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ORIGINAL PAPER

Genomic selection in wheat: optimum allocation of test resources and comparison of breeding strategies for line and hybrid breeding

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Abstract

Key message **The implementation of genomic selection in breeding programs can be recommended for hybrid and line breeding in wheat.**

Abstract High prediction accuracies from genomic selection (GS) were reported for grain yield in wheat asking for the elaboration of efficient breeding strategies applying GS. Our objectives were therefore, (1) to optimize the number of lines, locations and testers in different multi-stage breeding strategies with and without GS, (2) to elaborate the most efficient breeding strategy based on the selection gain and its standard deviation, and (3) to investigate the potential of GS to improve the relative efficiency of hybrid versus line breeding in wheat. We used the open source software package "selectiongain" to optimize the allocation of resources in different breeding strategies by predicting the expected selection gain for a fixed budget. Classical two-stage phenotypic selection was compared with three GS breeding strategies for line and hybrid breeding in wheat. The ranking of the alternative breeding strategies varied largely in dependence of the GS prediction accuracy. Fast-track breeding strategies based solely on GS were only advantageous for high GS prediction accuracies that is >0.50 and >0.65 for hybrid and line breeding, respectively. However, a GS prediction accuracy across breeding cycles of 0.3 or even less must be assumed as realistic for grain yield in wheat. For this low GS prediction accuracy, the use of GS is advantageous for line but especially for hybrid breeding in wheat. Furthermore, the use of GS in hybrid wheat breeding increased the relative efficiency of hybrid versus line breeding and, thus, might be an important pillar for the establishment of hybrid wheat.

Introduction

Genomic selection (GS) is a promising approach to improve complex traits like grain yield with genome-wide molecular markers. Intensive work has been performed to elaborate appropriate statistical models maximizing the prediction accuracy of GS in animal and plant breeding. Although these models differ considerably in basic assumptions, most of them yielded similar prediction accuracies (for wheat, cf. Heslot et al. 2012; Zhao et al. 2013; Storlie and Charmet 2013; Heslot et al. 2015). For grain yield in wheat, GS prediction accuracies ranging from 0.36 to 0.64 were reported in the literature (cf. Heslot et al. 2012). In contrast to line breeding, different prediction scenarios must be distinguished in hybrid wheat breeding: prediction of hybrids for which no (T0), one (T1), or both parents (T2) have already been phenotyped for their general combining ability (GCA) in other hybrid combinations. For grain yield, only one study is available in the literature thus far, reporting a GS prediction accuracy ranging from 0.28 in the T0 to 0.63 in the T2 scenario (Zhao et al. 2013 as reported in Zhao et al. 2014).

While further research is required to validate these findings, these prediction accuracies suggest that the inclusion of GS in breeding programs bears the potential to maximize the selection gain per unit time (Heffner et al. 2010). First

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model calculations have been performed investigating the optimal size of training and estimation sets compared to the intensity of phenotyping. Except very large budgets are available, it seems to be beneficial to increase the number of genotyped individuals and to phenotype all of them, but at a reduced number of locations or replications (Riedelsheimer and Melchinger 2013; Lorenz 2013; Endelman et al. 2015). However, only one-stage selection was considered in these studies but classical breeding programs are based on multi-stage selection (cf. Gordillo and Geiger 2008).

Heffner et al. (2010) compared multi-stage phenotypic selection including a couple of informative marker with a one-stage GS breeding scheme for line breeding in wheat. The authors clearly showed the high potential of GS by speeding up the breeding process. However, the allocation of resources was not optimized and hybrid wheat breeding was not considered. To the best of our knowledge, no study is available for wheat where different multi-stage breeding strategies with and without GS are compared at their respective optimum allocation of resources for a fixed budget for either line or hybrid breeding. Therefore, we used the open source software package "selectiongain" (Mi et al. 2014) to compare two-stage phenotypic selection with different multi-stage GS breeding strategies based on the expected selection gain for line and hybrid breeding in wheat. Our objectives were to (1) determine the optimum number of lines, locations and testers in each breeding strategy, (2) elaborate the most efficient breeding strategy for line and hybrid breeding, and (3) investigate the potential of GS to improve the relative efficiency of hybrid versus line breeding in wheat.

Materials and methods

Breeding strategies

We investigated line and hybrid breeding separately, that is the full budget is either used for line or hybrid breeding (Fig. 1). For all breeding strategies, we assumed the production of N_1 DH lines from numerous line crosses entering the first test and the final selection of the five best lines, which will proceed to further field evaluation in (pre)registration trials. The selection criterion was grain yield. In all strategies, the first selection was based on GS. For the breeding strategies $Line_{GSonly}$ and $Hybrid_{GSonly}$, no further phenotypic selection is performed representing a one-stage selection based only on markers which is the most rapid breeding strategy possible with GS. In contrast, for the other strategies, this genomic selection was followed by one-stage (breeding strategies $Line_{GSrapid}$ or $Hybrid_{GSrapid}$) or two-stage phenotypic selection (breeding strategies $Line_{GSstandard}$ or $Hybrid_{GSstandard}$). To compare the use of GS with breeding strategies based only on phenotypic data, we used the line and hybrid breeding strategy "GS standard" simply without genomic selection in test stage one, *i.e.,* two-stage phenotypic selection (breeding strategies $Line_{PSstandard}$, $Hybrid_{PSstandard}$). These two strategies represent the status quo in Central European wheat breeding and were considered in our treatise as the standard for all comparisons.

