

Genetic Correlations among Haploids, Doubled Haploids, and Testcrosses in Maize

Hartwig H. Geiger,[★] G. Andrés Gordillo, and Silvia Koch

ABSTRACT

Doubled haploid (DH) lines produced via *in vivo* haploid induction have become an indispensable tool in maize (*Zea mays* L.) breeding and research. To determine the predictive value of the *per se* performance of haploid and DH lines in population and hybrid breeding, genetically balanced sets of haploid and DH lines along with testcrosses of the DH lines were evaluated in field trials across four locations over 2 yr in Germany. Suitable material sets were provided by three collaborating breeders. Each set comprised 54 to 58 DH lines developed from a proprietary elite dent single cross. These DH lines were crossed with one or two flint testers and subjected to haploid induction for production of corresponding haploid versions. Haploid lines, DH lines, and testcrosses were grown in separate but adjacent blocks. Haploid lines were surrounded by a mixture of inbred lines functioning as pollen source. Most haploid plants were male sterile but showed a certain degree of female fertility. Highly significant genetic variation reflected by high heritability coefficients existed in all material sets for all traits. Genetic correlations between haploid and DH lines were moderate to strong ($0.5 < \text{genetic correlation coefficient } [r_g] < 0.9$) for early vigor, silking date, plant height, and stover weight per plant. Correlations between DH lines and testcrosses varied from nonsignificant to moderately strong for grain yield but were strong for silking date, plant height, stover yield, and grain moisture content. Silking date, early vigor, and plant height of haploid lines were moderately but significantly associated with grain yield of testcrosses. Somewhat higher estimates were obtained for the corresponding correlations between DH lines and testcrosses. In conclusion, selection for silking date, early vigor, plant height, and stover weight at the haploid level is expected to result in positive correlated genetic gain for various traits not only at the DH but also at the testcross level. Likewise, selection at the DH level may substantially speed up progress in combining ability.

Univ. of Hohenheim, Institute of Plant Breeding, Seed Science, and Population Genetics, 70593 Stuttgart, Germany. G.A. Gordillo, KWS-Lochow GmbH, 29303 Bergen, Germany. Received 12 Mar. 2013.
[★]Corresponding author (geigerhh@uni-hohenheim.de).

Abbreviations: DH, doubled haploid; HRS, haploid recurrent selection; KWS, KWS Saat AG, Einbeck, Germany; MON, Monsanto Agrar Deutschland GmbH, Borken, Germany; SWS, Südwestsaat GbR, Rastatt, Germany.

IN VIVO induced haploids and doubled haploids are routinely used in modern maize breeding for production of homozygous parent lines (for reviews see Geiger, 2009; Chang and Coe, 2009; Geiger and Gordillo, 2009). Standard hybrid breeding schemes include a first stage of selection among the doubled haploid (DH) lines *per se* followed by one or several stages of testcross selection (Seitz, 2005; Röber et al., 2005; Longin et al., 2006; Gordillo and Geiger, 2008). Chromosome doubling procedures are generally performed in the coleoptile or early seedling stage using colchicine or equivalent substances (Prigge and Melchinger, 2012; Prasanna et al., 2012). However, this upregulates only part of the meristematic cells so that treated haploid plants generally consist of haploid tissue with a variable number of DH segments. Moreover, the stressful chromosome doubling treatment and transplanting the treated plants into the field are largely masking the heritable variation among those plants. Therefore, treated haploid plants are not suited for selection. In contrast, first studies with untreated haploid plants indicated that various morphological and developmental characteristics of those plants may successfully be used as indirect selection criteria for improving open-pollinated diploid populations (Rotarencu et al., 2007, 2012).

Published in Crop Sci. 53:2313–2320 (2013).

doi: 10.2135/cropsci2013.03.0163

© Crop Science Society of America | 5585 Guilford Rd., Madison, WI 53711 USA

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher.

Obviously, selection among haploid plants or DH lines can only contribute to progress in combining ability if strong enough genetic correlations exist between these selection units per se and their testcross progenies (Bernardo, 2002; Hallauer et al., 2010). Quite a number of studies indicate that correlations between selfed lines and their testcrosses may be rather tight for morphological and developmental characteristics but only moderate or loose for reproductive traits, in particular for grain yield (Seitz, 1989; Mihaljevic et al., 2005; Grieder et al., 2012). However, to our knowledge, no estimates of correlations between haploid plants and testcrosses have been reported in the literature so far. Estimates of genetic correlations between haploid and corresponding DH lines are missing in the literature as well. Chase (1964) produced haploid versions of several homozygous diploid donor lines and observed that the haploid lines were much smaller and less vigorous than the corresponding diploid donor lines. Nevertheless, the haploid and DH versions strongly resembled each other for various morphological traits. Studies of Chalyk and Ostrovsky (1993) corroborated these findings.

In this study we are presenting estimates of genetic correlations between three types of entries: (i) haploid lines, (ii) corresponding DH lines, and (iii) testcrosses of the DH lines with two unrelated inbred lines. Performance data were assessed in multilocation, 2-yr yield trials in Germany. The discussion is focusing on the relevance of our results for current maize breeding schemes.

