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ORIGINAL PAPER

Optimum allocation of test resources and comparison of breeding strategies for hybrid wheat

C. Friedrich H. Longin · Xuefei Mi · Albrecht E. Melchinger · Jochen C. Reif · Tobias Würschum

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Abstract

Key message **The use of a breeding strategy combining the evaluation of line per se with testcross performance maximizes annual selection gain for hybrid wheat breeding.**

Abstract Recent experimental studies confirmed a high commercial potential for hybrid wheat requiring the design of optimum breeding strategies. Our objectives were to (1) determine the optimum allocation of the type and number of testers, the number of test locations and the number of doubled haploid lines for different breeding strategies, (2) identify the best breeding strategy and (3) elaborate key parameters for an efficient hybrid wheat breeding program. We performed model calculations using the selection gain for grain yield as target variable to optimize the number of lines, testers and test locations in four different breeding strategies. A breeding strategy (BS2) combining the evaluation of line per se performance and general combining ability (GCA) had a far larger annual selection gain across all considered scenarios than a breeding strategy (BS1) focusing only on GCA. In the combined strategy, the production of testcross seed conducted in parallel with the first yield trial for line per se performance ($BS2_{rapid}$) resulted in a further increase of the annual selection gain. For the current situation in hybrid wheat, this relative superiority of the strategy $BS2_{rapid}$ amounted to 67 % in annual selection gain compared to BS1. Varying a large number of parameters, we identified the high costs for hybrid seed production and the low variance of GCA in hybrid wheat breeding as key parameters limiting selection gain in $BS2_{rapid}$.

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C. F. H. Longin (✉) · T. Würschum
State Plant Breeding Institute, University of Hohenheim, 70599 Stuttgart, Germany
e-mail: friedrich.longin@uni-hohenheim.de

X. Mi · A. E. Melchinger
Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, 70599 Stuttgart, Germany

J. C. Reif
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466 Gatersleben, Germany

Introduction

Worldwide crop production has to be greatly increased in the future to feed a growing world population but current yield trends are insufficient to meet this rising demand (Ray et al. 2013). For wheat, breeding of hybrids is currently seen as a very promising avenue to address this problem. Recent studies based on a large number of wheat hybrids reported a 1.00–1.86 ton per ha yield advantage of the best hybrids compared to the highest yielding line varieties (Gowda et al. 2012; Longin et al. 2013). Furthermore, yield stability across locations was higher for hybrids than for line varieties (Mühleisen et al. 2014) further underpinning the potential of hybrid wheat. Consequently, the design of optimum breeding strategies for hybrid wheat is of utmost importance.

In hybrid breeding, all lines developed in line breeding can serve as potential parents, rendering the number of factorial crosses rapidly prohibitive. Therefore, lines are usually tested for their general combining ability (GCA) using a tester from the opposite heterotic group (Hallauer et al. 1988). Specific combining ability (SCA) thereby acts as a masking effect. Its influence can be reduced using genetically broad and/or multiple testers (Hallauer and Miranda 1981). For hybrid breeding in maize, use of double-cross testers was highly recommended if the variance due to SCA is high (Longin et al. 2007). For wheat, high variances due to SCA were recently reported in the literature (Gowda et al. 2012; Longin et al. 2013) underlining the importance of an optimum choice of testers for hybrid wheat breeding. However, no study is yet available on the choice of testers in a hybrid wheat context.

Alternative breeding strategies are theoretically feasible in hybrid wheat breeding comprising classical multi-stage selection for GCA as well as selection for GCA combined with selection for line per se performance (LP). From a theoretical point of view, the latter scheme seems most promising because hybrid wheat breeding is currently pursued in parallel to line breeding. In addition, a combined breeding strategy can save 1 year compared with strategies focusing on GCA alone. In a previous study, the number of years required to complete one breeding cycle as well as the costs for hybrid seed production were found to be key parameters determining the efficiency of hybrid wheat breeding (Longin et al. 2014). However, results from this study were based on a simple one-stage selection scheme, neglecting the allocation of test resources and the choice of tester(s). Consequently, further studies are required for optimizing and comparing alternative breeding strategies for hybrid wheat breeding.

In this study, we calculated the selection gain of different breeding strategies for different allocations of test resources in hybrid wheat breeding. In particular, our objectives were to (1) determine the optimum type and the optimum number of testers, test locations, and doubled haploid (DH) lines under different breeding strategies, (2) elaborate the best breeding strategy for hybrid wheat breeding, and (3) assess key parameters for efficient breeding of hybrid wheat.

Materials and methods

Breeding strategies

Hybrid breeding in wheat requires different strategies for the male and female pool. For the male pool, the main emphasis is currently to identify among the vast number of available lines those few with exceptionally good pollen production, while GCA is only of secondary importance. Using chemical hybridization agents (CHA) for hybrid seed production, which is the current common practice, pollen production is not of interest for the female lines. Thus, all available lines can principally serve as female parents and their GCA is the most important selection criterion. However, in the future, genetic sterility systems like cytoplasmatic male sterility (CMS) might also be used in hybrid wheat seed production. Thereby, an economical maintenance of the A-line also requires efficient pollination by the B-line. Here, we focus on optimizing selection in the female pool neglecting pollen production and assume that N_1 DH lines generated from several intra-pool F_1 crosses are evaluated for their line per se and/or testcross performance. The target variable was a selection index based on GCA and line per se performance for grain yield. Four different strategies for evaluating line per se performance and GCA were compared. In all strategies, the DH lines are evaluated in two consecutive field tests (Fig. 1). In field test one, N_1 DH lines are evaluated either as hybrids produced with a tester, or as lines per se, and the subset of N_2 lines with highest rank then selected for evaluation in the second year. The $N_f = 5$ best DH lines are selected after both selection stages for further screenings in pre-registration trials.