Hybrid and line breeding strategies differ only in the need of hybrid seed production stages elongating the hybrid strategies. We assumed the use of a chemical hybridization agent (CHA) for hybrid seed production, which is the current common practice in wheat. In phenotypic selection, the line per se performance was evaluated at L_j locations in selection stage two and three for line breeding ($j = 2, 3$). Similarly for phenotypic selection in hybrid breeding, the testcross performance was evaluated at L_j locations with T_j testers in selection stage two and three ($j = 2, 3$). We assumed the use of inbred line 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

Calculation of ΔG is based on the well-known formula of Cochran (1951) with multivariate normal integrals for selected fractions and heritabilities. In contrast to stochastic simulations requiring several thousand of simulation runs, these numerical calculations deliver with "one run" the expected result. 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 the number of years required in the respective breeding strategy. For all our calculations, we used the open source R (R Development Core Team 2012) package "selection gain". For details, the reader is referred to Mi et al. (2014) and the package manual (http://cran.r-project.org/web/packages/selectiongain/selectiongain.pdf).

Calculation of the standard deviation of the selection gain

The standard deviation of the selection gain ($SD_{\Delta G}$) in one-stage selection is defined as

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

where σ^2_z is the variance of the target variable, *i.e.,* genotypic variance of lines or the variance due to GCA, ρ_{z,x_1} the correlation between test and target criteria, *i.e.,* prediction

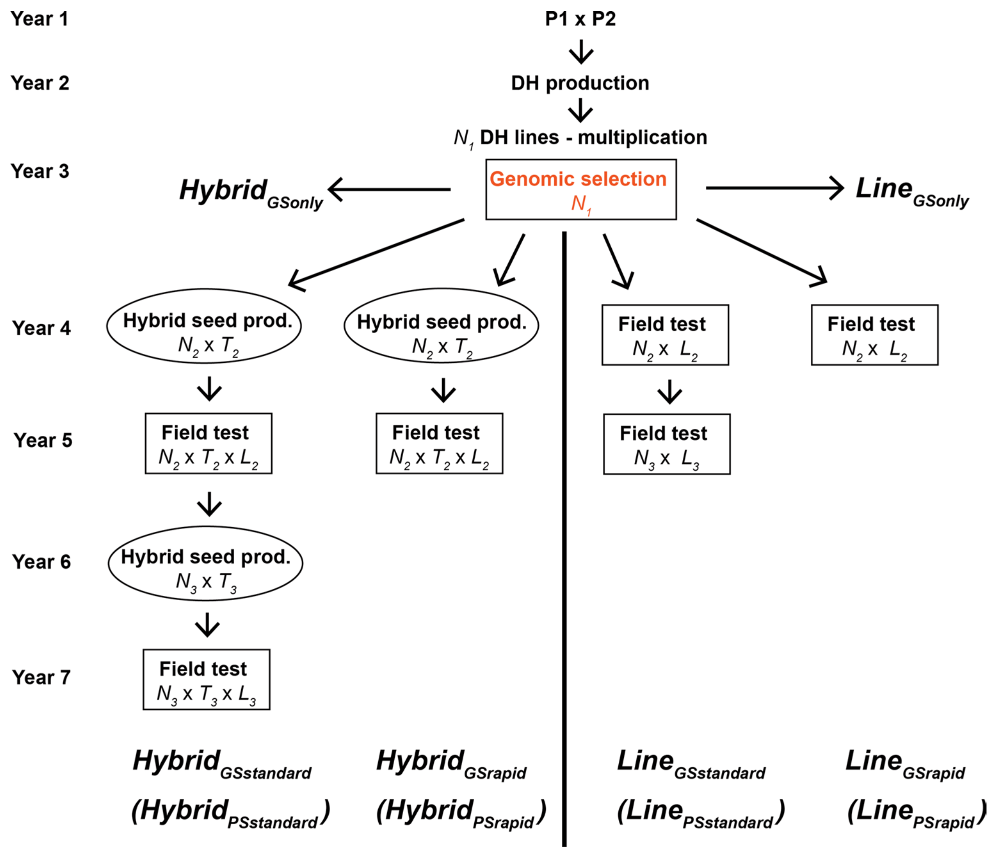

Fig. 1 Wheat breeding strategies with production of an initial number of N_1 DH lines from numerous line crosses, first selection based on GS followed by four different ways to evaluate their grain yield performance in the field with their respective allocation of test resources. In hybrid breeding, phenotypic selection is based on GCA, while in line breeding phenotypic selection is based on the line per se performance. Selection in stage 2 and 3 was performed after the respective field trials, which are indicated by the *black boxes*. In breeding strategies "rapid", the second field test is omitted speeding up the breeding process. In breeding strategies "GS only", only genomic selection is performed without any further field tests minimizing the breeding cycle length. In all breeding strategies, the final number of five lines was selected for internal preregistration trials. For comparison, the standard line and hybrid breeding strategy is also optimized without genomic selection, *i.e.,* phenotypic selection with $N_1 = N_2$. (N, L, T = number of DH lines, test locations and tester in selection stage one, two and three, respectively)

accuracy for GS, and N_f is the number of finally selected lines. In Eq. (1), v_1 is determined as

$$v_1 = 1 - i_1(i_1 - k_1) + (1 - \alpha_1)(i_1 - k_1)^2, \tag{2}$$

where α_1 is the selected fraction $= N_2/N_1$, i_1 the selection intensity and k_1 the truncation point of the selected fraction in the normal distribution. In the software R, the latter two variables can be calculated based on the quantile (qnorm) and density (dnorm) function of the normal distribution, respectively, as

