

Hybrid wheat: quantitative genetic parameters and consequences for the design of breeding programs

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Abstract

Key message Commercial heterosis for grain yield is present in hybrid wheat but long-term competitiveness of hybrid versus line breeding depends on the development of heterotic groups to improve hybrid prediction.

Abstract Detailed knowledge of the amount of heterosis and quantitative genetic parameters are of paramount importance to assess the potential of hybrid breeding. Our objectives were to (1) examine the extent of midparent,

better-parent and commercial heterosis in a vast population of 1,604 wheat (*Triticum aestivum* L.) hybrids and their parental elite inbred lines and (2) discuss the consequences of relevant quantitative parameters for the design of hybrid wheat breeding programs. Fifteen male lines were crossed in a factorial mating design with 120 female lines, resulting in 1,604 of the 1,800 potential single-cross hybrid combinations. The hybrids, their parents, and ten commercial wheat varieties were evaluated in multi-location field experiments for grain yield, plant height, heading time and susceptibility to frost, lodging, septoria tritici blotch, yellow rust, leaf rust, and powdery mildew at up to five locations. We observed that hybrids were superior to the mean of their parents for grain yield (10.7 %) and susceptibility to frost (−7.2 %), leaf rust (−8.4 %) and septoria tritici blotch (−9.3 %). Moreover, 69 hybrids significantly ($P < 0.05$) outyielded the best commercial inbred line variety underlining the potential of hybrid wheat breeding. The estimated quantitative genetic parameters suggest that the establishment of reciprocal recurrent selection programs is pivotal for a successful long-term hybrid wheat breeding.

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Introduction

Hybrid breeding is a remarkable success story in several allogamous crop species, but it is still not established on a large scale in autogamous cereals except in rice (Longin et al. 2012). Reasons are a low magnitude of heterosis in autogamous crops, need for high seed densities in small grain cereals coupled with the lack of a low cost hybridization system, and the lack of high yielding heterotic patterns (Edwards 2001; Singh et al. 2010). Recently, large public and commercial projects have been launched to

establish hybrid wheat (*Triticum aestivum* L.) breeding programs in Europe, Mexico, and Australia, mainly driven by the demand of an increase in grain yield per area.

The decision to start a hybrid breeding program depends on the long-term competitiveness of hybrid versus line breeding (Oettler et al. 2005; Longin et al. 2012). A stable yield surplus is required to justify the higher seed production costs of hybrids. The yield surplus is a function of the extent of heterosis and of the selection gain of hybrid versus line breeding. A recent study based on experimental data of the current market leader in hybrid wheat breeding reported 1.86 Mg ha⁻¹ yield advantage of the best hybrid compared to the highest yielding line variety (Gowda et al. 2012). This suggests that hybrid wheat breeding has the potential to be competitive with line breeding, but further data are needed to substantiate these findings.

The genetic variances, which can be exploited in line versus hybrid breeding, and the variances of genotypes-by-environments interactions are further crucial factors determining the competitiveness of hybrids (Longin et al. 2012). In particular, the magnitude and the ratio of variance due to general (GCA) and specific combining ability (SCA) are relevant to estimate the expected selection gain in hybrid breeding. Moreover, detailed knowledge of the correlation between line per se and hybrid performance, and between GCA effects and line per se performance is required to optimize hybrid breeding schemes (Longin et al. 2007; Gordillo and Geiger 2008). For wheat, only very limited information is available on these important quantitative genetic parameters in the literature (Borghi and Perenzin 1994; Perenzin et al. 1998; Gowda et al. 2012).

In our current treatise, we evaluated a vast population comprising 1,604 wheat hybrids and their 135 parental European elite wheat inbred lines in intensive field trials. Our objectives were to (1) study the extent of midparent, better-parent and commercial heterosis and (2) discuss the consequences of relevant quantitative parameters for the design of hybrid wheat breeding programs.

Materials and methods

Plant material and field experiments

Four wheat breeding companies provided 68 male inbred lines with known good pollination characters and 275 female lines. We used in preliminary work 24 SSR markers and selected 15 males and 120 females of the 343 elite lines based on genetic distances among the lines eliminating close relatives and maximizing the allelic diversity (>77 % of alleles maintained after selection, data not shown). For detailed molecular characterization of the parental lines, we refer to Würschum et al. (2013) and Zhao et al. (2013). The 120 female lines were crossed with the 15 male lines using a factorial mating design and 1,604 of the 1,800 potential single-cross hybrid combinations could be produced with sufficient amount of seeds. Females were emasculated using chemical hybridizing agents. The sterility was checked bagging 1–3 female plants. Sterility was high with mean values above 95 %. To avoid pollen contamination from other male parents, the separate crossing blocks, each containing one male and several female parental lines, were isolated by at least 16 m distance from adjacent crossing blocks. The 1,604 hybrids, their 120 female and 15 male parental lines, as well as 10 commercial varieties as checks, 8 inbred line cultivars (quality class E: *Genius*, quality class A: *JB Asano*, *Julius*, *Tuerkis*; quality class B: *Colonia*, *Kredo*, *Tobak*; quality class C: *Tabasco*) and 2 hybrids (*As de Coeur* and *Hystar*), were evaluated in multiple environments for 9 agronomic and disease resistance traits in Germany in the year 2012 (Table 1). The environments were Adenstedt, Bredstedt, Böhnshausen, Hohenheim, Hadmersleben, Harzhof, Rosenthal, and Seligenstadt.