The first breeding strategy (BS1) includes a two-stage selection based on testcross evaluation of N_j DH lines with T_j testers at L_j locations in selection stage j ($j = 1, 2$). The number and type of testers can vary in both stages. Our comparison included as tester type either inbreds alone ($M_j = 1$) or mixtures of two ($M_j = 2$), three ($M_j = 3$) or four inbred lines ($M_j = 4$). In strategy BS2, the N_1 DH lines were 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. For both breeding strategies, we additionally investigated an accelerated scheme in which hybrid seed for the second field test was already produced in parallel to the first field test ($BS1_{rapid}$ and $BS2_{rapid}$). This acceleration is at the expense of increased costs for testcross seed production because all N_1 DH lines must be crossed with the T_2 testers. Without restrictions on L_j, the selection gain (ΔG) is maximized for one replication per location (cf. Melchinger et al. 2005). Thus, we set the number of replications equal to one for all calculations. An overview of the abbreviations used throughout the manuscript is given in Table 1.

Calculation of selection gain

We adopted the formulas for the calculation of ΔG and the optimization of resources from maize breeding as described in detail by Longin et al. (2007). In brief, we used a selection index $H = a_{LP}g_{LP} + a_{GCA}g_{GCA}$ (Cochran 1951) as target variable, where a refers to the economic weight and g to the genotypic effect of line per se performance and GCA, respectively, with $a_{GCA} = 1 - a_{LP}$. Calculation of ΔG is based on the well-known formula of Cochran (1951) with multivariate normal integrals for selected fractions and heritabilities. The four investigated breeding strategies differ up to 2 years regarding their cycle length (Fig. 1). To account for this difference, we also determined the annual selection gain ΔG_a, which is the absolute ΔG divided by

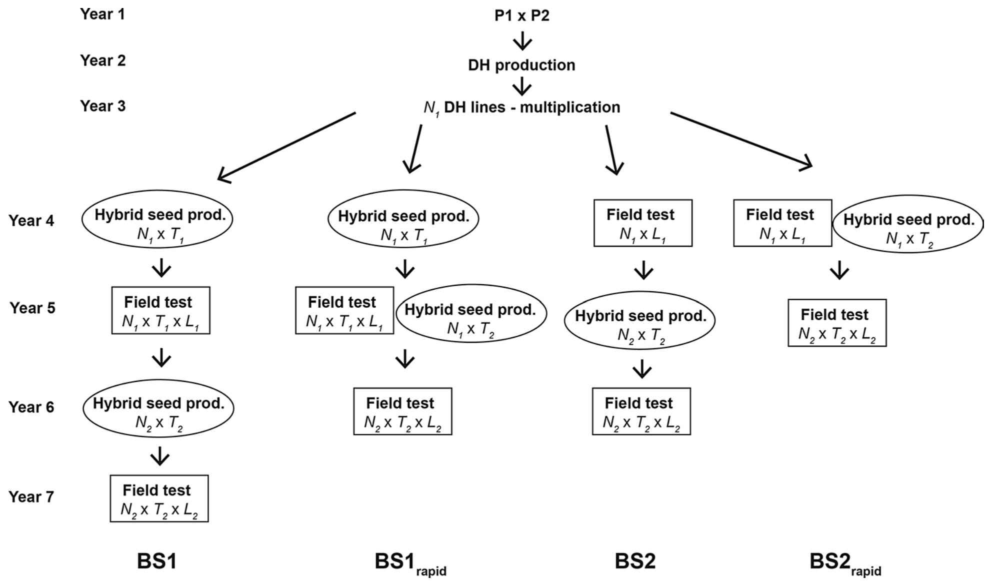

Fig. 1 Hybrid wheat breeding strategies with production of an initial number of N_1 DH lines followed by four different ways to evaluate their grain yield performance with their respective allocation of test resources. Breeding strategy BS1 comprises a two-stage selection for GCA, whereas in strategy BS2, the N_1 DH lines are first evaluated for their line per se performance followed by selection for GCA. In $BS1_{rapid}$ and $BS2_{rapid}$, hybrid seed production for the second field test is advanced by 1 year, parallel to the first field test. Selection in stage 1 and 2 was performed after the respective field trials, which were indicated by the black boxes (N, L, T = number of DH lines, test locations and tester in selection stage one and two, respectively)

Table 1 Abbreviations used in the manuscript

DH	Doubled haploid line
LP	Line per se performance of DH lines
GCA	General combining ability of DH lines
a_{LP}, a_{GCA}	Economic weight of LP and GCA
ρ(LP, GCA)	Genetic correlation between LP and GCA
M_j	Definition of tester type, i.e., number of unrelated inbred lines combined in a single tester in stage j
N_j, T_j, L_j	Allocation of test resources, i.e., number of DH lines, testers and locations in stage j of performance trials
N_f	Number of lines finally selected after two selection stages
ΔG, ΔG_a	Selection gain and annual selection gain
B	Budget available for the breeding scheme in testcross plot equivalents
Cost	Costs of producing hybrid seed with one DH line
BS1	Breeding strategy one with GCA evaluation in both selection stages
BS2	Breeding strategy two with evaluation of LP in stage one followed by GCA evaluation in stage two

the number of years required in the respective breeding strategy. For all our calculations, we used the open source R package "selectiongain" (Mi et al. 2014).

Optimum allocation of resources

The allocation of resources refers to triples (N_j, T_j, L_j) for each tester type in both selection stages j. A vector (N_j, T_j, L_j) is denoted as optimum allocation if it maximizes ΔG for all admissible allocations, which are valid for the budget, variance components, and tester type considered. The optimum allocation was determined by a grid search across all possible allocations for the scenario under consideration (for details, cf. Mi et al. 2014). We assumed that (i) each tester is evaluated at each location, (ii) the test locations in the first stage are a subset of those used in the second stage, and (iii) selection in stage two is based on an index of the phenotypic means of both selection stages (for details see Longin et al. 2007). In applied wheat breeding, a maximum number of $L_1 = 5$ and $L_2 = 10$ are normally available (E.

