Aus dem Institut für
Pflanzenzüchtung, Saatgutforschung und Populationsgenetik
der Universität Hohenheim
Fachgebiet Angewandte Genetik und Pflanzenzüchtung
Prof. Dr. A.E. Melchinger

Optimum Allocation of Test Resources and Comparison of Alternative Breeding Schemes for Hybrid Maize Breeding with Doubled Haploids

Dissertation
zur Erlangung des Grades eines Doktors
der Agrarwissenschaften
vorgelegt
der Fakultät Agrarwissenschaften

von
Diplom-Agrarbiologe
Carl Friedrich Horst Longin
aus Stuttgart

Stuttgart-Hohenheim
2007

Die vorliegende Arbeit wurde am 25.04.2007 von der Fakultät Agrarwissenschaften als “Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften (Dr. sc. agr.)” angenommen.

Tag der mündlichen Prüfung: 06.07.2007

1. Prodekan:	Prof. Dr. W. Bessei
Berichterstatter, 1. Prüfer:	Prof. Dr. A.E. Melchinger
Mitberichterstatter, 2. Prüfer:	Prof. Dr. S. Chen
3. Prüfer:	Prof. Dr. R. Doluschitz

Contents

---

[1] Longin, C.F.H., H.F. Utz, J.C. Reif, W. Schipprack, and A.E. Melchinger. 2006. Theor. Appl. Genet. 112:903–912

[2] Longin, C.F.H., H.F. Utz, A.E. Melchinger, and J.C. Reif. 2006. Acta Agronomica Hungarica 54:343–350

[3] Longin, C.F.H., H.F. Utz, A.E. Melchinger, and J.C. Reif. 2007. Theor. Appl. Genet. 114:393–402

[4] Longin, C.F.H., H.F. Utz, J.C. Reif, T. Wegenast, W. Schipprack, and A.E. Melchinger. 2007. Theor. Appl. Genet. Published online. DOI 10.1007/s00122-007-0585-2

1. General Introduction

The development of inbred lines with superior testcross performance is a major objective of hybrid breeding (Hallauer 1990). In maize, inbred lines have commonly been derived by recurrent selfing for five to six generations. The use of doubled haploids (DHs) enables the development of completely homozygous lines in one step and, thus, represents a promising alternative to recurrent selfing. Besides time saving, further advantages of using DHs in hybrid breeding are (i) the availability of the maximum additive variance for DHs in comparison with half the additive variance in the S_1 generation, (ii) the possibility to evaluate potential hybrid cultivars from the very beginning of the selection process, (iii) the reduction of masking effects due to remaining heterozygosity, (iv) a good *per se* performance of DH lines due to the elimination of deleterious mutants during DH development, (v) cost-savings due to reduced expenses for selfing and maintenance breeding, (vi) the possibility to protect outstanding lines early by plant variety rights, and (vii) simplified logistics (*cf.* Schmidt 2004, Röber et al. 2005).

Spontaneous haploids have early been reported in maize (Stadler 1929 unpublished, cited by Randolph 1932), but their occurrence is very low (0.1%, Chase 1947). Furthermore, spontaneous chromosome doubling is seldom in maize with rates below 10% (Beckert 1994). The identification of inbred lines that induced larger number of haploids (Coe 1959, Kermicle 1969) and intensive selection resulted up to now in the development of lines with haploid induction rates above 10% (*cf.* Röber et al. 2005). These lines are

used in the in-vivo haploid induction mostly as pollinators in crosses with elite germplasm to induce larger number of haploids in breeding materials. In combination with artificial chromosome doubling, *e.g.*, by the use of colchicine, in-vivo haploid induction can easily be integrated into existing breeding programs. Consequently, in-vivo haploid induction is currently adopted as a routine method in commercial maize breeding programs in Europe (Schmidt 2004) and North America (Seitz 2005). The implementation of the new DH technology in maize breeding requires an investigation of the optimum breeding scheme. This involves the optimization and comparison of different breeding schemes in order to maximize progress from selection, taking possible limits of the DH technique into account.

Target criteria for optimization of breeding schemes

Selection gain (ΔG)(Cochran 1951; Utz 1969) and the probability of identifying superior genotypes ($P(q)$) (Robson et al. 1967; Johnson 1989; Knapp 1998) were reported in the literature to quantify the progress from selection. Selection gain represents the difference between the genotypic mean of the selected fraction and the genotypic mean of the base population (Fig. 1.1). In contrast, $P(q)$ is the percentage of selected genotypes exceeding a given threshold, *e.g.*, the $(100 - q)\%$ quantile of the normal distribution.

For a given population, ΔG is a function of the heritability and selection intensity, and increases with larger values for both parameters (Bernardo 2002). Heritability increases with an increasing number of testers, test locations, years, and replications in performance trials. Selection intensity increases with a larger number of initial test candidates and/or a smaller number of selected test candidates. For a given population and a fixed number of selected test candidates, $P(q)$ is increased by an increasing number of initial test candidates and by an increasing heritability (Robson et al. 1967;

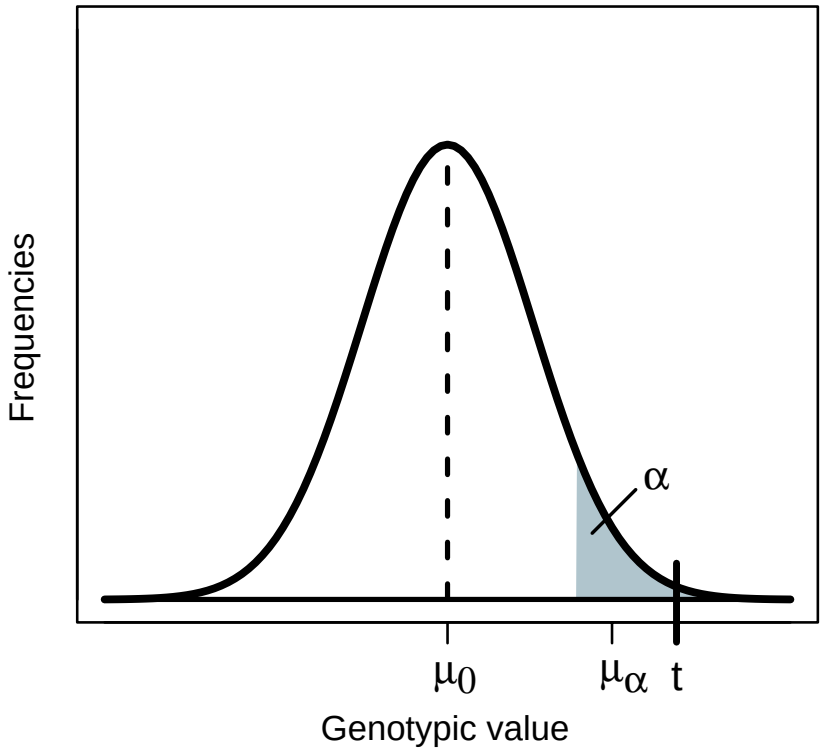

Figure 1.1 Expected distribution of genotypes in an infinite base population, where α refers to the selected fraction, μ_0 to the mean genotypic value of the base population, μ_α to the mean genotypic value of the selected fraction, and t to a given threshold.

Johnson 1989). Thereby, a large number of initial test candidates is required in order to have at least the number of desired superior genotypes in the initial sample. A large heritability warrants a high probability of detecting them. Hence, a plant breeder with a fixed budget has to find a compromise between (i) the number of candidates to be tested and (ii) the intensity of their testing as determined by the number of testers, test locations, years, and replications. This requires an optimization of the test resources for each breeding scenario.

Selection gain represents the most widely used target criterion in recurrent selection to optimize the allocation of test resources and to compare different methods (*cf.* Choo and Kannenberg 1988; Gallais 1991). In contrast, $P(q)$

represents a suitable target criterion to focus on rapid development of competitive varieties (Robson et al. 1967; Johnson 1989), *e.g.*, in second-cycle breeding, where new lines are developed by crossing elite inbreds within heterotic groups. An optimization of the allocation of test resources based on $P(q)$ seems especially promising for the use of DHs due to the possibility to evaluate potential hybrid cultivars from the beginning of the selection process. However, $P(q)$ has so far not been used to determine the optimum allocation of test resources. Furthermore, the diverse definitions of ΔG and $P(q)$ may lead to differences in the optimum allocation of test resources. Thus, a comparison of these target criteria is necessary with particular emphasis on the potential of $P(q)$ for hybrid maize breeding with DHs.

Selection theory was developed by assuming an infinite population size, although populations of medium size are commonly used in plant breeding (Cochran 1951; Hanson and Brim 1963; Utz 1969; Tomerius 2001; Grüneberg et al. 2004). This assumption simplifies the calculations considerably but affects the probability distribution of the test candidates. With stochastic simulations, selection gain was determined for a finite population size (Cochran 1951; Finney 1966; Utz 1969; Young 1976). Thereby, marginally reduced ΔG and similar optimum allocation of test resources were obtained in comparison with infinite population sizes. However, these studies were conducted more than 30 years ago and the limited computing power available at that time largely restricted the accuracy of simulations and the number of scenarios considered. Therefore, verification of the above conclusions with more accurate simulations in a broader range of scenarios is required.

Alternative breeding schemes for hybrid maize breeding with DHs

Owing to the necessity of five to six selfing generations in conventional line development, two selection stages on testcross performance can be realized

until homozygous lines are available. In contrast, with DHs the evaluation of potential hybrid cultivars is possible from the very beginning of the selection process. This enables an early registration of varieties, *e.g.*, after one stage of selection. However, for one-stage selection, a 20% smaller ΔG in comparison with two-stage selection was reported in the literature (Utz 1969). The assumptions about the budget and variance components in this study differed clearly from those applying to hybrid maize breeding. Thus, an investigation of the potential of one- and two-stage selection in hybrid maize breeding with DHs is required.

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).

Considering one-stage selection for GCA between inbred lines in maize, Federer and Sprague (1947) and Keller (1949) investigated the optimum number of testers, lines, and replications. They concluded that for a fixed budget, 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 generations in maize considering also the number of test locations. For a fixed budget corresponding to 1200 testcross plots, he suggested to use one tester in the first and seven testers in the second stage of selection. However, these studies used simplified genetic models for calculation of ΔG. Varying the type of tester, *e.g.*, use of inbred lines, single crosses, or double crosses as testers, may affect the optimum number of test candidates, testers, test locations, and replications. However, investigations on the type of testers within the context of optimum allocated test resources have not been reported in the literature. Consequently, a thorough study on the optimum type and number of testers is required for the optimum allocation of hybrid maize breeding schemes.

Testcross performance of experimental lines is the prime selection criterion in hybrid maize breeding (Mihaljevic et al. 2005). However, an economic production of hybrid seed requires an acceptable line *per se* performance of the seed parent (Sampoux and Gallais 1996, Gallais 1997). The possibility of a simultaneous improvement of line *per se* and testcross performance depends on the genetic correlation between both selection criteria (Mihaljevic et al. 2005). For nearly homozygous lines in maize, values for the genetic correlation between line *per se* and testcross performance of 0.5 are reported in the literature (Seitz et al. 1992, Mihaljevic et al. 2005). For these reasons, evaluation of line *per se* performance may be an interesting alternative to testcross evaluations in the first selection stage. Nevertheless, an assessment of this selection strategy based on line *per se* and testcross performance is not available in maize.

Alternatively to the evaluation of potential hybrid cultivars from the beginning of the selection process, an early test on testcross performance in the S_1 or S_2 selfing generation could be made before DH production. This elongates the breeding scheme but permits to restrict the production and testing of DH lines to those derived from segregation in the most promising families. The potential of early testing has been debated ever since it was first proposed by Jenkins (1935). Early testing was considered useful by Sprague (1946), Lonnquist (1950), Hallauer and Lopez-Perez (1979), and Jensen et al. (1983). In contrast, Richey (1945) and Payne and Hayes (1949) discouraged the use of early testing.

The aim of early testing is to select lines with above-average combining ability to concentrate the test resources on more promising material (*cf.* Hallauer et al. 1988). Thereby, 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). The genetic correlation for testcross performance between S_1 plants and inbreds is larger than 0.7 supporting the determination of combining ability in the early stages of selfing (Bernardo 1991). Therefore, the concentration of test resources on the most promising

families in early testing prior to DH production may be an interesting alternative to intensive evaluation of DH lines from the beginning of the selection process. However, an assessment of the potential of early testing in hybrid maize breeding with DHs is completely lacking in the literature.

Objectives

The goal of my thesis research was to examine the optimum implementation of DHs in hybrid maize breeding with emphasis on the optimum allocation of test resources. In particular, the objectives were to

1. compare two target criteria for the allocation of test resources with ΔG for infinite and finite population size as well as $P(q)$ by use of numerical integration and Monte Carlo simulations;
2. investigate the impact of varying budget, variance components, and number of finally selected test candidates on the optimum allocation of test resources and the different target criteria;
3. examine the potential and limitations of the current technique of DH production and its impact on the target criteria, choice of breeding schemes, and optimum allocation of test resources;
4. compare one- versus two-stage selection on testcross performance in hybrid maize breeding with DHs;
5. assess two-stage breeding schemes with evaluation of (i) testcross performance in both stages, or (ii) line *per se* performance in the first stage followed by testcross performance in the second stage;
6. investigate the potential of early testing in hybrid maize breeding with DHs and identify the optimum number of families and DH lines within families;

7. determine the optimum allocation of the number of test candidates, test locations, as well as number and type of testers for the investigated breeding schemes; and

8. give recommendations for the optimum implementation of DHs in commercial hybrid maize breeding.

References

Beckert M (1994) Advantages and disadvantages of the use of in vitro / in situ produced DH maize plants. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, Vol 25, Springer-Verlag, Berlin, Heidelberg, Germany, pp 201–213

Bernardo R (1991) Correlation between testcross performance of lines in early and late selfing generations. Theor Appl Genet 82:17–21

Bernardo R 2002 Breeding for quantitative traits in plants. Stemma Press, Woodbury, USA, pp 204–205

Cochran WG (1951) Improvement by means of selection. Proc Second Berkeley Symp Math Stat Prob, 449–470

Coe EH (1959) A line of maize with high haploid frequency. Am Nat 93:381–382

Chase SS (1947) Techniques for isolating monoploid maize plants. J Bot 34:582

Choo TM, Kannenberg LW (1988) Selection response and efficiency of doubled-haploid recurrent selection in a cross-fertilized species. Theor Appl Genet 75:410–414

Federer WT, Sprague GF (1947) A comparison of variance components in corn yield trials: I. Error, tester × line, and line components in top-cross experiments. J Am Soc Agron 39:453–463

Finney DJ (1966) An experimental study of certain screening processes. Jour Roy Stat Soc B 28:88–109

Gallais A (1991) A general approach for the study of a population of test-cross progenies and consequences for recurrent selection. Theor Appl Genet 81:493–503

Gallais A (1997) Combined testcross and S_1 selection for the improvement of testcross and inbred performances. Crop Sci 37:1126–1133

Grüneberg WJ, Abidin E, Ndolo P, Pereira CA, Hermann M (2004) Variance component estimations and allocation of resources for breeding sweetpotato under East African conditions. Plant Breeding 123:311–315

Hallauer AR, Lopez-Perez E (1979) Comparison among testers for evaluating lines of corn. Proc Corn Sorghum Industry Res Conf 34:57–75

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, USA, pp 267–298

Hallauer AR, Russell WA, Lamkey KR (1988) Corn breeding. In: Sprague GF, Dudley JW (eds) Corn and corn improvement, 3rd edn. Am Soc Agronomy, Madison, Wisconsin, USA, pp 463–564

Hallauer AR (1990) Methods used in developing maize inbreds. Maydica 35:1–16

Hanson WD, Brim CA (1963) Optimum allocation of test material for two-stage testing with an application to evaluation of soybean lines. Crop Sci 3:43–49

Jenkins MT (1935) The effect of inbreeding and of selection within inbred lines of maize upon the hybrids made after successive generations of selfing. Iowa State Coll J Sci 3:429–450

Jensen SD, Kuhn WE, McConnell RL (1983) Combining ability studies in elite U.S. maize germ plasm. Proc Corn Sorghum Industry Res Conf 38:87–96

Johnson B (1989) The probability of selecting genetically superior S_2 lines from a maize population. Maydica 34:5–14

Keller KR (1949) A comparison involving the number of, and relationship between, testers in evaluating inbred lines of maize. Agron J 41:323–331

Kermicle JL (1969) Androgenesis conditioned by a mutation in maize. Science 166:1422–1424

Knapp SJ (1998) Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci 38:1164–1174

Lonnquist JH (1950) The effect for selecting for combining ability within segregating lines of corn. Agron J 42:503–508

Mihaljevic R, Schön CC, Utz HF, Melchinger AE (2005) Correlations and QTL correspondence between line per se and testcross performance for agronomic traits in four populations of European maize. Crop Sci 45:114–122

Payne KT, Hayes HK (1949) A comparison of combining ability in F_3 and F_2 lines of corn. Agron J 41:383–388

Randolph LF (1932) Some effects of high temperature on polyploidy and other variations in maize. Genetics 18:222–229

Richey FD (1945) Isolating better foundation inbreds for use in corn hybrids. Genetics 30:455–471

Robson DS, Powers Leroy, Urquhart NS (1967) The proportion of genetic deviates in the tails of a normal population. Theor Appl Genet 37:205–216

Röber F, Gordillo GA, Geiger HH (2005) In vivo haploid induction in maize - performance of new inducers and significance of doubled haploid lines in hybrid breeding. Maydica 50:275–283

Sampoux JP, Gallais A (1996) Expected genetic gains in testcross and S_1 performances for several populations of silage maize with combined S_1-testcross selection. Maydica 41:167–179

Schmidt W (2004) Hybridmaiszüchtung bei der KWS SAAT AG. (In German) In: Bericht über die 54. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2003, Gumpenstein, Austria, pp 1–6

Schnell FW (1996) Über Zuchtplanung und die Entscheidungsspielräume des Pflanzenzüchters. (In German) Vorträge Pflanzenzüchtung 33:227-244

Seitz G, Geiger HH, Schmidt GA, Melchinger AE (1992) Genotypic correlations in forage maize II: Relationship between inbred line and testcross performance. Maydica 37:101–105

Seitz G (2005) The use of doubled haploids in corn breeding. In: Proceedings of the Forty First Annual Illinois Corn Breeders' School 2005, Urbana-Champaign, Illinois, USA, pp 1–8

Sprague GF (1946) Early testing of inbred lines of corn. J Am Soc Agron 38:108–117

Tomerius AM (2001) Optimizing the development of seed-parent lines in hybrid rye breeding. PhD thesis University of Hohenheim, Stuttgart, Germany (*http://opus-ho.uni-stuttgart.de/hop/volltexte/2001/10/pdf/tomerius.pdf*).

Utz HF (1969) Mehrstufenselektion in der Pflanzenzüchtung. (In German) Arbeiten der Universität Hohenheim, Vol 49, Verlag Eugen Ulmer, Stuttgart, Germany

Young JC (1976) Varietal screening from finite normal populations. J Am Stat Assoc 71:87–92

Theor Appl Genet (2006) 112: 903–912
DOI 10.1007/s00122-005-0192-z

ORIGINAL PAPER

C. Friedrich H. Longin · H. Friedrich Utz
Jochen C. Reif · Wolfgang Schipprack
Albrecht E. Melchinger

Hybrid maize breeding with doubled haploids: I. One-stage versus two-stage selection for testcross performance

Received: 29 July 2005 / Accepted: 2 December 2005 / Published online: 25 January 2006

Abstract Optimum allocation of resources is of fundamental importance for the efficiency of breeding programs. The objectives of our study were to (1) determine the optimum allocation for the number of lines and test locations in hybrid maize breeding with doubled haploids (DHs) regarding two optimization criteria, the selection gain ΔG_k and the probability P_k of identifying superior genotypes, (2) compare both optimization criteria including their standard deviations (SDs), and (3) investigate the influence of production costs of DHs on the optimum allocation. For different budgets, number of finally selected lines, ratios of variance components, and production costs of DHs, the optimum allocation of test resources under one- and two-stage selection for testcross performance with a given tester was determined by using Monte Carlo simulations. In one-stage selection, lines are tested in field trials in a single year. In two-stage selection, optimum allocation of resources involves evaluation of (1) a large number of lines in a small number of test locations in the first year and (2) a small number of the selected superior lines in a large number of test locations in the second year, thereby maximizing both optimization criteria. Furthermore, to have a realistic chance of identifying a superior genotype, the probability P_k of identifying superior genotypes should be greater than 75%. For budgets between 200 and 5,000 field plot equivalents, $P_k > 75\%$ was reached only for genotypes belonging to the best 5% of the population. As the optimum allocation for $P_k(5\%)$ was similar to that for ΔG_k, the choice of the optimization criterion was not crucial. The production costs of DHs had only a minor effect on the optimum number of locations and on values of the optimization criteria.