MATERIALS AND METHODS

The DH lines descend from three unrelated proprietary dent single crosses owned by the seed companies KWS Saat AG, Einbeck, Germany (KWS), Südwestsaat GbR, Rastatt, Germany (SWS) (which is now Dow AgroSciences GmbH), and Monsanto Agrar Deutschland GmbH, Borken, Germany (MON). All parent lines of the single crosses were conventionally developed inbred lines adapted to the temperate Central European climate. The two KWS lines belong to the heterotic groups Iodent and Iowa Stiff Stalk Synthetic (BSSS), the two SWS lines to Iodent and Lancaster, and both MON lines to Iodent.

The DH lines were produced by *in vivo* haploid induction using line RWS (Röber et al., 2005) or related genotypes as inducers. Random samples of 54 to 58 DH lines per donor single cross were (i) subjected to *in vivo* haploid induction for establishing haploid versions of each line and (ii) testcrossed with two unrelated flint maize lines of the respective partner company. All these materials were built up by the partner companies and kindly provided to the authors for the present study. A graphical representation of these activities is illustrated in Fig. 1 for one of the three material sets.

All materials were evaluated in field trials at three public experimental stations (Hohenheim, Eckartsweier, and Frankendorf) in southern Germany in the years 2004 and 2005. In addition, each of the partner companies tested their own material at one of their German breeding stations (KWS at Bernburg, SWS at Lichtenau, and MON at Künzing). Thus,

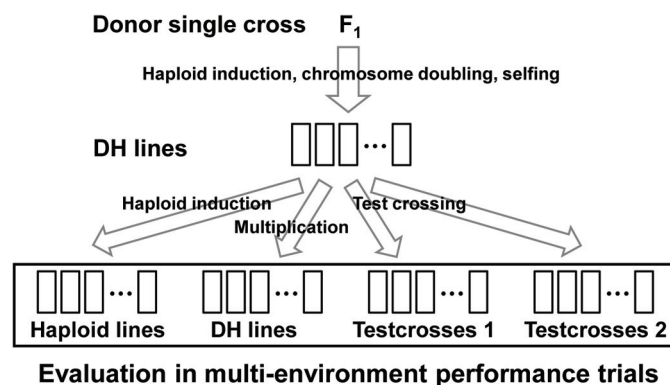


Figure 1. Experimental design used to build up doubled haploid (DH) lines, their haploid (H) versions, and testcrosses of the DH lines to two testers illustrated for one material set.

in total each material set was evaluated in eight environments. In 2004 early growth was impaired by unusually cool temperatures whereas warm weather prevailed during the grain filling period. In 2005 weather conditions did not greatly deviate from the long-term data (Supplemental Table S1). The different genetic structures (haploid lines, DH lines, and testcrosses) of a given material set were evaluated in separate but neighboring experiments in the same field (Supplemental Fig. S1 and S2). The haploid-line blocks were bordered by four rows of a mixture of inbred lines differing in flowering date to warrant pollination of the haploid lines throughout the flowering period. Each trial was laid out as a partially balanced 8 by 8 or 6 by 10 lattice with two replicates. Free entry places were filled up with appropriate checks. Seed limitations required two plot types: one-row plots of 4 m length for the haploid and DH lines and two-row plots of 6 m length for the testcrosses. Row distance was 0.75 m. Stand density varied between locations from 9.0 to 9.5 plants m⁻² for the testcrosses and from 8.0 to 9.0 plants m⁻² for the DH lines. The latter densities were also intended for the haploid lines but were not always achieved due to low emergence rates or seed misclassification leading to off-types that had to be pulled out during early development. Plant husbandry was practiced as common at the respective locations.

The following plant traits were analyzed: early vigor (visual rating of vitality at a given date when the majority of plants have reached the two to four node stage of stem elongation; score 1 indicates poor and 9 indicates excellent), silking (days from planting till 50% female flowering), plant height (cm from the ground to the first tassel branch), grain weight (g) per plant, grain yield (t ha⁻¹) adjusted to 140 g kg⁻¹ moisture content, stover dry matter weight (g) per plant, stover dry matter yield (t ha⁻¹), and grain moisture content at harvest (g kg⁻¹). Stover comprised all aboveground plant parts except ears at grain maturity. Adequate samples of chopped stover were taken directly after harvest for determining moisture content. Due to biological and labor capacity constraints, not all traits could be assessed for each of the three genetic structures in each of the eight environments. For details see Supplemental Table S2.

Statistical data analyses were performed by means of the software packages PLABSTAT version 2 (Utz, 2009) and SAS version 9.1.2 (SAS Institute, 2006) for Windows. Computations were performed in two successive steps (Piepho, 1999; Smith et al., 2001). First, PLABSTAT was used to calculate

Table 1. Means of three sets of maize material (KWS, SWS, and MON)[†] each consisting of a sample of doubled haploid (DH) lines, their haploid versions, and testcrosses of the DH lines to one or two testers (testcross 1 [TC1] and testcross 2 [TC2]) evaluated in field trials at four locations in 2004 and 2005 for various quantitative traits. (Some traits, in particular stover weight per plant and stover yield, could not be assessed in all eight environments; for details see Supplemental Table S2).