Ebmeyer, V. Lein, pers. comm.), which were used as upper boundaries in our calculations.

Economic frame and quantitative genetic parameters

We assumed a fixed total budget for producing hybrid seed of the DH lines and evaluating their line per se performance or GCA in two selection stages in field plot equivalents. The budget was defined as $B = N_1T_1\text{Cost} + N_1T_1L_1 + N_2T_2\text{Cost} + N_2T_2L_2$, where Cost refers to the cost of producing hybrid seed with one DH line. An example of components of 'cost' might include cost for the CHA, the staff and machinery applying the CHA and checking the sterility as well as consumables for the field. Owing to the difficulties in hybrid seed production in wheat (cf., Kempe and Gils 2011), these costs currently amount to about four times the costs of one field plot (Cost = 4; V. Lein, pers. comm.). In addition, we also assumed Cost = 1 to evaluate the impact of the costs for hybrid seed production. For each scenario, we determined the optimum allocation of test resources. The different breeding strategies required modifications in the formula for the budget, i.e., for BS2 Cost = 0 in selection stage 1. For $\text{BS1}_{\text{rapid}}$ as well as for $\text{BS2}_{\text{rapid}}$ the hybrid seed production costs for the second selection stage were set to N_1T_2Cost. We compared two budgets with 5,000 and 10,000 plot equivalents, which reflects the current situation of wheat breeders in Central Europe (E. Ebmeyer, pers. comm.).

Variance components were taken from a vast experimental study comprising 1,604 hybrids and their 135 parental lines phenotyped for grain yield in eleven German locations (cf., Longin et al. 2013). In particular, we used $\sigma^2_{\text{GCA}} = 3.65$, $\sigma^2_{\text{GCA}\times L} = 5.19$, $\sigma^2_{\text{SCA}} = 1.88$, $\sigma^2_{\text{SCA}\times L} = 2.94$, $\sigma^2_{\text{error}} = 24.37$, and $\sigma^2_{\text{LP}} = 14.06$, $\sigma^2_{\text{LP}\times L} = 22.27$, where σ^2_{GCA}, σ^2_{SCA}, and σ^2_{LP} refer to the variances of GCA, specific combining ability (SCA) and line per se performance, respectively, and $\sigma^2_{\text{GCA}\times L}$, $\sigma^2_{\text{SCA}\times L}$, and $\sigma^2_{\text{LP}\times L}$ to the variances of the interaction of these factors with locations and σ^2_{error} to the variance of the plot error. The genetic correlation between line per se performance and GCA of $\rho(\text{LP, GCA}) = 0.75$ was assumed on the basis of the above-mentioned study. With further intensification of hybrid wheat breeding, the GCA variance might increase and ρ(LP, GCA) might be reduced. Thus, we additionally determined the optimum allocation of test resources assuming $\sigma^2_{\text{GCA}} = 7.30$ but otherwise the same variances components and $\rho(\text{LP, GCA}) = 0.5$. To simplify our comparisons, we defined a standard scenario with the following parameters: $B = 5{,}000$, Cost = 4, $a_{\text{GCA}} = a_{\text{LP}} = 0.5$, $\sigma^2_{\text{GCA}} = 3.65$, $\rho(\text{LP, GCA}) = 0.75$ and $N_{\text{f}} = 5$. The impact of each single parameter on the optimum allocation of test resources and the selection gain ΔG was investigated under ceteris paribus conditions, i.e., by varying only this parameter and comparing the result with the results of the standard scenario.

Results

The maximum absolute selection gain ΔG was observed for BS2 in all investigated scenarios followed in decreasing order by $\text{BS2}_{\text{rapid}}$, BS1 and $\text{BS1}_{\text{rapid}}$ (Table 2). However, the four investigated breeding strategies differ up to 2 years regarding their cycle length (Fig. 1). To account for this difference, we will focus in the following on the annual ΔG_{a}, which is the absolute ΔG divided by the number of years required in the respective breeding strategy.

The size of ΔG_{a} in all breeding strategies depended on the economic weight for line per se performance (a_{LP}) as well as the strength of the correlation between line per se performance and GCA (ρ(LP, GCA) (Fig. 2). For BS2 and $\text{BS2}_{\text{rapid}}$, ΔG_{a} increased with increasing ρ(LP, GCA) with the slope of the response curves being similar for the different economic weights. The larger a_{LP}, the larger was ΔG_{a}. In contrast, for BS1 and $\text{BS1}_{\text{rapid}}$, the slope of the response curves of ΔG_{a} for increasing ρ(LP, GCA) differed, depending on the economic weights. For $a_{\text{LP}} = 0$, ΔG_{a} remained constant with increasing ρ(LP, GCA). In contrast, for $a_{\text{LP}} > 0$, ΔG_{a} increased strongly with increasing ρ(LP, GCA) indicating a strong interaction of ρ(LP, GCA) with a_{LP} on ΔG_{a} in BS1 and $\text{BS1}_{\text{rapid}}$.

For the standard scenario defined in the last paragraph of the "Material and methods", ΔG_{a} amounted to 0.70, 0.73, 1.06, and 1.16 decitons per ha and year for BS1, $\text{BS1}_{\text{rapid}}$, BS2 and $\text{BS2}_{\text{rapid}}$, respectively (Table 2). By comparison, a reduction in hybrid seed production costs by factor four (Cost = 1) led to an increase in ΔG_{a} of 6, 11, 1 and 5 % for BS1, $\text{BS1}_{\text{rapid}}$, BS2 and $\text{BS2}_{\text{rapid}}$, respectively. A reduction of ρ(LP, GCA) from 0.75 to 0.5 resulted in reduced ΔG_{a} for all breeding strategies. This reduction was much larger for BS1 and $\text{BS1}_{\text{rapid}}$ compared with BS2 and $\text{BS2}_{\text{rapid}}$. An increased number of finally selected lines of $N_{\text{f}} = 10$ reduced ΔG_{a} in all breeding strategies. Putting all economic weight on GCA ($a_{\text{LP}} = 0$) led to largely reduced ΔG_{a} in all breeding strategies, especially for BS2 and $\text{BS2}_{\text{rapid}}$. Nevertheless, $\text{BS2}_{\text{rapid}}$ had the highest annual ΔG_{a} for all considered scenarios.