Keywords Optimum allocation · Selection gain · Probability · Superior genotype · Monte Carlo simulation

Communicated by H. Becker

C. Friedrich H. Longin and H. Friedrich Utz contributed equally to this work.

C. F. H. Longin · H. F. Utz · J. C. Reif · W. Schipprack
A. E. Melchinger (✉)
Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany
E-mail: melchinger@uni-hohenheim.de
Fax: +49-711-4592343

Introduction

Optimum allocation of financial and breeding resources is of fundamental importance for the efficiency of breeding programs and selection strategies. Advances in the production of doubled haploids (DHs) by in vivo haploid induction (Bordes et al. 1997; Röber 1999) offer a promising alternative to recurrent selfing for rapid inbred line development in hybrid maize breeding. Currently, DHs are adopted as a routine method in commercial maize breeding programs in North America (Seitz 2005) and Europe (Schmidt 2004). Their efficient use requires an optimization of the entire breeding scheme in order to maximize progress from selection.

A selection strategy may involve one or several stages of selection. In the latter case, the initial population of lines is evaluated in 1 year and a superior subset is selected for further evaluation and selection in subsequent year(s). To quantify the progress from k selection stages, various criteria have been used such as (1) the selection gain (ΔG_k) (Cochran 1951; Utz 1969) and (2) the probability of identifying superior genotypes (P_k) (Keuls and Sieben 1955; Robson et al. 1967; Johnson 1989; Knapp 1998). In recurrent selection, ΔG_k represents the most widely used criterion to compare different methods and optimize the selection progress in population improvement (cf. Choo and Kannenberg 1988; Gallais 1991). For a given population, ΔG_k is a function of the heritability (h^2) and selection intensity (i_α), and increases with larger values for both parameters (Bernardo 2002). Heritability increases with an increasing number of test locations, years, and replications in performance trials, whereas i_α depends on the selected fraction (α) and the

probability distribution of the lines. With a fixed number of finally selected lines (N_f), i_α increases with a larger number of initial lines. Hence, a plant breeder with a fixed budget has to find a compromise between (a) the number of initial lines and (b) the intensity of their testing as determined by the number of test locations, years, and replications. This requires an optimization of the test resources for each breeding scenario.

For ΔG_k the optimum allocation of test resources for a fixed budget was investigated with numerical integration, assuming an infinite population size (Utz 1969), and with stochastic simulations, assuming a finite population size (Finney 1966; Young 1976). However, production costs of DHs have so far not been taken into account. In addition, most studies on the optimum allocation of resources were conducted more than 30 years ago and the limited computing power available at that time restricted the number of scenarios considered.

In addition to medium- and long-term germplasm improvement, plant breeders are forced to focus on rapid development of competitive varieties. For the latter purpose, P_k represents a suitable criterion (Johnson 1989). For a given population and a fixed number of N_f, P_k is increased by (1) an increasing number of lines in order to have at least the number of desired superior genotypes in the initial sample and (2) an increasing h^2 to warrant a high probability of detecting them. For one-stage selection, Robson et al. (1967) and Johnson (1989) investigated the impact of h^2, the initial sample size, and N_f on the probability (P_1) that all N_f have genotypic values exceeding a given threshold. Knapp (1998) extended this approach to marker-assisted selection. Nevertheless, these studies investigated P_k only for given values of h^2 and α, disregarding the optimum allocation of resources. Furthermore, ΔG_k and P_k have not yet been compared for one- and two-stage selection.

In this study, we optimized the allocation of test resources in hybrid maize breeding with DHs under one- and two-stage selection for testcross performance with a given tester by using Monte Carlo simulations. For different assumptions regarding the budget, ratio of variance components, and value of N_f, we (1) determined the optimum allocation of the number of lines and test locations for ΔG_k and P_k, (2) compared both optimization criteria including their standard deviations, and (3) investigated the influence of production costs of DHs on the optimum allocation of test resources.

Materials and methods

Selection strategies

In a standard maize breeding scheme (Fig. 1), a total of N_1 DH lines generated from one or several F_1 crosses via in vivo haploid induction are available at the beginning of the evaluation and selection process. A certain number N_f of phenotypically best DH lines are selected. We compared $N_f = 1$ and $N_f = 5$. The target variable Y is the genotypic value of testcross performance with a given tester T for a certain trait or index of traits. The tester can be any population with an arbitrary structure such as an inbred line, single cross, or random mating population. With one-stage selection, selection is based on field tests in a single year. With two-stage selection, field tests are conducted in 2 years with a subset of the most superior lines N_2 selected after the first year being evaluated in the second year. At stage j ($j = 1, 2$), selection among N_j DH lines is based on variable X_j, the phenotypic mean of testcross performance at this stage with tester T evaluated in L_j locations with R_j replications. At stage $j = 2$, the selection among lines could alternatively be based on an index of their performance in the first and second year. However, this would affect the optimum allocation and the selection gain only marginally (Utz 1969; Young 1976). Without an upper limit on L_j, $R_j = 1$ is optimal regarding ΔG_k (Sprague and Federer 1951; Utz 1969). Thus, we set $R_j = 1$.

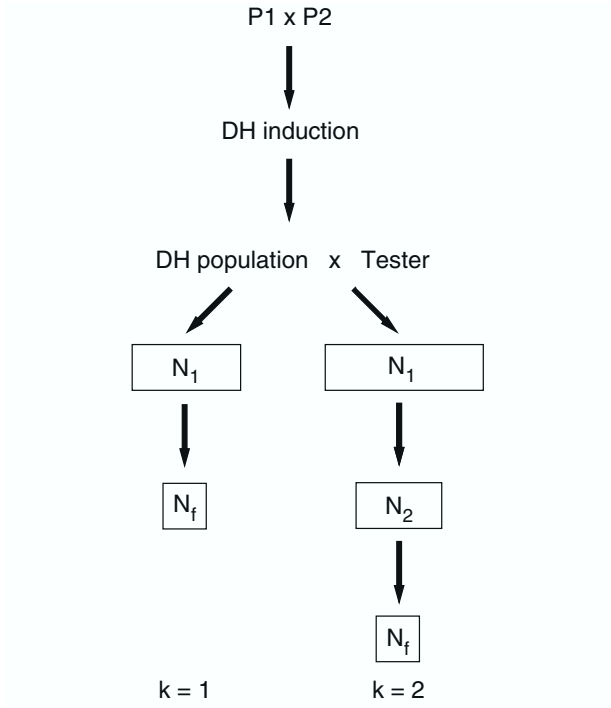

Fig. 1 Hybrid maize breeding scheme with production of doubled haploid (*DH*) lines, their testcross progenies and testcross evaluation in several test locations with one-stage ($k = 1$) or two-stage selection ($k = 2$). (N_1 = number of initial lines; N_2 = subset of superior lines selected after the first stage of two-stage selection; N_f = number of finally selected lines)

Economic frame and quantitative-genetic parameters

We investigated three assumptions ($C = 0$, 0.5, 1) concerning the production cost of one DH line relative to

the cost of one field plot for evaluating testcross progenies. For instance, $C=0.5$ means that the production cost of one DH line is equal to half the cost of one field plot. $C=0.5$ corresponds to the actual costs of DH production in breeding companies most advanced in the DH technique (Seitz, personal communication). $C=1$ is a realistic assumption at the beginning of establishing the DH technique in a breeding program. With further improvements in the DH technique, the costs of DH production may become negligible in the future ($C=0$).

A fixed total budget B for (1) producing the DH lines and (2) evaluating their testcross progenies in k selection stages was defined in terms of testcross plot equivalents as

$$B = N_1 C + \sum_{j=1}^{k} N_j L_j R_j \qquad (1)$$

assuming equal plot sizes in all selection stages. We compared three budgets with $B=200$, 1,000, 5,000 plot equivalents. An overview of the notation used throughout this treatise is given in Table 1.

Three ratios of variance components (σ^2_g:σ^2_{gl}:σ^2_{gy}:σ^2_{gly}:σ^2_e) were considered, where σ^2_g refers to the genotypic variance, σ^2_{gl} to the variance of genotype × location interactions, σ^2_{gy} to the variance of genotype × year interactions, σ^2_{gly} to the variance of genotype × location × year interactions, and σ^2_e to the error variance. We set VC1 = 1:0.25:0.25:0.5:1, VC2 = 1:0.5:0.5:1:2, and VC3 = 1:1:1:2:4, resulting in a heritability on a plot basis of 0.33, 0.20, and 0.11, respectively. These ratios were chosen based on combined analyses of variance of grain yield in (1) recent official maize variety performance tests in Germany (VC1, Laidig, personal communication), (2) DH populations of commercial breeding programs (VC2, Gordillo and Geiger 2004), and (3) official maize variety performance tests in Southwest Germany (VC3, P. Herrmann, unpublished data).

Table 1 Notation used in this treatise

h^2	Heritability
i_α	Selection intensity for a certain selected fraction $\alpha = N_f / N_1$
j	Selection stage
B	Fixed total budget in field plot equivalents
C	Production costs of one DH line relative to the costs of one field plot for evaluating testcross progenies
DH	Doubled haploid
ΔG_k	Selection gain after k stages of selection
$\Delta\hat{G}_k$	ΔG_k estimated by Monte Carlo simulations
$\Delta\hat{G}^*_k$	Value of $\Delta\hat{G}_k$ at the corresponding optimum allocation (N_j^*, L_j^*)
N_f	Number of finally selected lines
N_j, L_j, R_j	Number of lines, locations, or replications at stage j in performance trials
N_j^*, L_j^*	Optimum number of lines and locations maximizing the optimization criterion in the set of admissible allocations
$P_k(q)$	Probability of identifying lines with genotypic values exceeding a fixed $(100-q)$% quantile of the corresponding normal distribution $N(0, \sigma^2_g)$ after k stages of selection
$\hat{P}_k(q)$	$P_k(q)$ estimated by Monte Carlo simulations
$\hat{P}^*_k(q)$	Value of $\hat{P}_k(q)$ at the corresponding optimum allocation (N_j^*, L_j^*)
VC	Ratio of variance components σ^2_g: σ^2_{gl}: σ^2_{gy}: σ^2_{gly}: σ^2_e

Simulation model

Genotypic and phenotypic values were generated separately for each combination of the above factors. Genotypic values were sampled from a normal distribution $N(0, \sigma^2_g)$. Non-genetic values were sampled from a normal distribution $N(0, \sigma^2_{m_j})$, with

$$\sigma^2_{m_j} = \sigma^2_{gy} + \frac{\sigma^2_{gl}}{L_j} + \frac{\sigma^2_{gly}}{L_j} + \frac{\sigma^2_e}{L_j R_j} \qquad (2)$$

representing the non-genetic variance. Phenotypic values were then generated by adding non-genetic values to the genotypic values. For two-stage selection, genotypic and phenotypic values were sampled out of a multivariate normal distribution MVN(μ, V) with $\mu^T = (0, 0, 0)$ and

$$V = \begin{pmatrix} \sigma^2_g & \sigma^2_g & \sigma^2_g \\ \sigma^2_g & \sigma^2_{x_1} & \mathrm{cov}_{x_1x_2} \\ \sigma^2_g & \mathrm{cov}_{x_1x_2} & \sigma^2_{x_2} \end{pmatrix}. \qquad (3)$$

The covariance between the phenotypic values at stage $j=1$ and $j=2$ was determined as $\mathrm{cov}_{x_1x_2} = \sigma^2_g + (L_c\sigma^2_{gl})/(L_1L_2)$, with L_c representing the number of locations common to both selection stages (Utz 1969). We assumed $L_c = L_1$. The two optimization criteria and their SDs were then calculated and stored. This procedure was repeated for each factor combination and choice of N_j and L_j, with a new set of realizations of random variables (further referred to as runs). The number of runs required to warrant an accuracy of 0.01 for the optimization criterion was calculated based on the standard error of the arithmetic mean as $(3\mathrm{SD}/0.01)^2$ (Berry and Lindgren 1996). Between 7,000 and 70,000 simulation runs were required for the different scenarios.

Optimum allocation and optimization criteria

An admissible allocation of test resources refers to tuples (N_j, L_j) for all stages j, such that Eq. 1 is satisfied. An element (N_j^*, L_j^*) is denoted as an optimum allocation if it maximizes the optimization criterion in the set of admissible allocations. For each run, the mean genotypic value of the N_f selected lines was calculated and the selection gain was estimated by averaging over all Monte Carlo runs for the allocation considered $(\Delta\hat{G}_k)$. The variance among these runs was used to calculate the

corresponding SD $\left(\mathrm{SD}_{\Delta\hat{G}_k}\right)$. In addition, the number of selected lines with genotypic values exceeding a fixed $(100-q)\%$ quantile of the corresponding normal distribution $N(0, \sigma_g^2)$ was determined for each run and divided by N_f. The probability of identifying superior genotypes was estimated by averaging these values over all Monte Carlo runs for the allocation considered $\left(\hat{P}_k(q)\right)$. The variance among these runs was used to calculate the corresponding SD $\left(\mathrm{SD}_{\hat{P}_k(q)}\right)$. We examined q values of 25, 5, 1, and 0.1%, with corresponding standardized genotypic thresholds of 0.67449, 1.64485, 2.32635, and 3.09023, respectively.

The optimum allocation of test resources for each scenario was obtained by a grid search in $\mathbf{Z}$, the space of admissible resource allocations. For instance, for $B=200$, $N_f=1$, VC1, $C=0$, and one-stage selection, the optimum choice of N_1 was determined by varying the selected fraction α_1 between 0.01 and 0.30 for each L_1 between one and a number that allowed a clear identification of the optimum of L_1. Thus, at least 100 calculations were performed to identify the optimum allocation for each scenario. Let OC represent the optimization criterion ΔG_k or $P_k(q)$. Let (N_j^o, L_j^o) be the allocation, where OC assumes its numerical maximum value in the simulations

$$\mathrm{OC}\left(N_j^o, L_j^o\right) = \max_{(N_j,L_j)\epsilon\mathbf{Z}} \mathrm{OC}\left(N_j, L_j\right). \quad (4)$$

Since OC is only estimated with a precision of 0.01, the optimum allocation (N_j^*, L_j^*) was determined following Utz (1969) such that the number of locations was minimum among all allocations within 0.01 drop-off of $\mathrm{OC}(N_j^o, L_j^o)$, i.e.,

$$L_j^* = \min_{L_j}\left\{\left(N_j, L_j\right)\epsilon\mathbf{Z} | \mathrm{OC}\left(N_j^o, L_j^o\right) - \mathrm{OC}\left(N_j, L_j\right) < 0.01\right\}. \quad (5)$$

The reason being that breeders prefer for technical reasons tests in fewer locations if this affects the OC only marginally.

The values of each optimization criterion at its corresponding optimum allocation (N_j^*, L_j^*) were denoted as $\Delta\hat{G}_k^*$ and $\hat{P}_k^*(q)$. Simulation programs were written in C and implemented in the statistical software R (R Development Core Team 2004).

Results

The optimization criteria were similarly affected by deviations from the optimum allocation of test resources for one- and two-stage selection, $N_f=1$ or 5, and production costs of DHs. Thus, only response curves for $\Delta\hat{G}_1$ and $\hat{P}_1(1\%)$ as a function of L_1 were presented for varying budgets and ratio of variance components assuming one-stage selection, $N_f=1$, and $C=0.5$ (Fig. 2). With increasing L_1, the optimization criteria $\Delta\hat{G}_1$ and $\hat{P}_1(1\%)$ increased up to an optimum and decreased slightly thereafter. Both response curves were flat in the vicinity of the maximum. The increase in $\Delta\hat{G}_1$ and $\hat{P}_1(1\%)$ was largest between $L_1=1$ and $L_1=4$. Curves for $\mathrm{SD}_{\hat{P}_1(1\%)}$ displayed similar trends as those for $\hat{P}_1(1\%)$, with a maximum at the optimum allocation of $\hat{P}_1(1\%)$. In contrast, curves for $\mathrm{SD}_{\Delta\hat{G}_k}$ decreased with increasing L_1.

The consequences of one-stage versus two-stage selection, varying N_f, and budgets on the optimum allocation of test resources and optimization criteria were hardly affected by the ratio of variance components and production costs of DHs (data not shown). Hence, the results on the influence of the former group of factors were presented exemplarily for intermediate values VC2 and $C=0.5$ (Table 2). The optimum number of initial lines N_1^* and test locations for two-stage selection was about twice as large as for one-stage selection. This was due to the optimum allocation of two-stage selection, which comprised a large number of initial lines N_1^* tested in a small number of test locations L_1^* at the first stage, and a small number of selected lines N_2^* tested in a large number of test locations L_2^* at the second stage. Furthermore, under the same allocation of resources $\Delta\hat{G}_k^*$, and values of $\hat{P}_k(5\%)$, $\hat{P}_k(1\%)$, and $\hat{P}_k(0.1\%)$ were on average 20, 30, 50, and 80%, respectively, higher than for one-stage selection. Reducing N_f from five to one resulted in (1) smaller values of N_j^* but larger values of L_j^* in the last selection stage, and (2) an increase in $\Delta\hat{G}_k^*$ and corresponding values for $\hat{P}_k(5\%)$, $\hat{P}_k(1\%)$, and $\hat{P}_k(0.1\%)$ of 20, 30, 60, and 110%, respectively. However, SD of these estimates were also increased by more than 60% on average. For one-stage selection, increasing the budget from $B=200$ to $B=5{,}000$ resulted in a more than 10-fold increase in N_1^* and a twofold increase in L_1^*. For two-stage selection, N_1^* and N_2^* increased more than 15- and 5-fold, whereas L_1^* and L_2^* increased twofold and threefold, respectively. In addition, the average increase in $\Delta\hat{G}_k^*$ and corresponding values for $\hat{P}_k(5\%)$, $\hat{P}_k(1\%)$, and $\hat{P}_k(0.1\%)$ was 65, 125, 300, and 650%, respectively.