All genotypes were evaluated in yield trials and also in observation plots. In the yield trials, all hybrids and lines were split into three adjacent trials linked with 10 common checks. The experimental designs were partially replicated alpha designs (Williams et al. 2010), where all parents, checks as well as 29 % of the hybrids were used in both

Table 1 Agronomic and disease resistance traits and number of locations assessed in our study

Traits	Abbreviation	Unit of measurement	No. of locations
Grain yield	GY	Mg ha ⁻¹	5
Heading time	HT	Days in the year	4
Plant height	PH	cm	4
Frost susceptibility	FS	1 = no, 9 = highly susceptible	3
Lodging susceptibility	LO	1 = no, 9 = highly susceptible	3
Yellow rust susceptibility	YR	1 = no, 9 = highly susceptible	4
Leaf rust susceptibility	LR	1 = no, 9 = highly susceptible	4
Powdery mildew susceptibility	PM	1 = no, 9 = highly susceptible	3
Septoria tritici blotch susceptibility	STB	1 = no, 9 = highly susceptible	2

replications. Sowing density ranged from 230 to 290 seeds m^{-2} and plot size ranged from 5 to 7.4 m^2 . The yield trials were treated with fertilizers, fungicides and herbicides according to farmers practice for intensive wheat production. Data were recorded for natural occurring frost and lodging susceptibility on a ordinal scale of 1–9, where 1 refers to low susceptibility and 9 to high susceptibility, for plant height in cm between soil surface and tip of the ear, for heading time in days between the beginning of the year and heading, and for grain yield in $Mg\ ha^{-1}$ with a moisture content of 140 $g\ H_2O\ kg^{-1}$.

In the observation plots, all genotypes were tested in an alpha design without replications using micro-plots of 0.5–1.5 m^2 . The observation plots were treated with fertilizers and herbicides like the yield trials, but fungicides were not applied. Disease susceptibility was recorded for yellow rust, leaf rust, and septoria tritici blotch based on artificial inoculation with a mixture of different races and for powdery mildew based on natural infection. All diseases were scored in an ordinal scale of 1–9, where 1 refers to healthy plants and 9 to fully infected plants (Table 1).

Phenotypic data analyses

Residuals for all traits in each environment were normally distributed except for yellow rust, where data were skewed more toward resistance. Therefore, yellow rust data were transformed by applying $\arcsin(\sqrt{x/100})$. In the yield trials, the phenotypic data of each environment were first analyzed separately based on the statistical model

$$y_{ijklm} = \mu + g_{ij} + t_k + r_{lk} + b_{mlk} + e_{ijklm},$$

where y_{ijklm} is the phenotypic performance for the ij th genotype (parental line $i = j$, or hybrid $i \neq j$) in the m th incomplete block of the l th replication in the k th trial, μ an intercept term, g_{ij} the genetic effect of the ij th genotype, t_k the effect of the k th trial, r_{lk} the effect of the l th replication in the k th trial, b_{mlk} the effect of the m th incomplete block in the l th replication of the k th trial, and e_{ijklm} is the residual. Except b_{mlk} , all effects were treated as fixed.

The adjusted means of each genotype and one divided by the squared standard error as weighting factor were used to estimate the genetic variance components across environments (Möhring and Piepho 2009) with the following model:

$$y_{ijn} = \mu + a + l_n + p_{ij} + g'_i + g''_j + s_{ij} + (pl)_{ijn} + (g'l)_{in} + (g''l)_{jn} + (sl)_{ijn} + e_{ijn},$$

where y_{ijn} is the phenotypic performance of the ij th entry (line $i = j$, or hybrid $i \neq j$) at the n th environment, μ an intercept term, a the group effect for lines, hybrids and checks, l_n the effect of the n th environment, p_{ij} the genetic effect of the parental lines, g'_i the GCA effect of the i th

female line, g''_j the GCA effect of the j th male line, s_{ij} the SCA effect of crosses between lines i and j , $(pl)_{ijn}$ the interaction effect of the ij th parental line with the n th environment, $(g'l)_{in}$ and $(g''l)_{jn}$, are GCA \times environment effects of female and male lines, $(sl)_{ijn}$ the SCA \times environment interaction effect, and e_{ijn} is the residual. Except group effects, all other effects were treated as random.

For the observation plots, statistical analysis was performed assuming that each environment corresponds to one replication (Payne 2006). Thus, the phenotypic data were analyzed with following linear model

$$y_{ijnm} = \mu + a + l_n + p_{ij} + g'_i + g''_j + s_{ij} + (g'l)_{in} + (g''l)_{jn} + b_{mn} + e_{ijnm},$$

where y_{ijnm} is the phenotypic performance of the ij th entry (line $i = j$, or hybrid $i \neq j$) in the m th incomplete block at the n th environment, μ an intercept term, a the group effect for lines, hybrids and checks, l_n the effect of the n th environment, p_{ij} the genetic effect of the parental lines, g'_i the GCA effect of the i th female line, g''_j the GCA effect of the j th male line, s_{ij} the SCA effect of crosses between lines i and j , $(g'l)_{in}$ and $(g''l)_{jn}$, are GCA \times environment interaction effects of female and male lines, respectively, and e_{ijnm} is the residual confounded with SCA \times environment interaction effects. Except group effects, all other effects were treated as random.