The optimum allocation of test resources differed largely for the four breeding strategies (Table 2). For instance, the optimum allocation of test resources for BS1 under the standard scenario was $N_1 = 361$, $N_2 = 25$, $T_1 = 1$, $T_2 = 5$, $L_1 = 5$, and $L_2 = 10$. By comparison, BS2 and $\text{BS2}_{\text{rapid}}$ generally had a much higher optimum number of N_1 and a slightly reduced optimum number of N_2. In addition, the optimum number of T_2 was lower in $\text{BS1}_{\text{rapid}}$ and $\text{BS2}_{\text{rapid}}$. For almost all scenarios, the upper limit of $L_1 = 5$ and $L_2 = 10$ was optimum;

Table 2 Optimum allocation of test resources maximizing selection gain (ΔG) under alternative breeding strategies for varying hybrid seed production costs (Cost), economic weights for line per se performance (a_{LP}) and GCA ($a_{GCA} = 1 - a_{LP}$), correlation between line per se performance and GCA (ρ(LP, GCA)) and numbers of finally selected lines (N_f)

Strategy	Cost	a_{LP}	a_{GCA}	ρ(LP, GCA)	N_f	M_1	M_2	Optimum allocation of test resources						ΔG	
								N_1	N_2	T_1	T_2	L_1	L_2	Absolute	Annual
BS1	4	0.5	0.5	0.75	5	4	1	361	25	1	5	5	10	4.87	0.70
$BS1_{rapid}$	4	0.5	0.5	0.75	5	4	1	248	39	1	2	5	10	4.39	0.73
BS2	4	0.5	0.5	0.75	5		1	787	19		4	5	10	6.38	1.06
$BS2_{rapid}$	4	0.5	0.5	0.75	5		1	530	23		1	5	10	5.82	1.16
BS1	1	0.5	0.5	0.75	5	4	1	615	35	1	5	4	10	5.18	0.74
$BS1_{rapid}$	1	0.5	0.5	0.75	5	4	1	432	37	1	3	5	10	4.89	0.81
BS2	1	0.5	0.5	0.75	5		1	997	23		4	4	10	6.43	1.07
$BS2_{rapid}$	1	0.5	0.5	0.75	5		1	648	23		2	5	10	6.13	1.23
BS1	4	0.5	0.5	0.5	5	4	1	361	25	1	5	5	10	3.91	0.56
$BS1_{rapid}$	4	0.5	0.5	0.5	5	4	1	248	39	1	2	5	10	3.52	0.59
BS2	4	0.5	0.5	0.5	5		1	823	21		3	5	10	5.81	0.97
$BS2_{rapid}$	4	0.5	0.5	0.5	5		1	530	32		1	5	10	5.32	1.06
BS1	4	0.5	0.5	0.75	10	4	1	378	38	1	3	5	10	4.25	0.61
$BS1_{rapid}$	4	0.5	0.5	0.75	10	4	1	240	46	1	2	5	10	3.86	0.64
BS2	4	0.5	0.5	0.75	10		1	935	30		3	4	10	5.77	0.96
$BS2_{rapid}$	4	0.5	0.5	0.75	10		1	520	32		1	5	10	5.27	1.05
BS1	4	0	1	0.75	5	4	1	361	25	1	5	5	10	3.94	0.56
$BS1_{rapid}$	4	0	1	0.75	5	4	1	248	39	1	2	5	10	3.55	0.59
BS2	4	0	1	0.75	5		1	938	39		4	3	10	4.30	0.72
$BS2_{rapid}$	4	0	1	0.75	5		1	315	45		2	5	10	3.78	0.76

Assumptions: $B = 5{,}000$; standard variance components; use of a mixture of four inbred lines as tester in selection stage one and one inbred tester in selection stage two for reasons explained in detail in the discussion. For abbreviations, see Table 1