The influence of different ratios of variance components and production costs of DHs on the optimum allocation of test resources and optimization criteria was hardly affected by the number of selection stages, N_f, and budget. Therefore, representative results on the influence of both factors were given for two-stage selection, $B=1{,}000$, and $N_f=1$ (Table 3). An increase in the non-genetic variance from VC1 to VC3 resulted in a reduction in N_1^* and an increase in L_j^* for $\Delta\hat{G}_k^*$, $\hat{P}_k^*(5\%)$, and $\hat{P}_k^*(1\%)$. For $\hat{P}_k^*(0.1\%)$, N_1^* was also reduced with increasing non-genetic variance, but N_2^* increased and L_j^* was fairly stable. The optimum allocation of test resources based on the same VC but different optimization criteria differed largely for small values of q ($q=0.1\%$) and large non-genetic variance (VC3). For instance, for VC3 and $C=0.5$ the optimum number of lines N_j^* was approximately doubled and the optimum number of locations L_j^* was halved for

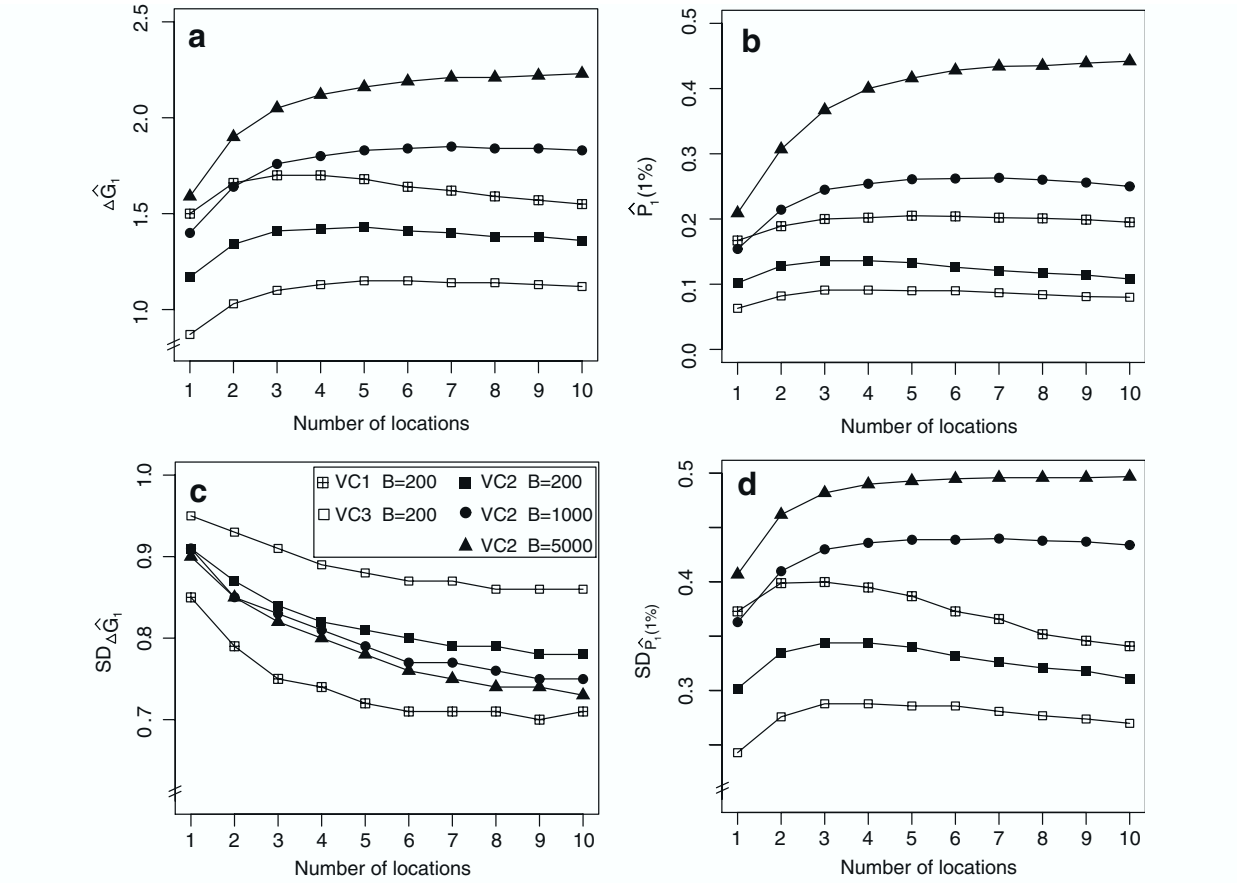

Fig. 2 **a** Selection gain $\Delta\hat{G}_1$, **b** probability $\hat{P}_1(1\%)$ of identifying one line with a genotypic value belonging to the 1% best genotypes of the population, **c**, **d** corresponding standard deviation $SD_{\Delta\hat{G}_1}$ and $SD_{\hat{P}_1(1\%)}$, respectively, as a function on the number of locations for one-stage selection assuming $C=0.5$, and $N_f=1$. For explanation of abbreviations, see Table 1

Table 2 Optimum allocation of test resources maximizing selection gain ($\Delta\hat{G}_k^*$), values of $\Delta\hat{G}_k^*$, and corresponding probabilities $\hat{P}_k(q)$ of identifying N_f lines with genotypic values belonging to the 5, 1, and 0.1% best genotypes of the population assuming $C=0.5$ and VC2. For explanation of abbreviations, see Table 1

Assumptions			Optimum allocation				Selection gain		Corresponding probabilities $\hat{P}_k(q)$					
k[a]	N_f	B	N_1^*	N_2^*	L_1^*	L_2^*	$\Delta\hat{G}_k^*$	SD[b]	$\hat{P}_k(5\%)$	SD[b]	$\hat{P}_k(1\%)$	SD[b]	$\hat{P}_k(0.1\%)$	SD[b]
1	1	200	44	–	4	–	1.42	0.81	0.39	0.49	0.13	0.34	0.02	0.14
1	1	1,000	133	–	7	–	1.85	0.76	0.60	0.49	0.27	0.44	0.05	0.22
1	1	5,000	588	–	8	–	2.22	0.74	0.78	0.42	0.44	0.50	0.12	0.32
1	5	200	57	–	3	–	1.08	0.36	0.25	0.20	0.07	0.11	0.01	0.04
1	5	1,000	222	–	4	–	1.52	0.36	0.43	0.23	0.16	0.16	0.03	0.07
1	5	5,000	769	–	6	–	1.92	0.35	0.64	0.22	0.30	0.21	0.06	0.11
2	1	200	93	10	1	6	1.68	0.78	0.52	0.50	0.21	0.40	0.04	0.19
2	1	1,000	298	17	2	15	2.20	0.70	0.79	0.41	0.42	0.49	0.10	0.30
2	1	5,000	1,560	50	2	22	2.64	0.67	0.94	0.06	0.68	0.22	0.25	0.19
2	5	200	90	16	1	4	1.25	0.37	0.31	0.21	0.09	0.13	0.01	0.05
2	5	1,000	461	44	1	7	1.80	0.35	0.58	0.23	0.24	0.20	0.04	0.09
2	5	5,000	1,502	83	2	15	2.30	0.32	0.84	0.17	0.48	0.23	0.12	0.15

[a] $k=1$, one-stage selection; $k=2$, two-stage selection
[b] SD = standard deviation of estimates among runs

$\hat{P}_k^*(0.1\%)$ in comparison with $\Delta\hat{G}_k^*$. In addition, $\Delta\hat{G}_k^*$, $\hat{P}_k^*(5\%)$, $\hat{P}_k^*(1\%)$, and $\hat{P}_k^*(0.1\%)$ were reduced by approximately 25, 35, 65, and 70%, respectively, with increasing non-genetic variance. For $C=1$ compared with $C=0$, N_j^* decreased about 50%, whereas L_j^* changed only slightly. The reduction in $\Delta\hat{G}_k^*$ and $\hat{P}_k^*(q)$ for

Table 3 Optimum allocation of test resources maximizing selection gain $\Delta\hat{G}_k^*$ or probability $\hat{P}_k^*(q)$ of identifying one line ($N_f = 1$) with a genotypic value belonging to the 5, 1, and 0.1% best genotypes of the population for two-stage selection assuming $B = 1{,}000$. For explanation of abbreviations, see Table 1

Assumptions		Optimum allocation					
VC	C	N_1^*	N_2^*	L_1^*	L_2^*	OC[a]	SD[b]
$\Delta\hat{G}_k^*$							
1[c]	0	739	29	1	9	2.59	0.63
	0.5	498	28	1	9	2.51	0.63
	1	396	23	1	9	2.44	0.64
2[d]	0	660	34	1	10	2.26	0.73
	0.5	298	17	2	15	2.20	0.70
	1	251	19	2	13	2.16	0.70
3[e]	0	346	22	2	14	1.89	0.80
	0.5	224	12	3	18	1.85	0.78
	1	199	12	3	17	1.82	0.78
$\hat{P}_k^*(5\%)$							
1	0	760	30	1	8	0.93	0.25
	0.5	480	31	1	9	0.92	0.27
	1	350	30	1	10	0.90	0.30
2	0	620	38	1	10	0.80	0.40
	0.5	293	19	2	14	0.78	0.41
	1	257	19	2	12	0.76	0.43
3	0	580	35	1	12	0.61	0.49
	0.5	271	23	2	14	0.60	0.49
	1	254	17	2	14	0.58	0.49
$\hat{P}_k^*(1\%)$							
1	0	739	29	1	9	0.66	0.47
	0.5	480	31	1	9	0.61	0.49
	1	392	27	1	8	0.56	0.50
2	0	690	31	1	10	0.46	0.50
	0.5	312	20	2	11	0.42	0.50
	1	257	19	2	12	0.40	0.49
3	0	667	37	1	9	0.28	0.45
	0.5	288	28	2	10	0.27	0.44
	1	271	17	2	11	0.26	0.44
$\hat{P}_k^*(0.1\%)$							
1	0	832	24	1	7	0.21	0.41
	0.5	538	32	1	6	0.17	0.38
	1	427	29	1	5	0.15	0.35
2	0	760	40	1	6	0.12	0.32
	0.5	526	35	1	6	0.10	0.30
	1	412	35	1	5	0.09	0.28
3	0	730	45	1	6	0.06	0.24
	0.5	480	35	1	8	0.06	0.23
	1	395	42	1	5	0.05	0.21

[a]OC = optimization criterion
[b]SD = standard deviation of estimates among runs
[c]VC1 = 1:0.25:0.25:0.5:1
[d]VC2 = 1:0.5:0.5:1:2
[e]VC3 = 1:1:1:2:4

$C = 1$ versus $C = 0$ was small, and ranged from 5% $(\Delta\hat{G}_k^*)$ to 30% $(\hat{P}_k^*(0.1\%))$.

Discussion

The selection gain (ΔG_k) is the most widely used criterion to optimize selection processes, but an infinite sample size was assumed in most studies (Cochran 1951; Hanson and Brim 1963; Utz 1969; Tomerius 2001; Grüneberg et al. 2004). As breeding populations normally are relatively small, we determined ΔG_k for finite sample sizes. However, both assumptions result in similar optimum allocations and marginally reduced gains for the finite sample case (Cochran 1951; Finney 1966; Utz 1969). We compared ΔG_k with an alternative optimization criterion, the probability $P_k(q)$ of identifying superior genotypes. Both ΔG_k and $P_k(q)$ were estimated by Monte Carlo simulations for one- and two-stage selection, assuming a Gaussian normal distribution of (1) genotypic and (2) phenotypic values. Experimental verification of the latter assumption requires a large population size in view of the low power of statistical tests for deviations from a Gaussian normal distribution. However, an extremely extensive QTL mapping experiment in maize (Schön et al. 2004) with testcross

progenies of 976 F_5 lines evaluated in 19 locations provided no evidence that phenotypic means for yield deviated from a Gaussian normal distribution. Likewise, the large number of detected QTL with small effects resulted in an approximative Gaussian normal distribution of genotypic values due to the Central Limit Theorem (Schön et al. 2004). Nevertheless, further research is needed to check the assumptions on probability distributions. DH populations should be an excellent tool for this purpose, because natural selection during inbreeding is minimized, if selection during in vivo haploid induction can be neglected.

We chose an accuracy of 0.01 for the optimization criteria to limit the number of simulation runs to a manageable number. Increasing the accuracy up to 0.0001 would require 5,000,000–600,000,000 simulation runs. However, the length of the resulting optimum allocation interval for an accuracy of 0.01 is only a minor problem for practical breeding purposes due to the extremely flat response curves.

Comparison of optimization criteria

In a first step, we compare the two optimization criteria under the assumption of no non-genetic variance ($h^2 = 1$) and one-stage selection (Fig. 3), because two-stage selection offers advantages only for $h^2 < 1$. Our simulation results for $\Delta\hat{G}_1$ and $SD_{\Delta\hat{G}_1}$ were in harmony with means and standard deviations of order statistics (Pearson and Hartley 1972). For $\hat{P}_1(q)$ the results were in agreement with those reported by Robson et al. (1967, Appendix 6 and Table 2). Thus, our Monte Carlo simulations were sufficiently accurate to estimate ΔG_k and $P_1(q)$. Furthermore, simulations can provide estimates for $SD_{\Delta G_2}$, $P_2(q)$, $SD_{P_1(q)}$, and $SD_{P_2(q)}$, which were not reported in previous studies.

The response curves of both optimization criteria illustrated that the slopes decreased with an increasing number of lines (Fig. 3). This corroborates the well-known relationship that a linear increase in ΔG_1 requires an exponential increase in N_1 (Becker 1993). The choice

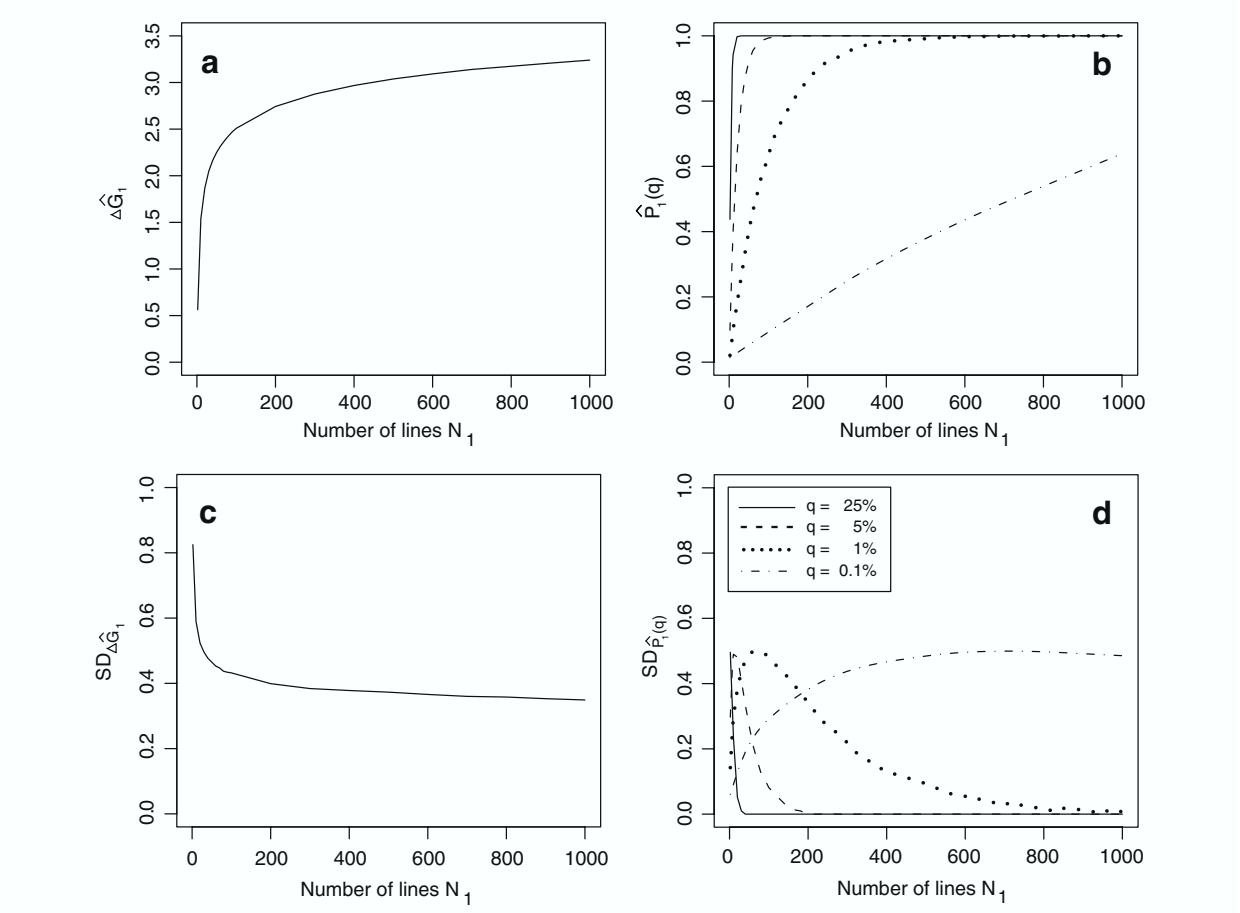

Fig. 3 **a** Selection gain $\Delta\hat{G}_1$, **b** probability $\hat{P}_1(q)$ of identifying one line with a genotypic value belonging to the q% best genotypes of the population, **c**, **d** corresponding standard deviation $SD_{\Delta\hat{G}_1}$ and $SD_{\hat{P}_1(q)}$, respectively, as a function of the number of lines assuming $h^2 = 1$. For explanation of abbreviations, see Table 1

of q had a strong influence on the curve of $\hat{P}_1(q)$, especially its slope. Response curves of $\hat{P}_1(5\%)$ and $\hat{P}_1(1\%)$ were similar in shape to the curve of $\Delta\hat{G}_1$. $\hat{P}_1(25\%)$ increased rapidly between 2 and 20 lines reaching for $N_1 > 20$ a 100% probability that the selected genotype belongs to the best 25% of the population. In contrast, the response curve of $\hat{P}_1(0.1\%)$ was almost linear with a low slope. Thus, for $N_1 = 1{,}000$ the probability that the selected genotype belongs to the best 0.1% of the population was still smaller than 65%. Consequently, for obtaining DHs with large genotypic values, a very large number N_1 of initial lines must be tested, which is in harmony with results of Robson et al. (1967), Johnson (1989), and Knapp (1998). In addition to rarely occurring positive recombinants, this result may explain that outstanding inbreds are identified only seldom in practice because the choice of N_1 is commonly much smaller than required. $\hat{P}_k(25\%)$ will be disregarded in our further discussion, because of being close to one.

In practice, selection is based on phenotypic and not on genotypic values and, thus, heritability is smaller than one. The influence of the different optimization criteria on the optimum allocation of test resources was hardly affected by the number of selection stages. Hence, only results for two-stage selection are discussed. Optimum allocation of test resources differed for the two optimization criteria and also for values of q, especially under large non-genetic variance (Table 3). The closest agreement between the optimum allocation of test resources maximizing $\hat{P}_k(q)$ and $\Delta\hat{G}_k$ was observed for $q = 5\%$. With decreasing values of q, an increased N_1^* and a decreased L_j^* were observed. Nevertheless, values of $\hat{P}_k^*(q)$ differed only slightly from $\hat{P}_k(q)$ at the optimum allocation of test resources with regard to $\Delta\hat{G}_k$. For instance, $\hat{P}_2^*(0.1\%) - \hat{P}_2(0.1\%)$ was below 0.01 for two-stage selection, $B = 1{,}000$, $N_f = 1$, VC2, and $C = 0.5$ (Tables 2, 3). This can be explained by the flat response curves of $\Delta\hat{G}_k$ and $\hat{P}_k(q)$ in the vicinity of the maximum (Fig. 2). For $\Delta\hat{G}_k$, it is attributable to the small slopes of the curves of h^2 for increased L_j, and i_α for decreased α (Becker 1993). For $\hat{P}_k(q)$, these findings are due to the small slopes of the curves of (1) h^2 and (2) the probability that genotypes belonging to the q% best genotypes of the population are among the lines for decreased α (Fig. 3).

The concept of ΔG_k is based on the superiority of the selected genotypes in comparison with their unselected base population. In contrast, $P_k(q)$ reflects the chance of developing competitive varieties that are better than the existing ones. To have a realistic chance of identifying a superior genotype, $P_k(q)$ should be greater than 75%, permitting only q values of about 5% for the budgets considered. The choice of the optimization criterion for these q values is not crucial, because the optimum allocation of test resources differed only slightly from those obtained by applying $\Delta\hat{G}_k$. For small values of q, different allocation optima were obtained for $\Delta\hat{G}_k$ and $\hat{P}_k(q)$, but probabilities $\hat{P}_k(q)$ were too low to be recommended as optimization criterion for the budgets investigated. Extending the formula of $P_1(q)$ given by Robson et al. (1967) to multi-stage selection could facilitate the optimum allocation of resources based on $P_k(q)$ due to a drastic reduction in computation time.

Standard deviations of optimization criteria

The choice of q had a large influence on the curves of $SD_{\hat{P}_1(q)}$ (Fig. 3). For instance, $SD_{\hat{P}_1(25\%)}$ decreased rapidly between 2 and 20 lines and reached zero for $N_1 = 40$, whereas $SD_{\hat{P}_1(0.1\%)}$ increased up to a maximum at $N_1 = 700$ and decreased slightly thereafter. These differences can be explained by the binomial nature of $P_k(q)$ with genotypes surpassing the defined threshold or not. Thus, $SD_{\hat{P}_k(q)}$ assumed its maximum for $\hat{P}_k(q) = 0.5$. In contrast, the response curve of $SD_{\Delta\hat{G}_1}$ decreased continuously with an increasing number of lines (Fig. 3) and test locations (Fig. 2). The small differences between values of $SD_{\Delta\hat{G}_1}$ for varying budgets (Fig. 2) can be explained by the small negative slope of $SD_{\Delta\hat{G}_1}$ for increasing values of N_1 (Fig. 3). As the curves of the optimization criteria were flat in the vicinity of the maximum (Fig. 2), their respective SD could serve as a secondary optimization criterion. However, curves of SD were also flat in the vicinity of the maximum of the optimization criteria, thus limiting their usefulness as additional optimization criterion.