Significance of the variance components was tested by model comparison with likelihood ratio tests, where the halved P values were used as approximation (Stram and Lee 1994). In addition, we assumed fixed genetic effects and estimated the best linear unbiased estimates (BLUEs) of the 1,749 genotypes. Pair-wise Pearson's correlation coefficients (r) were calculated among the traits for parental lines and F_1 hybrids, and their significance was tested using tabulated values based on Fisher z transformation (Fisher 1921). Broad-sense heritability on an entry-mean basis was calculated as the ratio of genotypic to phenotypic variance, $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{G \times E}^2 / l + \sigma_e^2 / lr)$, where σ_G^2 and $\sigma_{G \times E}^2$ refer to the total genotypic variance of hybrids (or lines) and their interaction with environments, respectively, l to the number of environments, r to the average number of replications and σ_e^2 to residual variance. For the observation plots, $\sigma_{G \times E}^2$ is confounded with σ_e^2 .

For each combination of parental lines, midparent value (MP), relative midparent heterosis (MPH), relative betterparent heterosis (BPH) and commercial heterosis (CH) were calculated using hybrid performance (HYB) as follows: $MP = (P1 + P2)/2$, $MPH = [(HYB - MP)/MP] \times 100$, $BPH = [(HYB - P_{max})/P_{max}] \times 100$, and $CH = [(HYB - \text{best check})/\text{best check}] \times 100$, where P_{max} is the better performing parent. We tested the Pearson's

correlation of HYB with MP, $r(\text{MP}, \text{HYB})$, and the GCA effects with per se performance of parents, $r(\text{GCA}, \text{per se})$. Further, we also tested the Pearson's correlation of HYB with the sum of GCA effects of both the parents $r(\text{GCA}, \text{HYB})$ using a leave-one-out crossvalidation as explained in detail by Schrag et al. (2009). All analyses were performed using the software ASReml-R 3.0 (Butler et al. 2009).

To combine the single traits in a base index (Baker 1986), we transformed the data by $y_{ij} = (x_{ij} - x_k)/s_j$, where x_{ij} is the i th genotypic value for the j th trait, x_j and s_j the mean, and standard deviation of the j th trait. The sign of the weight of a single trait in the index depended on the aim to increase or decrease the trait.

Results

Genotypic variances σ_G^2 were significantly ($P < 0.05$) larger than zero for parents and hybrids for all nine traits (Table 2). Parents and hybrids displayed a similar amount of σ_G^2 for susceptibility to lodging, leaf rust, yellow rust and powdery mildew. In contrast, we observed larger σ_G^2 for the parents compared to the hybrids for grain yield, heading time, plant height, and susceptibility to frost as well as septoria tritici blotch. For grain yield, $\sigma_{G \times E}^2$ was larger than σ_G^2 , and vice versa for the other traits for both lines and hybrids. Estimates of σ_{GCA}^2 were significantly ($P < 0.01$) larger than zero for all traits, except for lodging susceptibility of the females. For all traits, σ_{GCA}^2 was larger for female than that for male lines. Estimates of σ_{SCA}^2 were significantly ($P < 0.01$) larger than zero for all traits, except for septoria tritici blotch susceptibility. Relative to σ_G^2 of the hybrids, the amount of σ_{SCA}^2 was close to zero for plant height, heading time, and susceptibility to frost, leaf rust, powdery mildew, and septoria tritici blotch. In contrast, for grain yield and yellow rust susceptibility, we observed that the proportion of σ_{SCA}^2 of the total genotypic variance σ_G^2 amounted to 33 and 28 %, respectively. Heritabilities were moderate to high, showed only small discrepancies for parents and hybrids, and ranged from 0.33 for lodging susceptibility to 0.89 for frost susceptibility.

In contrast to grain yield, negative values for heterosis are preferable for the remaining traits as the goal was to develop early sturdy hybrid varieties with low susceptibility against abiotic and biotic stresses. Thus, lower heading time, plant height and susceptibility to frost, lodging and disease are desired (Table 1). Compared to the midparent performance, hybrids had on average a higher grain yield (10.7 %), plant height (9.16 %) and lodging susceptibility (21.7 %), but lower susceptibility to frost (−7.2 %), leaf rust (−8.42 %), and septoria tritici blotch (−9.27 %; Table 2). Midparent

heterosis for grain yield ranged from −7.2 to 28.6 %. Compared to the better-parent, hybrids had on average a 7.66 % higher grain yield, were taller, later heading time, and less susceptibility to frost and all diseases scored.

Midparent performance was significantly ($P < 0.01$) correlated with hybrid performance for all traits (Table 2). The lowest correlation was observed for lodging susceptibility with a value of $r = 0.28$ ($P < 0.01$) and the largest value for frost susceptibility of $r = 0.85$ ($P < 0.01$). The correlation between GCA predicted and observed hybrid performance was generally higher than the correlation between midparent and hybrid performance. Correlation between GCA effects and line per se performance was moderate to high ranging from $r = 0.49$ ($P < 0.01$) for lodging susceptibility to $r = 0.86$ ($P < 0.01$) for frost susceptibility.

The pattern of phenotypic trait correlations was similar for parents and hybrids (Table 3). We observed a significant ($P < 0.01$) and moderate correlation between grain yield and plant height with values of $r = 0.43$ for the parents and $r = 0.26$ for the hybrids. A significant ($P < 0.01$) and moderate correlation was further detected between plant height and frost susceptibility as well as between plant height and septoria tritici blotch susceptibility.