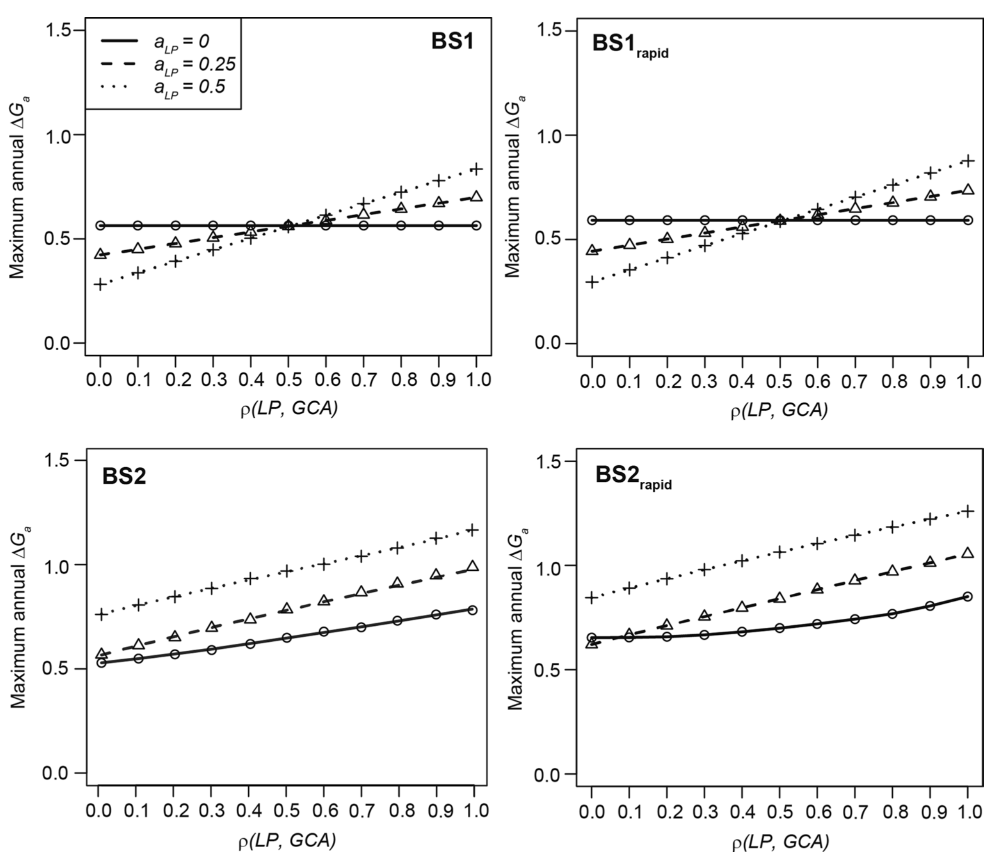

Fig. 2 Maximum annual selection gain (ΔG_a) in the different breeding strategies as a function of the economic weights for line per se performance (a_{LP}) and GCA ($a_{GCA} = 1 - a_{LP}$) as well as the correlation between line per se performance and GCA (ρ(LP, GCA)). (Assumptions: $B = 5{,}000$; Cost = 4; standard variance components; the use of a mixture of four inbred lines as tester in selection stage one and one inbred tester in selection stage two for reasons explained in detail in the discussion. For abbreviations, see Table 1)

Table 3 Optimum allocation of test resources maximizing selection gain (ΔG) in breeding strategy $BS2_{rapid}$ for varying budget, hybrid seed production costs (Cost), economic weights for line per se performance (a_{LP}) and GCA ($a_{GCA} = 1 - a_{LP}$), amount of GCA variance (σ^2_{GCA}), correlation between line per se performance (LP) and GCA (ρ(LP, GCA)), and tester type (M_2)

Budget	Cost	a_{LP}	a_{GCA}	σ^2_{GCA}	ρ(LP, GCA)	N_f	M_2	Optimum allocation of test resources					ΔG	
								N_1	N_2	T_2	L_1	L_2	Absolute	Annual
5,000	4	0.5	0.5	3.65	0.75	5	1	530	23	1	5	10	5.82	1.16
5,000	4	0.5	0.5	3.65	0.75	5	2	530	23	1	5	10	5.88	1.18
5,000	4	0.5	0.5	3.65	0.75	5	3	525	27	1	5	10	5.91	1.18
5,000	4	0.5	0.5	3.65	0.75	5	4	523	29	1	5	10	5.93	1.19
5,000	4	0.5	0.5	3.65	0.75	5	1	352	21	2	5	10	5.67	1.13
5,000	4	0.5	0.5	3.65	0.75	5	1	262	18	3	5	10	5.51	1.10
5,000	4	0.5	0.5	3.65	0.75	5	1	205	17	4	5	10	5.35	1.07
5,000	1	0.5	0.5	3.65	0.75	5	1	790	26	1	5	10	6.11	1.22
5,000	1	0.5	0.5	3.65	0.75	5	1	648	23	2	5	10	6.13	1.23
5,000	1	0.5	0.5	3.65	0.75	5	1	546	21	3	5	10	6.09	1.22
5,000	1	0.5	0.5	3.65	0.75	5	1	471	19	4	5	10	6.01	1.20
5,000	4	0	1	3.65	0.75	5	1	315	45	2	5	10	3.78	0.76
5,000	4	0	1	3.65	0.50	5	1	315	61	2	4	10	3.47	0.69
5,000	4	0.5	0.5	3.65	0.50	5	1	530	23	1	5	10	5.32	1.06
10,000	4	0.5	0.5	3.65	0.75	5	1	1,074	33	1	5	10	6.33	1.27
10,000	4	0.5	0.5	3.65	0.75	10	1	1,294	47	2	5	10	6.13	1.23
5,000	4	0.5	0.5	7.30	0.75	5	1	516	35	1	5	10	6.83	1.37

For abbreviations, see Table 1

thus, the number of test locations is disregarded in our further discussion. A reduction in hybrid seed production costs by a factor of four (Cost = 1) led to higher optimum numbers of N_1 in all breeding strategies. In addition, the optimum number of T_2 increased for $BS1_{rapid}$ and $BS2_{rapid}$.

The choice of tester type (M_j) hardly influenced the optimum allocation of test resources but strongly affected ΔG_a, as illustrated for $BS2_{rapid}$ (Table 3). With increasing M_j, i.e., increasing the number of lines combined in each tester by a seed mix, ΔG_a was increased. For instance for $BS2_{rapid}$, increasing M_j from 1 to 4 led to an increase in ΔG_a of 1.8 %. Similar results were found for the other breeding strategies (data not shown). Owing to concerns from breeders discussed later, we set $M_1 = 4$ and $M_2 = 1$ in all breeding strategies. Consequently, complex testers were only used in BS1 and $BS1_{rapid}$, because the first selection stage in BS2 and $BS2_{rapid}$ focuses only on line per se performance. Doubling the budget to 10,000 testcross plot equivalents led to a largely increased optimum number of N_1 and an increase in ΔG_a of 8 %. Doubling the GCA variance to $\sigma^2_{GCA} = 7.30$ hardly influenced the optimum allocation of test resources but increased ΔG_a by 18 %.