$$k_1 = \text{qnorm}(1 - \alpha_1,\ 0,\ 1) \tag{3}$$

$$i_1 = \text{dnorm}(k_1,\ 0,\ 1)/\alpha_1 \tag{4}$$

To our knowledge, for two and more stages of selection, no formula exists in the literature to estimate the $\text{SD}_{\Delta G}$. However, Longin (2007) showed by computer simulations, that $\text{SD}_{\Delta G}$ for two-stage selection can be roughly approximated with

$$\text{SD}_{\Delta G} = \sqrt{(1 - \rho'^2_{z,x_2}(1 - v_2))\frac{\sigma'^2_z}{N_f}}, \tag{5}$$

where v_2 is calculated by applying formulas (2), (3) and (4) simply adjusting their values to the second selection stage. Furthermore σ'^2_z is approximated after Cochran (1951) as

$$\sigma'^2_Z = \sigma^2_Z(1 - \rho^2_{z,x_1}\gamma), \tag{6}$$

where $\gamma = i_1(i_1 - k_1)$ as well as ρ'_{z,x_2} after Dickerson and Hazel (1944) with

$$\rho'_{z,x_2} = \frac{\rho_{z,x_2} - \rho_{z,x_1}\rho_{x_1,x_2}\gamma}{\sqrt{(1 - \rho^2_{z,x_1}\gamma)(1 - \rho^2_{x_1,x_2}\gamma)}}, \tag{7}$$

Table 1 Abbreviations used in the manuscript

DH	Doubled haploid line
GCA	General combining ability of DH lines
SCA	Specific combining ability of DH lines
GS	Genomic selection
PS	Phenotypic selection
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 1–3 selection stages
ΔG, ΔG_a	Selection gain and annual selection gain
$SD_{\Delta G}$	Standard deviation of ΔG after the last selection stage
$Hybrid_{PSstandard}$	Hybrid breeding strategy with two-stage phenotypic selection
$Hybrid_{PSrapid}$	Hybrid breeding strategy with one-stage phenotypic selection
$Hybrid_{GSstandard}$	Hybrid breeding strategy with GS followed by two-stage phenotypic selection
$Hybrid_{GSrapid}$	Hybrid breeding strategy with GS followed by one-stage phenotypic selection
$Hybrid_{GSonly}$	Hybrid breeding strategy only with GS
$Line_{PSstandard}$	Line breeding strategy with two-stage phenotypic selection
$Line_{PSrapid}$	Line breeding strategy with one-stage phenotypic selection
$Line_{GSstandard}$	Line breeding strategy with GS followed by two-stage phenotypic selection
$Line_{GSrapid}$	Line breeding strategy with GS followed by one-stage phenotypic selection
$Line_{GSonly}$	Line breeding strategy only with GS

where ρ_{z,x_2} is the square root of the heritability for phenotypic selection at the second selection stage and $\rho_{x_1,x_2} = \rho_{z,x_1}\rho_{z,x_2}$. The extension to three stages is straightforward and analogous to the two-stage extension (Utz 1984).

Optimum allocation of resources

The allocation of resources refers in selection stage one to the number of produced and genotyped DH lines (N_1). In stage two and three, it refers to a number of lines N_j, tested at L_j locations with *Tj* testers (for hybrids only). A vector [N_1, N_j, L_j, (T_j)] is denoted as optimum allocation if it maximizes ΔG in the respective breeding strategy for a given GS prediction accuracy. The optimum allocation was determined by a grid search across all possible allocations for the scenario under consideration (for details see Mi et al. 2014). 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 consequently were used as upper boundaries in our calculations.

Economic frame and quantitative genetic parameters

We assumed a fixed total budget for producing seed of the DH lines and hybrids, genotyping and evaluating their line per se or testcross performance in field trials in field plot equivalents. A total budget available for line or hybrid breeding of 10,000 field plots was assumed, reflecting the current basis of Central European breeders (Longin et al. 2014b). As an example, the budget for the breeding strategy $Hybrid_{GSstandard}$ is illustrated with:

$$B = N_1(Cost_{DH} + Cost_{Genotyping}) + N_2T_2Cost_{Hybridseed} + N_2T_2L_2 + N_3T_3Cost_{Hybridseed} + N_3T_3L_3,$$

where $Cost_{DH}$, $Cost_{Genotyping}$ and $Cost_{Hybridseed}$ refer to the cost of producing, genotyping and hybrid seed production of one DH line, respectively. Thus, the term $N_2T_2\mathrm{Cost}_{Hybridseed}$ represents the costs for hybrid seed production in test stage two, *i.e.,* the costs for producing hybrids from N_2 DH lines crossed with T_2 testers. Similarly, the term $N_2T_2L_2$ represents the costs for phenotyping these hybrids. Based on discussions with different breeders and service providers for genotyping, we assume the following scenario as currently realistic in wheat: $Cost_{DH} = 1$ field plot, $Cost_{Genotyping} = 2$ field plots and $Cost_{Hybridseed} = 4$ field plots (Longin et al. 2014a, b). We further assumed that one hybrid seed production delivers enough seeds for 2-year phenotyping. For instance, in two-stage phenotyping, the tester used in the first stage is normally also used in the second stage with additional new testers. Thus, only for the additional testers, new hybrid seeds have to be produced. For line breeding, harvested kernels of the field plots of phenotypic selection can serve as seeds for the next generation, thereby minimizing seed production costs in this breeding method. We assumed for various general maintenance effort costs of 0.1 field plots per line and selection stage. For a reduced number of selection stages or line breeding, respective variables in the budget formula above were set to zero.