Trait	KWS				SWS				MON		
	Haploid	DH	TC1	TC2	Haploid	DH	TC1	TC2 [‡]	Haploid	DH	TC1 [§]
Early vigor, score 1–9 [¶]	5.9	6.2	–	–	5.0	5.8	–	–	5.5	5.9	–
Silking, dap [#]	87.5	89.5	79.9	82.3	90.8	91.3	81.5	76.4	95.6	97.2	85.7
Plant height, cm	95	168	221	244	104	189	263	256	126	193	273
Grain weight, g per plant	9.4	56.8	–	–	2.0	55.0	–	–	2.8	72.4	–
Stover weight, g per plant	17.4	51.0	–	–	29.1	68.2	–	–	42.8	70.3	–
Grain moisture content, g kg ⁻¹	–	–	294	315	–	–	309	298	–	–	345
Grain yield, t ha ⁻¹	–	–	9.8	10.6	–	–	10.3	10.3	–	–	11.5
Stover yield, t ha ⁻¹	–	–	6.9	7.0	–	–	7.3	–	–	–	8.0

[†]KWS, KWS Saat AG, Einbeck, Germany; SWS, Südwestsaat GbR, Rastatt, Germany; MON, Monsanto Agrar Deutschland GmbH, Borken, Germany.

[‡]TC2 evaluated in 2005 only.

[§]Set MON contained only one testcross series.

[¶]Score 1 indicates poor; score 9 indicates excellent.

[#]dap, days after planting.

adjusted entry means for each individual experiment in each environment. In a second step, SAS was applied for subjecting the adjusted values to a mixed model analysis combined over genetic structures and environments within material sets. **Outliers identified according to Anscombe and Tukey (1963) were treated as missing values.** The following model terms were fitted for each genetic structure within each material set: genotype main effects (random), environment effects (fixed), and genotype × environment interaction effects (random). **Heritability and genetic correlation coefficients were calculated according to Knapp and Bridges (1987) and Holland (2006), respectively. Parameter estimates exceeding twice their standard errors were considered significant.** To avoid scaling effects caused by differences in vigor between the three genetic structures, observed values for plant height, grain weight, grain yield, stover weight, and stover yield were transformed to percentage values (environmental means of the respective experiment set equal to 100) for computing second order statistics.

RESULTS

In all three material sets, silking (Supplemental Fig. S3) was earliest in the testcrosses and latest in the DH lines (Table 1). On average, the testcrosses flowered 9.6 d and the haploid lines 1.4 d earlier than the DH lines. The haploid lines were much smaller and had narrower leaves than the DH lines. In all three haploid line populations, several lines contained a few or even many plants with some seed setting. In the KWS material a few ears displayed almost complete seed setting. However, seeds were much smaller than in the DH lines. Low mean values of the haploids were also observed for stover weight. Among the three material sets, MON was latest in maturity and highest in testcross performance.

Highly significant genotypic variation existed in all material sets and all genetic structures for all traits analyzed (Table 2). Estimated variances of the haploid lines

were similar to those of the DH lines and several times larger than those of the testcrosses. No consistent differences in the genotypic variances of the haploid and DH lines existed between the three material sets. At the testcross level, greater variation was observed in the KWS and SWS sets than in the MON set.

Genotype × environment interaction was higher in the haploid and DH material sets than in the testcross sets (Table 2). Generally the interaction variance was smaller than the genotypic variance in all material sets. Entry-mean heritability estimates were high to very high for all traits.

Significant and mostly strong genetic correlations occurred between haploid and DH lines for early vigor, silking, plant height, and stover weight (Table 3). In this regard, no important differences existed between the three material sets. Similarly strong correlations were observed between testers in the KWS and the SWS testcross series (Table 4). Only for grain yield the latter correlations were somewhat lower.

Strong correlations were generally obtained between DH lines and testcrosses for silking date, plant height, stover weight, and grain moisture content (Table 5). Moderate correlations occurred for grain yield in the KWS set whereas no significant correlation was observed in the SWS and MON materials. Significant correlations also existed between haploid lines and testcrosses (Table 5). Estimates were moderate to high for silking date and somewhat lower for plant height and stover weight. Correlation coefficients ranged from 0.53 to 0.84 for silking date, from 0.28 to 0.69 for plant height, and from 0.33 to 0.72 for stover weight.

Low or nonsignificant “cross-correlations” existed between early vigor of the haploid lines and stover yield of the testcrosses (Table 6). In contrast, moderate to strong

Table 2. Estimated variance components[†] and heritability coefficients in three material sets (KWS, SWS, and MON)[‡] for various quantitative traits assessed in field trials at four locations in 2004 and 2005.

