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ORIGINAL PAPER

Hybrid maize breeding with doubled haploids: II. Optimum type and number of testers in two-stage selection for general combining ability

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Abstract Optimum allocation of test resources is of crucial importance for the efficiency of breeding programs. Our objectives were to (1) determine the optimum allocation of the number of lines, test locations, as well as number and type of testers in hybrid maize breeding using doubled haploids with two breeding strategies for improvement of general combining ability (GCA), (2) compare the maximum selection gain (ΔG) achievable under both strategies, and (3) give recommendations for the optimum implementation of doubled haploids in commercial hybrid maize breeding. We calculated ΔG by numerical integration for two two-stage selection strategies with evaluation of (1) testcross performance in both stages (BS1) or (2) line per se performance in the first stage followed by testcross performance in the second stage (BS2). Different assumptions were made regarding the budget, variance components (VCs), and the correlation between line per se performance and GCA. Selection gain for GCA increased with a broader genetic base of the tester. Hence, testers combining a large number of divergent lines are advantageous. However, in applied breeding programs, the use of single- or double-cross testers in the first and inbred testers in the second selection stage may be a good compromise between theoretical and practical requirements. With a correlation between line per se performance and GCA of 0.50, ΔG for BS1 is about 5% higher than for BS2, if an economic weight of line per se performance is neglected. With increasing economic weight of line per se performance, relative efficiency of BS2 increased rapidly resulting in a superiority of BS2 over BS1 already for an economic weight for line per se performance larger than 0.1. Considering the importance of an economic seed production, an economic weight larger than 0.1 seems realistic indicating the necessity of separate breeding strategies for seed and pollen parent heterotic groups.

Introduction

Inbred line development by doubled haploid technology is currently adopted as a routine method in commercial hybrid maize breeding programs in North America (Seitz 2005) and Europe (Schmidt 2004). The use of doubled haploids offers the possibility to evaluate potential hybrid cultivars from the very beginning of the selection process. With a large number of lines in each heterotic group, the number of factorial crosses among them becomes rapidly prohibitive. Hence, new lines are usually tested in combination with one or several testers to evaluate their general combining ability (GCA, Hallauer et al. 1988). Specific combining ability (SCA) acts as a masking effect in determining GCA. Its influence can be reduced by using genetically broad testers and/or an increased number of testers (Hallauer and Miranda 1981). However, choice of type and number of testers also affect the optimum alloca-

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tion of test resources. As plant breeders have only a fixed budget available, they must find a compromise between (1) the number of initial lines to be tested and (2) the intensity of their testing as determined by the number of testers, test locations, years, and replications.

A selection strategy may involve one or several stages of selection. With multi-stage selection, the initial population of lines is evaluated in one year and based on the test results, a superior subset is selected for further evaluation and selection in subsequent year(s). Considering one-stage selection for GCA between inbred lines in maize, Federer and Sprague (1947) and Keller (1949) investigated the optimum allocation of the number of testers, lines, and replications. They concluded that for a fixed budget, the selection gain (ΔG) was increased by increasing the number of testers even at the expense of the number of lines and replications. Schnell (1996) extended these investigations to two-stage selection for early testing in maize considering also the number of test locations. For a fixed budget corresponding to 1,200 testcross plots, he suggested to use one tester in the first and seven testers in the second stage of selection. However, simplified genetic models and covariances were used for calculation of selection gain. In addition, a larger genetic variance is expected with doubled haploids in comparison with segregating lines.

Several experimental studies examined the impact of testers with narrow versus broad genetic base (for review see Hallauer and Miranda 1981). To our knowledge, investigations on the type of testers within the context of optimum allocated test resources have not been reported in the literature. An economic production of hybrid seed requires an acceptable line per se performance of the seed parent. For this and other reasons, evaluation of line per se performance may be an interesting alternative to testcross evaluations in the first selection stage. An assessment of this alternative selection strategy based on line per se performance and testcross performance is not available in maize.

We calculated the maximum ΔG by numerical integration to optimize the allocation of test resources in hybrid maize breeding using doubled haploids under two two-stage selection strategies with evaluation of (1) testcross performance in both stages, or (2) line per se performance in the first stage followed by testcross performance in the second stage. Different assumptions were made regarding the budget, variance components, correlation between line per se performance and GCA, and economic weight of line per se performance and GCA. Our objectives were to (1) determine the optimum allocation of the number of lines, test locations, as well as number and type of testers for each strategy, (2) compare the maximum ΔG achievable under both strategies, and (3) give recommendations for the optimum implementation of doubled haploids in commercial hybrid maize breeding.