Before routinely using GS, calibration experiments are required to develop the prediction models. Furthermore, recalibrations of the models need to be done regularly in the breeding program. We assumed, however, that these GS calibrations were already developed outside the breeding programs. Furthermore, we assumed that recalibrations of the prediction models were done based on the available data from the routine breeding, thus without requiring additional budget. The choice of the prediction models is for our context here not important, and, thus, the reader is referred to the literature on this topic (cf. Zhao et al. 2014).

Variance components were taken from a vast experimental study comprising 1604 hybrids and their 135 parental lines phenotyped for grain yield in eleven German locations (cf., Longin et al. 2013). In particular, we used $\sigma^2_{\text{GCA}} = 5.7, \sigma^2_{\text{GCA}\times L} = 5.19, \sigma^2_{\text{SCA}} = 1.88, \sigma^2_{\text{SCA}\times L} = 2.94$, $\sigma^2_{\text{error}} = 24.37$, $\sigma^2_{\text{LP}} = 14.06$, $\sigma^2_{\text{LP}\times L} = 22.27$, where σ^2_{GCA}, σ^2_{SCA}, σ^2_{LP} refer to the variances of GCA, specific combining ability (SCA) and line per se performance, respectively, and $\sigma^2_{\text{GCA}\times L}$, $\sigma^2_{\text{SCA}\times L}$, $\sigma^2_{LP\times L}$ to the variances of the interaction of these factors with locations, respectively, and σ^2_{error} to the variance of the plot error. Using only one inbred tester and one test locations, these variance components led to a 0.79 for lines and 0.73 for hybrids, respectively. With increasing number of locations and/or testers, this single plot-based heritability is increased (cf. Longin et al. 2007).

Results

For hybrid and line breeding, the investigated alternative breeding strategies differed considerably in the number of years required to finish one breeding cycle (Fig. 1). To account for these differences, we concentrate in the following on the annual selection gain ΔG_a. For hybrid and line breeding, the ranking of the alternative breeding strategies largely depended on the GS prediction accuracy (Fig. 2). The breeding strategy with first-stage GS followed by only one-stage phenotypic selection ($Hybrid_{GSrapid}$, $Line_{GSapid}$) was superior to the classical two-stage phenotypic selection ($Hybrid_{PSstandard}$, $Line_{PSstandard}$) for the whole range of theoretically possible GS prediction accuracies. In contrast, breeding strategies based solely on one-stage GS selection ($Hybrid_{GSonly}$, $Line_{GSonly}$) were only advantageous for high GS prediction accuracies. For instance, in hybrid breeding with prediction accuracies <0.5, the annual selection gain ΔG_a was maximized using the breeding strategy $Hybrid_{GSrapid}$, while for higher prediction accuracies, ΔG_a was maximized using the breeding strategy $Hybrid_{GSonly}$. For line breeding, a similar trend was observed, but the breeding strategy $Line_{GSonly}$ was only better than the breeding strategy $Line_{GSrapid}$ for prediction accuracies >0.65.

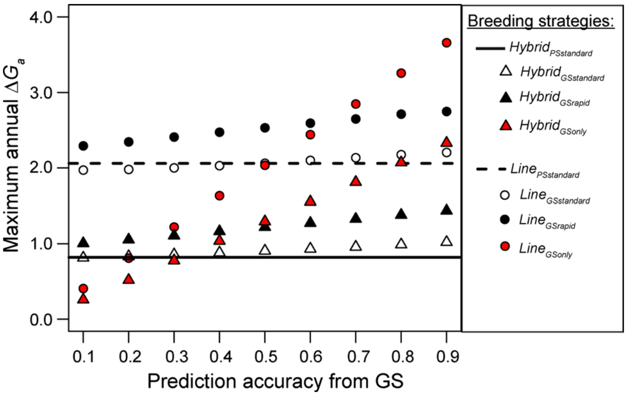

Fig. 2 Maximum annual selection gain (ΔG_a) in dependence of an increasing prediction accuracy from genomic selection (GS) for (1) line breeding without GS ($Line_{PSstandard}$; *dashed line*), with GS in the standard breeding strategy ($Line_{GSstandard}$; *open circle*), the rapid line breeding strategy ($Line_{GSrapid}$; *filled circle*) and selection based only on GS ($Line_{GSonly}$; *circles* filled by *red color*) as well as for (2) hybrid breeding without GS ($Hybrid_{PSstandard}$; *black line*), with GS in the standard breeding strategy ($Hybrid_{GSstandard}$; *open triangle*), the rapid breeding strategy ($Hybrid_{GSrapid}$; *filled triangle*) and selection based only on GS ($Hybrid_{GSonly}$; *triangles* filled with *red color*). For details regarding the breeding strategies, see Fig. 1 (color figure online)

According to recent literature, we assume that the GS prediction accuracy across different breeding cycles is rather low. Assuming a prediction accuracy of 0.3, we observed different rankings of the alternative breeding strategies in hybrid and line breeding according to the annual selection gain ΔG_a (Fig. 2). For hybrid breeding, the ranking was $Hybrid_{GSonly} < Hybrid_{PSstandard} < Hybrid_{GSstandard} < Hybrid_{GSrapid}$ while for line breeding it was $Line_{GSonly} < Line_{GSstandard} < Line_{PSstandard} < Line_{GSrapid}$.