Distribution of phenotypic values of the hybrids for each trait approximated a normal distribution (Fig. 1). The commercial line cultivar with the highest grain yield in our study was *Tobak* with an average of 11.30 Mg ha^{−1}. Sixty-nine hybrids had a significantly ($P < 0.05$) higher grain yield than *Tobak* and the best hybrid had an average grain yield of 12.79 Mg ha^{−1}. Positive commercial heterosis was further identified for all other traits. Ranking of the commercial checks and hybrids, however, changed substantially depending on the trait of interest. For grain yield, 11 further hybrids were not significantly ($P < 0.05$) different than the best hybrid (Fig. 2). Nearly all of these 12 hybrids had a female parent with very high GCA effects, while only 7 of the 12 hybrids had a male parent with high GCA value. Interestingly, the 12 hybrids comprised combinations among but also within particular breeding programs (data not shown).

Discussion

The decision to embark on a hybrid breeding program strongly depends on the long-term competitiveness of hybrid versus line breeding. Detailed knowledge of the amount of heterosis and the ratio of genetic variance components are of paramount importance to study the long-term selection gain of hybrid wheat breeding. Only a few experimental surveys investigated quantitative genetic parameters, which are relevant to gain insights into the

Table 2 Estimates of variance components, heritabilities, mean and range of midparent (MPH) and better-parent heterosis (BPH), as well as correlations between midparent values and hybrid performance $r(\text{MP, HYB})$, general combining ability effects and hybrid performance $r(\text{GCA, HYB})$, as well as between general combining ability effects and line per se performance $r(\text{GCA, per se})$

Source	GY (Mg ha ⁻¹)	HT (days)	PH (cm)	FS (1-9)	LO (1-9)	YR (1-9)	LR (1-9)	PM (1-9)	STB (1-9)
Parents									
σ_G^2	0.136***	3.58***	22.54*	3.14***	0.15**	0.007***	1.40***	0.52***	0.83**
$\sigma_{G \times L}^2$	0.249***	1.26***	6.58**	0.73***	0.13**	-	-	-	-
h^2	0.64	0.87	0.85	0.89	0.33	0.76	0.85	0.75	0.69
F₁ hybrids									
σ_G^2	0.085***	2.04***	17.79**	1.66***	0.13**	0.005***	1.36***	0.57***	0.41**
$\sigma_{GCA-Females}^2$	0.033***	1.74***	15.14**	1.51***	0.20**	0.002***	1.05***	0.48***	0.23**
$\sigma_{GCA-Males}^2$	0.025***	0.31***	2.46**	0.26***	0.02 ^{ns}	0.001***	0.07***	0.06***	0.17**
σ_{SCA}^2	0.028***	0.05***	1.16**	0.08***	0.04**	0.001***	0.08***	0.04***	0.02
$\sigma_{G \times E}^2$	0.125***	0.58***	5.39**	0.38***	0.08**	-	-	-	-
$\sigma_{GCA-F \times E}^2$	0.054***	0.39***	2.69**	0.36***	0.20**	0.002***	0.59***	0.08***	0.22**
$\sigma_{GCA-M \times E}^2$	0.033***	0.11***	3.44**	0.07***	0.17**	0.001***	0.02***	0.01***	0.03**
$\sigma_{SCA \times E}^2$	0.045***	0.13***	1.13**	0.04***	0.01 ^{ns}	-	-	-	-
σ_e^2 [†]	0.260	1.13	12.71	0.52	1.06	0.009	0.99	0.51	0.74
h^2	0.56	0.85	0.82	0.87	0.40	0.59	0.75	0.77	0.52
$\sigma_{SCA}^2 / (\sigma_{GCA-M}^2 + \sigma_{GCA-F}^2 + \sigma_{SCA}^2)$	0.33	0.02	0.06	0.04	0.14	0.28	0.07	0.07	0.04
MPH (%)	10.70 (-7.2 to 28.6)	-0.74 (-2.6 to 1.3)	9.16 (-10.2 to 23.8)	-7.2 (-51.9 to 120.5)	21.70 (-93.2 to 314.8)	0.35 (-68.0 to 122.3)	-8.42 (-93.5 to 102.1)	2.52 (-63.1 to 102.1)	-9.27 (-72.5 to 76.7)
BPH (%)	7.66 (-15.7 to 25.7)	0.21 (-1.9 to 2.9)	19.10 (-2.3 to 38.0)	30.23 (-39.1 to 262.2)	47.41 (-91.2 to 395.2)	40.07 (-45.9 to 290.4)	25.69 (-91.7 to 285.9)	24.06 (-55.4 to 162.0)	6.12 (-68.6 to 165.0)
$r(\text{MP, HYB})$	0.49**	0.84**	0.72**	0.85**	0.28**	0.60**	0.70**	0.68**	0.51**
$r(\text{GCA, HYB})^a$	0.60**	0.92**	0.90**	0.93**	0.58**	0.68**	0.87**	0.84**	0.71**
$r(\text{GCA, per se})$	0.72**	0.85**	0.65**	0.86**	0.49**	0.73**	0.79**	0.76**	0.70**

For abbreviations, see Table 1

ns not significantly different from zero at the 0.05 level of probability

*, **, *** Significantly different from zero at the 0.05, 0.01, and 0.001 level of probability, respectively

[†] for disease traits, $\sigma_{G \times E}^2$ is confounded with σ_e^2

^a Determined by leave-one-out cross validation

Table 3 Phenotypic correlation coefficients among 9 traits determined for 135 parental lines (below diagonal) and their 1,604 hybrids (above diagonal)

