5 : 1 : 2$ (VC2), where σ^2_{DH} refers to the genotypic variance among testcross progenies of DH lines, $\sigma^2_{DH\times y}$ to the variance of genotype × year interactions, $\sigma^2_{DH\times l}$ to the variance of genotype × location interactions, $\sigma^2_{DH\times l\times y}$ to the variance of genotype × location × year interactions, and σ^2_e to the plot error variance. Two additional scenarios were considered with interactions and error variances being halved (VC1) and doubled (VC3) in comparison with σ^2_{DH}. These ratios were chosen based on combined analyses of variance of grain yield in (1) recent official maize variety performance tests in Germany including early and late germplasm (VC1, Laidig, personal communication), (2) DH populations in maize programs of Central European breeding companies (VC2, Gordillo and Geiger 2004), and (3) official maize variety performance tests of early germplasm in Southwest Germany (VC3, P. Herrmann, unpublished data). Variance components for traits with less complex genetic architecture than yield, e.g., dry matter content, are expected to be close to VC1 or even with smaller non-genetic variances. However, the study focused only on grain yield of maize and, thus, the chosen variance components warrants the inclusion of a wide range of maize breeding populations.

The total genotypic variance among testcross progenies of DH lines from different S_1 families in breeding scheme S_1TC-DHTC was the sum of the genotypic variance among testcross progenies of S_1 families (σ^2_F) plus the genotypic variance among testcross progenies of DH lines within S_1 families ($\sigma^2_{DH/F}$), i.e., $\sigma^2_{DH} = \sigma^2_F + \sigma^2_{DH/F}$. In the absence of epistasis, $\sigma^2_F = \sigma^2_{DH/F} = 0.5\ \sigma^2_{DH}$ for the use of S_1 families and DH lines within S_1 families according to quantitative genetic expectations (Melchinger 1988; Bernardo 2002). In both stages, we assumed that the ratio of σ^2_F or $\sigma^2_{DH/F}$ to corresponding interaction variances was identical to the ratio of σ^2_{DH} to interaction variances described above. However, σ^2_e was assumed to be equal for testcrosses of DH lines and S_1 families. For example, for S_1 families and VC2, we assumed $\sigma^2_F:\sigma^2_{F\times y}:\sigma^2_{F\times l}:\sigma^2_{F\times l\times y}:\sigma^2_e = 0.5:0.25:0.25:0.5:2$, where $\sigma^2_{F\times y}$, $\sigma^2_{F\times l}$, and $\sigma^2_{F\times l\times y}$ refer to the interaction variances of testcross progenies of S_1 families with years, locations, as well as locations × years.

Results

For parameters only marginally affected by varying budget and variance component ratios, representative results were shown for intermediate values of the budget (1,000 testcross plot equivalents) and variance components (VC2). Deviations from these assumptions are explicitly stated. With production costs of one DH line equal to half the cost of one testcross plot ($C_{DH} = 1/2$), maximum selection gain ΔG^* was approximately 10% larger in breeding scheme S_1TC-DHTC than in DHTC (Table 1). For S_1TC-DHTC, the optimum allocation was $L_1^* = 5$ and $L_2^* = 6$ test locations

Table 1 Optimum allocation of test resources maximizing selection gain (ΔG^*) in two-stage selection with evaluation of testcross progenies of (1) DH lines in both stages (breeding scheme DHTC) and (2) S_1 families in the first stage and DH lines within S_1 families in the second stage (breeding schemes S_1TC-DHTC and S_1TC-DHTC$_{fast}$) and its dependence on production costs of DH lines (C_{DH}) assuming a budget of 1,000 testcross plot equivalents, variance components VC2, and four finally selected DH lines. For explanation of abbreviations, see "Materials and methods"

Breeding scheme	C_{DH}	Optimum allocation					$\Delta G^{*,a}$ (%)
		N_1^*	N_2^*		L_1^*	L_2^*	
DHTC	1/2	272	26		2	11	89.7
S_1TC-DHTC	1/2	82	84	$=2 \times 42^b$	5	6	100.0
S_1TC-DHTC$_{fast}$	1/2	53	30	$=3 \times 10^c$	7	11	87.0
DHTC	0	583	42		1	9	92.8
S_1TC-DHTC	0	81	106	$= 2 \times 53$	5	5	100.8
S_1TC-DHTC$_{fast}$	0	138	40	$= 4 \times 10^c$	4	10	92.4
S_1TC-DHTC$_{fast}$	0	73	104	$= 2 \times 52$	6	5	100.8