The optimum allocation of test resources for hybrid and line breeding also depended on the GS prediction accuracies (Tables 2, 3). With decreasing GS prediction accuracy, the optimum number of N_1 was reduced in favor of an increased number of N_2. That is, the lower the GS prediction accuracy, the more GS becomes the character of a pre-test with reduced selection intensity. This effect was especially pronounced for line breeding.

For hybrid breeding without GS ($Hybrid_{PSstandard}$), the selection gain was maximized by testing the testcross performance of $N_2 = 680$ DH lines at $L_2 = 5$ locations with $T_2 = 1$ inbred tester and selecting the best $N_3 = 40$ DH lines for a final testcross evaluation with $T_3 = 6$ inbred testers at $L_3 = 10$ locations (Table 2). For breeding strategies applying GS ($Hybrid_{GSstandard}$, $Hybrid_{GSrapid}$, $Hybrid_{GSonly}$), the optimum number of test locations and testers was similar to that in classical phenotypic selection ($Hybrid_{PSstandard}$). However, the number of DH lines evaluated in the field (N_2, N_3) was considerably smaller than in the classical breeding strategy ($Hybrid_{PSstandard}$). Thus the available budget was

Table 2 Optimum allocation of test resources maximizing selection gain (ΔG) under alternative hybrid breeding strategies for varying prediction accuracies from genomic selection; for prediction accuracy = NA, no genomic selection is performed, *i.e.,* classical phenotypic selection (N, L, T = number of DH lines, test locations and tester in selection stage one, two, and three, respectively; $SD_{\Delta G}$ = standard deviation of the selection gain; ΔG_a = annual selection gain)

Breeding strategy	Accuracy	Optimum allocation of test resources							ΔG	$SD_{\Delta G}$	ΔG_a
		N_1	N_2	N_3	L_2	L_3	T_2	T_3			
$Hybrid_{PSstandard}$	NA	N_2	680	40	5	10	1	6	5.76	0.65	0.82
$Hybrid_{PSrapid}$	NA	N_2	344	–	10	–	2	–	4.95	0.74	0.99
$Hybrid_{GSstandard}$	0.2	1198	427.6	38.7	5	10	1	5	5.83	0.66	0.83
$Hybrid_{GSstandard}$	0.3	1444	328.2	33.9	5	10	1	6	5.99	0.65	0.86
$Hybrid_{GSstandard}$	0.4	1682	248.7	33.9	5	10	1	6	6.17	0.64	0.88
$Hybrid_{GSrapid}$	0.2	962	169.3	–	10	–	3	–	5.27	0.75	1.05
$Hybrid_{GSrapid}$	0.3	1519	129.6	–	10	–	3	–	5.54	0.75	1.11
$Hybrid_{GSrapid}$	0.4	1797	109.7	–	10	–	3	–	5.81	0.74	1.16
$Hybrid_{GSonly}$	0.2	3333.3	–	–	–	–	–	–	1.55	1.05	0.52
$Hybrid_{GSonly}$	0.3	3333.3	–	–	–	–	–	–	2.33	1.03	0.78
$Hybrid_{GSonly}$	0.4	3333.3	–	–	–	–	–	–	3.11	0.99	1.04

Table 3 Optimum allocation of test resources maximizing selection gain (ΔG) under alternative line breeding strategies for varying prediction accuracies from genomic selection; for prediction accuracy = NA, no genomic selection is performed, *i.e.,* classical phenotypic selection (N, L = number of DH lines and test locations in selection stage one, two and three, respectively; $SD_{\Delta G}$ = standard deviation of the selection gain; ΔG_a = annual selection gain)

Breeding strategy	Accuracy	Optimum allocation of test resources					ΔG	$SD_{\Delta G}$	ΔG_a
		N_1	N_2	N_3	L_2	L_3			
$Line_{PSstandard}$	NA	N_2	3521	292.7	1	10	10.34	0.57	2.07
$Line_{PSrapid}$	NA	N_2	909	–	10	–	9.30	1.05	2.32
$Line_{PSrapid}$	NA	N_2	2000	–	$4^{\S}$	–	8.61	1.24	2.15
$Line_{GSstandard}$	0.2	1934	1361.6	132.5	2	10	9.90	1.01	1.98
$Line_{GSstandard}$	0.3	2217	1003.9	122.7	2	10	10.00	1.01	2.00
$Line_{GSstandard}$	0.4	2422	645.3	72.5	3	10	10.15	0.98	2.03
$Line_{GSrapid}$	0.2	1492	546.8	–	10	–	9.38	1.06	2.35
$Line_{GSrapid}$	0.3	1826	447.4	–	10	–	9.63	1.05	2.41
$Line_{GSrapid}$	0.3	2395	685.9	–	$4^{\S}$	–	8.66	1.23	2.17
$Line_{GSrapid}$	0.4	2161	348.1	–	10	–	9.88	1.04	2.47
$Line_{GSonly}$	0.2	3333.3	–	–	–	–	2.44	1.65	0.81
$Line_{GSonly}$	0.3	3333.3	–	–	–	–	3.66	1.61	1.22
$Line_{GSonly}$	0.4	3333.3	–	–	–	–	4.88	1.55	1.63

§ Amount of seed, which is available for each line at this stage limits the number of test locations to 4

invested to evaluate a large number of N_1 DH lines by GS. Consequently, a considerably higher number of DH lines can be tested in the breeding strategies applying GS than in breeding schemes based solely on phenotypic selection. Similar trends were observed for line breeding (Table 3).