Materials and methods

Breeding strategies

Doubled haploid lines generated from several F_1 crosses via in vivo haploid induction are evaluated for line per se performance and/or testcross performance. The target variable is GCA or a selection index of line per se performance and GCA. We investigated two strategies to evaluate the doubled haploid lines. In both strategies, the lines are evaluated in two consecutive years. In the first year, N_1 lines are evaluated and a subset N_2 of the most superior lines are selected for evaluation in the second year. The five best doubled haploid lines are selected after these two selection stages to give opportunity to further selection also on SCA. Breeding strategy one (BS1) represents two-stage selection based on testcross evaluation of N_j lines with T_j testers at L_j locations in stage j ($j = 1, 2$). Tester number and tester type can vary in both stages. The investigated tester types were inbred lines, single-crosses, double-crosses, or double-double crosses. In breeding strategy two (BS2), the lines are evaluated for line per se performance in the first stage and for testcross performance with T_2 testers in the second stage at L_j locations, respectively. Without restrictions on L_j, ΔG is maximum for one replication per location (Sprague and Federer 1951; Utz 1969; Melchinger et al. 2005). For this reason, we set the number of replications to one for all calculations. An overview of the notation used throughout this treatise is given in Table 1.

Calculation of selection gain

Our target variable was the selection index $H = a_{\mathrm{GCA}}\, g_{\mathrm{GCA}} + a_{\mathrm{LP}}\, g_{\mathrm{LP}}$ (Cochran 1951), where a refers to the economic weight and g to the genotypic effect of GCA and line per se performance (LP), respectively. We used mostly $a_{\mathrm{LP}} = 0$ restricting the target variable to GCA. For comparison, we also calculated $a_{\mathrm{LP}} = 0.1$ and 0.2 with $a_{\mathrm{GCA}} = 1 - a_{\mathrm{LP}}$. The selection criterion in the second stage is an optimum index of the phenotypic means of the lines evaluated in the first and second stage with $I = b_1 x_1 + b_2 x_2$, where x refers to the phenotypic mean and b to its weight in stage one or two.

Table 1 Notation used in this treatise

a_{LP}, a_{GCA}	Economic weight of line per se performance (LP) and GCA of the doubled haploid lines
h^2	Heritability on an entry-mean basis
ρ(LP, GCA)	Genetic correlation between line per se performance and GCA
x_j	Phenotypic mean in stage j with corresponding variance $\sigma^2_{x_j}$
BS1	Breeding strategy one representing two-stage selection with evaluation of testcross performance in both stages
BS2	Breeding strategy two representing two-stage selection with evaluation of line per se performance in the first stage followed by testcross performance in the second stage
ΔG	Selection gain in two-stage selection, where the second selection is based on an optimum index combining the phenotypic means of both selection stages
ΔG^*	Value of ΔG at the corresponding optimum allocation (T_j^*, L_j^*, N_j^*)
M_j	Number of unrelated inbred lines combined in a single tester in stage j
T_j, L_j, N_j,	Number of testers, locations, and lines in stage j in performance trials
T_j^*, L_j^*, N_j^*	Optimum number of testers, locations, and lines maximizing selection gain in the set of admissible allocations
T_c, L_c	Number of T and L common to both selection stages
VC	VCs, for details see Table 2

Calculation of ΔG is based on the well-known formula of Cochran (1951) with uni- and bivariate normal integrals for selected fractions and the square root of heritabilities of x_1 and x_2. For a detailed description of the calculation of ΔG, the reader is referred to Wricke and Weber (1986). For BS1, heritability is calculated by $h^2_{x_j} = \sigma^2_{\text{GCA}}/\sigma^2_{x_j}$ with

$$\sigma^2_{x_j} = \sigma^2_{\text{GCA}} + \sigma^2_{\text{GCA}\times y} + \frac{\sigma^2_{\text{GCA}\times l}}{L_j} + \frac{\sigma^2_{\text{GCA}\times l\times y}}{L_j} + \frac{\sigma^2_{\text{SCA}}}{T_jM_j} + \frac{\sigma^2_{\text{SCA}\times y}}{T_jM_j} + \frac{\sigma^2_{\text{SCA}\times l}}{T_jM_jL_j} + \frac{\sigma^2_{\text{SCA}\times l\times y}}{T_jM_jL_j} + \frac{\sigma^2_e}{T_jL_j}, \tag{1}$$

where σ^2_{GCA} and σ^2_{SCA} refer to the variance of GCA and SCA effects, $\sigma^2_{\text{GCA}\times y}$ to the variance of GCA × year interactions, $\sigma^2_{\text{GCA}\times l}$ to the variance of GCA × location interactions, $\sigma^2_{\text{GCA}\times l\times y}$ to the variance of GCA × location × year interactions, $\sigma^2_{\text{SCA}\times y}$, $\sigma^2_{\text{SCA}\times l}$, and $\sigma^2_{\text{SCA}\times l\times y}$ to the respective interactions with SCA, as well as σ^2_e to the variance of the plot error. Tester type is defined by M_j, the number of inbred lines combined in a tester. We assumed an equal contribution of the gametes of the inbred lines combined in the tester to the testcross progenies, with $M_j = 1, 2, 4, 8$ referring to an inbred line, a single-cross, a double-cross, or a double-double cross tester, respectively. The covariance between testcross means of doubled haploid lines evaluated in two years was calculated as