[a] Relative to ΔG^* in S_1TC-DHTC assuming $C_{DH} = 1/2$

[b] Number of S_1 families × DH lines within S_1 families

[c] With current limitations in DH technique a maximum of 10 DH lines can be produced from a single S_1 plant

in stage one and two, $N_1^* = 82$ S_1 families in the first stage, and $N_{2_F}^* = 2$ S_1 families as well as $N_{2_{DH/F}}^* = 42$ DH lines within each of the two S_1 families in the second stage. In DHTC, N_1^* and L_2^* were larger and N_2^* and L_1^* were smaller in comparison with S_1TC-DHTC. Assuming negligible production costs for DH lines ($C_{DH} = 0$), ΔG^* in S_1TC-DHTC was 8% larger than in DHTC. For $C_{DH} = 0$ compared with $C_{DH} = 1/2$, ΔG^* was increased in S_1TC-DHTC by 1% and DHTC by 3%.

The impact of varying budget and variance component ratios on the optimum allocation and selection gain was hardly affected by the production costs of DH lines (data not shown). Thus, results in Fig. 2 and Table 2 were presented only for $C_{DH} = 1/2$ referring to actual costs in breeding companies most advanced in DH technology (G. Seitz, personal communication). For all considered variance component ratios in S_1TC-DHTC, selection gain ΔG increased strongly up to a maximum and thereafter decreased slightly with increasing $N_{2_{DH/F}}$ at the expense of decreasing N_{2_F} (Fig. 2). Deviations from $N_{2_{DH/F}}^*$ by de- or increasing N_{2_F} led to reductions in selection gain of more than 2%.

Breeding scheme S_1TC-DHTC was superior to DHTC for a large range of budgets and variance components (Table 2). Increasing the budget from 200 to 5,000 testcross plot equivalents in breeding scheme S_1TC-DHTC resulted in a more than eightfold increase in N_1^* and $N_{2_{DH/F}}^*$, in tripled values of L_j^*, and an increase in the maximum selection gain ΔG^* of about 80%. An increased budget for DHTC led to larger increases in N_1^* and L_2^* and smaller increases in L_1^* in comparison with S_1TC-DHTC. A fourfold increase in the non-genetic variance from VC1 to VC3 resulted for S_1TC-DHTC in roughly halved values of N_1^* and $N_{2_{DH/F}}^*$, doubled values of L_j^*, and a reduction in ΔG^* of 30%. Increased non-genetic variances (VC3) had a smaller impact on the optimum number of N_2^* and L_2^* in DHTC than in S_1TC-DHTC. For S_1TC-DHTC, the final selection of one DH line in each of the top four S_1 families instead of selecting four DH lines within the top S_1 family led to an increase in N_1^* and $N_{2_F}^*$ of 30 and 250%, respectively. Furthermore, a slight reduction in L_j^*, and reductions in $N_{2_{DH/F}}^*$ by 70% and ΔG^* by 13% were revealed. The final selection of only one DH line reduced the superiority of S_1TC-DHTC over DHTC. For a budget of 200 field plots, ΔG^* was smaller in S_1TC-DHTC than in DHTC. In addition, the optimum number of DH lines N_2^* and $N_{2_{DH/F}}^*$ in the second stage was reduced in favor of a larger optimum number of test locations L_2^*.

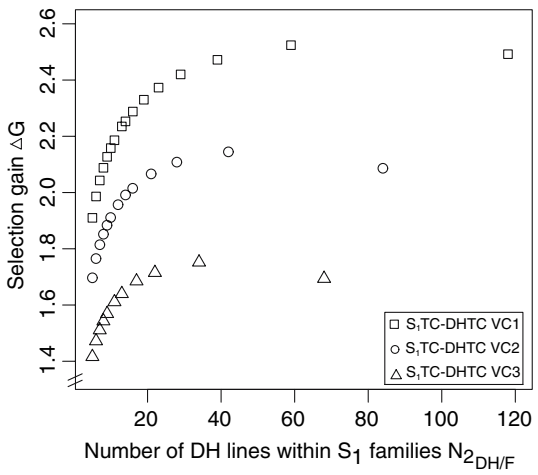

Fig. 2 Selection gain (ΔG) in breeding scheme S_1TC-DHTC as a function of the number of DH lines within S_1 families evaluated in the second stage for varying variance components (VC) assuming a budget of 1,000 testcross plot equivalents, production costs for DH lines of $C_{DH} = 1/2$, and optimum numbers of S_1 families in the first stage and test locations in both stages for the respective VC. Values of ΔG were shown for all possible integer allocation combinations possible for the scenario considered

Discussion

We focused on second-cycle breeding with selection within one cross of two homozygous lines. Therefore, short-term success of different breeding schemes achieved in one breeding cycle was of interest. Comparison among breeding schemes with different length by per-cycle selection gain becomes feasible under the assumption that breeding is a continuous process and every year a new breeding cycle is initiated. Under this assumption, the annually available budget for all breeding cycles running in parallel is equal to the budget available for one entire breeding cycle (Utz 1969). Consequently, we used per-cycle selection gain, which is further referred to as selection gain.