Besides the selection gain, the standard deviation of the selection gain $SD_{\Delta G}$ was also largely influenced by the choice of the breeding strategy. For hybrid breeding, we determined a $SD_{\Delta G} = 0.65$ for $Hybrid_{PSstandard}$ and similar values for $Hybrid_{GSstandard}$ (Table 2). Higher values of $SD_{\Delta G}$ were determined for breeding strategy $Hybrid_{GSrapid}$ ($SD_{\Delta G} = 0.75$) and especially for breeding strategy $Hybrid_{GSonly}$ ($SD_{\Delta G} = 1.03$). For line breeding without markers ($Line_{PSstandard}$), we observed $SD_{\Delta G} = 0.57$ (Table 3) but all other breeding strategies resulted in much higher values of $SD_{\Delta G}$. Compared with $Line_{PSstandard}$, nearly doubled values of $SD_{\Delta G}$ were determined for $Line_{GSstandard}$ and $Line_{GSrapid}$ as well as a tripled value of $SD_{\Delta G}$ for $Line_{GSonly}$.

Discussion

The implementation of genomic selection (GS) in breeding programs is currently intensively discussed in the scientific literature as well as in breeding companies (cf. Zhao et al. 2014; Heslot et al. 2015). To our knowledge, this discussion is mainly driven by estimating prediction accuracies and speculating about future marker costs but not based on evaluating possible breeding schemes under given budgets and logistical constraints. Furthermore, the allocation of test resources, that is the number of lines to be genotyped and phenotyped as well as the number of locations and testers to be used, is completely disregarded. In wheat, the breeding companies currently consider shifting from the classical line towards hybrid breeding. It is therefore of high interest, whether GS could improve the efficiency of hybrid compared to line breeding. This motivated us to evaluate the efficiency of three breeding strategies for line and hybrid wheat breeding with GS regarding the expected selection gain and its standard deviation. These breeding strategies were compared with a strategy based on two-stage phenotypic selection without GS ($Hybrid_{PSstandard}$, $Line_{PSstandard}$), which is common practice in Central European wheat breeding.

The GS prediction accuracy largely influences the ranking of the breeding strategies

With increasing GS prediction accuracy, the selection gain increased linearly in all breeding strategies except those applying only phenotypic selection (Fig. 2). Furthermore, the ranking of the alternative breeding strategies changed with increasing GS prediction accuracy. This can be explained by the different weight put on GS in the alternative breeding strategies. For instance, in breeding strategies using GS followed by two-stage phenotypic selection ($Hybrid_{GSstandard}$, $Line_{GSstandard}$), the impact of the GS prediction accuracy on the total selection gain is balanced across three selection stages. In contrast, breeding strategies $Hybrid_{GSonly}$ and $Line_{GSonly}$ are based solely on one-stage GS. Thus, all weight is put on GS leading to a strong dependency of the selection gain on the GS prediction accuracy.

This reduction of three to one-stage selection largely reduced the absolute selection gain. However, the use of $Hybrid_{GSonly}$ and $Line_{GSonly}$ enables a reduction in breeding cycle length of up to 4 years (Fig. 1) which counterbalances the lower absolute selection gain to a large extent. For instance, for hybrid breeding, the highest annual selection gain was observed for breeding strategy $Hybrid_{GSonly}$ as long as the GS prediction accuracy was higher than 0.5 (Fig. 2). For lower prediction accuracies, however, the breeding strategy $Hybrid_{GSrapid}$ should be chosen as it maximized the annual selection gain across all compared strategies. Similar trends were observed for line breeding with the exception that the fast-track breeding strategy $Line_{GSonly}$ was only better than $Line_{GSrapid}$ for a GS prediction accuracy higher than 0.65 (Fig. 2). Summarizing, breeders should base their choice of the breeding strategy carefully, taking the expected GS prediction accuracies for their traits of interest in their germplasm into account.

GS increases annual selection gain in line and hybrid wheat breeding

For line breeding in wheat, the few published GS prediction accuracies for grain yield differed largely, ranging from 0.36 to 0.64 (cf. Heslot et al. 2012). These prediction accuracies were observed for estimation and prediction of lines in the same population, *i.e.,* in the same cycle of selection. However, in breeding programs, the newly developed lines must be predicted based on calibrations from previous cycles of selection which largely reduces the GS prediction accuracy (Hofheinz et al. 2012; Storlie and Charmet 2013). In hybrid wheat breeding, three prediction scenarios must be distinguished: prediction of hybrids for which no (T0), one (T1), or both parents (T2) have already been phenotyped for GCA in other hybrid combinations. Thereby, T0 reflects the situation where lines in the training and prediction set are unrelated as it is the case for prediction in a breeding program where new lines must be predicted based on calibrations developed in previous cycles of selection. For grain yield of hybrid wheat, only one study is available in the literature reporting a GS prediction accuracy for T0 of 0.28 (Zhao et al. 2013). Consequently, we assume for line and hybrid breeding a GS prediction accuracy across breeding cycles of 0.3 as realistic for wheat grain yield and focus our following considerations on this assumption. Nevertheless, intensive research is needed in wheat especially to quantify the prediction accuracy across breeding cycles for grain yield and further traits of economic importance.