Trait	KWS				SWS				MON		
	Haploid	DH [§]	TC1 [§]	TC2 [§]	Haploid	DH	TC1	TC2	Haploid	DH	TC1
<u>Genotypic variance</u>											
Early vigor, score 1–9 [¶]	0.28	0.22	–	–	0.30	0.15	–	–	0.38	0.38	–
Silking, dap [#]	5.55	2.39	0.95	0.66	8.51	8.27	1.71	1.28	1.80	2.03	0.39
Relative plant height	207.5	115.4	27.1	17.5	118.8	53.0	6.9	10.8	202.1	52.9	2.8
Relative grain weight per plant	–	129.5	–	–	–	547.1	–	–	–	131.5	–
Relative stover weight per plant	491.1	291.5	–	–	387.1	290.8	–	–	378.5	182.5	–
Grain moisture content, g kg ^{–1}	–	4.58	0.98	1.17	–	9.89	1.33	1.94	–	4.49	0.95
Relative grain yield	–	–	23.6	18.3	–	–	15.9	22.6	–	–	7.0
Relative stover yield	–	–	74.9	66.3	–	–	48.6	–	–	–	5.9*
<u>Genotype × environment interaction variance</u>											
Early vigor, score 1–9	0.18	0.18	–	–	0.17	0.20	–	–	0.19	0.07	–
Silking, dap	1.27	0.95	0.15	0.22	1.42	1.68	0.55	0.19	0.68	0.56	0.15
Relative plant height	52.5	34.8	2.5	1.6	17.6	12.2	1.6	2.8	296.0	55.8	0.7
Relative grain weight per plant	–	64.1	–	–	–	355.5	–	–	–	61.6	–
Relative stover weight per plant	182.4	86.3	–	–	110.1	92.5	–	–	121.8	28.2	–
Grain moisture content, g kg ^{–1}	–	2.32	0.42	0.27	–	2.52	0.38	0.51	–	0.67	0.15
Relative grain yield	–	–	13.3	8.1	–	–	10.6	16.3	–	–	3.0
Relative stover yield	–	–	21.5	11.3*	–	–	6.81 ns ^{††}	–	–	–	8.6
<u>Heritability</u>											
Early vigor, score 1–9	0.84	0.77	–	–	0.74	0.60	–	–	0.75	0.82	–
Silking, dap	0.96	0.93	0.94	0.91	0.96	0.97	0.94	0.84	0.90	0.93	0.89
Relative plant height	0.96	0.95	0.97	0.96	0.96	0.96	0.93	0.87	0.82	0.87	0.87
Relative grain weight per plant	–	0.78	–	–	–	0.91	–	–	–	0.90	–
Relative stover weight per plant	0.91	0.92	–	–	0.92	0.92	–	–	0.90	0.91	–
Grain moisture content, g kg ^{–1}	–	0.93	0.95	0.93	–	0.96	0.95	0.91	–	0.96	0.95
Relative grain yield	–	–	0.88	0.88	–	–	0.84	0.73	–	–	0.83
Relative stover yield	–	–	0.77	0.83	–	–	0.79	–	–	–	0.40

*Significant at the 0.05 probability level.

[†]All nonmarked estimates significant at $P = 0.01$ (F -test of appropriate mean squares).

[‡]KWS, KWS Saat AG, Einbeck, Germany; SWS, Südwestsaat GbR, Rastatt, Germany; MON, Monsanto Agrar Deutschland GmbH, Borken, Germany.

[§]DH, doubled haploid; TC1, testcross 1; TC2, testcross 2.

[¶]Score 1 indicates poor; score 9 indicates excellent.

[#]dap, days after planting.

^{††}ns, nonsignificant.

associations were found between DH lines and testcrosses in four out of five testcross series. Generally, moderate correlations occurred between early vigor of the haploid and DH lines and grain yield of the testcrosses. In most cases, the correlation estimates were somewhat higher for the DH lines. Remarkably, early vigor of the haploid and DH lines correlated stronger with grain yield than with stover yield of the testcrosses. No or only weak relationships existed between early vigor of the haploid and DH lines and grain moisture content of the testcrosses. Silking date of the haploid and DH lines was strongly correlated with stover yield of the testcrosses in the KWS set whereas low to medium correlations occurred in the SWS and MON materials.

Silking of the haploid and DH lines was also associated with grain yield of the testcrosses. Most correlation coefficients varied closely around 0.5. Lower values were only obtained in the MON set and for the haploid lines

in the SWS set. Testcrosses of late flowering haploid and DH lines generally had higher grain moisture content at harvest. However, correlation coefficients were moderate, ranging from 0.19 to 0.52 in the haploid lines and from 0.38 to 0.54 in the DH lines.

Low to moderate correlations were observed between plant height of the haploid lines and stover yield of the testcrosses. Similar or higher estimates were obtained for the DH lines. The relationship between plant height of the haploid and DH lines and grain yield of the testcrosses largely resembled that between plant height and stover yield. No association existed between plant height of the haploid and DH lines and grain moisture content of the testcrosses. However, mostly moderate correlations occurred between stover weight of the haploid lines and grain yield of the testcrosses. Estimates ranged from 0.32 to 0.50. For the DH lines, these correlations were mostly somewhat higher ranging from 0.29 to 0.61.