In $BS2_{rapid}$, only the N_2 DH lines were tested in hybrid combinations and the optimum number of $N_2 = 23$ and $T_2 = 1$ was found to be low (Tables 2, 3). Increasing T_2 led to losses in $\Delta G_a > 2.5$ % (Table 3; Fig. 3). By contrast, increasing N_2 at the expense of reduced N_1 led only to slight reductions in ΔG_a (Fig. 3). For instance for $T_2 = 1$, using the allocation $N_1 = 444$ and $N_2 = 100$ instead of $N_1 = 530$ and $N_2 = 23$ reduced ΔG_a only by 1.7 %.

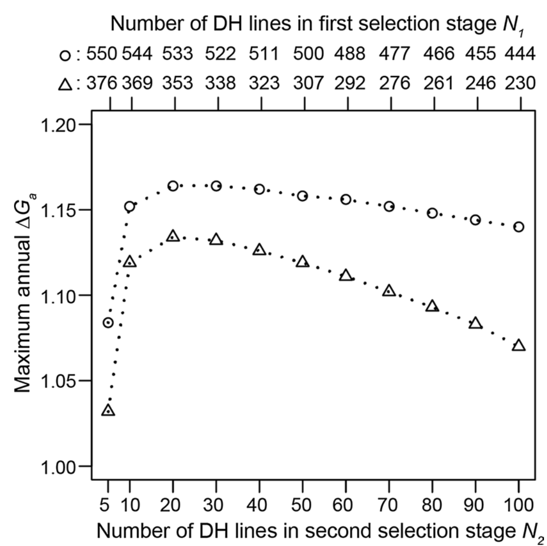

Fig. 3 Maximum annual selection gain (ΔG_a) in breeding strategy $BS2_{rapid}$ for the use of $T_2 = 1$ inbred tester (*circles*) or $T_2 = 2$ inbred testers (*triangles*) in dependence of an increasing number of DH lines in the second selection stage (N_2) at the expense of decreased number of DH lines in the first selection stage (N_1) (Assumptions: $B = 5{,}000$, Cost = 4, standard variance components, $a_{LP} = 0.5$ and ρ(LP, GCA) = 0.75. For abbreviations, see Table 1)

Discussion

The growing interest in hybrid breeding in wheat requires the elaboration of efficient breeding strategies and optimum allocation of test resources. Selection gain (ΔG) is the most widely used criterion to compare and optimize breeding strategies (cf. Cochran 1951; Longin et al. 2007; Wegenast et al. 2008; Gordillo and Geiger 2008). We consequently adapted the open source software 'selectiongain' (http://www.R-project.org, package 'selectiongain') to a hybrid wheat context. This enabled us to optimize the allocation of test resources and to compare different breeding strategies based on ΔG for the female pool. In particular, we compared a breeding strategy including two-stage selection for GCA (BS1, Fig. 1) with a breeding strategy combining one-stage selection on line per se performance followed by one-stage selection for GCA (BS2). In addition, we investigated a modification of both schemes in which production of hybrid seed for the second field test is already performed in parallel to the first field test ($BS1_{rapid}$ and $BS2_{rapid}$) to shorten the length of the breeding cycle.

Relative efficiency of alternative breeding strategies

For the annual selection gain ΔG_a, we obtained across all investigated scenarios the following ranking of the four breeding strategies: $BS2_{rapid} > BS2 \gg BS1_{rapid} > BS1$ (Fig. 2; Table 2). For instance regarding the standard scenario, we found a superiority of 5, 52 and 67 % for $BS1_{rapid}$, BS2, and $BS2_{rapid}$, respectively, as compared with BS1 (Table 2). The large superiority of $BS2_{rapid}$ compared to the other strategies is surprising owing to the high number of test hybrids which have to be produced in this strategy but which are discarded before the second selection stage due to their poor line per se performance. For instance in the standard scenario of $BS2_{rapid}$, the optimum allocation of test resources requires the production of hybrid seed for $N_1 = 530$ DH lines, while only $N_2 = 23$ of them were tested in the field (Table 2). Thus, 40 % of the total budget was invested in producing hybrid combinations which were not advanced and evaluated in the field. However, the utilization of seed yield data from testcross seed production can be used to drive understanding and improvement of pollen production in wheat for hybrid seed yield. Furthermore, our results clearly indicate that the possibility to speed up the breeding cycle by far counterbalances this disadvantage, thus underlining the importance of short breeding cycles.

The relative superiority of BS2 and $BS2_{rapid}$ over BS1 and $BS1_{rapid}$ depends on several parameters. Across all considered scenarios, the economic weights for line per se performance (a_{LP}) and GCA ($a_{GCA} = 1 - a_{LP}$) as well as the correlation ρ(LP, GCA) were identified as having the largest impact on this comparison (Table 2). This can be explained by a more detailed look at the models and breeding strategies used in our study. Although our aim is to select for lines with high GCA, a good line per se performance is also of interest for different reasons: First, a high line per se performance of female lines used in hybrid combinations is a crucial prerequisite to maximize the yield in hybrid seed production and second, most wheat breeders are currently starting hybrid programs in parallel to their ongoing long-time breeding programs for line varieties. If these breeders identify lines with promising line per se performance in the hybrid program, they will most likely use them not only as hybrid parents, but also as potential new line varieties. To account for these possibilities, our target variable was the index $H = a_{LP}g_{LP} + a_{GCA}g_{GCA}$ combining line per se performance and GCA and allowing for different economic weights for both selection criteria. In this formula, the economic weights strictly depend on the specific market and seed production situation of the breeder. The high necessity to improve yield in hybrid seed production fields (cf. Kempe and Gils 2011) precludes the choice of $a_{LP} = 0$ in wheat. Focusing for instance only on hybrid wheat without the aim to sell any line variety, the economic weight for line per se performance might range between $a_{LP} = 0.1$ and $a_{LP} = 0.3$. However, most wheat breeders currently run line and hybrid programs in parallel suggesting the use of a_{LP} in the range of 0.4–0.6. It must be noted that for $a_{LP} > 0$, ΔG_a reflects the sum of the selection gain achieved in the hybrid breeding program for GCA and line per se performance. Thereby the proportion of ΔG_a due to line per se performance was larger for BS2 compared to BS1 and strongly depended on a_{LP} and ρ(LP, GCA).