Lines/hybrids	GY	HT	PH	FS	LO	YR	LR	PM	STB
GY		0.16**	0.26**	-0.06*	-0.01	0.07**	0.03	-0.05*	-0.02
HT	0.38**		0.25**	-0.01	-0.01	0.08**	-0.20**	-0.22**	-0.03
PH	0.43**	0.39**		-0.29**	0.42**	0.03	0.16**	0.01	-0.40**
FS	-0.23**	-0.14	-0.50**		-0.13**	0.07**	-0.09**	0.01	0.27**
LO	-0.01	0.03	0.30**	-0.10		-0.03	0.12**	-0.01	-0.27**
YR	-0.02	0.08	-0.01	0.11	-0.04		-0.21**	-0.09**	0.13**
LR	0.05	-0.01	0.22**	-0.17*	-0.04	-0.17*		0.15**	-0.06*
PM	-0.04	-0.21*	0.16	-0.02	0.15	-0.08	0.10		0.04
STB	-0.11	-0.23**	-0.36**	0.33**	-0.25**	0.08	-0.01	0.07	

For abbreviations of the traits, see Table 1

*, ** Significantly different from zero at the 0.05, and 0.01 level of probability, respectively

potential of hybrid wheat breeding. Previous reports have the major shortcomings of either the use of unbalanced and often small data sets or the consideration of a limited set of traits (for review, see Longin et al. 2012). This stimulated us to initiate a joint public–private partnership to study a large population of wheat hybrids and their parental inbred lines.

Heterosis and hybrid performance

We observed an average midparent heterosis for grain yield of 10.7 % (Table 2), which is in the same range as previous findings in wheat (Barbosa-Neto et al. 1996; Perenzin et al. 1998; Oury et al. 2000). Average better-parent heterosis for grain yield was slightly smaller than midparent heterosis, but maximum values amounted up to 23.8 %. This value for better-parent heterosis is higher than any one previously reported in the literature (Borghì and Perenzin 1994; Barbosa-Neto et al. 1996; Perenzin et al. 1998; Corbellini et al. 2002) and might reflect the first success in the search for excellent male parental lines in European hybrid breeding programs.

Schachschneider (1997) as well as Weißmann and Weißmann (2002) estimated the yield surpluses needed to counterbalance the higher seed costs of hybrid versus line varieties. Both studies regarded a commercial heterosis of about 1 Mg ha⁻¹ as sufficient to justify higher seed production costs. Eleven hybrids had a commercial heterosis for grain yield exceeding this threshold of 1 Mg ha⁻¹ with a maximum grain yield advantage of 1.3 Mg ha⁻¹ (Fig. 1). Consequently, our result suggests that a competitive hybrid breeding program seems feasible in wheat.

For heading time, heterosis was close to zero (Table 2), which is also in accordance with the literature (Borghì and Perenzin 1994; Perenzin et al. 1998; Corbellini et al. 2002). For plant height, we observed a substantial amount of

heterosis, which was considerably higher than reported in the literature (Borghì and Perenzin 1994; Perenzin et al. 1998; Corbellini et al. 2002). Plant height is often associated with early vigor (Spielmeyer et al. 2007). Increased vigor is desired, especially in the first growing phase, because the plant rapidly covers the soil, thus preventing the growth of herbs and exploiting the photosynthetic potential per area in an efficient manner. During later stages of plant development, this can cause lodging susceptibility as reflected by the moderate but positive correlation between plant height and lodging susceptibility (Table 3). This correlation, however, does not prevent to select hybrids combining lodging tolerance and high grain yield. For instance, of the 20 hybrids with the highest grain yield, only 4 hybrids showed an increased lodging susceptibility (>3, data not shown).

Predicting hybrid performance

In hybrid breeding, a high number of parental lines are available and, therefore, not all potential factorial crosses can be evaluated in field trials. Thus, efficient methods are required to predict the hybrid performance. In allogamous species, new lines were usually selected as hybrid parents based on their line per se performance or their GCA effects (Hallauer et al. 1988). The efficiency of this selection strongly depends on the correlation between hybrid performance and midparent value $r(\text{MP}, \text{HYB})$ or the sum of GCA effects of both parents $r(\text{GCA}, \text{HYB})$. Further aspects on the implementation of estimation of GCA effects in top cross tests are discussed in detail elsewhere (Gowda et al. 2012). For plant height, heading time and susceptibility to frost and leaf rust, we observed a correlation between midparent and hybrid performance of $r > 0.70$ (Table 2). Thus, for these traits, line per se performance serves as an accurate predictor of the hybrid performance.