Optimum allocation of test resources

For a given target variable, selection gain is increased by a higher selection intensity and a closer correlation between the phenotypic mean of testcross performance and the target variable (ρ_{x_j}) (cf. Bernardo 2002). We used the term selection intensity in our multi-stage selection approach in a more general sense than its strict definition for one-stage selection, where it refers to the standardized selection differential (cf. Falconer and Mackay 1996; Wricke and Weber 1986). Selection intensity can be increased by increasing the number of test candidates and/or decreasing the number of selected test candidates. The correlation between the phenotypic mean of testcross performance and the target variable is increased with a higher heritability. Heritability can be increased by larger numbers of test locations, years, and replications in performance trials. In both breeding schemes, variation in the budget had a stronger impact on the number of test candidates than the

Table 2 Optimum allocation of test resources maximizing selection gain (ΔG^*) in two-stage selection with evaluation of testcross progenies of (1) DH lines in both stages (breeding scheme DHTC) and (2) S_1 families in the first stage and DH lines within S_1 families in the second stage (breeding scheme S_1TC-DHTC) and its dependence on the budget, variance components, and number of finally selected DH lines (N_f) assuming production costs for DH lines of C_{DH} = 1/2. For explanation of abbreviations, see "Materials and methods"

Assumptions			Optimum allocation					ΔG^*
Budget	Variance components[a]	N_f	N_1^*	N_2^*		L_1^*	L_2^*	
Breeding scheme DHTC								
200	VC2	4	79	15		1	5	1.375
5,000	VC2	4	1,422	64		2	20	2.412
1,000	VC1	4	460	35		1	8	2.219
1,000	VC2	4	272	26		2	11	1.924
1,000	VC3	4	252	28		2	12	1.605
200	VC2	1	53	6		2	10	1.848
1,000	VC2	1	286	14		2	18	2.348
5,000	VC2	1	1,463	38		2	31	2.780
Breeding scheme S_1TC-DHTC								
200	VC2	1×4[b]	24	34	$=2 \times 17$[c]	3	3	1.527
5,000	VC2	1×4	264	282	$=2 \times 141$	8	9	2.725
1,000	VC1	1×4	106	118	$=2 \times 59$	4	4	2.524
1,000	VC2	1×4	82	84	$=2 \times 42$	5	6	2.145
1,000	VC3	1×4	56	68	$=2 \times 34$	8	7	1.752
1,000	VC2	2×2	82	84	$=3 \times 28$	5	6	2.032
1,000	VC2	4×1	104	98	$=7 \times 14$	4	5	1.902
200	VC2	1×1	30	18	$=2 \times 9$	3	5	1.812
1,000	VC2	1×1	81	58	$=2 \times 29$	5	9	2.417
5,000	VC2	1×1	278	190	$=2 \times 95$	8	13	2.974

[a] VC1 = σ^2_{DH}:$\sigma^2_{DH\times y}$:$\sigma^2_{DH\times l}$:$\sigma^2_{DH\times l\times y}$:$\sigma^2_e$ = 1:0.25:0.25:0.5:1; VC2 = 1:0.5:0.5:1:2; VC3 = 1:1:1:2:4

[b] Number of finally selected S_1 families × DH lines within selected S_1 families

[c] Number of S_1 families × DH lines within S_1 families

number of test locations (Table 2), thus, affecting mainly selection intensity and, to a smaller extent, heritability. With larger non-genetic variance, heritability is strongly reduced. This can be counterbalanced by a larger number of test locations. However, for a given budget, this requires a simultaneous reduction in the number of test candidates, thus reducing selection gain considerably (Table 2). Smaller number of finally selected DH lines resulted in a decreased number of test candidates and an increased number of test locations in the second stage increasing both the selection intensity and heritability.