Table 3. Estimated genetic correlations[†] between haploid and doubled haploid (DH) lines in three material sets (KWS, SWS, and MON)[‡] for various quantitative traits assessed in field trials at four locations in 2004 and 2005.

Trait	KWS	SWS	MON
Early vigor	0.87	0.78	0.57
Silking	0.81	0.89	0.68
Plant height	0.88	0.84	0.72
Stover weight per plant	0.63	0.82	0.63

[†]All estimates are larger than twice their standard errors.

[‡]KWS, KWS Saat AG, Einbeck, Germany; SWS, Südwestsaat GbR, Rastatt, Germany; MON, Monsanto Agrar Deutschland GmbH, Borken, Germany.

Table 4. Estimated genetic correlations[†] between two testcross series testcross 1 [TC1] and testcross 2 [TC2] in two material sets (KWS and SWS)[‡] estimated for various quantitative traits assessed in field trials at four locations in 2004 and 2005.

Trait	KWS			SWS
	2004	2005	Combined	2005 [§]
Silking	0.91	0.85	0.88	0.83
Plant height	0.95	0.94	0.94	0.95
Grain moisture content	0.88	0.97	0.92	0.89
Grain yield	0.81	0.74	0.78	0.68
Stover yield	0.91	–	–	–

[†]All estimates are larger than twice their standard errors.

[‡]KWS, KWS Saat AG, Einbeck, Germany; SWS, Südwestsaat GbR, Rastatt, Germany.

[§]Series TC2 of set SWS was evaluated in 2005 only.

DISCUSSION

Trait means and ranges of the haploid, DH, and testcross materials had the same relative order of magnitude as comparable estimates reported in the literature (Chase, 1969; Chalyk and Ostrovsky, 1993; Rotarenco et al., 2007). Interestingly, the haploid lines flowered somewhat earlier than the DH lines in all three material sets. This may be explained by the high sensitivity of the haploid lines to stress, which probably causes an earlier transition into the reproductive phase.

Estimated genetic variances generally were considerably higher in the haploid and DH lines per se than in their testcrosses (Table 2). This is in agreement with quantitative genetic theory (Griffing, 1975; Gallais, 2009). However, the higher variances were not reflected in higher heritability estimates due to relatively large genotype × environment and error variances. Nevertheless, substantial progress can be expected from selection for per se performance among haploid or DH lines.

Strong correlations existed between testers in the KWS and SWS testcross series (Table 4). This is in agreement with various other studies in which testers were taken from an “opposite” heterotic group (Hallauer et al., 2010). Such strong correlations indicate that in the present material the general combining ability component of

Table 5. Estimated genetic correlations[†] of haploid and doubled haploid (DH) lines with testcrosses in three material sets (KWS, SWS, and MON)[‡] for various quantitative traits assessed in field trials at four locations in 2004 and 2005.

Type of lines	Trait	KWS		SWS		MON
		TC1 [§]	TC2 [§]	TC1	TC2	TC
Haploid	Silking	0.53	0.58	0.84	0.77	0.58
	Plant height	0.69	0.61	0.54	0.46	0.28
	Stover weight [#]	0.39	0.60	0.72	–	0.33
DH	Silking	0.81	0.80	0.86	0.84	0.77
	Plant height	0.89	0.80	0.75	0.79	0.42
	Grain moisture content	0.83	0.82	0.71	0.65	0.82
	Grain yield	0.52	0.51	–0.13 ns ^{††}	0.10 ns	–0.08 ns
	Stover weight [§]	0.77	0.57	0.71	–	0.77

[†]All nonmarked estimates are larger than twice their standard errors.

[‡]KWS, KWS Saat AG, Einbeck, Germany; SWS, Südwestsaat GbR, Rastatt, Germany; MON, Monsanto Agrar Deutschland GmbH, Borken, Germany.

[§]TC1, testcross 1; TC2, testcross 2.

^{||}Series TC2 of set SWS was evaluated in 2005 only.

[#]Stover weight was assessed plantwise in the haploid and DH lines and plotwise in the testcrosses.

^{††}ns, nonsignificant.

the genotypic variance is much more important than the specific combining ability component.

To our knowledge, this is the first study reporting estimates of genetic correlations between balanced sets of maize haploid lines, corresponding DH lines, and testcrosses of the DH lines. In maize breeding, selection among haploid plants or DH lines per se may be practiced for four purposes: (i) to rid deleterious recessive alleles from the gene pool of breeding materials (Chalyk, 1999), (ii) to indirectly (in case of haploid plants) or directly (in case of DH lines) improve the usefulness of DH lines in hybrid seed production (Chase, 1951), (iii) to enhance the performance of open-pollinated diploid populations (Chalyk and Rotarenco, 1999, 2001; Eder and Chalyk, 2002), or (iv) to develop high-yielding hybrid varieties (for review see Chang and Coe, 2009). In what follows we will focus on the latter three purposes.