Economic frame, quantitative-genetic parameters, and selection strategies

To assess their relative importance, the economic frame and quantitative-genetic parameters were varied in a range relevant for maize. Production costs C of DHs covered the entire range from recently established ($C = 1$) to further improved ($C = 0$) DH technology, with $C = 0.5$ corresponding to the actual costs in breeding companies advanced in the DH technique (Seitz, personal communication). The budget in our study can either refer to the resources available for evaluating the progenies of one cross ($B = 200 - 1{,}000$) or a complete breeding program ($B = 5{,}000$). For instance, considering the evaluation of 100 DH lines for each cross in two locations, 200 plots are required for 1 cross, and 5,000 plots for 25 crosses. The optimization of a complete breeding program would, however, require the assumption of equal means and segregation variances for progenies from different crosses. As these parameters usually differ among crosses (cf. Mihaljevic et al. 2004), optimization of breeding programs including these population parameters would be very promising but requires additional research.

The choice of N_f in this study reflects two situations. Commonly, numerous crosses are completely rejected before final evaluation and only few lines are selected in each of the remaining crosses. Thus, $N_f = 1$ represents a reasonable compromise for one specific cross. In contrast, in a complete breeding program, typically several

lines are finally selected. Consequently, $N_f = 1$ seems appropriate for $B = 200 - 1{,}000$ but $N_f = 5$ for $B = 5{,}000$.

For selection among genetically fixed lines, both optimization criteria depend on $\alpha = N_f/N_1$ and h^2. Variation in the budget or production costs of DHs mainly influenced α and, to a lesser extent, h^2. The budget was the major factor influencing values of both optimization criteria by its strong influence on α (Table 2). In contrast, production costs of DHs had only a minor effect on both optimization criteria. This can be explained by small changes in (1) α in comparison to changes of α for different budgets and (2) h^2 (Tables 2, 3). The slight trend towards larger values of L_j^* for $C = 1$ versus $C = 0$ reflects the fact that rejection of more expensive lines should be based on more reliable information.

The variance components were chosen according to recent estimates from large series of experiments within a broad sample of Central European maize breeding populations including DH populations (VC2, Gordillo and Geiger 2004), reflecting the typical situation for breeding programs with adapted maize populations. Variance components affect h^2 directly, and with VC3 the reduced h^2 could only partly be compensated by increased values of L_j^* with a parallel reduction in N_1^*. Altogether, we found a large reduction in values of $\hat{P}_k(1\%)$ and $\hat{P}_k(0.1\%)(> 50\%)$ with increased non-genetic variance. This is in accordance with previous studies (Keuls and Sieben 1955; Robson et al. 1967; Johnson 1989; Knapp 1998) analyzing the problem to identify superior genotypes under high non-genetic variance. Summarizing, our results underline the high impact of VC on the optimum allocation of resources with alternative breeding strategies.

Breeding is a continuous process and every year a new breeding cycle is initiated. Under this assumption, the annually available budget, for all cycles running in parallel is equal to the budget available for one entire cycle (Utz 1969). Consequently, comparisons between one- and two-stage selection can be made directly without dividing the optimization criteria by the years required in the selection strategy. Two-stage selection with optimum allocation of resources allows the evaluation of a large number of lines N_1 in a small number of test locations L_1. The N_2 lines selected in stage one are further evaluated in a large number of test locations L_2 to ascertain a high accuracy of the test results. This guarantees a low α and high h^2 and increases consequently both optimization criteria. In addition, response curves of the optimization criteria were flatter for two-stage selection than for one-stage selection, reducing the risk of choosing a non-optimal allocation. However, with one-stage selection breeders could exploit 1 year earlier the progress of selection by improved DH lines and hybrids developed from them.

Values of $\Delta\hat{G}_k$ and $\hat{P}_k(q)$ increased roughly to the same extent by (1) two-stage instead of one-stage selection, (2) a fivefold increase in the budget ($B = 200$ to $B = 1{,}000$), (3) a reduction in N_f from five to one, or (4) a quarter reduction in the non-genetic variance (VC1 instead of VC3). Except for the last factor, which is determined by the breeding material and target environments, all other factors can be chosen in favor of an increased selection response, but at the expense of a longer duration of the selection strategy (two-stage selection), higher costs (larger budget), and a higher risk of the final outcome (larger SD for $N_f = 1$). In particular, our results demonstrate that employing two-stage instead of one-stage selection represents a promising alternative to an increased budget.

Conclusions

The production costs of DHs had only a minor effect on the optimum allocation of breeding resources. Even if the current DH production using in vivo haploid induction is still relatively expensive, the compensation obtained through a reduced number of initial lines recommends their application. As DH costs are decreasing owing to expected improvements in the DH technique in the future, they will be only of secondary importance regarding the optimum allocation of resources.

For two-stage selection, a budget of approximately 1,000 field plot equivalents, and actual production costs of DHs, the allocation of test resources is roughly close to its optimum, if (1) the selected fraction $\alpha_1 = N_2/N_1$ is smaller than 0.10, (2) the number of test locations at the final selection stage exceeds at least six, and (3) about three quarters of the budget are invested in the first stage.

We attained a reasonable probability of success with continuous breeding for q values of about 5%. In these cases, the choice of the optimization criterion was relatively unimportant. However, for very large budgets the small probability of identifying outstanding genotypes is maximized if the number of lines is increased at the expense of the number of test locations. Optimization of complete breeding programs based on DHs is very promising, but selection theory must be extended for selection among and within crosses, consideration of different number and types of testers, and tests for line per se performance.

Acknowledgements This research was supported by funds from DFG, Grant No 1070/1, International Research Training Group "Sustainable Resource Use in North China" to C. F. H. Longin. The authors appreciate the editorial work of Dr. J. Muminović whose suggestions considerably improved the style of the manuscript. In addition, the authors thank Dr. F. Laidig, Bundessortenamt Hannover, Germany, and Dr. G. Seitz, AgReliant Genetics, Westfield, IN, USA for their valuable suggestions. We greatly appreciate the helpful comments and suggestions of two anonymous reviewers.

References

Becker H (1993) Pflanzenzüchtung (in German). Verlag Eugen Ulmer, Stuttgart, pp 129–132

Bernardo R (2002) Breeding for quantitative traits in plants. Stemma Press, Woodbury, p 205

Berry DA, Lindgren BW (1996) Statistics. Duxbury Press, New York, p 371

Bordes J, de Vaulx RD, Lapierre A, Pollacsek M (1997) Haplodiploidization of maize (*Zea mays* L.) through induced gynogenesis assisted by glossy markers and its use in breeding. Agronomie 17:291–297

Choo TM, Kannenberg LW (1988) Selection response and efficiency of doubled-haploid recurrent selection in a cross-fertilized species. Theor Appl Genet 75:410–414

Cochran WG (1951) Improvement by means of selection. In: Proc second Berkeley symp math stat prob, pp 449–470

Finney DJ (1966) An experimental study of certain screening processes. J Roy Stat Soc B 28:88–109

Gallais A (1991) A general approach for the study of a population of test-cross progenies and consequences for the recurrent selection. Theor Appl Genet 81:493–503

Gordillo GA, Geiger HH (2004) Estimating quantitative-genetic parameters of European maize populations to optimize hybrid breeding methods by model calculations. In: Poster abstract XVIIth EUCARPIA general congress 2004, Tulln, Austria

Grüneberg WJ, Abidin E, Ndolo P, Pereira CA, Hermann M (2004) Variance component estimations and allocation of resources for breeding sweetpotato under East African conditions. Plant Breed 123:311–315

Hanson WD, Brim CA (1963) Optimum allocation of test material for two-stage testing with an application to evaluation of soybean lines. Crop Sci 3:43–49

Johnson B (1989) The probability of selecting genetically superior S_2 lines from a maize population. Maydica 34:5–14

Keuls M, Sieben JW (1955) Two statistical problems in plant selection. Euphytica 4:34–44

Knapp SJ (1998) Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci 38:1164–1174

Mihaljevic R, Utz HF, Melchinger AE (2004) Congruency of quantitative trait loci detected for agronomic traits in testcrosses of five populations of european maize. Crop Sci 44:114–124

Pearson ES, Hartley HO (1972) Biometrika tables for statisticians, 2nd edn. Cambridge University Press, London

R Development Core Team (2004) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, URL http://www.R-project.org

Röber FK (1999) Fortpflanzungsbiologische und genetische Untersuchungen mit RFLP-Markern zur in-vivo Haploideninduktion bei Mais. (in German). Ph.D. thesis, University of Hohenheim, Stuttgart, Germany

Robson DS, Powers L, Urquhart NS (1967) The proportion of genetic deviates in the tails of a normal population. Theor Appl Genet 37:205–216

Schmidt W (2004) Hybridmaiszüchtung bei der KWS SAAT AG (in German). In: Bericht über die 54. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2003, Gumpenstein, Austria, pp 1–6

Schön CC, Utz HF, Groh S, Truberg B, Openshaw S, Melchinger AE (2004) Quantitative trait locus mapping based on resampling in a vast maize testcross experiment and its relevance to quantitative genetics for complex traits. Genetics 167:485–498

Seitz G (2005) The use of doubled haploids in corn breeding. In: Proceedings of the forty first annual Illinois Corn Breeders' School 2005, Urbana—Champaign, Illinois, USA, pp 1–8

Sprague GF, Federer WT (1951) A comparison of variance components in corn yield trials: II. Error, year × variety, location × variety and variety components. Agron J 42:535–541

Tomerius AM (2001) Optimizing the development of seed-parent lines in hybrid rye breeding. Ph.D. thesis, University of Hohenheim, Stuttgart, Germany (http://opus-ho.uni-stuttgart.de/hop/volltexte/2001/10/pdf/tomerius.pdf)

Utz HF (1969) Mehrstufenselektion in der Pflanzenzüchtung (in German). Arbeiten der Universität Hohenheim, vol 49. Verlag Eugen Ulmer, Stuttgart, Germany

Young JC (1976) Varietal screening from finite normal populations. J Am Stat Assoc 71:87–92

Hybrid Maize Breeding with Doubled Haploids: Comparison Between Selection Criteria

C. Friedrich H. Longin, H. Friedrich Utz, Albrecht E. Melchinger, and Jochen C. Reif

Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany.

ABSTRACT

The optimum allocation of breeding resources is crucial for the efficiency of breeding programmes. The objectives were to (i) compare selection gain (ΔG_k) for finite and infinite sample sizes, (ii) compare ΔG_k and probability of identifying superior hybrids (P_k), and (iii) determine the optimum allocation of the number of hybrids and test locations in hybrid maize breeding using doubled haploids. Infinite compared to finite sample sizes led to almost identical optimum allocation of test resources, but to an inflation of ΔG_k. This inflation decreased as the budget and the number of finally selected hybrids increased. A reasonable P_k was reached for hybrids belonging to the q = 1% best of the population. The optimum allocations for $P_k(q)$ and ΔG_k were similar indicating that $P_k(q)$ is promising for optimizing breeding programmes.

THE optimum allocation of financial and breeding resources is of fundamental importance for the efficiency of breeding programmes. Currently, doubled haploids (DHs) are adopted as a routine method in commercial maize breeding programmes (Seitz 2005). Their efficient use requires the optimization of the entire breeding strategy in order to maximize progress from selection. To quantify the progress from k selection stages, various criteria have been used.

Selection gain ΔG_k is the most widely used criterion to optimize selection processes. The selection theory for ΔG_k was developed assuming an infinite sample size, although populations of medium size are commonly used in plant breeding (Cochran 1951; Hanson and Brim 1963; Utz 1969; Grüneberg et al. 2004). This assumption simplifies the calculations considerably. Inflated ΔG_k and slightly different optimum allocation of test resources for infinite compared to finite sample size were reported in the literature (Cochran 1951; Finney 1966; Utz 1969; Young 1976). However, these studies were conducted more than 30 years ago and the limited computing power available at that time restricted the accuracy of simulations. The probability of identifying superior genotypes (P_k) represents an interesting alternative to ΔG_k for optimizing the allocation of test resources (Robson et al. 1967; Johnson 1989; Longin et al. 2006).

The allocation of test resources in hybrid maize breeding with DHs was optimized under one- and two-stage selection for testcross performance with a given tester by using Monte Carlo simulations and numerical integration. The objectives were to (i) compare ΔG_k for finite and infinite sample sizes, (ii) compare ΔG_k and P_k, and (iii) determine the optimum allocation of the number of hybrids and test locations.

Corresponding author: Albrecht E. Melchinger, Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany. Email: melchinger@uni-hohenheim.de

MATERIALS AND METHODS

Selection strategies

A total of N_1 hybrids generated by crosses of DH lines to a given tester are available each year to start selection. The tester can be any population with an arbitrary structure, such as an inbred line, single cross, or random mating population. The N_f phenotypically best hybrids are finally selected. A value of $N_f = 1$ was assumed to emphasize the interest in the very best hybrid. The target variable is the genotypic value of testcross performance with a given tester for a certain trait or index of traits. With one-stage selection, selection is based on field tests in a single year. With two-stage selection, field tests are conducted in two years with a subset of the most superior hybrids N_2 selected after the first year being evaluated in the second year. At stage j ($j = 1, 2$), selection among N_j hybrids is based on the phenotypic mean of testcross performance at this stage with a given tester evaluated in L_j test locations with R_j replications. Without an upper limit on L_j, $R_j = 1$ is optimal for ΔG_k (Sprague and Federer 1951; Utz 1969). The R_j value was thus set to 1.

Economic frame and quantitative-genetic parameters

A fixed total budget B for (i) producing the DH lines and (ii) evaluating their testcross progenies in two selection stages was defined in terms of testcross plot equivalents as $B = N_1C + N_1L_1R_1 + N_2L_2R_2$ assuming equal plot sizes in both selection stages. Therein, the production cost C of one DH line was assumed to equal half the cost of one field plot ($C = 0.5$), corresponding to the actual costs of DH production in breeding companies most advanced in the DH technique (Seitz, pers. comm.). The focus was generally on a budget of $B = 20\,000$ field plot equivalents. Three ratios of variance components ($\sigma_g^2 : \sigma_{gl}^2 : \sigma_{gy}^2 : \sigma_{gly}^2 : \sigma_e^2$) were considered, where σ_g^2

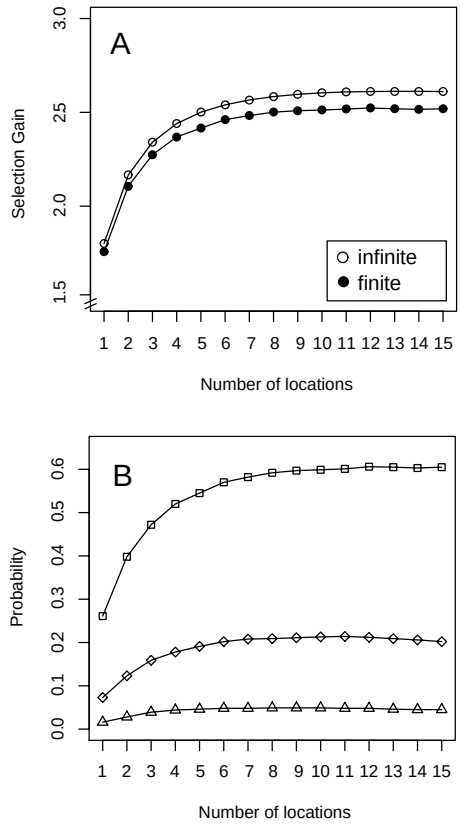

Figure 1 (**A**) Selection gain for infinite $\Delta G(inf)_1$ and finite sample size $\Delta\hat{G}_1$ and (**B**) probability $\hat{P}_1(q)$ of identifying one hybrid with a genotypic value belonging to the 1%(□), 0.1%(◊), and 0.01%(△) best genotypes of the population as a function of the number of test locations for one-stage selection, assuming a budget of 20 000 field plot equivalents and a ratio of variance components of 1 : 0.5 : 0.5 : 1 : 2.

refers to the genotypic variance, σ^2_{gl} to the variance of genotype × location interactions, σ^2_{gy} to the variance of genotype × year interactions, σ^2_{gly} to the variance of genotype × location × year interactions, and σ^2_e to the error variance. Values were set to $VC1$ = 1 : 0.25 : 0.25 : 0.5 : 1, $VC2$ = 1 : 0.5 : 0.5 : 1 : 2, and $VC3$ = 1 : 1 : 1 : 2 : 4, resulting in a heritability on a plot basis of 0.33, 0.20, and 0.11, respectively. These ratios were chosen based on combined analyses of variance of testcrosses of DH populations from commercial breeding programmes (Longin et al. 2006).

Calculation of optimization criteria

Selection gain for finite sample size ($\Delta\hat{G}_k$) and probability of identifying superior hybrids ($\hat{P}_k$) were estimated by Monte Carlo Simulations according to Longin et al. (2006) assuming a standard normal distribution of the hybrids in a whole breeding programme. The calculation of selection gain for infinite sample sizes ($\Delta G(inf)_k$) with numerical integration is based on uni- and bivariate normal integrals for selected fractions $\alpha_j = N_{j+1}/N_j$ and the square root of heritability of phenotypic means at stage j (*cf.* Cochran 1951). An admissible allocation of test resources refers to tuples (N_j, L_j) for all stages j. An element $(N^*_j; L^*_j)$ is denoted as an optimum allocation if it maximizes the optimization criterion in the set of admissible allocations. The values of each optimization criterion at its corresponding optimum allocation $(N^*_j; L^*_j)$ were denoted as $\Delta\hat{G}^*_k$ and $\hat{P}^*_k(q)$ for the Monte Carlo simulations and $\Delta G(inf)^*_k$ for the numerical integration. The optimization criteria $\Delta\hat{G}_k$ and $\hat{P}_k(q)$ are estimated with a precision of 0.01 to limit the number of simulation runs to a manageable number (Longin et al. 2006). Thus, the optimum allocation (N^*_j, L^*_j) was determined following Utz (1969) such that the number of test locations was minimum among all allocations within a 0.01 drop-off of all optimization criteria, since breeders prefer tests in fewer test locations for technical reasons if this only affects the optimization criteria marginally.

Table 1 Optimum allocation of test resources maximizing selection gain for infinite ($\Delta G(inf)^*_1$) and finite sample size ($\Delta\hat{G}^*_1$) and their standard deviation (SD) for one-stage selection assuming a ratio of variance components of 1 : 0.5 : 0.5 : 1 : 2. (B = budget in field plot equivalents, N_f = number of finally selected hybrids, N^*_1, L^*_1 = optimum number of hybrids and test locations, OC = optimization criterion).

Assumptions		Optimum allocation			
B	N_f	N^*_1	L^*_1	OC	SD^a
$\Delta G(inf)^*_1$					
200	1	44	4	1.54	0.83
	5	57	3	1.11	0.39
	20	80	2	0.71	0.21
20 000	1	1904	10	2.60	0.73
	5	2352	8	2.26	0.34
	20	3076	6	1.95	0.18
$\Delta\hat{G}^*_1$					
200	1	44	4	1.42	0.80
	5	57	3	1.08	0.36
	20	133	1	0.69	0.21
20 000	1	1739	11	2.52	0.72
	5	2352	8	2.25	0.34
	20	3076	6	1.94	0.17

[a] Approximated for infinite sample size after Burrows (1975)

RESULTS

With increasing L_1, the optimization criteria $\Delta G(inf)_1$, $\Delta\hat{G}_1$, and $\hat{P}_1(q)$ increased up to an optimum and decreased slightly thereafter (Fig. 1). The increase in $\Delta G(inf)_1$, $\Delta\hat{G}_1$, and $\hat{P}_1(q)$ was largest between $L_1 = 1$ and $L_1 = 6$. All response curves were flat in the vicinity of the maximum. With decreasing q, the slope of $\hat{P}_1(q)$ decreased. The optimum allocation and the standard deviations (SDs) of the optimization criteria were similar for $\Delta G(inf)^*_1$ and $\Delta\hat{G}^*_1$ (Table 1). Differences were observed for $\Delta G(inf)^*_1$ compared to $\Delta\hat{G}^*_1$. With increasing N_f, the ratio $\Delta G(inf)^*_1/\Delta\hat{G}^*_1$ decreased from 8.5 to 2.9% for B = 200 and from 3.2 to 0.5% for B = 20 000. With increasing B, the ratio $\Delta G(inf)^*_1/\Delta\hat{G}^*_1$ decreased from 8.5% to 3.2% for N_f = 1 and

Table 2 Optimum allocation of test resources maximizing selection gain $\Delta\hat{G}_k^*$ or probability $\hat{P}_k^*(q)$ of identifying one hybrid with a genotypic value belonging to the q = 1, 0.1, and 0.01% best genotypes in the population assuming a budget of 20 000 field plot equivalents. (k = number of selection stages, VC = ratio of variance components, N_j^*, L_j^* = optimum number of hybrids and test locations at stage j, OC = optimization criterion, SD = standard deviation of estimates among runs).