$$\text{Cov}(x_1, x_2) = \sigma^2_{\text{GCA}} + \frac{L_c\sigma^2_{\text{GCA}\times l}}{L_1L_2} + \frac{T_c\sigma^2_{\text{SCA}}}{T_1M_1T_2M_2} + \frac{T_cL_c\sigma^2_{\text{SCA}\times l}}{T_1M_1L_1T_2M_2L_2}, \tag{2}$$

where L_c and T_c refer to the number of locations and tester lines ($T_j \times M_j$) common to both selection stages. For BS2, $h^2_{x_1} = \sigma^2_{\text{Line}}/\sigma^2_{x_1}$ with

$$\sigma^2_{x_1} = \sigma^2_{\text{Line}} + \sigma^2_{\text{Line}\times y} + \frac{\sigma^2_{\text{Line}\times l}}{L_j} + \frac{\sigma^2_{\text{Line}\times l\times y}}{L_j} + \frac{\sigma^2_e}{L_j}, \tag{3}$$

where σ^2_{Line} refers to the genetic variance among lines, $\sigma^2_{\text{Line}\times y}$ to the variance of line × year interactions, $\sigma^2_{\text{Line}\times l}$ to the variance of line × location interactions, $\sigma^2_{\text{Line}\times l\times y}$ to the variance of line × location × year interactions, as well as σ^2_e to the variance of the plot error. In the second stage, heritability was calculated as for BS1. The covariance between line and testcross means of doubled haploid lines in the two years was calculated as

$$\text{Cov}(x_1, x_2) = \rho(\text{LP}, \text{GCA})\sigma_{\text{Line}}\sigma_{\text{GCA}} + \frac{L_c\text{Cov}(\text{Line}\times l, \text{GCA}\times l)}{L_1L_2}, \tag{4}$$

where ρ(LP, GCA) refers to the genetic correlation between line per se performance and GCA. We assumed Cov(Line × l, GCA × l) = 0, because experimental values are lacking and a small value is expected from theory. The extension of the formulas for ΔG expected for an optimum index in the second stage and $a_{\text{LP}} > 0$ is straightforward in multivariate selection (Baker 1986).

Optimum allocation of resources

The allocation of test resources refers to triples (T_j, L_j, N_j) for each tester type in all stages j. An element (T_j^*, L_j^*, N_j^*) is denoted as an optimum allocation if it

Table 2 Variance components used in this study with σ^2_{GCA} and σ^2_{SCA} referring to the variance of general (GCA) and specific combining ability (SCA) effects, $\sigma^2_{\mathrm{GCA} \times y}$ to the variance of GCA × year interactions, $\sigma^2_{\mathrm{GCA} \times l}$ to the variance of GCA × location interactions, $\sigma^2_{\mathrm{GCA} \times l \times y}$ to the variance of GCA × location × year interactions, $\sigma^2_{\mathrm{SCA} \times y}$, $\sigma^2_{\mathrm{SCA} \times l}$, and $\sigma^2_{\mathrm{SCA} \times l \times y}$ to the respective interactions with SCA, σ^2_e to the variance of the plot error, σ^2_{Line} to the genetic variance among lines per se, $\sigma^2_{\mathrm{Line} \times y}$ to variance of line × year interactions, $\sigma^2_{\mathrm{Line} \times l}$ to the variance of line × location interactions, as well as $\sigma^2_{\mathrm{Line} \times l \times y}$ to the variance of line × location × year interactions

Testcross performance

Acronym	Variance components									
	$\sigma^2_{\mathrm{SCA}}/\sigma^2_{\mathrm{GCA}}$	σ^2_{GCA}	$\sigma^2_{\mathrm{GCA} \times y}$	$\sigma^2_{\mathrm{GCA} \times l}$	$\sigma^2_{\mathrm{GCA} \times l \times y}$	σ^2_{SCA}	$\sigma^2_{\mathrm{SCA} \times y}$	$\sigma^2_{\mathrm{SCA} \times l}$	$\sigma^2_{\mathrm{SCA} \times l \times y}$	σ^2_e
VC1	1/4	0.40	0.20	0.20	0.40	0.10	0.05	0.05	0.10	1.80
VC2.1	1/2	0.40	0.10	0.10	0.20	0.20	0.05	0.05	0.10	1.00
VC2.2	1/2	0.40	0.20	0.20	0.40	0.20	0.10	0.10	0.20	2.00
VC2.3	1/2	0.40	0.40	0.40	0.80	0.20	0.20	0.20	0.40	4.00
VC3	1/1	0.40	0.20	0.20	0.40	0.40	0.20	0.20	0.40	2.40