The gain from indirect selection can be predicted by the formula $G_I = i^* h^* \rho \sigma$, in which i^* is the selection intensity, h^* the square root of the heritability of the selection criterion, ρ the genetic correlation between the selection criterion and the target criterion, and σ the genetic standard deviation of the target criterion (Bernardo, 2002; Hallauer et al., 2010). An asterisk indicates that the respective parameter refers to the population in which the selection criterion is assessed. For comparison, the formula for the expected gain from direct selection is $G = i h \sigma$, in which all parameters refer to the target criterion. As can be seen by comparing the formulas, a high genetic correlation between the selection criterion and the target criterion is a prerequisite for the success of indirect selection. Accordingly, the genetic correlations estimated in

Table 6. Estimated genetic correlations[†] between different traits of lines and testcrosses (cross-correlations) in field trials at four locations in 2004 and 2005.

Trait combination		KWS [‡]		SWS [‡]		MON [‡]
Lines vs. testcrosses	Type of lines	TC1 [§]	TC2 [§]	TC1	TC2	TC2
Early vigor vs. stover yield	Haploid	0.13 ns [¶]	0.47	0.21 [#]	–	0.35 [#]
	DH ^{††}	0.04 ns	0.85	0.61	–	0.48 [#]
Early vigor vs. grain yield	Haploid	0.43	0.24 [#]	0.44	0.48	0.54
	DH	0.77	0.56	0.70	0.57	0.36
Early vigor vs. grain moisture content	Haploid	0.03 ns	–0.18 [#]	0.09 ns	0.03 ns	0.18 ns
	DH	0.20 [#]	0.07 ns	0.28 ns	0.27 [#]	0.00 ns
Silking vs. stover yield	Haploid	0.74	0.74	0.42	–	0.56
	DH	0.71	0.74	0.36	–	0.39 [#]
Silking vs. grain yield	Haploid	0.43	0.51	0.56	0.36	0.20 [#]
	DH	0.63	0.64	0.48	0.49	0.33
Silking vs. grain moisture content	Haploid	0.21	0.19 [#]	0.52	0.45	0.44
	DH	0.38	0.46	0.54	0.45	0.49
Plant height vs. stover yield	Haploid	0.49	0.49	0.27 [#]	0.41	0.38 [#]
	DH	0.74	0.59	0.44	0.45	0.37 [#]
Plant height vs. grain yield	Haploid	0.50	0.36	0.36	0.18 [#]	0.26 [#]
	DH	0.71	0.51	0.38	0.52	0.20 [#]
Plant height vs. grain moisture content	Haploid	0.02 ns	–0.15 [#]	–0.01 ns	0.01 ns	0.11 ns
	DH	0.12 ns	–0.01 ns	–0.04 ns	0.34	0.09 ns
Stover weight vs. grain yield	Haploid	0.50	0.30	0.48	0.42	0.32
	DH	0.61	0.47	0.57	0.61	0.29 ^{††}

[†]All nonmarked estimates are larger than twice their standard errors.

[‡]KWS, KWS Saat AG, Einbeck, Germany; SWS, Südwestsaat GbR, Rastatt, Germany; MON, Monsanto Agrar Deutschland GmbH, Borken, Germany.

[§]TC1, testcross 1; TC2, testcross 2.

[¶]ns, nonsignificant.

[#]Estimate larger than its standard error.

^{††}DH, doubled haploid.

this study can be considered as indicators of the progress achievable at the DH level from preselection at the haploid level and for progress at the testcross level by preselection at the haploid or DH level or at both. In addition, the advantage of indirect selection increases if the heritability of the selection criterion is higher than that of the target criterion and/or if a higher selection intensity can be realized for the selection criterion than for the target criterion.

Strong to moderate correlations were found between haploid and DH lines (Table 3). This is in support of early observations of Chase (1964) and Chalyk and Rotarenco (2001). Preselection among untreated haploid plants directly sown in the field should therefore result in improved per se performance at the DH level. However, production of DH lines from untreated haploid plants requires a sufficient degree of spontaneous female and male fertility in the haploid plants such that the selected plants can be selfed. While partial female fertility of haploid plants is quite common in many breeding populations (for review see Chang and Coe, 2009), partial male fertility occurs at low frequency only. Yet haploid plants producing a few anthers with traces of pollen (Supplemental Fig. S4) were found in various genetic materials (Geiger and Schönleben, 2011; Kleiber et al., 2012). Moreover, Chase (1952) and Zabirowa et al. (1993) demonstrated that

partial fertility of haploids is a heritable quantitative trait and thus can be improved by selection. In addition, one may expect that selection for responsiveness to artificial chromosome doubling at the coleoptile stage, as practiced in commercial DH-line-based hybrid breeding, will indirectly improve the degree of fertility of untreated haploid plants. Therefore, hope is permitted that after a few breeding cycles the use of doubling agents for the development of DH lines will become obsolete in elite breeding materials.