The use of index selection, in which several directly measured traits are combined for the final selection, is common practice in wheat breeding. However, the applied index in our manuscript differs from these indices. That is, neither in BS1 nor in BS2 the two selection criteria, line per se performance and GCA, are measured in parallel field tests. In BS1, in both stages, only GCA is measured and any improvement in the line per se performance results from the correlation ρ(LP, GCA), i.e., classical indirect selection. In BS2, only line per se performance is measured in the first selection stage and any improvement of the GCA in this selection stage results from the correlation ρ(LP, GCA), and vice versa for the second selection stage. Thus, BS2 represents a combination of directly selecting for both selection criteria in different stages with indirect selection within each stage on the non-tested selection criteria.

These model circumstances have severe consequences on the maximum ΔG_a under the different breeding strategies and its dependency on the economic weight for line per se performance (a_{LP}) and the correlation between line per se performance and GCA (ρ(LP, GCA)). As expected, the relative superiority of BS2, which places much greater emphasis on line per se performance as compared to BS1, was largely reduced with decreasing a_{LP} (Fig. 2; Table 2). By contrast, with decreasing ρ(LP, GCA), the relative superiority of BS2 over BS1 was strongly increased, which is due to an interaction of a_{LP} and ρ(LP, GCA) on ΔG_a in the different breeding strategies. For instance, for an economic weight of line per se performance of $a_{LP} = 0$ ΔG_a remained constant with increasing ρ(LP, GCA) in BS1 and $BS1_{rapid}$ (Fig. 2). This can be explained by the fact that in this situation neither any field test nor any economic interest is given to line per se performance. In contrast, in BS2 and $BS2_{rapid}$, ΔG_a increased with increasing ρ(LP, GCA) for $a_{LP} = 0$ owing to the use of line per se performance as indirect selection criterion for GCA in the first stage of these breeding strategies.

Furthermore, for BS2 and $BS2_{rapid}$, we determined an increasing ΔG_a with increasing ρ(LP, GCA) with similar slopes of the response curves for the different economic weights (Fig. 2). In contrast, for BS1 and $BS1_{rapid}$, the slope of the response curve of ΔG_a with increasing ρ(LP, GCA) depended largely on the size of the economic weights. For $a_{LP} = 0$, ΔG_a remained constant with increasing ρ(LP, GCA) but increased strongly for $a_{LP} > 0$. This led to the apparently paradox situation that for ρ(LP, GCA) <0.5, ΔG_a was reduced with increasing a_{LP} while for ρ(LP, GCA) >0.5, ΔG_a was increased with increasing a_{LP}. Consequently, the efficiency of breeding strategies using selection indices depends on the correlation among the selection criteria, their respective economic weights, and the interplay of these two parameters. The latter can vary across breeding strategies requiring a careful choice of the selection method applied in each breeding strategy.

Hybrid seed production costs must be reduced

Hybrid seed production in wheat is complicated and expensive (for a detailed review see Kempe and Gils 2011; Longin et al. 2012). Currently, the production of seed for one test hybrid amounts to four times the costs of one yield plot. Intensive research activities to improve hybrid seed production in wheat have been initiated in the last years in the private and public sector with first important outcomes (cf. Whitford et al. 2013; Langer et al. 2014). Consequently, a reduction of hybrid seed production costs can be expected in the future. To account for this fact, we also compared the breeding strategies assuming strongly reduced hybrid seed production costs (Cost = 1, Tables 2, 3). This led to an increase in ΔG_a of >5 % across all breeding strategies except for BS2. Especially ΔG_a of the breeding strategies $BS1_{rapid}$ and $BS2_{rapid}$ was increased, which is due to the large number of test hybrids produced in these schemes underlining the importance to improve hybrid seed production. In conclusion, breeding strategy $BS2_{rapid}$ had the by far largest annual selection gain for all considered scenarios and is consequently recommended for applied hybrid wheat breeding. If not stated otherwise, we concentrate our considerations in the following on this breeding strategy.

Parameters strongly influencing the selection gain

Doubling the budget from 5,000 to 10,000 testcross plot equivalents led to an increase in ΔG_a of 8 % for a final number of selected DH lines of 5 ($N_f = 5$, Table 3). For $N_f = 10$, the doubled budget led to an increase in ΔG_a of 16 %. These findings are in line with reports for maize (Longin et al. 2006, 2007; Wegenast et al. 2008). Nevertheless, an increase in ΔG_a of <16 % by doubling the budget is a rather low return of investment. It thus seems worthwhile to consider alternatives for investing an available budget.

Recently, the size of σ^2_{GCA} was identified as the most important parameter when comparing the efficiency of line versus hybrid breeding in wheat (Longin et al. 2014). The authors reported that already small increases in σ^2_{GCA} led to a large increase in the efficiency of hybrid wheat breeding. In line with these findings, we observed an increase in ΔG_a of 18 % when doubling σ^2_{GCA} (Table 3), which is comparable to the return of investments for doubling the budget. One major reason for the relative low amount of σ^2_{GCA} determined in experimental studies for hybrid wheat is the low number of lines investigated for GCA so far. In contrast to line breeding, where tens of thousands of lines have been screened for their line per se performance in the last decades, only a few dozen lines have been tested for their GCA. Consequently, the probability is high that prebreeding activities screening higher numbers of lines for their GCA to defined elite testers and subsequent recurrent selection will increase the amount of σ^2_{GCA} available in hybrid wheat breeding. As small increases of σ^2_{GCA} have already a large impact on ΔG_a, the investment of an available budget in these activities is highly recommended.