Line per se performance

		Variance components								
		σ^2_{Line}	$\sigma^2_{\mathrm{Line} \times y}$	$\sigma^2_{\mathrm{Line} \times l}$	$\sigma^2_{\mathrm{Line} \times l \times y}$	σ^2_e	–	–	–	–
VC4	–	1	0.15	0.15	0.50	0.50	–	–	–	–
VC5	–	1	0.30	0.30	1.00	1.00	–	–	–	–
VC6	–	1	0.60	0.60	2.00	2.00	–	–	–	–

maximizes ΔG in the set of admissible allocations, which are valid for the budget, variance components, and tester type considered. The value of ΔG at its corresponding optimum allocation (T_j^*, L_j^*, N_j^*) was denoted as ΔG^*. The optimum allocation of test resources for each scenario was obtained by a grid search in the space of admissible resource allocations by increasing N_1 by one between its minimum and maximum possible value under the allocation considered.

Economic frame and quantitative-genetic parameters

A fixed total budget for (1) producing the doubled haploid lines and (2) evaluating their testcross progenies in two selection stages was defined in terms of testcross plot equivalents as $N_1\ C + N_1T_1L_1 + N_2T_2L_2$, assuming equal plot sizes in all selection stages. Therein, the production cost C of one doubled haploid line was assumed to equal half the cost of one testcross plot equivalent ($C = 0.5$), corresponding to the actual costs of doubled haploid production in breeding companies most advanced in the doubled haploid technique (G. Seitz, personal communication). We compared three budgets with 500, 1,000, and 5,000 testcross plot equivalents. We assumed that each tester is evaluated at each location. Alternatively, we considered that each tester × line combination is evaluated only in a single location. With that assumption, T_j L_j is reduced to T_j in Eqs. 1, 2 and the calculation of the budget.

We determined the optimum allocation for different scenarios of variance component for line per se performance and testcross performance (Table 2). These variance components were chosen based on combined analyses of variance in testcrosses of doubled haploid populations in commercial breeding programs and in elite germplasm of the maize breeding program of the University of Hohenheim (Longin et al. 2006a; Schrag et al. 2006). In addition, variance components were varied to cover a wide range of scenarios. The reference scenarios VC2.2 for testcross performance and VC5 for line per se performance resulted in heritabilities on a plot basis of 0.11 and 0.28, respectively. The larger h^2 for line per se performance in comparison with testcross performance is in accordance with results of experimental studies (Seitz 1989; Gallais 1997; Mihaljevic et al. 2005). This is due to similar non-genetic variances but larger genetic variances for line per se performance than for testcross performance. We investigated three assumptions concerning the genetic correlation between line per se performance and GCA with $\rho(\mathrm{LP, GCA}) = 0.25$, 0.50, and 0.75, which were based on results published by Mihaljevic et al. (2005) and Weiss (1981).

Results

For all parameters being only marginally affected by varying budget and variance components, representative results were presented for intermediate values of the budget (1,000) and variance components (VC2.2). Deviations from these assumptions are explicitly stated. A fourfold increase in the ratio $\sigma^2{}_{\mathrm{SCA}}/\sigma^2_{\mathrm{GCA}}$ from VC1 to VC3 resulted in an approximately doubled optimum number T_2^*, a 50% reduction in L_2^*, slightly decreased N_1^*, and a reduction in ΔG^* of more than 7% (Table 3). For a given ratio $\sigma^2{}_{\mathrm{SCA}}/\sigma^2{}_{\mathrm{GCA}}$, the use of double-double cross instead of inbred testers resulted in a substantial reduction in T_2^* and a parallel increase in L_2^*, a minor increase in N_1^*, and an increase in ΔG^* of at least 6%. Restricting the tester type in the second stage to inbreds resulted in fairly stable values of T_j^* and L_j^* for all tester types. However, N_1^* decreased with the use of genetically broad testers in the first stage. In addition, the possibility of using genetically broad testers only in the first stage reduced their superiority over inbred testers in comparison with non-restricted tester types in both stages.

Further results were presented for single-cross testers in the first stage and inbred testers in the second stage, because these tester types are most commonly used in applied maize breeding programs. With increasing L_2 or T_2, ΔG increased strongly up to a maximum and decreased thereafter (Fig. 1). In the vicinity of the maximum, all response curves of ΔG were flat for varying values of L_2, T_1, and T_2. The optimum number L_2^* depended strongly on T_2 with smaller values of L_2^* being obtained with larger values of T_2 (Fig. 1a). The optimum number T_1^* was always one (Fig. 1b). For $T_1 > 1$, the reduction in ΔG depended on the ratio σ^2_{SCA} / σ^2_{GCA} with a bigger loss for smaller values of σ^2_{SCA}.