In this context, it needs to be considered that in standard DH-line-based hybrid breeding, selection would be practiced among haploid plants rather than haploid lines. This means that the heritability would be considerably smaller than the estimates obtained in the present study. On the other hand, a higher selection intensity can be achieved with plants than with lines. Hence, much of the decrease in heritability could be compensated by an increase of the selection intensity resulting in a similar size of the product of i^* times h^* in the above formula for the gain from indirect selection.

Correlations between the DH lines and their testcrosses for the highly heritable traits silking, plant height, and grain moisture content were as strong as those between testers and were moderate to strong for stover yield but

were medium to low or nonsignificant for grain yield (Table 5). Similar results were obtained by Mihaljevic et al. (2005) and Grieder et al. (2012) in studies with selfed inbred lines and their testcrosses. In contrast, mostly low correlations were reported in the early maize breeding literature (for reviews see Seitz, 1989; Hallauer et al., 2010). Possibly the per se performance of those older inbred lines was strongly affected by the manifestation of deleterious recessive genes, which were masked by favorable dominant alleles at the testcross level (Smith, 1986).

Remarkable results were obtained for the relationship between haploid lines and testcrosses. Despite the reduced vigor of the haploid lines and their high sensitivity to abiotic stress, the correlations were significant for all traits (silking, plant height, and stover weight) in all three material sets (Table 5). However, in most cases the correlations between haploid lines and testcrosses were somewhat lower than those between DH lines and testcrosses. Hence, it may be concluded that selection among haploid lines or untreated haploid plants should lead to significant correlated genetic gain for developmental and morphological traits not only at the DH but also at the testcross level.

Interestingly, even significant cross-correlations could be observed (Table 6). For example, early vigor and silking of the haploid and DH lines were moderately to strongly associated with stover and grain yield of the testcrosses in the majority of test series. Significant cross-correlations also existed between plant height of the haploid and DH lines with stover yield and even grain yield of the testcrosses. Moreover, stover weight of the DH lines was moderately to strongly associated with grain yield of the testcrosses. This reinforces our above conclusions regarding the efficacy of selection at the haploid and DH level.

In the haploid recurrent selection (HRS) method of population improvement suggested by Chalyk and Rotarenco (1999), mass selection is applied to untreated haploid plants grown in an isolated field plot surrounded by the diploid source population as pollinator. Seed from selected haploid plants is bulked to start the next HRS cycle. A remarkable increase in grain yield was obtained by applying this method to a synthetic composed of four elite inbred lines (Rotarenco et al., 2007, 2012). By using highly effective modern haploid inducers for HRS, mass selection may be substituted by between-and-within-family selection. The increased induction rate of those inducers deliver a high enough number of haploid kernels per donor plant for growing them in an ear-to-row manner. This way the breeder can take advantage of the higher heritability of family-based performance data and thus further improve the efficacy of the HRS approach.

Selection at the haploid and DH level may be considered as the most effective method of reducing the mutational load of breeding materials (Chalyk, 1999). This is

particularly relevant in prebreeding programs aiming at the exploitation of landraces and nonadapted open-pollinated source populations (Wilde et al., 2010). Moreover, observations of Chase (1951) and of the present authors revealed that haploid lines react even more sensitively than DH lines to biotic and abiotic stress and therefore effectively uncover susceptibility to diseases and environmental constraints. In this regard, selection at the haploid level may be especially interesting for the improvement of threshold traits. For example, at Frankendorf in 2005 a distinct differentiation for green snap occurred among the haploid lines but not among the corresponding DH lines or testcrosses. To take advantage of this phenomenon, breeders may create haploid versions of superior DH lines and evaluate these haploid lines per se in specific stress-prone environments during the final testcross selection stage. We hypothesize that the threshold required to induce a certain phenotypic reaction is lower in haploid than in DH plants for diverse traits.

In conclusion, results of our correlation study indicate that preselection for per se performance at the haploid and DH level may significantly contribute to progress in maize hybrid and population breeding.

Supplemental Information Available

Supplemental material is available at <http://www.crops.org/publications/cs>.

Acknowledgments

The authors are grateful to KWS Saat AG, Einbeck, Südwestsaat GbR (SWS), Rastatt, Monsanto Agrar Deutschland GmbH, Düsseldorf, and Bavarian State Institute for Agronomy (BLP), Freising-Weihenstephan, for providing field testing capacities. Furthermore, we thank Walter Schmidt and Milena Ouzunova from KWS, Frank K. Röber and Nicole Schultze from SWS, Eckard Hozhausen and Jürgen Engler from Monsanto, Joachim Eder, Birte Krützfeldt, and Regina Volkhausen from BLP, and Wolfgang Schippreck, Jochen Jesse, Henriette Burger, and Bastian Csiky from University of Hohenheim for valuable scientific advice and excellent technical assistance.