Assumptions		Optimum allocation					
k	VC	N_1^*	N_2^*	L_1^*	L_2^*	OC	SD
$\Delta\hat{G}_k^*$							
1	1[a]	2666	-	7	-	2.87	0.63
1	2[b]	1739	-	11	-	2.25	0.72
1	3[c]	1481	-	13	-	2.11	0.81
2	1	10440	217	1	20	3.33	0.56
2	2	6090	129	2	37	2.99	0.64
2	3	3660	84	4	42	2.57	0.73
$\hat{P}_k^*(1\%)$							
1	1	2666	-	7	-	0.81	0.40
1	2	2105	-	9	-	0.60	0.49
1	3	1739	-	11	-	0.39	0.49
2	1	7224	102	2	19	0.97	0.19
2	2	4926	89	3	31	0.85	0.36
2	3	3967	58	4	37	0.63	0.48
$\hat{P}_k^*(0.1\%)$							
1	1	3636	-	5	-	0.35	0.48
1	2	2666	-	7	-	0.21	0.41
1	3	2666	-	7	-	0.10	0.30
2	1	10440	217	1	20	0.66	0.47
2	2	6627	143	2	24	0.43	0.50
2	3	5001	96	3	26	0.23	0.42
$\hat{P}_k^*(0.01\%)$							
1	1[c]	5714	-	3	-	0.09	0.08
1	2	4444	-	4	-	0.04	0.04
1	3	5714	-	3	-	0.01	0.01
2	1	10750	298	1	13	0.23	0.42
2	2	7104	140	2	16	0.12	0.33
2	3	5450	66	3	14	0.05	0.23

[a] $VC1$ = 1: 0.25: 0.25: 0.5: 1; [b] $VC2$ = 1: 0.5: 0.5: 1: 2; [c] $VC3$ = 1: 1: 1: 2: 4

from 2.9 to 0.5% for N_f = 20. The optimum allocation of test resources based on the same VC but different optimization criteria differed largely for small values of q (q = 0.1, 0.01%) and large non-genetic variance ($VC3$, Table 2). For instance, for $VC3$, the optimum number of hybrids N_j^* was approximately doubled and the optimum number of test locations L_j^* was more than halved for $\hat{P}_k^*(0.01\%)$ in comparison with $\Delta\hat{G}_k^*$.

The optimum number of initial hybrids N_1^* and test locations for two-stage selection was about twice as large as for one-stage selection (Table 2). This was due to the optimum allocation of two-stage selection, which comprised a large number of initial hybrids N_1^* tested in a small number of test test locations L_1^* at the first stage, and a small number of selected hybrids N_2^* tested in a large number of test locations L_2^* at the second stage. Furthermore, values of $\Delta\hat{G}_k^*$, and of $\hat{P}_k(1\%)^*$, $\hat{P}_k(0.1\%)^*$, and $\hat{P}_k(0.01\%)^*$ were 18%, 40%, 100%, and 250%, respectively, higher on average than for one-stage selection.

DISCUSSION

Comparison of selection gain for infinite *vs.* finite sample size The optimum allocation of test resources regarding selection gain for infinite ($\Delta G(inf)_k^*$) *vs.* finite sample size ($\Delta\hat{G}^*$) was almost identical for all the scenarios considered (Table 1). This is in accordance with a previous study (Utz 1969) and can be explained by the similar response curves for $\Delta G(inf)_k$ and $\Delta\hat{G}$ as a function of the number of test locations (Fig. 1). The similar response curves are due to similar slopes of the selection intensity for infinite and finite sample size and to the fact that heritability is not affected by the sample size of the population.

For small budgets and number of finally selected hybrids, $\Delta G(inf)_k^*$ was clearly inflated in comparison to $\Delta\hat{G}_k^*$ (Table 1), which is in harmony with results reported in the literature (Utz 1969). The inflation of $\Delta G(inf)_k^*$ decreased with increasing B and/or N_f. This can be explained by the distribution of the hy-

brids. With increasing population size (increasing B), the deviation of the realized distribution for finite sample sizes from the expected standard normal distribution for infinite sample sizes decreases. The impact of N_f can be explained by the fact that the deviation from the standard normal distribution for small sample sizes mainly affects the tails of the distribution.

Comparison between several breeding alternatives is normally based on the use of either $\Delta G(inf)_k$ or $\Delta\hat{G}_k$. Thus, the alternatives are equally affected by the inflation of $\Delta G(inf)_k$. For comparison between different B and N_f values, the small bias caused by $\Delta G(inf)_k$ can be neglected in comparison to the large impact of B and N_f on selection gain (Table 1). Consequently, the simplifying assumption of infinite sample sizes for determining the optimum allocation of test resources is justifiable as long as a reduction in computing time and effort is warranted.

Comparison of selection gain with probability of identifying superior hybrids

The optimum allocation of test resources differed for $\Delta\hat{G}_k^*$ and $\hat{P}_k(q)^*$ especially for small values of q and large non-genetic variance (Table 2). For one-stage selection, the closest agreement between the optimum allocation of test resources maximizing $\hat{P}_k(q)$ and $\Delta\hat{G}_k$ was observed for $q = 5\%$ (data not shown). With decreasing values of q, an increased N_1^* and a decreased L_1^* were observed. This can be explained by the fact that the probability that genotypes belonging to the q% best genotypes of the population are among the initial hybrids decreases rapidly with decreasing N_1 and q (Longin et al. 2006). In addition, the slope of the response curves of $\hat{P}_k(q)$ decreased with smaller q (Fig. 1), favoring allocations with smaller L_1.

For two-stage selection, the optimum allocation of test resources maximizing $\hat{P}_2(q)$ and $\Delta\hat{G}_2$ was only comparable for $VC1$ and $q = 0.1\%$. For large non-genetic variances and small q, an increased N_1^* and a decreased L_2^* were observed. However, for $q = 1\%$, $VC1$, and $VC2$, a decreased N_j^* and increased L_1^* were observed in comparison to $\Delta\hat{G}_2^*$. This may be due to the considerable increased N_1^* in two-stage selection compared to one-stage selection and the consequent increase in the importance of heritability. Nevertheless, values of $\hat{P}_k^*(q)$ differed only slightly from values of $\hat{P}_k(q)$ at the optimum allocation of test resources with regard to $\Delta\hat{G}_k^*$ (Longin et al. 2006), which can be explained by the flat response curves of $\Delta\hat{G}_k$ and $\hat{P}_k(q)$ in the vicinity of the maximum (Fig. 1).

To have a realistic chance of identifying a superior genotype, $P_k(q)$ should be greater than 75%, permitting only q values of about 1% even for the large budget considered. The choice of the optimization criterion for these q values is not crucial, because the optimum allocation of test resources differed only slightly from those obtained by applying $\Delta\hat{G}_k$. Therefore, the use of $P_k(q)$ seems appealing for the optimization of breeding programmes, favoring a reduction in the number of test locations with a parallel increase of the number of initial hybrids for the selection of very outstanding hybrids.

Two-stage selection – promising method to increase $P_k(q)$

The possibilities to increase $\hat{P}_k(q)$ are limited especially for small values of q (Longin et al. 2006). An increasing budget increases $\hat{P}_k(q)$, but the return from investment is rather low. Increasing the number of selection stages from one to two, considerably increased $\Delta\hat{G}_k$ and $\hat{P}_k(q)$ (Table 2). This is due to the optimum allocation of test resources in two-stage selection involving the evaluation of (i) a large number of hybrids in a small number of test locations in the first year and (ii) a small number of the selected superior hybrids in a large number of test locations in the second year. The probability of identifying superior hybrids for small values of q was particularly improved by two-stage selection in comparison to one-stage selection, which may mainly be due to the increased N_1^*. With one-stage selection, breeders could exploit the progress of selection by improved hybrids one year earlier. However, the limited possibilities for increasing $\hat{P}_k(q)$ make the use of two-stage instead of one-stage selection very appealing to identify a hybrid with outstanding performance.

This research was supported by funds from DFG, Grant No 1070/1, International Research Training Group "Sustainable Resource Use in North China" to C. F. H. Longin.

LITERATURE CITED

Burrows PM (1975) Variances of selection differentials in normal samples. Biometrics 31:125–133

Cochran WG (1951) Improvement by means of selection. Proc Second Berkeley Symp Math Stat Prob, pp 449–470

Finney DJ (1966) An experimental study of certain screening processes. J Roy Stat Soc B 28:88–109

Grüneberg WJ, Abidin E, Ndolo P, Pereira CA, Hermann M (2004) Variance component estimations and allocation of resources for breeding sweetpotato under East African conditions. Plant Breeding 123:311–315

Hanson WD, Brim CA (1963) Optimum allocation of test material for two-stage testing with an application to evaluation of soybean lines. Crop Sci 3:43–49

Johnson B (1989) The probability of selecting genetically superior S_2 lines from a maize population. Maydica 34:5–14

Longin CFH, Utz HF, Reif JC, Schipprack W, Melchinger AE (2006) Hybrid maize breeding with doubled haploids: I. One-stage versus two-stage selection for testcross performance. Theor Appl Genet 112:903–912

Robson DS, Powers LR, Urquhart NS (1967) The proportion of genetic deviates in the tails of a normal population. Theor Appl Genet 37:205–216

Seitz G (2005) The use of doubled haploids in corn breeding. In: Proceedings of the Forty First Annual Illinois Corn Breeders' School 2005, Urbana-Champaign, Illinois, USA, pp 1–8

Sprague GF, Federer WT (1951) A comparison of variance components in corn yield trials: II. Error, year × variety, location × variety and variety components. Agron Jour 42:535–541

Utz HF (1969) Mehrstufenselektion in der Pflanzenzüchtung. (In German) Arbeiten der Universität Hohenheim, Vol 49, Verlag Eugen Ulmer, Stuttgart, Germany

Young JC (1976) Varietal screening from finite normal populations. J Am Stat Assoc 71:87–92

Theor Appl Genet (2007) 114:393–402
DOI 10.1007/s00122-006-0422-z

ORIGINAL PAPER

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

C. Friedrich H. Longin · H. Friedrich Utz · Albrecht E. Melchinger · Jochen C. Reif

Received: 11 May 2006 / Accepted: 29 September 2006 / Published online: 16 December 2006

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.

Communicated by H. C. Becker.

C. Friedrich H. Longin and H. Friedrich Utz have contributed equally to this work.

C. F. H. Longin · H. F. Utz · A. E. Melchinger (✉) · J. C. Reif
Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany
e-mail: melchinger@uni-hohenheim.de

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-

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 $H = a_{GCA}\, g_{GCA} + a_{LP}\, g_{LP}$ (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 $a_{LP} = 0$ restricting the target variable to GCA. For comparison, we also calculated $a_{LP} = 0.1$ and 0.2 with $a_{GCA} = 1 - a_{LP}$. 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 $h^2_{x_j} = \sigma^2_{GCA}/\sigma^2_{x_j}$ with

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

where σ^2_{GCA} and σ^2_{SCA} refer to the variance of GCA and SCA effects, $\sigma^2_{GCA\times y}$ to the variance of GCA × year interactions, $\sigma^2_{GCA\times l}$ to the variance of GCA × location interactions, $\sigma^2_{GCA\times l\times y}$ to the variance of GCA × location × year interactions, $\sigma^2_{SCA\times y}$, $\sigma^2_{SCA\times l}$, and $\sigma^2_{SCA\times l\times y}$ 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

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

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, $h^2_{x_1} = \sigma^2_{Line}/\sigma^2_{x_1}$ with

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

where σ^2_{Line} refers to the genetic variance among lines, $\sigma^2_{Line\times y}$ to the variance of line × year interactions, $\sigma^2_{Line\times l}$ to the variance of line × location interactions, $\sigma^2_{Line\times l\times y}$ 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

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

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 $a_{LP} > 0$ 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, $\sigma^2_{GCA \times y}$ to the variance of GCA × year interactions, $\sigma^2_{GCA \times l}$ to the variance of GCA × location interactions, $\sigma^2_{GCA \times l \times y}$ to the variance of GCA × location × year interactions, $\sigma^2_{SCA \times y}$, $\sigma^2_{SCA \times l}$, and $\sigma^2_{SCA \times l \times y}$ 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, $\sigma^2_{Line \times y}$ to variance of line × year interactions, $\sigma^2_{Line \times l}$ to the variance of line × location interactions, as well as $\sigma^2_{Line \times l \times y}$ to the variance of line × location × year interactions

Testcross performance										
Acronym	Variance components									
	$\sigma^2_{SCA}/\sigma^2_{GCA}$	σ^2_{GCA}	$\sigma^2_{GCA \times y}$	$\sigma^2_{GCA \times l}$	$\sigma^2_{GCA \times l \times y}$	σ^2_{SCA}	$\sigma^2_{SCA \times y}$	$\sigma^2_{SCA \times l}$	$\sigma^2_{SCA \times l \times y}$	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}	$\sigma^2_{Line \times y}$	$\sigma^2_{Line \times l}$	$\sigma^2_{Line \times l \times y}$	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 $N_1 C + N_1 T_1 L_1 + N_2 T_2 L_2$, 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 ρ(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 $\sigma^2_{SCA}/\sigma^2_{GCA}$ 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 $\sigma^2_{SCA}/\sigma^2_{GCA}$, 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 $\sigma^2_{SCA}/\sigma^2_{GCA}$. Thus, results were presented only for $\sigma^2_{SCA}/\sigma^2_{GCA} = 1/2$. 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 $\sigma^2_{SCA}/\sigma^2_{GCA}$ 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

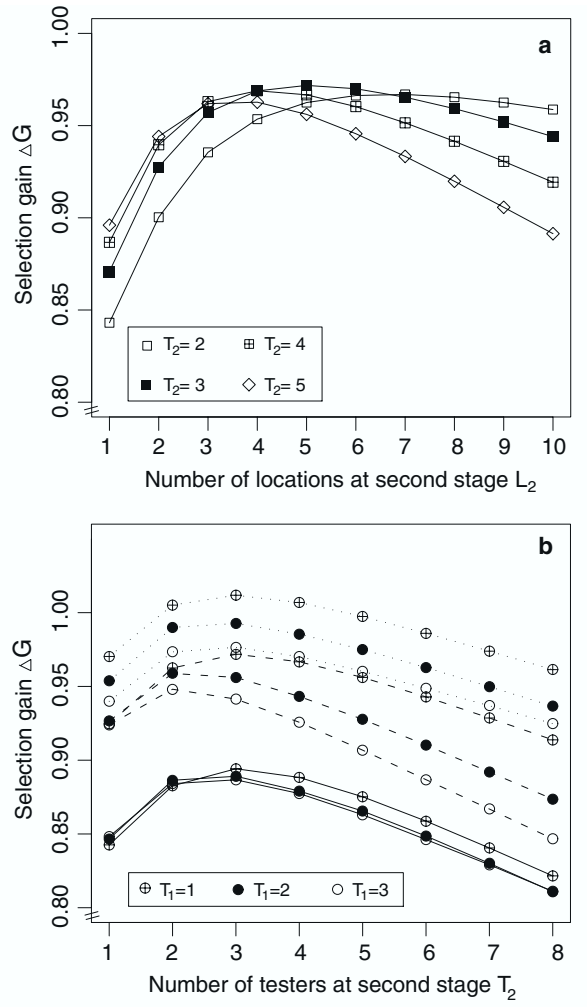

Fig. 1 Selection gain (ΔG) in two-stage selection for GCA as a function of **a** the number of test locations and inbred testers in the second stage, assuming T_1^* and L_1^*, and **b** the number of inbred testers in the second stage for different numbers of single-cross testers in the first stage and ratios of $\sigma^2_{SCA}/\sigma^2_{GCA} = 1/4$ (*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 $\sigma^2_{SCA}/\sigma^2_{GCA} = 1/2$, $T_c = \min(T_1 \times M_1, T_2 \times M_2)$, and tester type of T_2 restricted to inbred testers. For explanation of abbreviations, see Table 1

Budget	Variance components		ρ(LP, GCA)	Optimum allocation						ΔG^*
	TC[a]	LP		T_1^*	T_2^*	L_1^*	L_2^*	N_1^*	N_2^*	
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 $\sigma^2_{SCA}/\sigma^2_{GCA}$. 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, $\rho_1 = \sqrt{h^2_{\bar{x}_1}}$, whereas for BS2, $\rho_1 = \sqrt{h^2_{\bar{x}_1}} \times \rho(\mathrm{LP}, \mathrm{GCA})$. 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.

Acknowledgments This research was supported by funds from DFG, grant no. 1070/1, International Research Training Group ''Sustainable Resource Use in North China'' to C. F. H. Longin. The authors appreciate the editorial work of Dr. J. Muminović, whose suggestions considerably improved the style of the manuscript. In addition, the authors thank Dr. G. Seitz, AgReliant Genetics, Westfield, IN, USA and Dr. W. Schipprack, Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany for their valuable suggestions We greatly appreciate the helpful comments and suggestions of two anonymous reviewers.

References

Baker RJ (1986) Selection indices in plant breeding. CRC Press, Boca Raton, pp 97–108

Becker H (1993) Pflanzenzüchtung. (In German) Verlag Eugen Ulmer, Stuttgart, pp 129–132

Cochran WG (1951) Improvement by means of selection. In: Proc 2nd Berkeley Symp Math Stat Prob, pp 449–470

Federer WT, Sprague GF (1947) A comparison of variance components in corn yield trials: I. Error, tester × line, and line components in top-cross experiments. J Am Soc Agron 39:453–463

Finney DJ (1966) An experimental study of certain screening processes. J R Stat Soc B 28:88–109

Gallais A (1997) Combined testcross and S_1 selection for the improvement of testcross and inbred performances. Crop Sci 37:1126–1133

Grüneberg WJ, Abidin E, Ndolo P, Pereira CA, Hermann M (2004) Variance component estimations and allocation of resources for breeding sweetpotato under East African conditions. Plant Breed 123:311–315

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, pp 267–298

Hallauer AR, Russell WA, Lamkey KR (1988) Corn breeding. In: Sprague GF, Dudley JW (eds) Corn and corn improvement, 3rd edn. Am Soc Agronomy, Madison, pp 463–564

Hanson WD, Brim CA (1963) Optimum allocation of test material for two-stage testing with an application to evaluation of soybean lines. Crop Sci 3:43–49

Keller KR (1949) A comparison involving the number of, and relationship between, testers in evaluating inbred lines of maize. Agron J 41:323–331

Longin CFH, Utz HF, Reif JC, Schipprack W, Melchinger AE (2006a) Hybrid maize breeding with doubled haploids: I. One-stage versus two-stage selection for testcross performance. Theor Appl Genet 112:903–912

Longin CFH, Utz HF, Melchinger AE, Reif JC (2006b) Hybrid maize breeding with doubled haploids: Comparison between selection criteria. Acta Agron Hung. DOI 10.1556/AAgr.54.2006.3

Melchinger AE, Longin CF, Utz HF, Reif JC (2005) Hybrid maize breeding with doubled haploid lines: quantitative genetic and selection theory for optimum allocation of resources. In: Proceedings of the 41st annual Illinois Corn Breeders' School 2005, Urbana-Champaign, pp 8–21

Mihaljevic R, Schön CC, Utz HF, Melchinger AE (2005) Correlations and QTL correspondence between line per se and testcross performance for agronomic traits in four populations of European maize. Crop Sci 45:114–122

Schmidt W (2004) Hybridmaiszüchtung bei der KWS SAAT AG. (In German) In: Bericht über die 54. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2003, Gumpenstein, pp 1–6

Schnell FW (1996) Über Zuchtplanung und die Entscheidungsspielräume des Pflanzenzüchters (in German). Vorträge Pflanzenzüchtung 33:227–244

Schrag TA, Melchinger AE, Sørensen AP, Frisch M (2006) Prediction of single-cross hybrid performance for grain yield and grain dry matter content in maize using AFLP markers associated with QTL. Theor Appl Genet, in press

Seitz G (1989) Experimentelle und theoretische Untersuchungen zur Beziehung zwischen Linieneigenleistung und allgemeiner Kombinationsfähigkeit bei Silomais. (in German) PhD Thesis, University of Hohenheim, Stuttgart

Seitz G (2005) The use of doubled haploids in corn breeding. In: Proceedings of the 41st annual illinois corn Breeders' School 2005, Urbana-Champaign, pp 1–7

Seitz G, Geiger HH, Schmidt GA, Melchinger AE (1992) Genotypic correlations in forage maize. II: relationship between inbred line and testcross performance. Maydica 37:101–105

Sprague GF, Federer WT (1951) A comparison of variance components in corn yield trials: II. Error, year × variety, location × variety and variety components. Agron J 42:535–541

Tomerius AM (2001) Optimizing the development of seed-parent lines in hybrid rye breeding. PhD Thesis, University of Hohenheim (http://opus-ho.uni-stuttgart. de/hop/volltexte/2001/10/pdf/tomerius.pdf)

Utz HF (1969) Mehrstufenselektion in der Pflanzenzüchtung. (In German) Arbeiten der Universität Hohenheim, vol 49, Verlag Eugen Ulmer, Stuttgart

Weiss KMR (1981) Beziehungen zwischen Linienleistung, Heterosis und Hybridleistung bei Mais. PhD Thesis, University of Hohenheim

Theor Appl Genet
DOI 10.1007/s00122-007-0585-2

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

C. Friedrich H. Longin · H. Friedrich Utz · Jochen C. Reif · Thilo Wegenast · Wolfgang Schipprack · Albrecht E. Melchinger

Received: 9 March 2007 / Accepted: 30 May 2007

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.