Optimum allocation of test resources

The optimum number of testers was smallest in $BS2_{rapid}$ compared with the other breeding strategies (Table 2). For the standard scenario, the use of only one tester ($T_2 = 1$) was optimum and an increase in T_2 led to a large reduction in ΔG_a (Table 3; Fig. 3). For instance, using $T_2 = 2$ instead of $T_2 = 1$ resulted in a loss of ΔG_a of more than 2.5 % with a similar trend for further increasing T_2. This is in contrast to findings in maize, where modifications of T_2 showed only little effects on ΔG (Longin et al. 2007). One reason is the current high costs of hybrid seed production. For instance, a strong reduction of these costs (Cost = 1) led to a flat optimum regarding T_2 with an optimum number of $T_2 = 2$ and negligible losses in ΔG_a using either $T_2 = 3$ or $T_2 = 1$. Nevertheless, given the current high seed production costs, our results clearly suggest the use of $T_2 = 1$, which must be questioned for practical reasons.

In hybrid breeding, each inbred line can serve as parental line and the potential number of hybrids is a quadratic function of the number of available lines. Thus, the crossing of each line to numerous alternative lines is impossible. In maize for instance, the following compromise is widely used with small modifications across the breeders (Kramer 2003; Schmidt 2003). Within each breeding program, two-stage selection on GCA with very few testers is performed and a small number of interesting new lines with high GCA is finally selected. These lines are then passed to the product development teams, which perform several years of pre-registration trials. These pre-registration trials are spanned across several breeding programs in which the small number of new lines with high GCA is tested with a higher number of testers. Interesting hybrid combinations are then tested in total 3–4 years before entering official registration trials. However, for outstanding hybrid combinations, the process is accelerated by entering official registration trials already after 2 years of trials. Thus, breeders are demanded to screen as early as possible for potential hybrid combinations.

As a compromise from these differing demands, maize breeders often use a low number of complex testers in the first GCA trial but several inbred testers already in the second GCA test (cf. Longin et al. 2007). In wheat, however, this strategy is prohibitive due to the large superiority of the combined breeding strategy $BS2_{rapid}$. Therein, hybrids are only tested in the second field test just before entering the pre-registration trials. Consequently, the use of $T_2 > 1$ also in $BS2_{rapid}$ might be required in applied wheat breeding to identify promising hybrid combinations. Furthermore, the use of $T_2 > 1$ in $BS2_{rapid}$ balances on several testers the risk of losing hybrid seed production fields, which might easily happen when flowering time of males and females differ significantly or weather conditions have hampered correct application of the CHA.

Instead of inbred testers, the use of single-cross or double-cross testers or seed mixtures was recommended for maize especially for situations with a large amount of variance due to SCA (Longin et al. 2007). For hybrid wheat breeding, ΔG_a is also increased with increasing numbers of lines combined in one tester (M_j, Table 3). However, the realized increase in ΔG_a was relatively low compared to the high variance due to SCA present in current hybrid wheat programs (cf. Longin et al. 2013). Furthermore, as discussed above, in $BS2_{rapid}$ only one selection stage is based on hybrid tests before pre-registration trials making the use of complex testers in this strategy prohibitive as their use would only allow to obtain robust GCA estimates but not to identify promising hybrid combinations.

Besides a low number of testers used in $BS2_{rapid}$, the optimum number of DH lines tested in hybrid trials in the second stage ($N_2 = 23$) also appears low (Tables 2, 3). In $BS2_{rapid}$, only the N_2 DH lines were tested in hybrid combinations and thus, increasing their number increases the probability to identify promising hybrid combinations. Interestingly, reduced hybrid seed production costs did not influence the optimum number of N_2. However, doubling the number of N_2 by reducing in parallel the number of N_1 led to a reduction in ΔG_a of only 1 % for the standard scenario (Fig. 3). Nevertheless, for $BS2_{rapid}$ we have discussed above the practical necessity of $T_2 > 1$. For $T_2 = 2$, ΔG_a decreased more strongly with increasing N_2 compared with $T_2 = 1$ (Fig. 3). Thus, if at all, only moderate increases of N_2 were recommended for $BS2_{rapid}$.

Conclusion

Breeding strategy $BS2_{rapid}$ combining the evaluation of line per se performance and GCA in a short breeding cycle was identified as the strategy with the highest annual selection gain across all considered scenarios by far. Consequently, we recommend its use for hybrid wheat breeding with slight modifications of our findings on the optimum allocation of test resources due to practical concerns. In particular, we suggest to use two instead of one inbred testers ($T_2 = 2$, $M_2 = 1$). Some breeders might even want to use $T_2 > 3$ before pre-registration trials. For this case, similar values of the selection gain were determined for BS2 and $BS2_{rapid}$. Compared to $BS2_{rapid}$, a higher number of N_1 are optimum in BS2 and all produced hybrid combinations were also tested in the field making its use appealing. Nevertheless, for this strategy the annual selection gain is >6 % smaller than in the recommended strategy $BS2_{rapid}$.

Hybrid seed production costs and the low variance due to GCA currently available in hybrid wheat breeding were identified as key parameters for future research activities. The identification of wheat lines with significantly increased pollen amount and dispersal as well as the screening of wheat lines for GCA in combination with the establishment of heterotic groups requires very large series of field trials across several years. Furthermore, to warrant a stable and high long-term selection gain in hybrid wheat breeding, an exchange of material among breeders would be beneficial. Consequently, joint efforts in these pre-breeding activities among breeders and public institutes are highly recommended.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The authors declare that the experiments comply with the current laws of Germany.

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