The impact of varying budget and variance components on the optimum allocation and ΔG was hardly affected by the ratio $\sigma^2_{\mathrm{SCA}}/\sigma^2_{\mathrm{GCA}}$. Thus, results were presented only for $\sigma^2_{\mathrm{SCA}}/\sigma^2_{\mathrm{GCA}} = 1/2$. In both breeding strategies, increasing the budget from 500 to 5,000 testcross plot equivalents resulted in a more than sixfold increase in N_1^*, approximately doubled values of T_2^*, L_2^*, and N_2^*, as well as a 50% higher ΔG^* (Table 4). For BS1, a fourfold increase in the non-genetic variance from VC2.1 to VC2.3 resulted in (1) an increase in L_j^* of at least 50%, (2) a decrease in N_1^* of 30%, (3) a slight reduction in T_2^*, and (4) a reduction in ΔG^* of more than 30%. For BS2, a fourfold increase in the non-genetic variance of the first selection stage from VC4 to VC6 had only a minor effect on T_2^*, L_1^*, and L_2^*, but resulted in decreased N_1^*, increased N_2^*, and a 6% reduction in ΔG^*. In BS1, evaluating each tester × line combination only at a single location resulted in (1) doubled T_1^*, (2) tripled T_2^* and L_2^*, and (3) an increase of 6% in ΔG^*. Similar results were obtained for BS2 (data not shown). With increasing ρ(LP, GCA) in BS2, the optimum number of N_2^* was approximately halved,

Table 3 Optimum allocation of test resources in two-stage selection for GCA of doubled haploid lines for maximizing selection gain (ΔG^*) with several ratios of $\sigma^2_{\mathrm{SCA}}/\sigma^2_{\mathrm{GCA}}$ and their dependence on the tester type assuming a budget of 1,000 testcross plot equivalents and $T_c = \min(T_1 \times M_1, T_2 \times M_2)$. For explanation of abbreviations, see Table 1

Variance components	Tester type		Optimum allocation						
	Selection stage 1	Selection stage 2	T_1^*	T_2^*	L_1^*	L_2^*	N_1^*	N_2^*	ΔG^*
VC1	Inbred	Inbred	1	2	2	7	247	27.3	1.010
	Single-cross	Single-cross	1	1	2	12	258	29.6	1.038
	Double-cross	Double-cross	1	1	2	12	256	30.0	1.061
	Double-double cross	Double-double cross	1	1	2	12	255	30.2	1.073
	Single-cross	Inbred	1	2	2	7	252	26.4	1.019
	Double-cross	Inbred	1	2	2	7	253	26.3	1.029
	Double-double cross	Inbred	1	2	3	7	200	21.4	1.034
VC2.2	Inbred	Inbred	1	3	2	5	238	27.0	0.956
	Single-cross	Single-cross	1	2	2	7	244	27.9	0.998
	Double-cross	Double-cross	1	1	2	12	255	30.2	1.025
	Double-double cross	Double-double cross	1	1	2	12	253	30.6	1.047
	Single-cross	Inbred	1	3	2	5	246	25.7	0.972
	Double-cross	Inbred	1	2	3	7	201	21.2	0.985
	Double-double cross	Inbred	1	2	3	7	201	21.2	0.997
VC3	Inbred	Inbred	1	4	2	4	224	27.5	0.882
	Single-cross	Single-cross	1	3	2	5	233	27.8	0.937
	Double-cross	Double-cross	1	2	2	7	239	28.8	0.976
	Double-double cross	Double-double cross	1	1	2	13	244	30.0	1.001
	Single-cross	Inbred	1	4	2	4	236	25.6	0.905
	Double-cross	Inbred	1	5	3	4	182	18.2	0.921
	Double-double cross	Inbred	1	3	3	5	198	20.5	0.940