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

Communicated by H. C. Becker.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-007-0585-2) contains supplementary material, which is available to authorized users.

C. F. H. Longin · H. F. Utz · J. C. Reif · T. Wegenast · W. Schipprack · A. E. Melchinger (✉)
Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany
e-mail: melchinger@uni-hohenheim.de

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

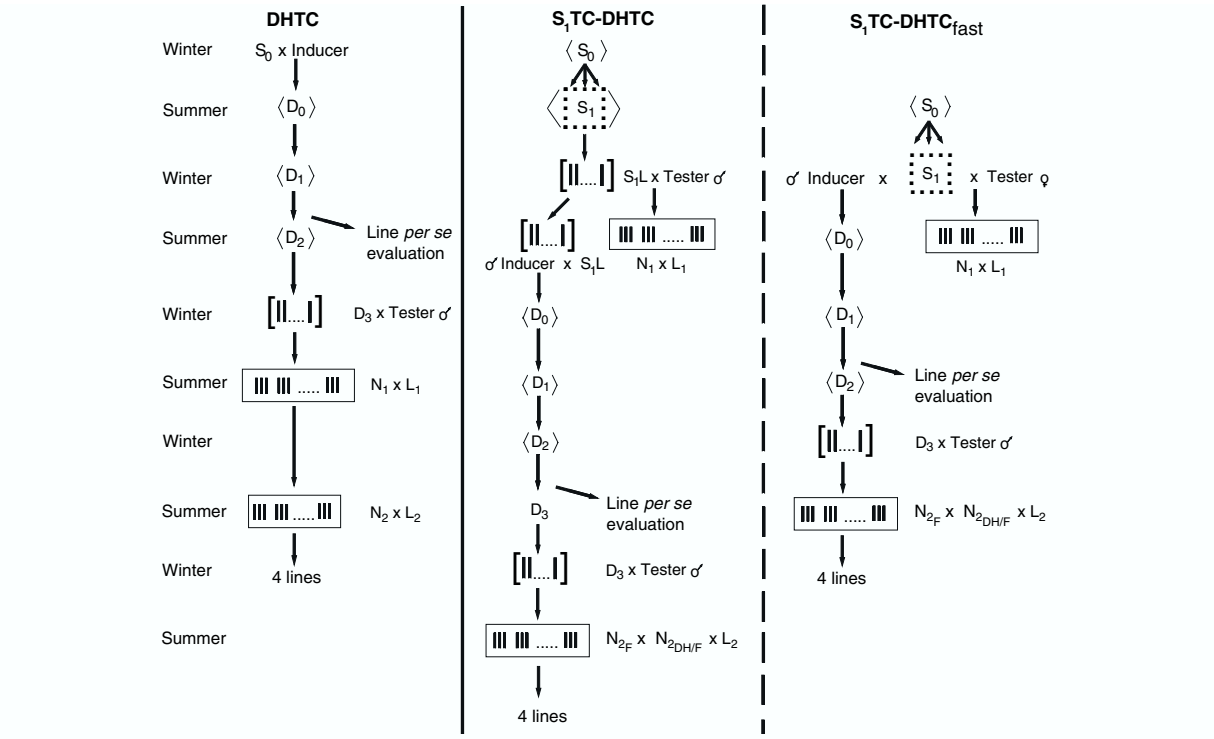

Fig. 1 Hybrid maize breeding schemes using DH lines under two-stage selection with test candidates generated within one cross of two homozygous lines. In breeding scheme DHTC, testcross progenies of N_1 doubled haploid lines produced from S_0 plants by in vivo haploid induction are evaluated in the first 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, ⟨ ⟩ = 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^*.

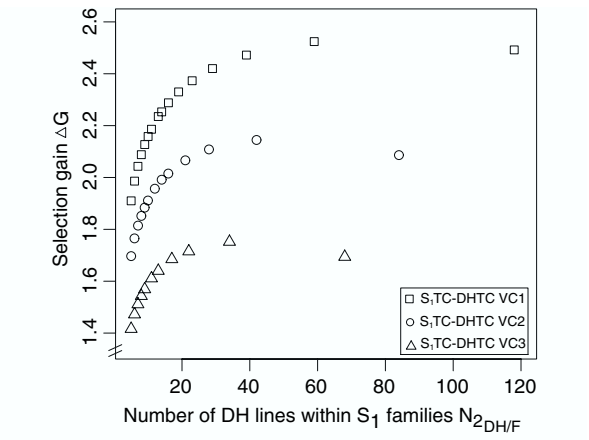

Fig. 2 Selection gain (ΔG) in breeding scheme S_1TC-DHTC as a function of the number of DH lines within S_1 families evaluated in the second stage for varying variance components (VC) assuming a budget of 1,000 testcross plot equivalents, production costs for DH lines of $C_{DH} = 1/2$, and optimum numbers 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 = $\sigma^2_{DH}:\sigma^2_{DH\times y}:\sigma^2_{DH\times l}:\sigma^2_{DH\times l\times y}:\sigma^2_e$ = 1:0.25:0.25:0.5:1; 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.

Acknowledgments This research was supported by funds from DFG, Grant No 1070/1, International Research Training Group "Sustainable Resource Use in North China" to C. F. H. Longin. The authors thank Dr. F. Laidig, Bundessortenamt Hannover, Germany, and Dr. G. Seitz, AgReliant Genetics, Westfield, IN, USA, for their valuable suggestions. In addition, the authors appreciate the editorial work of Dr. J. Muminović, whose suggestions considerably improved the style of the manuscript. We greatly appreciate the helpful comments and suggestions of one anonymous reviewer.

References

Bauman LF (1981) Review of methods used by breeders to develop superior inbreds. Proc Corn Sorghum Industry Res Conf 36:199–208

Bernardo R (1991) Correlation between testcross performance of lines in early and late selfing generations. Theor Appl Genet 82:17–21

Bernardo R (2002) Breeding for quantitative traits in plants. Stemma Press, Woodbury, pp 184, 205

Cochran WG (1951) Improvement by means of selection. In: Proceedings Second Berkeley Symposium on Math Stat Prob, pp 449–470

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman Scientific & Technical Ltd, Essex, pp 188–189, 232–234

Gordillo GA, Geiger HH (2004) Estimating quantitative-genetic parameters of European maize populations to optimize hybrid breeding methods by model calculations. In: Poster Abstract XVIIth EUCARPIA General Congress 2004, Tulln, Austria

Hallauer AR, Lopez-Perez E (1979) Comparison among testers for evaluating lines of corn. Proc Corn Sorghum Industry Res Conf 34:57–75

Hallauer AR, Russell WA, Lamkey KR (1988) Corn breeding. In: Sprague GF, Dudley JW (eds) Corn and corn improvement, 3rd edn. Am Soc Agronomy, Madison, Wisconsin, USA, pp 463–564

Jensen SD, Kuhn WE, McConnell RL (1983) Combining ability studies in elite U.S. maize germ plasm. Proc Corn Sorghum Industry Res Conf 38:87–96

Longin CFH, Utz HF, Reif JC, Schipprack W, Melchinger AE (2006) Hybrid maize breeding with doubled haploids: I. One-stage versus two-stage selection for testcross performance. Theor Appl Genet 112:903–912

Lonnquist JH (1950) The effect for selecting for combining ability within segregating lines of corn. Agron J 42:503–508

Melchinger AE (1988) Means, variances, and covariances between relatives in hybrid populations with disequilibrium in the parent

population. In: Weir BS, Eisen EJ, Goodman MM, Namkoong G (eds) Proc Second Int Conf Quantit Genet. Sinnauer, Sunderland, pp 400–415

Melchinger AE, Longin CF, Utz HF, Reif JC (2005) Hybrid maize breeding with doubled haploid lines: quantitative genetic and selection theory for optimum allocation of resources. In: Proceedings of the Forty First Annual Illinois Corn Breeders' School 2005, Urbana-Champaign, Illinois, USA, pp 8–21

Payne KT, Hayes HK (1949) A comparison of combining ability in F_3 and F_2 lines of corn. Agron J 41:383–388

Richey FD (1945) Isolating better foundation inbreds for use in corn hybrids. Genetics 30:455–471

Röber F, Gordillo GA, Geiger HH (2005) In vivo haploid induction in maize - performance of new inducers and significance of doubled haploid lines in hybrid breeding. Maydica 50:275–283

Schmidt W (2004) Hybridmaiszüchtung bei der KWS SAAT AG (In German). In: Bericht über die 54. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2003, Gumpenstein, Austria, pp 1–6

Seitz G (2005) The use of doubled haploids in corn breeding. In: Proceedings of the Forty First Annual Illinois Corn Breeders' School 2005, Urbana-Champaign, Illinois, USA, pp 1–7

Sprague GF (1946) Early testing of inbred lines of corn. J Am Soc Agron 38:108–117

Sprague GF, Federer WT (1951) A comparison of variance components in corn yield trials: II. Error, year × variety, location × variety and variety components. Agron J 42:535–541

Utz HF (1969) Mehrstufenselektion in der Pflanzenzüchtung. (In German) Arbeiten der Universität Hohenheim, Vol 49. Verlag Eugen Ulmer, Stuttgart

Utz HF (1981) Comparison of three methods of line selection. In: Proceedings of the fourth Meeting of the EUCARPIA Section Biometrics in Plant Breeding, Lusignan, France, pp 119–126

Weber WE (1981) Two stage selection in segregating generations of autogamous species. In: Proceedings of the fourth Meeting of the EUCARPIA Section Biometrics in Plant Breeding, Lusignan, France, pp 127–134

Wricke G, Weber WE (1986) Quantitative genetics and selection in plant breeding. Walter de Gruyter, Berlin, New York, pp 160–161, 186–189

Supplementary Table S1. Formulas required for calculation of selection gain for two-stage selection with evaluation of testcross progenies of (1) doubled haploid (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). Phenotypic variance at stage j ($\sigma^2_{x_j}$) is calculated for breeding scheme DHTC from variance components attributable to the (1) genotypic variance among DH lines (σ^2_{DH}), (2) variance of genotype × year interactions ($\sigma^2_{DH\times y}$), (3) variance of genotype × location interactions ($\sigma^2_{DH\times l}$), (4) variance of genotype × location × year interactions ($\sigma^2_{DH\times l\times y}$), and (5) plot error variance (σ^2_e). In S_1TC-DHTC, genetic variance was $\sigma^2_{DH} = \sigma^2_F + \sigma^2_{DH/F}$ with $\sigma^2_F = \sigma^2_{DH/F}$, where σ^2_F refers to the genotypic variance among S_1 families and $\sigma^2_{DH/F}$ to the genotypic variance among DH lines within S_1 families. Selection criterion in the second stage is an optimum index of the phenotypic means of those test candidates evaluated in both stages with $I = b_1x_1 + b_2x_2$, where b_j refers to the weight of x_j. (L_j = number of test locations at stage j, L_c = number of test locations common to both stages, $N_{2_{DH/F}}$ = number of DH lines within S_1 families)

$$\rho_{x_j} = \frac{Cov(z,x_j)}{\sigma_z\sigma_{x_j}} \text{ with } \sigma^2_z = \sigma^2_{DH} = \sigma^2_F + \sigma^2_{DH/F}$$

Breeding scheme DHTC

$$\sigma^2_{x_j} = \sigma^2_{DH} + \sigma^2_{DH\times y} + \frac{\sigma^2_{DH\times l}}{L_j} + \frac{\sigma^2_{DH\times l\times y}}{L_j} + \frac{\sigma^2_e}{L_j}$$

$$\sigma^2_I = b_1^2\sigma^2_{x_1} + b_2^2\sigma^2_{x_2} + 2b_1b_2Cov(x_1,x_2)$$

$$Cov(x_1,x_2) = \sigma^2_{DH} + \frac{L_c\sigma^2_{DH\times l}}{L_1L_2}$$

Breeding scheme S_1TC-DHTC

$$\sigma^2_{x_1} = \sigma^2_F + \sigma^2_{F\times y} + \frac{\sigma^2_{F\times l}}{L_1} + \frac{\sigma^2_{F\times l\times y}}{L_1} + \frac{\sigma^2_e}{L_1}$$

$$\sigma^2_{x_{2_F}} = \sigma^2_F + \sigma^2_{F\times y} + \frac{\sigma^2_{F\times l}}{L_2} + \frac{\sigma^2_{F\times l\times y}}{L_2} + \frac{\sigma^2_{DH/F} + \sigma^2_{DH/F\times y} + \frac{\sigma^2_{DH/F\times l}}{L_2} + \frac{\sigma^2_{DH/F\times l\times y}}{L_2} + \frac{\sigma^2_e}{L_2}}{N_{2_{DH/F}}}$$

$$\sigma^2_{x_{2_{DH/F}}} = \left(\sigma^2_{DH/F} + \sigma^2_{DH/F\times y} + \frac{\sigma^2_{DH/F\times l}}{L_2} + \frac{\sigma^2_{DH/F\times l\times y}}{L_2} + \frac{\sigma^2_e}{L_2}\right)\frac{N_{2_{DH/F}} - 1}{N_{2_{DH/F}}}$$

$$\sigma^2_I = b_1^2\sigma^2_{x_1} + b_2^2\sigma^2_{x_{2_F}} + 2b_1b_2Cov(x_1,x_{2_F})$$

$$Cov(x_1,x_{2_F}) = \sigma^2_F + \frac{L_c\sigma^2_{F\times l}}{L_1L_2}$$

6. General Discussion

The development of inbred lines with superior testcross performance is of fundamental importance for hybrid breeding. Inbred line development can be accelerated with doubled haploids (DHs) enabling the evaluation of completely homozygous lines from the very beginning of the selection process. The implementation of this promising technology in maize breeding requires the optimization and comparison of different breeding schemes in order to maximize progress from selection.

Choice of the model framework

The optimization and comparison of breeding schemes is commonly performed in plant breeding by model calculations (*cf.* Bouchez and Gallais 2000, Wang et al. 2003, Bordes et al. 2006). These calculations represent a non-linear optimization, which require the numerical computation of multivariate integrals for specific probability distributions. Alternatively to the numerical computation, the multivariate integrals can be estimated by Monte Carlo simulations.

Our model calculations require the definition of a model framework (Fig. 7.1), which includes a "Basic level", a "Breeding level", and an "Optimization level". At the "Basic level", the target criterion, the trait, and the genetic model of the trait was defined. The efficiency of the different breeding

schemes was evaluated by two target criteria, the selection gain (ΔG) and the probability of identifying superior genotypes ($P(q)$). Both target criteria were determined by assuming the same fixed budget for each breeding scheme.

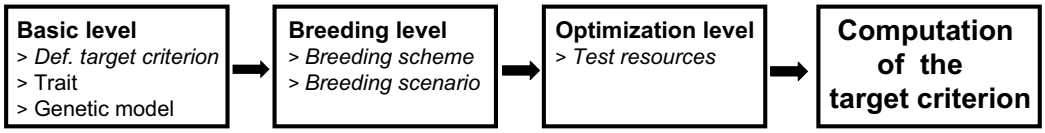

Figure 7.1 Model framework for the optimization and comparison of breeding schemes. The varied parameters are indicated in italics.

Breeding is a continuous process and, thus, 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, comparisons between breeding schemes can be made directly with per-cycle values of the target criteria, representing a constraint of our model framework.

We focused only on the trait grain yield assuming that this trait is controlled by many loci with small effects resulting approximately in a Gaussian normal distribution of genotypic and phenotypic values (*cf.* Dekkers and Hospital 2002). This assumption can be justified by results of an extremely extensive QTL mapping experiment in maize (Schön et al. 2004), which provided no evidence that phenotypic means for grain yield deviated from a Gaussian normal distribution. Likewise, the large number of detected QTL with small effects resulted in an approximative Gaussian normal distribution of genotypic values due to the Central Limit Theorem.

The different breeding schemes, which should be compared, were defined at the "Breeding level" by the number of selection stages, the types of test candidates, the time of DH production, and others. These assumptions are

described later in more detail. Furthermore, each breeding scheme was investigated for different breeding scenarios, *i.e.*, the budget, the variance components, the tester type, the number of finally selected DH lines, and the costs for DH production. At the "Optimization level", the allocation of test resources was defined, which was represented by the number of test candidates, testers, test locations, and replications.

In each model run, the target criterion was determined for a given allocation of test resources within a given breeding scenario in a given breeding scheme. The different breeding schemes were then compared at their optimum allocation of test resources, which maximized the target criterion under a given breeding scenario. Consequently, with this model framework, the optimum implementation of DHs into maize breeding requires three steps: the choice of the target criterion, the optimization of test resources for each breeding scheme under different breeding scenarios, and the comparison of different breeding schemes.

Comparison of the selection gain with the probability of identifying superior genotypes

Similar optimum allocations of test resources were obtained with regard to ΔG or $P(q)$, unless very small values of q were chosen (Longin et al. 2006a, b). For these small values of q, a larger optimum number of initial test candidates was observed at the expense of a reduced number of test locations for $P(q)$ in comparison with ΔG. However, the difference between values of $P(q)$ at its optimum allocation and those values of $P(q)$ obtained at the optimum allocation of test resources with regard to ΔG were very small. Furthermore, for extremely small values of q, the probability $P(q)$ was too low to be recommended as target criterion even for very large budgets. Consequently, the use of ΔG, $P(5\%)$, and $P(1\%)$ seem appropriate for optimization of breeding schemes.

The application of the new target criterion $P(q)$ allows important conclusions for hybrid maize breeding with DHs (Longin et al. 2006a, b). The large optimum number of test locations determined by ΔG for population improvement seems also very important to maximize the chances of identifying a superior genotype. A larger budget, smaller non-genetic variances, smaller production costs of DHs, and a smaller number of finally selected test candidates increased ΔG and $P(q)$. However, variation in these factors had a much larger impact on values of $P(1\%)$ than on ΔG indicating their importance for increasing the chances of identifying superior genotypes.

Comparison of the standard deviations of ΔG and $P(q)$ was hampered by the binomial nature of $P(q)$ with genotypes surpassing the defined threshold or not (Longin et al. 2006a, b). Thus, the standard deviation of $P(q)$ assumed by definition its maximum for $P(q) = 0.5$. In contrast, the standard deviation of ΔG decreased continuously with an increasing number of test candidates and test locations, and especially with an increasing number of finally selected test candidates. Knowledge of the standard deviation of the target criterion is important for plant breeders to maximize gain while reducing the risk of the final outcome. Thus, the number of finally selected DH lines should be carefully chosen, because it represents the factor with strongest impact on the standard deviation of the target criteria.

As breeding populations are commonly small, we used Monte Carlo simulations to determine ΔG and $P(q)$ for finite population size, which enables also the calculation of their standard deviations. However, Monte Carlo simulations require a large number of simulation runs to achieve a high accuracy, which results in prohibitive computing time (Longin et al. 2006a, b). The use of numerical formulas for calculation of ΔG and $P(q)$ would considerably reduce computing time. However, in populations with finite size, formulas for multi-stage selection are lacking in the literature and require further research. One possibility might be the extension of formulas for one-stage selection in finite populations given by Robson et al. (1967) and Hill (1976).