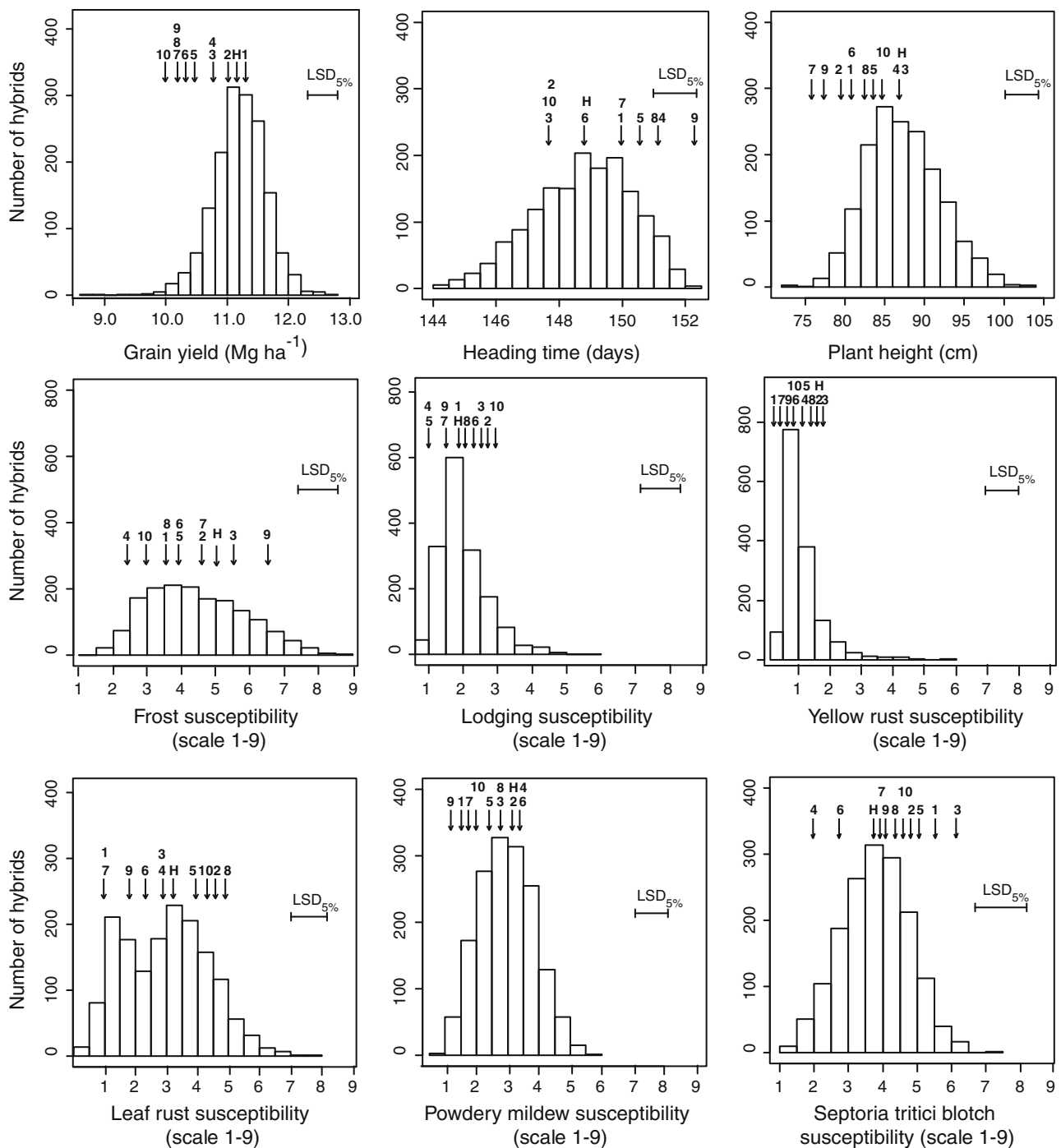


Fig. 1 Distribution of the phenotypic values of 1,604 hybrids compared to the 10 commercial checks for 9 traits (line checks: 1 = Tobak, 3 = JB Asano, 4 = Julius, 6 = Colonia, 7 = Kredo,

8 = Tuerkis, 9 = Tabasco, 10 = Genius; hybrid checks: 2 = As de Coeur, 5 = Hystar; H = hybrid mean)

For grain yield, we observed a correlation between midparent and hybrid performance of $r = 0.49$ ($P < 0.01$), while the correlation increased to $r = 0.60$ ($P < 0.01$) for GCA effects instead of line per se performance (Table 2; Supplementary Figure S2). These findings were in accordance with results reported for triticale (Oettler et al. 2005).

One explanation for this moderate correlation is the high extent of σ_{SCA}^2 compared to σ_{GCA}^2 observed for grain yield (Table 2), which corroborates previous findings in wheat (Gowda et al. 2012). The $\sigma_{GCA}^2/\sigma_{SCA}^2$ ratio varies depending on the allele frequencies between parental populations (Reif et al. 2007). Increasing genetic divergence of the two