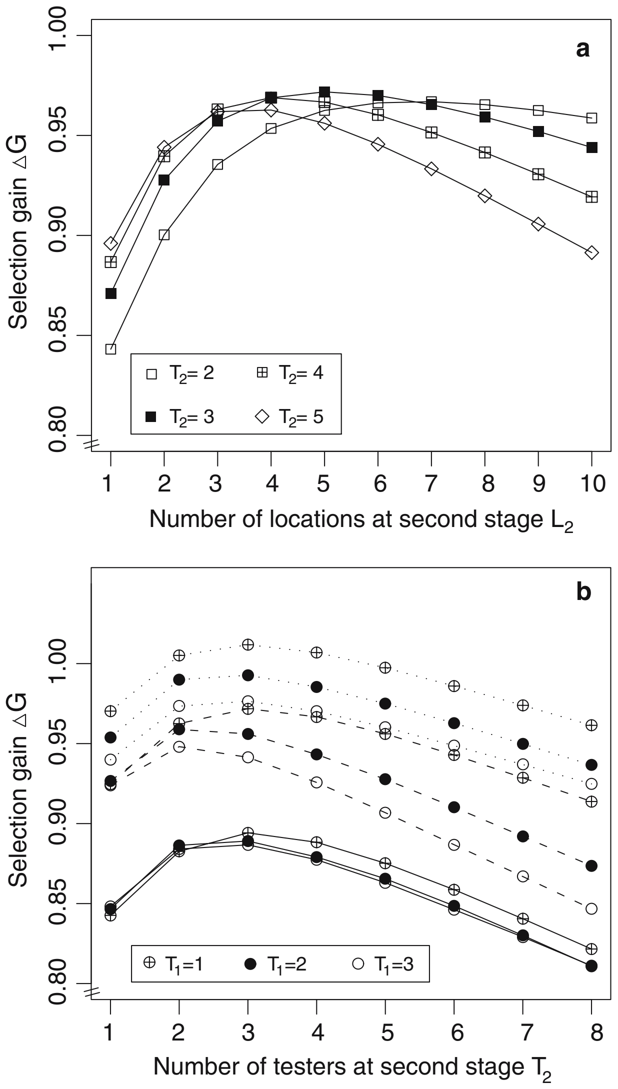

Fig. 1 Selection gain (ΔG) in two-stage selection for GCA as a function of **a** the number of test locations and inbred testers in the second stage, assuming T_1^* and L_1^*, and **b** the number of inbred testers in the second stage for different numbers of single-cross testers in the first stage and ratios of $\sigma^2_{\mathrm{SCA}}/\sigma^2_{\mathrm{GCA}} = 1/4$ (*dotted lines*), 1/2 (*dashed lines*), and 1 (*solid lines*), assuming L_1^* and L_2^* for each scenario. In both figures, a budget of 1,000 testcross plot equivalents and variance components VC2.2 were assumed. For explanation of abbreviations, see Table 1

T_2^*, L_1^*, and N_1^* were affected only slightly, and ΔG^* increased more than 19%.

Discussion

Selection gain is the most widely used criterion to optimize selection strategies. Selection theory was developed by assuming an infinite sample size, although populations of medium size are used commonly in plant breeding (Cochran 1951; Hanson and Brim 1963; Utz 1969; Tomerius 2001; Grüneberg et al. 2004). This assumption simplifies the calculations considerably and results only in marginally inflated ΔG and similar optimum allocation of test resources compared to finite sample sizes (Cochran 1951; Finney 1966; Utz 1969; Longin et al. 2006b).

Optimum use of resources is primarily a matter of heritability

We used one replication per location, which maximizes ΔG if the number of locations is unrestricted (Sprague and Federer 1951; Utz 1969; Melchinger et al. 2005). For instance, superiority in ΔG for one replication compared with two replications increased from 1.5% for $L_j = 1$ towards more than 5% for optimum L_j^* (data not shown). This can be explained by the fact that heritability is more increased by increasing L_j and/or T_j than by an increasing number of replications.

The use of different locations ($L_c = 0$) and tester lines ($T_c = 0$) either as inbred tester or in combination as single-crosses, double-crosses, or double-double crosses in both stages increased ΔG (data not shown). This is due to the reduction of the error part of the covariance between phenotypic means of the stages (Eqs. 2, 4). However, differences in ΔG^* between the extremes of using no common location ($L_c = 0$) or tester line ($T_c = 0$) or all locations ($L_c = L_1$) and tester lines of the first stage also in the second stage ($T_c = min(T_1 \times M_1, T_2 \times M_2)$) were small, ranging from 0.5 – 1% for L_c and 0.7–1.3% for T_c. In addition, the optimum allocation was affected only marginally. These small differences can be explained by the flat response curves of ΔG in the vicinity of the maximum (Fig. 1). Consequently, we limited our further discussion to the common practice in maize breeding of using the locations ($L_c = L_1$) and tester lines ($T_c = \min(T_1 \times M_1, T_2 \times M_2)$) of the first stage also in the second stage.

Evaluating progenies of each tester × line combination at a single location instead of evaluating progenies of tester × line combination at all locations led to an increased ΔG^* of up to 7.6% for large non-genetic variances (VC2.3, Table 4). This is due to a considerably increased h^2, which can be explained by a substantially larger optimum number of T_j^* and L_j^* and the fact that the reduced product $T_jL_j = T_j$ affects only three of the eight non-genetic variances (Eq. 1). Thus, this simple change in breeding policy represents a very promising method in first testcross evaluations of new lines.

The broader the genetic base of a tester, the higher is ΔG for GCA (Table 3). For instance, the

Table 4 Optimum allocation of test resources in two-stage selection for GCA of doubled haploid lines maximizing selection gain (ΔG^*) for both breeding strategies, varying budgets, variance components (VC), and correlation of line per se performance (LP) and GCA (ρ(LP, GCA)) assuming a ratio of $\sigma^2_{\mathrm{SCA}}/\sigma^2_{\mathrm{GCA}} = 1/2$, $T_c = \min(T_1 \times M_1, T_2 \times M_2)$, and tester type of T_2 restricted to inbred testers. For explanation of abbreviations, see Table 1