In DHTC, the optimum allocation of test resources involved evaluation of (1) a large number of DH lines in a small number of test locations in the first stage and (2) a small number of the selected DH lines in a large number of test locations in the second stage. Thus, a high selection intensity in the first stage is combined with a high heritability in the second stage. Thereby, selection gain was maximized by using about 70% of the budget for the initial screening of DH lines. These findings are in accordance with investigations of Utz (1969) on the optimum allocation of test resources in multi-stage selection.

In contrast, the optimum allocation of test resources in S_1TC-DHTC involved similar numbers of test locations and test candidates in both stages. Consequently, comparable parts of the budget were spent in both stages. This is due to the different types of test candidates in S_1TC-DHTC, with S_1 families in the first stage and DH lines within S_1 families in the second stage, where large number of test candidates and test locations are required in both stages. This compromise resulted in a smaller optimum number of test candidates in the first stage and test locations in the second stage in comparison with DHTC.

The optimum allocation of test resources in S_1TC-DHTC possesses two advantages over DHTC. First, a larger part of the budget is used for the evaluation of the more promising material in the second stage of S_1TC-DHTC. The possibility to use also a larger part of the budget in the second stage of DHTC is limited due to large reductions in selection gain. Second, the smaller optimum number of test locations in

S_1TC-DHTC compared with DHTC simplifies the logistics of breeding programs.

In S_1TC-DHTC, the optimum number of test candidates in the second stage was two S_1 families and a large number of DH lines within each of the two S_1 families for all budgets and variance components considered (Table 2; Fig. 2). For a small budget and small non-genetic variance, this is in accordance with results for self-pollinated crops (Utz 1981; Weber 1981). These findings can be explained by the different types of test candidates in both stages of S_1TC-DHTC and the consequences for the available amount of genetic variance. In the first stage, selection is made among S_1 families with genetic variance σ^2_F. In the second stage, new genetic variance is released due to DH lines within S_1 families with $\sigma^2_F = \sigma^2_{DH/F}$. Owing to the selection among S_1 families in the first stage, the variance among S_1 families in the second stage is smaller than $\sigma^2_{DH/F}$, favoring selection among DH lines.

Alternatively to the final selection of four DH lines from the top S_1 family in S_1TC-DHTC, one could finally select one DH line from each of the top four S_1 families. Consequently, $N_{2_F} \geq 4$ is required, but maximum selection gain ΔG^* is reduced by more than 10%, even though the total number of finally selected DH lines has not been changed (Table 2). An evaluation of varying numbers of DH lines within S_1 families according to the performance level of the S_1 family in the first stage and selecting the best DH line across all S_1 families tested in the second stage might increase $N^*_{2_F}$ and ΔG^*. However, to our knowledge no analytical results are available in the literature to cope with these more general situations and, hence, further research is warranted. Monte Carlo simulations may be a promising alternative for further investigations on the optimum number of families and lines within families.

Response curves of selection gain as a function of the number of DH lines within S_1 families were flat, close to the maximum (Fig. 2). However, deviations from the optimum number of DH lines within S_1 families by increasing N_{2_F} reduced the selection gain by more than 2%. This is in contrast to differences below 1% in the selection gain as a function of the number of (1) L_j in both breeding schemes (data not shown) and (2) N_j in DHTC (Longin et al. 2006). The difference may be due to the larger impact of N_{2_F} on the selection intensity and heritability in comparison with that of L_j, N_1, and N_2. In conclusion, with early testing prior to production of DH lines, an optimum allocation of the number of families is of crucial importance for maximizing the selection gain.

Relative efficiency of breeding schemes

For the final selection of four DH lines, maximum selection gain ΔG^* was largest in S_1TC-DHTC, with an advantage of about 10% over DHTC for all considered budgets and variance components (Tables 1, 2). A higher selection intensity and heritability are feasible in the first stage of S_1TC-DHTC compared with DHTC, which is due to the different amounts of genetic variance available in both breeding schemes. In DHTC, the total genetic variance σ^2_{DH} is available from the very beginning of the selection process. The genetic variance among the remaining DH lines in the second stage decreases with a smaller number of DH lines selected in the first stage. In S_1TC-DHTC, the same applies to σ^2_F. However, the newly released genetic variance due to DH lines within S_1 families in the second stage of S_1TC-DHTC with $\sigma^2_{DH/F} = \sigma^2_F = 0.5\ \sigma^2_{DH}$ sums up with the genetic variance among the remaining S_1 families. This allows a high selection intensity in the first stage of S_1TC-DHTC without exhausting the genetic variance for the second stage. Thus, the chances for obtaining superior DH lines by segregation within superior S_1 families far outweighs the smaller number of initial test candidates in comparison with DHTC and allows the use of a larger number of test locations in the first stage. The reduced heritability in the second stage of S_1TC-DHTC compared with DHTC is counterbalanced by a higher selection intensity due to the large number of test candidates in the second stage of S_1TC-DHTC. Consequently, early testing prior to production of DH lines largely increases selection gain, underpinning its importance for successful hybrid maize breeding.