For a GS prediction accuracy of 0.3, breeding strategies $Hybrid_{GSrapid}$ and $Line_{GSrapid}$ yielded the highest annual selection gain ΔG_a of all compared breeding strategies (Fig. 2; Tables 2, 3). For hybrid breeding, the use of breeding strategy $Hybrid_{GSrapid}$ increased the annual selection gain ΔG_a by more than 35 % relative to a breeding scheme based only on phenotypic selection ($Hybrid_{PSstandard}$). For line breeding, this advantage was less pronounced but still 16 %. This is surprisingly high taking the low GS prediction accuracy into account. This increase in annual selection gain ΔG_a can have two reasons: the incorporation of GS or the reduction in cycle length. To distinguish between these two confounded parameters, we additionally calculated the annual selection gain for breeding strategies which also had only one stage of phenotypic selection but without GS ($Hybrid_{PSrapid}$, $Line_{PSrapid}$, Fig. 1). For hybrid and line breeding, the breeding strategies "*PSrapid*" yielded a higher annual selection gain than the standard phenotypic two-stage selection strategies "*PSstandard*" (Tables 2, 3). However, the annual selection gain of the breeding strategies $Hybrid_{PSrapid}$ and $Line_{PSrapid}$ was still lower than the annual selection gain of the GS breeding strategies $Hybrid_{GSrapid}$ and $Line_{GSrapid}$. This illustrates that GS can increase ΔG_a and consequently, the use of GS appears interesting for line and hybrid breeding even with low prediction accuracies.

Besides a high selection gain, a low standard deviation of selection gain is desired for an optimum breeding strategy, because this standard deviation can be interpreted as a measure for the risk to realize the predicted selection gain. Compared to the breeding strategies, $Hybrid_{PSstandard}$ and $Line_{PSstandard}$, the standard deviation of selection gain $SD_{\Delta G}$ was larger for breeding strategies $Hybrid_{GSrapid}$ and $Line_{GSrapid}$ (Tables 2, 3). While this increase was only moderate for hybrid breeding, the use of breeding strategy $Line_{GSrapid}$ led to a doubled $SD_{\Delta G}$ compared to $Line_{PSstandard}$ (Table 3). Thus, the risk of selecting the wrong lines is considerably increased in line breeding using GS.

Furthermore, the optimum allocation of test resources of breeding strategy $Line_{GSrapid}$ requires the use of 10 test locations in selection stage two (L_2). To our knowledge on current DH technology in wheat, it seems unrealistic to obtain enough seeds for yield trials at 10 locations with only one DH multiplication step (Fig. 1). For instance, an average of about 20–40 kernels can be expected per generated DH line. With 1 year of seed multiplication, this cannot be increased to more than approximately 1000 g of seeds. Thus, in practice a maximum of four yield plots can be realized in the breeding strategy $Line_{GSrapid}$. Two options appear feasible to get around the impossible requirement of 10 test locations in the breeding strategy $Line_{GSrapid}$. First, a further seed multiplication can be done before yield tests which, however, prolongs the breeding strategy to the same length as $Line_{PSstandard}$ or $Line_{GSstandard}$ (Fig. 1). The latter two strategies realize both three instead of two test and selection stages, thus making this approach not appealing. Alternatively, the number of test locations in breeding strategy $Line_{GSrapid}$ can be reduced to four. This adjustment reduced the annual selection gain in the breeding strategy $Line_{GSrapid}$ from 2.41 to 2.17 (Table 3). Nevertheless, also with this large limitation, the annual selection gain of the breeding strategy $Line_{GSrapid}$ was still 5 % higher than in the standard two-stage phenotypic selection strategy $Line_{PSstandard}$.

Further drawbacks from the use of breeding schemes with a reduced number of phenotyping stages concern both line and hybrid breeding. For instance, grain yield is influenced by large genotype-by-year interactions. In addition, other traits like frost or severe disease epidemics like yellow rust in 2014 occur irregularly across years and must be taken into account. Thus, the use of multi-year phenotypic selection before expensive (pre) registration trials maximizes the chance of selecting genotypes well adapted to these events. Furthermore, additional traits with fully different genetic architecture, *i.e.*, with high heritability and cheap to phenotype, like disease resistance, plant height, or traits very expensive to phenotype like bread-making quality, are of high importance in wheat breeding. Their incorporation into the optimization might result in other optimum breeding strategies but requires further research especially on index selection.

Our results are in contrast to studies in wheat (cf. Heffner et al. 2010, Heslot et al. 2015) and maize (cf. Bernardo and Yu 2007) proposing the use of several cycles of GS before entering phenotyping. In our study, the use of $Hybrid_{GSonly}$ and $Line_{GSonly}$ was only advantageous for unrealistically high GS prediction accuracies but also largely increased the standard deviation of selection gain and can thus not be recommended. The difference to the previous studies in the literature is that to our opinion they rely partly on unrealistic breeding scenarios, did not optimize the allocation of resources and in addition, oftentimes did not assume a fixed budget. Thus, GS for several cycles can be done in the same time as phenotypic selection but consuming a by far larger budget. Summarizing, for a GS prediction accuracy around 0.3, we propose for hybrid breeding, the use of breeding strategy $Hybrid_{GSrapid}$. For line breeding, the use of breeding strategy $Line_{GSrapid}$ increased the annual selection gain by 5 % compared with breeding strategy $Line_{PSstandard}$ but at the expense of a nearly doubled standard deviation of the selection gain. Thus, the choice among breeding strategies in line breeding is not as clear as in hybrid breeding.