	Variance components			Optimum allocation						
Budget	TC[a]	LP	ρ(LP, GCA)	T_1^*	T_2^*	L_1^*	L_2^*	N_1^*	N_2^*	ΔG^*
Breeding strategy 1										
1,000	VC2.1	–	–	1 2W[b]	4	2	4	262	21.6	1.141
1,000	VC2.2	–	–	1 2W	3	2	5	246	25.7	0.972
1,000	VC2.3	–	–	1 2W	3	3	6	174	21.7	0.793
500	VC2.2	–	–	1 2W	2	2	5	127	18.3	0.831
5,000	VC2.2	–	–	1 2W	5	3	8	919	44.6	1.281
1,000	VC2.1	–	–	2[c] 2W	12	2	12	271	26.9	1.214
1,000	VC2.2	–	–	3[c] 2W	14	3	14	191	23.7	1.039
1,000	VC2.3	–	–	4[c] 2W	16	4	16	144	22.0	0.853
Breeding strategy 2										
1,000	VC2.2	VC4	0.25	–	2	1	5	237	64.5	0.833
1,000	VC2.2	VC4	0.50	–	3	1	4	344	40.3	0.959
1,000	VC2.2	VC4	0.75	–	3	2	5	285	19.2	1.108
1,000	VC2.2	VC5	0.25	–	2	1	5	206	69.1	0.811
1,000	VC2.2	VC5	0.50	–	2	1	5	317	52.5	0.905
1,000	VC2.2	VC5	0.75	–	3	2	5	257	23.8	1.027
1,000	VC2.2	VC6	0.25	–	2	1	5	175	73.8	0.793
1,000	VC2.2	VC6	0.50	–	2	1	5	267	60.0	0.858
1,000	VC2.2	VC6	0.75	–	3	2	4	238	33.8	0.946
500	VC2.2	VC5	0.50	–	2	1	4	158	32.9	0.779
5,000	VC2.2	VC5	0.50	–	4	2	7	997	89.6	1.193

[a] *TC* testcross performance

[b] Tester type is optimum of inbred lines and single-crosses (2W)

[c] Each tester × line combination was evaluated only at a single location

use of double-double cross testers instead of inbred testers resulted in a 9.5% higher ΔG for reference variance components VC2.2. This is in harmony with results of experimental studies (cf. Hallauer and Miranda 1981) and can be explained by an increase in h^2 without requiring more testcross plots (Eq. 1). However, in applied breeding programs, use of genetically broad testers is uncommon due to additional efforts required for their production, and the possibility of early identification of promising single-cross hybrids when using inbred testers. Thus, the use of inbred testers in the second stage of selection is very appealing in hybrid maize breeding with doubled haploids. However, the use of single-cross or double-cross instead of inbred testers in the first stage increased ΔG^* between 2.6 and 4.4% for larger ratios $\sigma^2_{\mathrm{SCA}}/\sigma^2_{\mathrm{GCA}}$. In applied breeding programs, intra-pool single-cross hybrids are frequently applied as testers in the first stage and inbred lines in the second selection stage (Schipprack, personal communication). Thus, we restricted our further discussion to single-cross and inbred testers in the first and second stage, respectively.

Use of previous information for selection

Results of previous selection stages are often neglected for further selection in applied plant breeding programs. For two-stage selection on GCA, superiority in ΔG by using results of the first stage in the second selection stage in comparison with neglecting this information was mostly around 1% (data not shown). This increase in ΔG was more than 2% with an increasing h^2 or decreasing selected fraction for first years' results. In addition, the optimum allocation of test resources was only marginally affected by using or neglecting previous information for selection. As databases are commonly used in modern plant breeding, the above discussed increase in ΔG of 1–2% can be accomplished without any experimental expenditures.

Relative efficiency of breeding strategies

Selection gain in BS1 was clearly larger than for BS2 except for ρ(LP, GCA) = 0.75, without any economic weight for line per se performance (Table 4). This is due to the differences in the correlation between selection and target criterion in first stage (ρ_1). For BS1, $\rho_1 = \sqrt{h^2_{x_1}}$, whereas for BS2, $\rho_1 = \sqrt{h^2_{x_1}} \times \rho(\mathrm{LP, GCA})$. Results of experimental studies suggest that ρ(LP, GCA) = 0.50 is realistic for grain yield (Seitz et al. 1992; Mihaljevic et al. 2005). Consequently, ΔG for BS1 is about 5% higher than for BS2.

Production costs of hybrid seed for single-crosses depends strongly on an acceptable yield level of the seed parent line. Thus, the assumption of no economic weight for line per se performance is not appropriate for the seed parent heterotic group. Therefore, we additionally calculated ΔG assuming an economic weight for line *per se* performance larger than zero (Table 5). For ρ(LP, GCA) = 0.50, the relative efficiency of BS2 increased rapidly with increasing economic weight for line per se performance, and resulted in a superiority of BS2 over BS1 already for an economic weight for line per se performance larger than 0.1. This is due to the change from direct to indirect selection in the first stage in BS1 and vice versa in BS2. Consequently, for the seed parent heterotic group, choice of BS2 improves the selection gain.