For the selection of only one DH line, the relative efficiency of S_1TC-DHTC was considerably decreased as compared with DHTC. In the extreme case of a budget of 200 field plots, S_1TC-DHTC resulted in a smaller maximum selection gain ΔG^* than for DHTC (Table 2). This can be explained by a strong reduction in the number of selected DH lines in the first stage of DHTC, which increased the selection intensity. In contrast, the already very small number of selected S_1 families in the first stage of S_1TC-DHTC could not be reduced any further. In addition, a sufficiently large number of test locations in the second stage is crucial for selecting the very best DH line, favoring DHTC. Nevertheless, S_1TC-DHTC was superior to DHTC for a large range of scenarios with the only exception for a combination of a very small number of finally selected DH lines and a very small budget.

Limitations in DH technique affect the efficiency of breeding schemes

Routine application of in-vivo haploid induction in hybrid maize breeding requires specific skills and equipment for chromosome doubling, transplanting of up-regulated plants in the field, as well as for raising and selfing of the up-regulated plants (cf. Röber et al. 2005). As these activities

are rather cost-intensive, we assumed that the costs for the production of one DH line are equal to half the costs of one testcross plot. This assumption corresponds to the actual costs for production of DH lines in breeding companies most advanced in the DH technique (G. Seitz, personal communication). In addition, the production of DH lines from a single plant is limited due to current rates of haploid induction (10–15%) and chromosome doubling (20–30%, cf. Röber et al. 2005). Thus, from individual S_1 ears with approximately 250 kernels, a maximum of 10 DH lines can be produced.

Breeding scheme S_1TC-DHTC has a longer cycle length than DHTC. The length of S_1TC-DHTC could be shortened by using individual S_1 plants as (1) males for production of testcross seed and in parallel as (2) females in crosses with the inducer. Furthermore, chromosome doubling must be performed simultaneously with early testing (S_1TC-DHTC$_{fast}$, Fig. 1). Therefore, test candidates are either S_1 single plants or DH lines derived from individual S_1 plants evaluated for their testcross performance.

With current costs and rates of success for production of DHs, maximum selection gain ΔG^* in S_1TC-DHTC$_{fast}$ was about 13% smaller than that in S_1TC-DHTC (Table 1). This can be explained by the necessity of producing DH lines from all S_1 plants of the first stage in S_1TC-DHTC$_{fast}$, which consumed about one third of the budget under current costs of DH production. Thus, the number of S_1 plants, which could be evaluated in the first stage of S_1TC-DHTC$_{fast}$, is limited. Furthermore, the number of DH lines, which can actually be produced per selected S_1 plant, is far below the theoretical optimum allocation of S_1TC-DHTC$_{fast}$, if there were no limitations in the DH technique (Table 1). Thus, substantial increases in the haploid induction rate and chromosome doubling rate as well as reductions in the costs for chromosome doubling and recovering of up-regulated plants are required to enable the use of an optimally allocated breeding scheme S_1TC-DHTC$_{fast}$.

Nevertheless, if more than 50 DH lines could be produced per individual S_1 plant at negligible costs, selection gain would most strongly be increased in breeding scheme S_1TC-DHTC$_{fast}$, resulting in a similar selection gain as for S_1TC-DHTC (Table 1). Thus, the high selection gain for breeding schemes with early testing prior to DH production could be combined with a cycle length similar to DHTC. Crossing DH lines with the tester already in the D_2 generation and performing per se and testcross evaluation in parallel may be another appealing alternative to shorten the breeding scheme. However, consideration of per se and testcross performance must be based on index selection, requiring more research on the optimum type of index and appropriate economic weights of the traits.

In conclusion, early testing prior to production of DH lines is very promising in hybrid maize breeding. However, its full potential can be exploited only by choice and optimization of an appropriate breeding scheme. With current limitations in the DH technique, S_1TC-DHTC seems most appealing for maximizing selection gain unless the available budget is extremely low. In order to take more advantage of early testing prior to DH production, enormous improvements in the DH technique are required to allow for an efficient use of S_1TC-DHTC$_{fast}$. Thus, time for inbred line development could be shortened and early testing prior to production of DH lines would become very attractive in hybrid maize breeding.

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