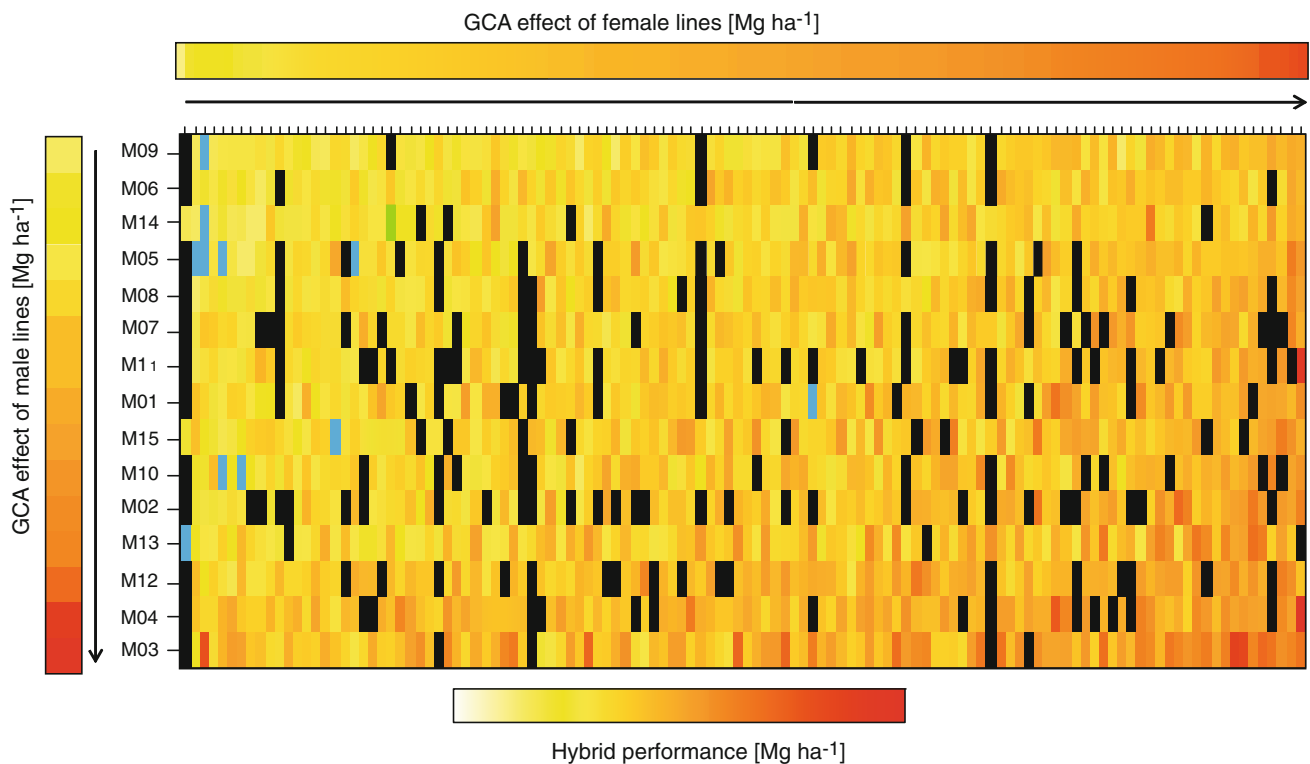


Fig. 2 Heat map of the grain yield performance of the 1,604 hybrids evaluated in our study. The *columns* and *rows* of the 15 male lines and the 120 female lines were ordered according to their GCA values for grain yield. For each male times female combination, hybrid values

were shown in *colored boxes* (yellow = good, red = poor; *black boxes* = missing hybrids, *blue boxes* = hybrids not significantly different from the best hybrid, which is marked in *green*) (color figure online)

parental populations tends to promote a high ratio of σ_{GCA}^2 versus σ_{SCA}^2 (Fischer et al. 2008). Consequently, the low $\sigma_{GCA}^2/\sigma_{SCA}^2$ ratio observed in wheat may be due to the lack of genetically diverse groups (Miedaner et al. 2010; Würschum et al. 2013). Applying inter-population improvement programs in wheat, keeping the male and female pool strictly separated, possesses a great potential to generate a long-term genetic divergence among heterotic pools and promotes, therefore, also a more favorable $\sigma_{GCA}^2/\sigma_{SCA}^2$ ratio (Labate et al. 1997).

The relevance of σ_{SCA}^2 makes it favorable to use a high number of tester lines or to use complex testers, like double crosses or line mixtures, as recommended for maize and rye hybrid breeding (Hallauer and Miranda 1981; Tomerius 2001; Longin et al. 2007). This advantage is overestimated due to the unrealistic assumption that tester lines would be unrelated in elite breeding. As the relatedness of additional tester lines increases, there is less reduction of the masking SCA effects (Appendix, Supplementary Figure S1). For instance, the efficiency in reducing SCA is reduced by >50 % for a coefficient of coancestry between tester T1 and T2 of 0.25, e.g., half sibs, compared to non-related testers. Furthermore, for wheat, practical concerns for the use of complex testers arise from the current hybrid seed

production system. Due to pollination problems, typically female lines cannot be used as pollinators like in maize. As commercial seed production is only feasible by chemical hybridization agents, homogenous flowering time and a smaller plant height than males is required for females complicating the use of complex testers. Nevertheless, the use of single-cross male tester for first GCA screenings of females is still an interesting option.

Additive genetic variance in populations of lines versus hybrids

If we assume absence of epistasis, σ_G^2 among inbred lines and σ_{GCA}^2 estimated for the hybrids reflect the additive genetic variation, which can be exploited in line or hybrid breeding in a recurrent manner. For grain yield, the magnitude of the σ_{GCA}^2 reached only 43 % of the genetic variance observed among the parental lines (Table 2). This is astonishing considering that the substantial magnitude of σ_{SCA}^2 observed for grain yield points toward the presence of significant dominance effects. Consequently, a ratio of σ_{GCA}^2 versus σ_G^2 of the lines higher than 0.5 is expected (Longin et al. 2012). A likely explanation for this low ratio is the presence of epistasis. Focusing exclusively on the

epistatic variance due to additive \times additive interactions (σ_{AA}^2) and an inbreeding coefficient of one for the inbred lines, the expected genetic variance among inbred lines in line breeding is $\sigma_G^2 = 2\sigma_A^2 + 4\sigma_{AA}^2$. In contrast, expected genetic variance among inbred lines in hybrid breeding is $\sigma_{GCA}^2 = 0.5\sigma_A^2 + 0.25\sigma_{AA}^2$ and $\sigma_{SCA}^2 = \sigma_D^2 + 0.5\sigma_{AA}^2$ (Wricke and Weber 1986; Reif et al. 2007). Thus, additive \times additive epistasis is a potential explanation for the low variance due to GCA effects compared to the variance among lines for grain yield. Consequently, further experimental studies are required with special field designs to gain more insights into the relative importance of variance components due to additive, dominance, and epistatic effects facilitating the comparison of the long-term efficiency of line versus hybrid breeding. Moreover, detailed knowledge of the relative importance of the different genetic effects is of great value to judge the potential to increase the prediction accuracy of hybrid performance including besides additive also dominance and epistatic effects (Zhao et al. 2013).