Optimum allocation of test resources

Optimum allocation of test resources for BS1 and BS2 was similar assuming no economic weight for line per se performance and ρ(LP, GCA) = 0.75 or an economic weight for line per se performance of 0.1 and ρ(LP, GCA) = 0.5 (Tables 4, 5). With decreasing economic weight for line per se performance or ρ(LP, GCA), the optimum allocation of BS2 changed towards a more intensive evaluation of testcross progenies in the second selection stage. This result indicates the importance of specific optimizations of test resources. For no economic weight for line per se performance and ρ(LP, GCA) < 0.75, optimum allocation for BS2 was $L_1^* = 1$ (Table 4). With the assumption of one replication per location, however, this includes a high risk in applied breeding because of possibility of failure at one location due to biotic or abiotic stresses and other hazards and, thus, complete loss of the first stage. Therefore, $L_1 = 2$ is advantageous for reducing this risk with only a small sacrifice in ΔG.

Response curves of ΔG revealed that a careful allocation of the test resources is important, if only a small number of L_2 and T_2 is available (Fig. 1). With larger values of L_2 and T_2, however, response curves become flatter and therefore strongly reduce the risk of choosing an unfavorable allocation of test resources. For instance, choice of $T_2 = 5$ instead of the optimum $T_2 = 3$ reduced ΔG only to a small extent, if the number of L_2 was reduced in parallel. These findings are in harmony with results of previous studies (Utz 1969; Melchinger et al. 2005; Longin et al. 2006a). Decreasing augmentation of ΔG with increasing L_2 and T_2 can be explained by decreasing slopes of (1) h^2 for increasing values of L_j and T_j and (2) selection intensity for increasing values of N_1 (Becker 1993).

For selection among genetically fixed lines, ΔG in both breeding strategies depends on the selected fraction and h^2. Variation in the budget or number of finally selected lines (data not shown) mainly affected the selected fraction and to a smaller degree h^2 (Table 4). The budget was the major factor affecting ΔG by its strong impact on the selected fraction. Variance components affect h^2 directly, and with larger non-genetic variance, h^2 was strongly reduced. Heritability can be increased

Table 5 Optimum allocation of test resources in two-stage selection for GCA of doubled haploid lines maximizing selection gain (ΔG^*) for both breeding strategies and varying economic weights of line per se performance (a_{LP}) assuming a budget of 1,000 testcross plot equivalents, variance components VC2.2 and VC5, ρ(LP, GCA) = 0.50, $T_c = min(T_1 \times M_1, T_2 \times M2)$, $a_{\mathrm{GCA}} = 1 - a_{\mathrm{LP}}$, and tester type of T_2 restricted to inbred testers. For explanation of abbreviations, see Table 1

	Optimum allocation						
a_{LP}	T_1^*	T_2^*	L_1^*	L_2^*	N_1^*	N_2^*	ΔG^*
Breeding strategy 1							
0	1 2W[a]	3	2	5	246	25.7	0.972
0.1	1 2W	3	2	5	246	25.7	0.951
0.2	1 2W	3	2	5	247	25.5	0.929
Breeding strategy 2							
0	–	2	2[b]	5	227	43.3	0.900
0.1	–	3	2	4	241	33.1	0.952
0.2	–	3	2	4	264	28.3	1.011

[a] Tester type is optimum of inbred lines and single-crosses (2W)

[b] We demanded a minimum of two plots per line and stage

most efficiently by larger numbers of L_j (Eq. 1). However, this requires a parallel reduction in N_j and T_j for BS1 and BS2, and reduces ΔG considerably (Table 4).

Implications for hybrid development

In second cycle breeding, where new lines were developed by crossing elite inbreds within heterotic groups, the number of initial lines is normally too large to be tested in factorial crosses with several testers. Therefore, a breeder must find a compromise between (1) selection for GCA to reduce the number of initial lines and (2) parallel selection for GCA and SCA to identify superior hybrids. Optimization of breeding strategies for GCA and SCA must be based on different definitions of the gain criterion, exploiting either σ^2_{GCA} or 2 σ^2_{GCA} + σ^2_{SCA}. This requires additional research.

Nevertheless, the findings of our study allow some conclusions to link GCA and SCA selection. For the seed parent heterotic group, the use of BS2 is most suitable with an allocation of resources adapted to the economic weight of line per se performance. For the pollen parent heterotic group, BS1 is most suitable with (1) use of several genetically broad testers, such as two-way or four-way intra-pool hybrids, and (2) evaluation of the progenies of each tester only at a single location in the first stage. The selection in the first stage strongly reduces the number of lines in the second stage, enabling an evaluation of factorial crosses with more than six testers in the second selection stage. Consequently, this strategy represents a good compromise between the large number of initial lines and early exploitation of GCA and SCA for rapid identification and economical seed production of superior hybrids.

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