For the simplifying assumption of infinite population size, exact formulas for multi-stage selection have been developed for ΔG (Cochran 1951, Utz

1969). The comparison of ΔG calculated for finite and infinite population size led to similar optimum allocation of test resources and values of ΔG for a large range of scenarios (Longin et al. 2006b). Consequently, the use of ΔG under the simplifying assumption of infinite population size seems justified as long as a reduction in computing time is warranted. Thus, our further discussion is restricted to results determined by ΔG assuming infinite population size. For practical breeders, approximations determining ΔG without multiple integration techniques may be easier applicable with standard software tools. Therefore, approximations for ΔG and its standard deviation in two-stage selection with infinite population size are shown in the Appendix. Furthermore, suggestions for approximating two-stage selection in finite populations are made, which are close to the results of our Monte Carlo simulations.

Optimum breeding schemes for hybrid maize breeding with DHs

We assumed that a maximum of two selection stages for testcross performance are used for line development in hybrid maize breeding. Thereby, we focused on selection within one cross of two homozygous lines, because means and variances normally differ among crosses (*cf.* Mihaljevic et al. 2004). We considered two breeding schemes with one-stage (Fig. 7.2A) or two-stage selection among DH lines (Fig. 7.2B), and two breeding schemes with early testing prior to DH production (Fig. 7.2C, D). The considered breeding schemes were based on the assumption that (i) two selfing generations of DH lines are required to have sufficient quantities of seeds for field evaluations and (ii) *per se* evaluation of DH lines before testcross evaluation is considered with regard to the time length of the breeding scheme but is neglected for the selection process on yield due to the low correlations between yield and traits with high heritability determined commonly in line *per se* evaluation.

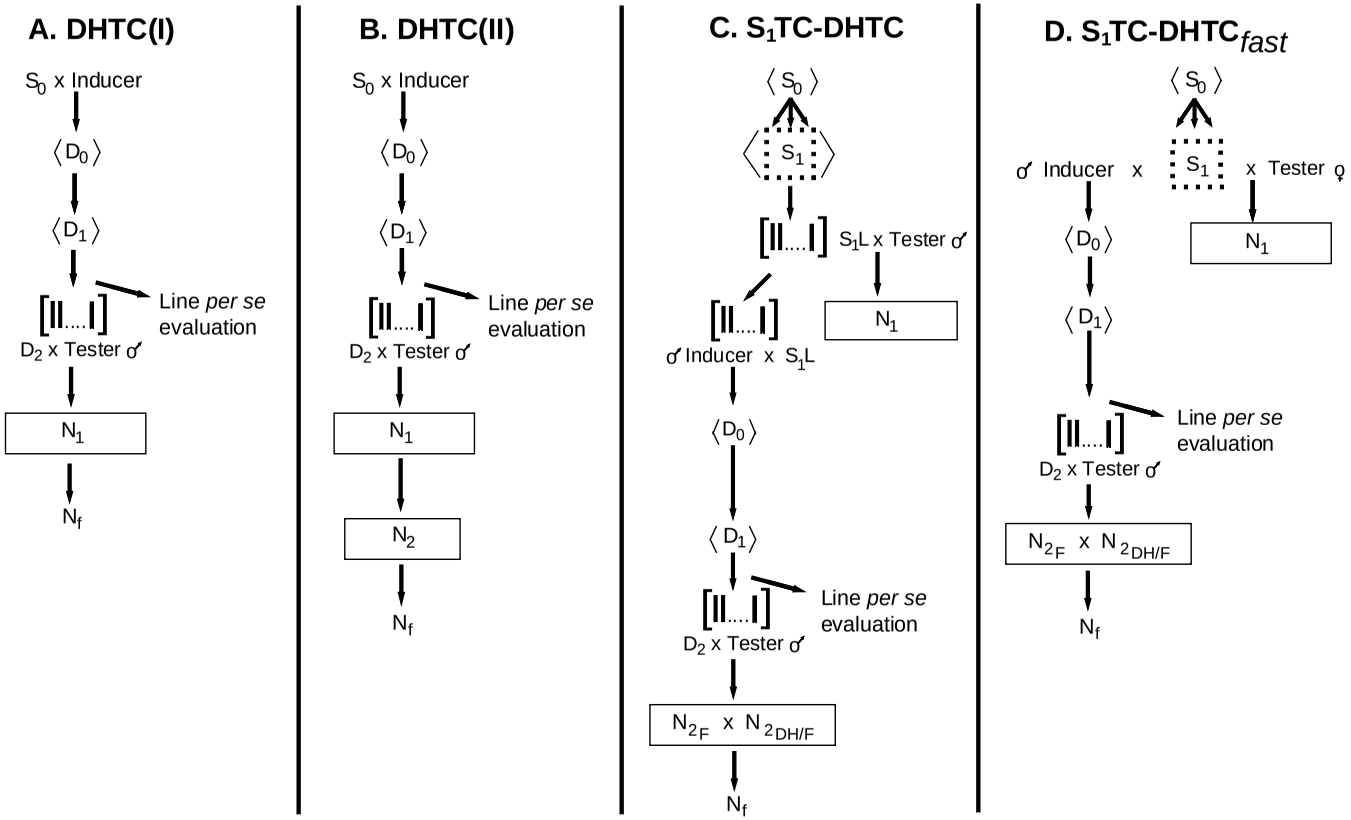

Figure 7.2 Hybrid maize breeding schemes with test candidates generated within one cross of two homozygous lines. In breeding schemes DHTC(I) and DHTC(II), testcross progenies of N_1 DH lines produced from S_0 plants by in-vivo haploid induction are evaluated in the first stage to select (i) the final number of DH lines (N_f) in breeding scheme DHTC(I) or (ii) the top N_2 DH lines for the second stage of breeding scheme DHTC(II), where the N_f DH lines are 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 to select N_f DH lines within the best S_1 family. ($D_i = i$th generation of DH multiplication, $\langle\rangle$ = selfing, [ ] = isolation plot, $\square$ = performance trial).

The impact of the allocation of test resources on ΔG

Selection gain is a function of the selection intensity, the heritability, and the variance of the target variable and increases with larger values for these parameters (Bernardo 2002). The variance of the target variable was constant in our study, because we focused on a given population derived from one cross of two homozygous lines. However, the selection intensity and the heritability must be chosen in order to maximize ΔG. Heritability can be increased by an increase in the number of test locations, testers, years, and replications in performance trials. Selection intensity can be increased by an increase in the number of initial test candidates and/or a decrease in the number of selected test candidates. Hence, a plant breeder with a fixed budget has to find a compromise between (i) the number of candidates to be tested and (ii) the intensity of their testing as determined by the number of test locations, testers, years, and replications.

The optimum number of replications was one for all considered scenarios (Melchinger et al. 2005), which is in accordance with theoretical studies in the literature (Sprague and Federer 1951, Utz 1969). This can be explained by the heritability, which is more affected by the number of test locations and testers than by the number of replications. However, these calculations were based on the assumptions that the number of test locations and testers is not limited and that the costs for one replication are equal to the costs for one location. These assumptions can be justified, because a breeder commonly uses a large network of test locations for screening of different diseases and for regionalization of test results. Thus, for the further discussion, the number of replications was set to one at the expense of requiring advanced statistical methods for the analysis of variance to separate the block effects from the plot error variance (nearest neighbor analysis, Moreau et al. 1999).

The type and number of testers had a crucial impact on the optimum allocation of test resources and values of ΔG for all considered breeding schemes (Longin et al. 2007a). The use of testers with broad genetic base

led to a reduced optimum number of testers allowing for a larger number of test locations. Consequently, ΔG was largely increased. However, the use of genetically broad testers is uncommon in applied breeding programs due to the additional effort required for their production. Furthermore, the use of inbred testers bears the possibility to early identify promising single-cross hybrids. A compromise between theoretical requirements and practical limitations might be the use of single-cross or double-cross testers in the first stage and inbred testers in the second stage. Instead of evaluating progenies of each tester at all test locations, the progenies of each tester could be investigated only at a single test location. This simple change in the breeding policy largely increased the optimum number of testers and test locations as well as ΔG. Consequently, the optimum type and number of testers is of utmost importance to maximize progress from selection.

Breeders can manipulate ΔG by varying economic and quantitative-genetic parameters. However, the large impact of these parameters on the optimum allocation of test resources must be considered (Longin et al. 2006a, b; Longin et al. 2007a, b). An increase in the budget led to increased optimum numbers of test locations and testers, but especially to an increased optimum number of test candidates. This increased ΔG, but reduced its standard deviation only slightly. A decrease in the number of finally selected DH lines mainly resulted in a decreased optimum number of test candidates and an increased optimum number of test locations and testers in the second stage. This increased ΔG, but also its standard deviation. With decreasing non-genetic variances, the optimum number of test locations was reduced in favor of an increased optimum number of test candidates. This increased ΔG and decreased its standard deviation.

Except for the variance components, which are determined by the breeding material and target environments, the other factors can be chosen in favor of an increased ΔG but at the expense of higher costs (larger budget) and a higher risk of the final outcome (larger standard deviation for a small number of finally selected DH lines). However, breeders should be aware of

the non-linear increase of ΔG with increasing budget and decreasing number of finally selected test candidates. This can be explained by the non-linear increase of (i) selection intensity with increasing number of test candidates and (ii) heritability with an increasing number of test locations, testers, and replications (*cf.* Becker 1993). Consequently, the possibility to increase ΔG by altering the above parameters is limited.

Response curves of ΔG revealed that a careful allocation of the test resources is important, if only a small number of test locations and testers is available (Longin et al. 2006a, b; Longin et al. 2007a, b). With larger numbers of test locations and testers, however, response curves become flatter, reducing the risk of choosing a non-optimal allocation. Practical requirements may lead to deviations from the theoretical optimum allocation of test resources. Loss in ΔG can be reduced by considering that the optimum allocation of test resources for a given budget is a compromise between a high selection intensity and a high heritability. Thus, a number of test locations exceeding its optimum can be counterbalanced by a reduction in the number of testers, years, and/or replications to minimize loss in selection intensity. A number of intial test candidates exceeding its optimum may be compensated in two-stage selection by a very small number of selected test candidates in the first stage to realize a higher heritability in the second stage. In contrast to these relatively flexible allocation options, two situations were identified in the considered breeding schemes, where even small deviations from the optimum allocation of test resources would clearly reduce ΔG. An increase in (i) the small optimum number of test locations in the first stage of breeding scheme DHTC(II) and (ii) the small optimum number of S_1 families in the second stage of breeding scheme S_1TC-DHTC cannot be compensated without substantial losses in ΔG. In conclusion, the optimum allocation of test resources is important to maximize ΔG under a given scenario, but flat response curves of ΔG reduce the risk of choosing suboptimal allocations.

Comparison of breeding schemes

The use of DHs allows the evaluation of potential hybrid cultivars from the very beginning of the selection process enabling an early registration of varieties, *e.g.*, after one stage of selection (breeding scheme DHTC(I), Fig. 7.2A). However, for breeding scheme DHTC(I), ΔG was about 20% smaller than for breeding scheme DHTC(II) (Fig. 7.2B) with two-stage selection of DH lines (Longin et al. 2006a). This is due to the optimum allocation of test resources in breeding scheme DHTC(II) with the evaluation of (i) a large number of DH lines in a small number of test locations in the first stage and (ii) 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. Furthermore, with breeding scheme DHTC(II), the standard deviation of ΔG was considerably reduced in comparison with breeding scheme DHTC(I). Consequently, breeding schemes with two-stage selection seem promising in line development with DHs and, thus, breeding scheme DHTC(I) is excluded from the further consideration.

A good line *per se* performance of DH lines is necessary for an economic seed production of the hybrids. For breeding scheme DHTC(II), the consideration of an economic seed production resulted in the necessity of different breeding strategies for seed and pollen parent heterotic groups (Longin et al. 2007a). Thereby, two-stage selection on testcross performance in both stages was most suitable for the pollen parent heterotic group. In contrast, for the seed parent heterotic group, evaluation of line *per se* performance in the first stage followed by evaluation of testcross performance in the second stage was most appealing. The importance of line *per se* evaluations is increased by considering further agronomically important traits like resistance to diseases and lodging. However, this requires index selection and warrants further research.

Alternatively to the evaluation of DHs from the very beginning of the selection process in breeding schemes DHTC(I) and DHTC(II), an early test

for testcross performance in generation S_1 or S_2 could be made before production of DHs (breeding scheme S_1TC-DHTC, Fig. 7.2C). This elongates the breeding scheme but permits to restrict the production and testing of DH lines to those derived from segregation in the most promising families. Selection gain for this breeding scheme S_1TC-DHTC was 10% higher than for breeding scheme DHTC(II) (Longin et al. 2007b). This can be explained by the different types of test candidates with S_1 families and DH lines in breeding scheme S_1TC-DHTC, enabling tremendously higher selection intensities in comparison with breeding scheme DHTC(II). This superiority in selection intensity decreased with a smaller selected fraction and resulted for the extreme case of a budget of ≤ 200 field plots and selection of only one DH line in a smaller ΔG for breeding scheme S_1TC-DHTC than for breeding scheme DHTC(II).

The optimum allocation of test resources in breeding scheme S_1TC-DHTC involved similar numbers of test locations and test candidates in both stages (Longin et al. 2007b). Furthermore, a similar number of testers was determined for both stages of breeding scheme S_1TC-DHTC, being approximately half as large as the respective optimum number of test locations (data not shown). This allocation of test resources in breeding scheme S_1TC-DHTC resulted in the use of more than 50% of the budget for evaluating the DH lines of the most promising S_1 families of the second stage. In contrast, in breeding scheme DHTC, less than 30% of the budget were used for the second stage. Consequently, the concentration of test resources on the most promising S_1 families in early testing prior to DH production was superior to the evaluation of DH lines from the beginning of the selection process with the only exception of the combination of a very small budget with a very small number of finally selected DH lines. This underpins the large potential of selection in early generations and "the chances of the segregating generations" (Schnell, pers. comm.).

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 is 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 (Seitz, personal communication). In addition, the production of DH lines from a single plant is limited due to low 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.

The efficiency of breeding scheme DHTC(II) was only marginally affected by the current limitations in DH technique (Longin et al. 2006a). Even for high costs of DH production, the decreased optimum number of DH lines can be compensated by an increased number of test locations without larger losses in ΔG. Owing to the small number of DHs that have to be produced, breeding scheme S_1TC-DHTC was even less affected by the current limitations in the DH technique (Longin et al. 2007b). The longer cycle length of breeding scheme S_1TC-DHTC compared with breeding scheme DHTC(II) can 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, and by performing chromosome doubling simultaneously with early testing (breeding scheme S_1TC-DHTC$_{fast}$, Fig. 7.2D). However, an efficient use of this breeding scheme will only be feasible, if more than 50 DH lines can be produced per individual S_1 plant at negligible costs (Longin et al. 2007b). With substantial increases in the haploid induction and chromosome doubling rate as well as reductions in the costs for DH production, the high selection gain for breeding schemes with early testing prior to DH production could be combined with a cycle length similar to breeding scheme DHTC(II), representing a promising breeding scheme for rapid line development in second-cycle breeding.

The focus of our study was to investigate the efficiency of different breeding schemes for short-term success in second-cycle breeding. We used per-cycle ΔG, because the valuation of different cycle length in the considered

breeding schemes is avoided. This is advantageous for the general assessments of breeding schemes, because the cycle length for the same breeding scheme may vary among breeders due to the availability of different test facilities. However, for the specific situation of a single breeder with given test facilities, the consideration of the cycle length by per-year ΔG might be of interest. Per-year ΔG can easily be obtained from our results by dividing per-cycle ΔG by the number of years required in the breeding scheme. For instance, for the assumption that two generations per year are possible and that field tests are only possible in summer season, largest per-year ΔG was obtained for breeding schemes DHTC(I) and S_1TC-DHTC$_{fast}$ with a superiority of more than 9 or 18% over breeding schemes DHTC(II) and S_1TC-DHTC, respectively. However, a change in the underlying assumptions may alter this ranking. In contrast to rapid line development in second-cycle breeding, the use of per-year ΔG in combination with the consideration of the effective population size seems necessary for the investigation of average population improvement in recurrent selection.

Prospects for model calculations

In breeding scheme S_1TC-DHTC, selection in the second stage was made first among and afterwards within S_1 families. However, modifications of the selection procedure of the second stage might increase the target criteria. For instance, the best DH lines could be selected across all S_1 families tested in the second stage without regard of the family structure. Furthermore, varying numbers of DH lines within S_1 families could be evaluated in the second stage according to the performance level of the S_1 family in the first stage. A consideration of best linear unbiased prediction (BLUP, *cf.* Bernardo 2002) might further improve selection among and within families. However, to our knowledge no analytical results are available in the literature to cope with these more general situations.

For the selection of the best DH lines across all S_1 families tested in the second stage of breeding scheme S_1TC-DHTC, preliminary calculations extending an approximation of Hill (1976) resulted in a more than 5% larger ΔG compared with selection first among and afterwards within S_1 families (data not shown). This indicates that the advantage of early testing prior to DH production is even underestimated by the selection procedure performed in our study. However, the used approximations are inaccurate for a small number of families (Hill 1976) and, hence, further research is warranted. Owing to the lack of analytical results, Monte Carlo simulations may be promising for further investigations on the optimum number of families and lines within families.

Our breeding schemes were limited to the selection within one cross of two homozygous lines. Extension of our results to populations from several crosses is feasible assuming that the same budget is spent and the same number of DH lines is finally selected in each population. However, both assumptions will most likely fail in applied breeding, where some of the populations are discarded at an early stage and promising populations might receive a larger budget. Consequently, further research is required by extending our formulas to selection among and within crosses considering the possibility to predict the average genotypic performance of a cross by the mean genotypic value of its parents (Utz 1982). The phenotypic values of the diverse parental lines are obtained with different test accuracy as determined by the number of test years, test locations, and testers used in the performance trials. Therefore, the consideration of this varying test accuracy could further improve the choice of parental lines.

We focused on grain yield assuming that this trait is controlled by a large number of loci each with a small effect. Results from the increasing number of QTL mapping experiments for yield (*cf.* Melchinger et al. 1998, Schön et al. 2004) could be used to specify more precise genetic models for the optimization of breeding schemes. For genetic models with small numbers of QTL each with a different effect, the optimum allocation of test resources

was similar to results achieved with a genetic model assuming a large number of QTL each with the same small effect (Longin 2004). However, ΔG was largely reduced for the considered breeding scheme indicating that a comparison of different breeding schemes might be affected by the specification of the genetic model. Furthermore, new, superior breeding schemes could be designed by using marker scores simultaneously with phenotypic data for selection, emphasizing the importance of further studies on optimum breeding schemes in hybrid maize breeding with DHs.