GS improves the efficiency of hybrid compared to line breeding in wheat

The use of breeding strategies $Hybrid_{GSrapid}$ and $Line_{GSrapid}$ increased the annual selection gain by 35 and 5 % compared to two-stage phenotypic selection for hybrid ($Hybrid_{PSstandard}$) and line breeding ($Line_{PSstandard}$), respectively (Fig. 2; Tables 2, 3). This large difference is mainly due to two reasons. First, the production of hybrid seed in wheat is very expensive compared to the production of inbred seed which largely hampers successful hybrid breeding. The use of GS in hybrid wheat breeding enables to considerably increase the number of tested DH lines. For instance, for a GS prediction accuracy of 0.3, $N_1 = 1444$ DH lines can be tested in the breeding strategy $Hybrid_{GSrapid}$ compared to only $N_2 = 680$ DH lines in the breeding strategy $Hybrid_{PSstandard}$ (Tables 2). Furthermore, for largely reduced hybrid seed production costs, the advantage in selection gain using GS was considerably reduced (data not shown) confirming the impact of the hybrid seed production costs on the efficiency difference between hybrid and line breeding using GS.

Second, the higher genetic variance available in line ($\sigma^2_{LP} = 14.06$) compared to hybrid breeding ($\sigma^2_{GCA} = 5.7$) also affected the different efficiencies of applying GS in hybrid and line breeding. For a doubled GCA variance, the advantage in selection gain using GS in hybrid breeding was more than halved (data not shown). However, unrealistically large changes of hybrid seed production costs or of the GCA variance are necessary to nullify the large superiority of the breeding strategy $Hybrid_{GSrapid}$.

The fact, that the use of GS at a prediction accuracy of 0.3 improves the relative annual selection gain more in hybrid than in line breeding also affects the efficiency of hybrid versus line breeding, which is intensively debated in the wheat community (cf. Longin et al. 2014a). Longin et al. (2014a) showed that for phenotypic selection based on the current situation regarding available budgets, hybrid seed production costs and variance components, the selection gain in hybrid breeding was around 33 % relative to that in line breeding. We determined a similar difference of line and hybrid breeding for the phenotypic breeding strategies $Hybrid_{PSstandard}$ and $Line_{PSstandard}$ (Tables 2, 3). However, using breeding strategy $Hybrid_{GSrapid}$, the relative efficiency versus line breeding using the breeding strategy $Line_{GSrapid}$ limited to four test locations was improved to 51 %. This improvement in efficiency is larger than the improvements achieved by either reducing the hybrid seed production costs to a quarter or by reducing the length of the phenotypic breeding cycle (Table 2, cf. Longin et al. 2014a) highlighting the potential of GS in hybrid wheat breeding.

Nevertheless, the advantage in annual selection gain of line compared to hybrid breeding is still large. However, an economic comparison of hybrid versus line breeding must also take the yield advantage of hybrids due to the exploitation of heterosis into account. Current estimates on heterosis for wheat grain yield are around 10 % (Longin et al. 2013). It has been shown in maize, however, that heterosis was higher under stress than under normal conditions (Duvick et al. 2004). Furthermore, hybrids are currently assumed to be more stress tolerant and in addition may possess a higher yield stability (Mühleisen et al. 2014). The effects of the predicted climate change as well as the expansion of wheat to less optimal growing areas may therefore further increase the yield advantage of hybrids compared to lines. In addition, with hybrid breeding, the stacking of major genes is facilitated and higher budgets than for line breeding are likely available due to a reduction of farm-saved seeds, thus further increasing the relative efficiency of hybrid versus line breeding (Longin et al. 2014a). Whether these increases are high enough to justify the shift from line to hybrid breeding must be decided on a case-by-case basis for each breeding company. However, if hybrid breeding is chosen, our results clearly recommend the use of genomic selection in the breeding strategy $Hybrid_{GSrapid}$.

Optimum allocation of test resources

The GS prediction accuracy largely influenced the optimum number of lines used in hybrid and line breeding. With decreasing GS prediction accuracy, the optimum number N_1 decreased in favor of an increased optimum number N_2 in hybrid (Table 2) and in line breeding (Table 3). This effect was especially apparent in the line breeding scheme. Thus, with decreasing prediction accuracy, GS gets the character of a pre-test with very low selection intensity, *i.e.*, removing only the worst lines. This phenomenon was less pronounced in hybrid breeding which might be explained by the high hybrid seed production costs requiring a strong reduction of DH lines before hybrid seed production. Interestingly, the use of GS rarely altered the optimum number of testers and locations determined for phenotypic selection (Tables 2, 3; cf. Longin et al. 2014b, 2006). Thus, it seems sufficient to adjust the number of lines across the different selection stages when including GS in the breeding logistics.

Conclusions

The GS prediction accuracy largely influenced the choice of the best breeding strategy. Based on very few experimental data in wheat, we assume a GS prediction accuracy for grain yield across breeding cycles of 0.3 as status quo. Based on this assumption, the use of a breeding strategy with one-stage genomic selection followed by one-stage phenotypic selection yielded the highest annual selection gain for hybrid and line breeding. The use of GS in hybrid wheat breeding largely improved the relative efficiency compared to line breeding and can consequently be seen as an important pillar for future hybrid wheat breeding. However, more research is necessary to investigate the influence of varying budgets and variance components on the above-discussed results.

Author contribution statement CFH Longin designed the study, CFH Longin performed data analyses, X Mi developed the software for optimizing breeding strategies, CFH Longin and T Würschum wrote the manuscript.

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

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