Multi-stage selection for hybrid wheat breeding

Breeders are often concerned that intensive selection in early generations for traits with high heritability, such as disease resistances, might reduce largely the genetic variability for grain yield. This fear is even more pronounced in hybrid wheat breeding, where parental lines are intensively pre-selected for high per se performance showing only moderate correlations with the hybrid performance, e.g., $r = 0.49$ for grain yield. We investigated the potential elimination of high yielding hybrids through early stage selection using an index combining line per se performance of grain yield, plant height, heading time, and susceptibility to frost, lodging, leaf rust, yellow rust, powdery mildew and septoria tritici blotch with equal weights (Supplementary Figure S3, open circles). In contrast to our concerns, no correlation between the index values and GCA for grain yield was determined.

Instead of using an index of all traits, a line might be already eliminated in early generations if it does not surpass a given threshold in one trait regardless of its performance in the other traits, i.e., selection of independent culling levels (Baker 1986). Thus, we eliminated all lines with a frost susceptibility >6.5 , disease susceptibility >5 and the 30 % poorest lines regarding grain yield per se performance (Supplementary Figure S3, filled circles). The resulting selected lines still cover the full range of GCA values for grain yield. Consequently, early selection of line per se performance does not result in a drastic reduction of σ_{GCA}^2 for grain yield in later stages of selection.

Choice of parents in hybrid wheat breeding programs

Selection of parents for the next breeding cycle represents one of the most crucial steps for maximizing selection gain (Wegenast et al. 2008). One relevant criterion for parental selection is the breeding value of a genotype (Bernardo 2002). Breeding values depend on the allele frequencies of the population, the inbreeding coefficient, as well as on the additive and dominance effects (Falconer and Mackay 1996). According to a companion study, differences in allele frequencies were not pronounced between the 120 female and the 15 male parental lines (Zhao et al. 2013). However, the presence of dominance effects may cause substantial differences in breeding values warranting further research.

Assuming absence of epistasis, either per se performance of a line or its GCA effect can be considered as an estimate of the breeding value. For grain yield, the correlation among GCA effects and line per se performance amounted to $r = 0.72$ ($P < 0.01$). This suggests that the decision for parental selection is similar for line and hybrid breeding when focusing exclusively on grain yield. Consequently, hybrid breeding can be conducted successfully as a spin-off from line breeding. Nevertheless, we speculate that with further development of heterotic groups, breeding values will diverge with ongoing reciprocal recurrent selection. In combination with the option to perform disease gene management in a synergistic manner between male and female parent pools, choice of parents will differ between line and hybrid breeding requiring a separate breeding program for hybrids.

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

Appendix

Assume two unrelated base populations π_1 (females) and π_2 (males) with two alleles, no epistasis, no linkage and equilibrium within and among loci in the base populations. For hybrid breeding, the total genetic variance is then defined after Schnell (1965, 1982) as $\sigma_G^2(\text{hybrid}) = \phi' \sigma_{GCA'}^2 + \phi'' \sigma_{GCA''}^2 + \phi' \phi'' \sigma_{SCA}^2$, where $\phi' = 1/2(1 + F_{\pi_1})$ and $\phi'' = 1/2(1 + F_{\pi_2})$ refer to the probability that test-cross lines received alleles identical by descent from π_1 and π_2 , and F is the inbreeding coefficient of the respective population. Regarding the reciprocal recurrent selection of GCA, each heterotic group is tested with few elite testers

from the opposite pool, e.g., numerous female parental lines with few male lines. Thus, for ϕ'' , we need to consider the number of tester lines. Imagine the homozygous lines J and K, which will be combined in a single-cross tester. Consequently, $\phi'' = 1/2 + 1/2f_{jk}$, where f_{jk} refers to the coefficient of coancestry among lines J and K. For three lines J, K, and L assuming theoretically an equal contribution of gametes to the tester, we get $\phi'' = 1/3 + 2/9(f_{jk} + f_{jl} + f_{lk})$ and for arbitrary numbers of n lines, $\phi'' = \frac{1}{n} + \frac{2}{n^2} \sum_{j=1}^{n-1} \sum_{k>1}^n f_{jk}$. The value n represents the product of the number of testers multiplied by the number of tester lines (gametes) used to build up each tester, e.g., $n = 4$ for either using four inbred testers or two single-cross testers. The efficiency of type and number of tester lines with different relatedness [$f_{jk} = 0$ (○), 0.25 (Δ) and 0.5 (×)] on the reduction of SCA is then determined relative to the use of one inbred tester as $\text{Eff} = 100 - [100 \times (\phi' \phi'' \sigma_{\text{SCA}}^2 + \phi' \phi'' \sigma_{\text{SCA} \times \text{E}}^2) / (\phi' \phi'' \sigma_{\text{SCA}}^2 + \phi' \phi'' \sigma_{\text{SCA} \times \text{E}}^2)] \cdot (\phi' \sigma_{\text{SCA}}^2 + \phi' \sigma_{\text{SCA} \times \text{E}}^2) = 100 - 100 \times \phi''$.

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