References

Abbramowitz M, Stegun IA (eds) (1964) Handbook of mathematical functions with formulas, graphs, and mathematical tables. Dover Publ, New York

Becker H (1993) Pflanzenzüchtung. (In German) Verlag Eugen Ulmer, Stuttgart, pp 129–132

Bernardo R (2002) Breeding for quantitative traits in plants. Stemma Press, Woodbury, USA, pp 204–205, pp 211–235

Bordes J, Charmet G, Dumas de Vaulx R, Pollacsek M, Beckert M, Gallais A (2006) Doubled haploid versus S_1 family recurrent selection for testcross performance in a maize population. Theor Appl Genet 112:1063–1072

Bouchez A, Gallais A (2000) Efficiency of the use of doubled-haploids in recurrent selection for combining ability. Crop Sci 40:23–29

Burrows PM (1972) Expected selection differentials for directional selection. Biometrics 28:1091–1100

Burrows PM (1975) Variances of selection differentials in normal samples. Biometrics 31:125–133

Cochran WG (1951) Improvement by means of selection. Proc Second Berkeley Symp Math Stat Prob, 449–470

Dekkers JCM, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. Nature Rev Genet 3:22–32

Dickerson GE, Hazel LN (1944) Effectiveness of selection on progeny performance as a supplement to earlier culling in livestock. J Agr Res 69:459–476

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman Scientific & Technical Ltd, Essex, England, pp 188–189

Hill WG (1976) Order statistics of correlated variables and implications in genetic selection programmes. Biometrics 32:889–902

Longin CFH (2004) Optimization of selection strategies using doubled haploids for hybrid breeding in maize. Master thesis, University of Hohenheim

Longin CFH, Utz HF, Reif JC, Schipprack W, Melchinger AE (2006a) Hybrid maize breeding with doubled haploids: I. One-stage versus two-stage selection for testcross performance. Theor Appl Genet 112:903–912

Longin CFH, Utz HF, Melchinger AE, Reif JC (2006b) Hybrid maize breeding with doubled haploids: Comparison between selection criteria. Acta Agronomica Hungarica, in press

Longin CFH, Utz HF, Melchinger AE, Reif JC (2007a) Hybrid maize breeding with doubled haploids: II. Optimum type and number of testers in two-stage selection on general combining ability. Theor Appl Genet 114:393–402

Longin CFH, Utz HF, Reif JC, Wegenast T, Schipprack W, Melchinger AE (2007b) Hybrid maize breeding with doubled haploids: III. Efficiency of early testing prior to doubled haploid production in two-stage selection for testcross performance. Theor Appl Genet, In review

Melchinger AE, Utz HF, Schön CC (1998) QTL mapping using different testers and independent population samples in maize reveals low power of QTL detection and a large bias in estimates of QTL effects. Genetics 149:383–402

Melchinger AE, Longin CF, Utz HF, Reif JC (2005) Hybrid maize breeding with doubled haploid lines: quantitative genetic and selection theory for optimum allocation of resources. In: Proceedings of the Forty First Annual Illinois Corn Breeders' School 2005, Urbana-Champaign, Illinois, USA, pp 8–21

Mihaljevic R, Utz HF, Melchinger AE (2004) Congruency of quantitative trait loci detected for agronomic traits in testcrosses of five populations of european maize. Crop Sci 44:114–124

Moreau L, Monod H, Charcosset A, Gallais A (1999) Marker-assisted selection with spatial analysis of unreplicated field trials. Theor Appl Genet 98:234–242

R Development Core Team (2004) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, URL http://www.R-project.org

Robson DS, Powers Leroy, Urquhart NS (1967) The proportion of genetic deviates in the tails of a normal population. Theor Appl Genet 37:205–216

Röber F, Gordillo GA, Geiger HH (2005) In vivo haploid induction in maize - performance of new inducers and significance of doubled haploid lines in hybrid breeding. Maydica 50:275–283

Schön CC, Utz HF, Groh S, Truberg B, Openshaw S, Melchinger AE (2004) Quantitative trait locus mapping based on resampling in a vast maize testcross experiment and its relevance to quantitative genetics for complex traits. Genetics 167:485–498

Sprague GF, Federer WT (1951) A comparison of variance components in corn yield trials: II. Error, year × variety, location × variety and variety components. Agron Jour 42:535–541

Utz HF (1969) Mehrstufenselektion in der Pflanzenzüchtung. (In German) Arbeiten der Universität Hohenheim, Vol 49, Verlag Eugen Ulmer, Stuttgart, Germany

Utz HF (1982) Der Selektionserfolg in spaltenden Generationen. (In German) Professorial dissertation, University of Hohenheim, Stuttgart, Germany

Utz HF (1984) Calculating and maximizing the gain from selection. Vortr Pflanzenzüchtg 7:30–40

Wang J, van Ginkel M, Podlich D, Ye G, Trethowan R, Pfeiffer W, DeLacy IH, Cooper M, Rajaram S (2003) Comparison of two breeding strategies by computer simulation. Crop Sci 43:1764–1773

7. Summary

A major objective in hybrid maize breeding is the development of inbred lines with superior testcross performance. Inbred lines have commonly been derived in maize by recurrent selfing for five to six generations. The use of doubled haploids (DHs) enables the generation of completely homozygous lines in one step, representing a promising alternative to recurrent selfing. The implementation of the new DH technique in maize breeding requires an optimization of the entire breeding scheme in order to maximize progress from selection.

The objectives of this study were to (i) compare selection gain (ΔG) per breeding cycle with the probability of identifying superior genotypes with respect to the optimum allocation of test resources, (ii) evaluate several breeding schemes for an optimum use of the DH technique, (iii) determine the optimum number of test candidates and test locations as well the optimum type and number of testers for the different breeding schemes, and (iv) investigate the potential and limitations in the current DH technique in hybrid maize breeding. Monte Carlo simulations and numerical integration techniques were used to calculate the optimization criteria.

The choice of ΔG and the probability of identifying superior genotypes seems not to be crucial for the optimization of breeding schemes. The use of the new probability criterion supported the large optimum number of test locations determined by ΔG. However, a larger impact of varying economic and quantitative-genetic parameters on the probability criterion than on ΔG

was found, emphasizing their importance to maximize the chances of identifying a superior genotype.

The use of Monte Carlo simulations for optimizing the allocation of test resources seems promising because of the possibility to calculate various optimization criteria for multi-stage selection in finite populations. However, the large computing power required for them can rapidly become prohibitive. Numerical integration techniques allow the calculation of ΔG in multi-stage selection under the simplified assumption of infinite population size. The differences between finite and infinite population size were negligible for both, ΔG and the optimum allocation of test resources. Thus, the simplifying assumption of infinite population size is justified as long as a tremendous reduction in computing time is warranted.

Two-stage selection of DH lines was important to increase ΔG and the probability of identifying superior genotypes, because it combines the evaluation of a large number of initial DH lines with the use of a large number of test locations. Consideration of an economic seed production indicated the necessity of separate breeding schemes for seed and pollen parent heterotic groups. For the pollen parent heterotic group, two-stage selection on testcross performance in both stages was most suitable, whereas for the seed parent heterotic group, line *per se* performance in the first stage followed by evaluation of testcross performance in the second stage was most appealing. The concentration of test resources on the most promising S_1 families in early testing prior to DH production was superior to the evaluation of DH lines from the beginning of the selection process.

The allocation of test resources was crucial to maximize ΔG for a given scenario. Testers with broad genetic base allow a reduction of the number of testers in favor of an increased number of test locations and a largely increased ΔG. An evaluation of progenies of each tester only in a single location instead of evaluating the progenies of each testers in all locations further increased ΔG. With early testing prior to DH production, similar

optimum numbers of testers and test locations were determined for evaluation of testcross performance of S_1 families and DH lines within S_1 families. This resulted in (i) a large optimum number of S_1 families for the first stage and (ii) a small optimum number of S_1 families but a large optimum number of DH lines within S_1 families for the second stage.

Current limitations in the DH technique with a low number of DH lines, which can be produced from a single maize plant, and high costs, affected the selection gain and the optimum allocation of test resources only marginally for breeding schemes with evaluation of DH lines from the beginning of the selection process. However, substantial improvements of the DH technique are required to realize the high potential of early testing prior to DH production in combination with a short cycle length.

In conclusion, the optimum allocation of test resources is of utmost importance to increase selection gain under given economic resources. The implementation of DHs into maize breeding enables to shorten the length of the breeding cycle, but a careful evaluation of the breeding alternatives is required to maximize progress from selection.

8. Zusammenfassung

Die Entwicklung von Inzuchtlinien mit überlegener Testkreuzungsleistung ist eine der bedeutendsten Aufgaben in der Hybridmaiszüchtung. Üblicherweise werden Maisinzuchtlinien durch fortgesetzte Selbstbefruchtung in fünf bis sechs aufeinanderfolgenden Generationen hergestellt. Eine vielversprechende Alternative stellt die Technik der Erzeugung von Doppelhaploiden (DH) dar, mit deren Hilfe vollständig homozygote Linien in einem Schritt entwickelt werden können. Um den Zuchtfortschritt zu maximieren, erfordert die Einführung der DH-Technik in die Maiszüchtung eine Optimierung des gesamten Züchtungsgangs.

Die Ziele unserer Studie waren: (i) die beiden Kriterien zur Bewertung des Zuchtfortschritts, nämlich den Selektionserfolg pro Zyklus und die Wahrscheinlichkeit, überlegene Genotypen zu identifizieren, hinsichtlich der optimalen Allokation von Testressourcen zu vergleichen, (ii) verschiedene Zuchtschemata für einen optimalen Einsatz der DH-Technik zu bewerten, (iii) die optimale Anzahl von Prüfkandidaten, Prüforten und Testern sowie den optimalen Testertyp zu bestimmen, und (iv) die Möglichkeiten und Grenzen der aktuellen DH-Technik zu untersuchen. Die Zielkriterien wurden mit Hilfe von Monte-Carlo-Simulationen und numerischen Integrationsverfahren berechnet.

Die Wahl des Kriteriums zur Bewertung des Zuchtfortschritts, nämlich der Selektionserfolg *vs.* die Wahrscheinlichkeit, überlegene Genotypen zu identifizieren, hatte nur einen geringen Einfluß auf die Optimierung von

Zuchtschemata. Die große Anzahl von Prüforten, die für den Selektionserfolg optimal war, wurde durch das Verwenden des Wahrscheinlichkeitskriteriums bestätigt. Allerdings wurde das Wahrscheinlichkeitskriterium stärker als der Selektionserfolg durch ökonomische und quantitativ-genetische Parameter beeinflusst, was deren Bedeutung für die Maximierung der Chancen, überlegene Genotypen zu identifizieren, unterstreicht.

Monte-Carlo-Simulationen sind für die Optimierung der Allokation von Testressourcen geeignet, weil sie ermöglichen, verschiedene Zielkriterien für die Mehrstufenselektion in Populationen mit finiter Größe zu bestimmen. Allerdings kann der damit einhergehende hohe Rechenaufwand schnell zum begrenzenden Faktor werden. Der Selektionserfolg in der Mehrstufenselektion kann unter der vereinfachenden Annahme einer infiniten Populationsgröße mittels numerischer Integrationsverfahren bestimmt werden. Die Unterschiede in der optimalen Allokation von Testressourcen und dem Selektionserfolg zwischen den Berechnungen für finite und infinite Populationsgrößen waren vernachlässigbar klein. Somit ist die vereinfachende Annahme einer infiniten Populationsgröße gerechtfertigt, solange damit eine deutliche Reduktion der Rechenzeit verbunden ist.

Der Selektionserfolg sowie die Wahrscheinlichkeit, überlegene Genotypen zu identifizieren, waren durch ökonomische und quantitativ-genetische Parameter nur begrenzt beeinflussbar. Dahingegen wurden beide Kriterien durch eine Zweistufenselektion von DH-Linien, bei der die Untersuchung einer großen Anzahl von Ausgangslinien mit dem Nutzen einer großen Anzahl von Prüforten kombiniert wird, beachtlich gesteigert. Die Berücksichtigung einer ökonomischen Saatgutproduktion erforderte die Verwendung unterschiedlicher Zuchtschemata für die heterotischen Gruppen der Saat- und Polleneltern. Für die Polleneltern war eine Zweistufenselektion auf Testkreuzungsleistung am besten geeignet, wohingegen sich für die Saateltern eine Selektion auf Linieneigenleistung in der ersten Selektionsstufe kombiniert mit einer Selektion auf Testkreuzungsleistung in der zweiten Selektionsstufe als überlegenes Zuchtschema erwies. Die Durchführung eines frühen Tests vor

der DH-Produktion ermöglichte eine Konzentration der Testressourcen auf die viel versprechendsten S_1-Familien, was der alleinigen Prüfung von DH-Linien während des gesamten Selektionsprozesses überlegen war.

Der Nutzen von genetisch breiten Testern ermöglichte eine Reduktion der Testerzahl zu Gunsten einer gesteigerten Anzahl an Prüforten und eines stark erhöhten Selektionserfolgs. Die Untersuchung der Nachkommen jedes Testers an jeweils nur einem Ort anstelle der Prüfung aller Testkreuzungsnachkommen an allen Orten steigerte zusätzlich den Selektionserfolg. Vergleichbare Anzahlen von Testern und Prüforten waren für die Untersuchung von S_1-Familien und DH-Linien optimal, wenn ein früher Test vor der Produktion von DH-Linien gemacht wurde. Dies führte dazu, dass in der ersten Selektionsstufe eine große Anzahl von S_1-Familien, in der zweiten Selektionsstufe allerdings nur eine kleine Anzahl von S_1-Familien mit jeweils einer großen Anzahl von DH-Linien innerhalb dieser S_1-Familien optimal waren.

Die Grenzen der aktuellen DH-Technik, insbesondere die geringe Anzahl von DH-Linien, die von einer Einzelpflanze produziert werden können, sowie die hohen Kosten beeinflussten den Selektionserfolg und die optimale Allokation der Testressourcen in Zuchtschemata, in denen ausschließlich DH-Linien getestet werden, kaum. Allerdings sind erhebliche Verbesserungen der DH-Technik nötig, um das große Potential des frühen Tests vor der DH-Produktion mit einer kurzen Zuchtzykluslänge zu vereinigen.

Das Fazit ist: Die optimale Allokation der Testressourcen ist für die Maximierung des Selektionserfolgs unter gegebenen ökonomischen Rahmenbedingungen von außerordentlich großer Bedeutung. Die Einführung von Doppelhaploiden in die Maiszüchtung ermöglicht zwar eine Verkürzung der Zuchtzykluslänge, allerdings ist für eine Maximierung des Zuchtfortschritts die sorgfältige Abwägung verschiedener Zuchtalternativen von Nöten.

9. Appendix

Approximating the selection gain

One-stage selection

For one-stage selection, selection gain (ΔG_1) is defined as

$$\Delta G_1 = i_1 \rho_{z,x_1} \sigma_z \text{ (\textit{cf.} Falconer and Mackay 1996),}$$

where i_1 refers to the selection intensity, ρ_{z,x_1} to the coefficient of correlation between the phenotypic mean of testcross performance x_1 and the target variable z, and σ_z to the standard deviation of the target variable. The calculation of the selection intensity requires knowledge of the genotypic and phenotypic distribution of the test candidates. Most often, a Gaussian normal distribution of phenotypic and genotypic values is assumed. For the assumption of infinite population size, i_1 can be approximated with good correspondence to exact computation by numerical integration for a selected fraction α of $0 \leq \alpha \leq 0.5$ with

$$i(infinite) = t + \frac{0.23204 - 1.7019\ t}{1 + 2.5143\ t + 0.5113\ t^2} \text{ (Utz 1984),}$$

assuming $t = \sqrt{\ln(1/\alpha^2)}$. For finite sample size, i_1 can be approximated by

$$i(finite) = i(infinite) - \frac{N - n}{2n(N+1)i(infinite)} \text{ (Burrows 1972),}$$

where N refers to the number of initial test candidates and n to the number of selected test candidates. In the statistic package R (R Development Core Team 2004), existing functions for numerical integration and order statistics can be used to determine exactly i_1 for finite and infinite population size. For infinite sample size, i_1 can be calculated as

$$i(infinite) = dnorm(qnorm(1-\alpha, 0, 1), 0, 1)/(\alpha).$$

For finite sample size, i_1 is calculated based on the expected values of the n largest order statistic with

$$i(finite) = sum(as.matrix(normOrder(N))[(N-n+1) : N])/n.$$

The function $normOrder()$ requires the use of $library(SuppDists)$.

The coefficient of correlation between the phenotypic mean of testcross performance x_1 and the target variable z is $\rho_{z,x_1} = Cov(z, x_1)/(\sigma_z \sigma_{x_1})$. Therein, the phenotypic variance $\sigma^2_{x_1}$ refers to the number of test locations and test candidates as well as the type of the test candidates, which are used in the study (*cf.* Longin et al. 2006a, b; Longin et al. 2007a, b).

The standard deviation of selection gain can be approximated with

$$SD_{\Delta G_1} = \sqrt{(1 - \rho^2_{z,x_1}(1 - v_1))\frac{\sigma^2_z}{n}} \text{ (Burrows 1975)}$$

with $v_1 = 1 - i_1(i_1 - k_1) + (1 - \alpha_1)(i_1 - k_1)^2$. Therein, k_1 can be approximated after Abbramowitz and Stegun (1964) as

$$k_1 = t - \frac{2.30753 - 0.27061t}{1 + 0.99229t + 0.4481t^2},$$

or calculated exactly in the statistic package R as $k_1 = qnorm(1 - \alpha_1, 0, 1)$.

Two-stage selection

Selection gain in two-stage selection (ΔG_2) is calculated as

$$\Delta G_2 = i_1 \rho_{z,x_1} \sigma_z + i_2 \rho'_{z,x_2} \sigma'_z \text{ (Utz 1984)}.$$

For the second stage, selection intensity i_2 is calculated like previously defined using α_2. However, ρ_{z,x_2} as well as σ_z^2 are reduced due to the skewness of the genotypic distribution after the first selection stage. The reduced correlation between the phenotypic mean of testcross performance x_2 and the target variable z can be approximated after Dickerson and Hazel (1944) as

$$\rho'_{z,x_2} = \frac{\rho_{z,x_2} - \rho_{z,x_1}\rho_{x_1,x_2}\lambda}{\sqrt{(1-\rho^2_{z,x_1}\lambda)(1-\rho^2_{x_1,x_2}\lambda)}},$$

with $\lambda = i_1(i_1 - k_1)$, $\rho_{x_1,x_2} = Cov(x_1, x_2)/(\sigma_{x_1}\sigma_{x_2})$, and ρ_{z,x_2} calculated as described for ρ_{z,x_1}. The variance of the target variable in the second stage is approximated as

$$\sigma'_z = \sqrt{\sigma^2_z(1-\rho^2_{z,x_1}\lambda)} \text{ (Cochran 1951).}$$

To my knowledge, definitions of the standard deviation of ΔG_2 are lacking in the literature. Results for calculating the standard deviation of ΔG_2 by a slight modification of the approximation for the standard deviation of ΔG_1 (Burrows 1975) were close to findings of our Monte Carlo simulations for a large range of scenarios (data not shown). Thus, to get a rough idea of the standard deviation of ΔG_2, I suggest the use of

$$SD_{\Delta G_2} = \sqrt{(1-\rho'^2_{z,x_1}(1-v_2))\frac{\sigma'^2_z}{n}}$$

using $i_2(infinite)$ for calculation of v_2.

10. Acknowledgements

I am very grateful to my academic supervisor Prof. Dr. A.E. Melchinger for his advise, suggestions, and support during this thesis work. Thanks to Prof. Dr. H.F. Utz for his valuable suggestions concerning the publications, his help in program development, and his words of encouragement. Sincere thanks to PD. Dr. J.C. Reif for awaking my interest in plant breeding, for many helpful discussions, and his never ending patience in proofreading. I appreciate the support in China and the serving on my graduate committee of Prof. Dr. S. Chen. Thanks to Prof. Dr. R. Doluschitz, Dr. D. Ebersberger, H. Beck, B. Boesig, and S. Meyer for being of great help in organizational matters. The helpful advice about applied plant breeding of Dr. W. Schipprack is acknowledged. Many thanks to S. Bhosale, C. Bolduan, Dr. S. Dreisigacker, C. Falke, Dr. C. Flachenecker, S. Fischer, PD. Dr. M. Frisch, Dr. M. Heckenberger, Dr. B. Kusterer, F. Mauch, H.P. Maurer, Prof. Dr. A.E. Melchinger, Dr. V. Mirdita, Dr. J.M. Montes, Dr. J. Muminović, H. Pöschl, PD. Dr. J.C. Reif, T. Schrag, Dr. B. Stich, Prof. Dr. H.F. Utz, T. Wegenast, Prof. Dr. P. Zhang and all unmentioned members of the institute of plant breeding, seed science, and population genetics for creating a pleasant work environment. I want to thank Li Liang and Sun Wentao for their support in China and their patience to introduce Chinese tradition to me. Thanks to R. Barning, J. Binder, Dr. F. Bode, M. Herndl, M. Hillebrand, J. Kopsch, R. Kröbel, S. Piotrowski, C. Rumbaur, and U. Schwarz for creating a pleasant work and life environment in China. The financial support from Deutsche Forschungsgemeinschaft is gratefully acknowledged. Finally, I want to thank my wife, my family, and my friends without their support I would never have succeeded this thesis.

Curriculum vitae

Name	Carl Friedrich Horst Longin geb. Hermann
Birth	04 August 1978 in Backnang
Marital status	married
School education	1985–1989, elementary school (Freudental and Stuttgart-Freiberg)
	1989–1998, high school (Stuttgart-Freiberg and Wangen i. Allg.) Abitur June 1998
Civil service	8/98–8/99, Bayrisches Rotes Kreuz, Lindau i.B.
University education	10/99–04/04, Agricultural Biology, University of Hohenheim, Stuttgart Diplom-Agrarbiologe April 2004
	06/04–04/07, Doctoral Student, Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart
Agricultural experience	08/02–10/02, Enza Zaden, Heidelberg
	07/03–09/03, Breeding stations of the Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart
	Since 06/04, Conduction of multi-location field trials at breeding stations of the National Maize Improvement Center of China, Beijing, China

Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst und lediglich unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Insbesondere erkläre ich, dass ich nicht früher oder gleichzeitig einen Antrag auf Eröffnung eines Promotionsverfahrens unter Vorlage der hier eingereichten Dissertation gestellt habe.

Stuttgart, im April 2007

Friedrich Longin