References

- Anscombe, F.J., and J.W. Tukey. 1963. The examination and analysis of residuals. *Technometrics* 5:141–160. doi:10.1080/00401706.1963.10490071
- Bernardo, R. 2002. Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.
- Chalyk, S.T. 1999. Use of maternal haploids for improving maize inbred lines. *Maize Genet. Newsl.* 73:54–56.
- Chalyk, S.T., and V.V. Ostrovsky. 1993. Comparison of haploid and diploid maize (*Zea mays* L.) plants with identical genotypes. *J. Genet. Breed.* 47:77–80.
- Chalyk, S.T., and V.A. Rotarenco. 1999. Using maternal haploid plants in recurrent selection in maize. *Maize Genet. Newsl.* 73:56–57.

- Chalyk, S.T., and V.A. Rotarenco. 2001. The use of matroclinous maize haploids for recurrent selection. *Russ. J. Genet.* 37:1382–1387. doi:10.1023/A:1013291711912
- Chang, M.-T., and E.H. Coe. 2009. Doubled haploids. In: A.L. Kriz and B.A. Larkins, editors, *Molecular genetic approaches to maize improvement: Biotechnology in agriculture and forestry*. Springer, Berlin, Germany. p. 127–142.
- Chase, S.S. 1951. Efficient methods of developing and improving inbred lines. The monoploid method of developing inbred lines. In: Report of the 6th Hybrid Corn Industry Research Conference, Chicago, IL. 28–29 Nov. 1951. American Seed Trade Association, Hybrid Corn Division, Chicago, IL. p. 29–34.
- Chase, S.S. 1952. Selection for parthenogenesis and monoploid fertility in maize. *Genetics* 37:573–574.
- Chase, S.S. 1964. Monoploids and diploids of maize: A comparison of genotypic equivalents. *Am. J. Bot.* 51:928–933. doi:10.2307/2440242
- Chase, S.S. 1969. Monoploids and monoploid-derivatives of maize (*Zea mays* L.). *Bot. Rev.* 35:117–167. doi:10.1007/BF02858912
- Eder, J., and S.T. Chalyk. 2002. In vivo haploid induction in maize. *Theor. Appl. Genet.* 104:703–708. doi:10.1007/s00122-001-0773-4
- Gallais, A. 2009. Full-sib reciprocal recurrent selection with the use of doubled haploids. *Crop Sci.* 49:150–152. doi:10.2135/cropsci2008.04.023sc
- Geiger, H.H. 2009. Doubled haploids. In: J.L. Bennetzen and S. Hake, editors, *Maize handbook – Volume II: Genetics and genomics*. Springer, New York, NY. p. 641–657.
- Geiger, H.H., and G.A. Gordillo. 2009. Doubled haploids in hybrid maize breeding. *Maydica* 54:485–499.
- Geiger, H.H., and M. Schönleben. 2011. Incidence of male fertility in haploid elite dent maize germplasm. *Maize Genet. Newsl.* 85:22–32.
- Griffing, B. 1975. Efficiency changes due to use of doubled-haploids in recurrent selection methods. *Theor. Appl. Genet.* 46:367–368.
- Gordillo, G.A., and H.H. Geiger. 2008. Alternative recurrent selection strategies using doubled haploid lines in hybrid maize breeding. *Crop Sci.* 48:911–922. doi:10.2135/cropsci2007.04.0223
- Grieder, C., B.S. Dhillon, W. Schipprack, and A.E. Melchinger. 2012. Breeding maize as biogas substrate in Central Europe. II. Quantitative-genetic parameters for inbred lines and correlations with testcross performance. *Theor. Appl. Genet.* 124:981–988. doi:10.1007/s00122-011-1762-x
- Hallauer, A.R., M.J. Carena, and J.B. Miranda. 2010. *Quantitative genetics in maize breeding*. Springer, New York, NY.
- Holland, J.B. 2006. Estimating genotypic correlations and their standard errors using multivariate restricted likelihood estimation with SAS Proc MIXED. *Crop Sci.* 46:642–654.
- Kleiber, D., V. Prigge, A.E. Melchinger, F. Burkard, F. San Vicente, G. Palomino, and G.A. Gordillo. 2012. Haploid fertility in temperate and tropical maize germplasm. *Crop Sci.* 52:623–630. doi:10.2135/cropsci2011.07.0395
- Knapp, S.J., and W.C. Bridges. 1987. Confidence interval estimates for heritability for several mating and experimental designs. *Theor. Appl. Genet.* 73:759–763. doi:10.1007/BF00260787
- Longin, C.F.H., H.F. Utz, J.C. Reif, W. Schipprack, and A.E. Melchinger. 2006. Hybrid maize breeding with doubled haploids: I. One-stage versus two stage selection for testcross performance. *Theor. Appl. Genet.* 112:903–912. doi:10.1007/s00122-005-0192-z
- Mihaljevic, R., C.C. Schön, H.F. Utz, and A.E. Melchinger. 2005. Correlations and QTL correspondence between line per se and testcross performance for agronomic traits in four populations in European maize. *Crop Sci.* 45:114–122. doi:10.2135/cropsci2004.0760
- Piepho, H.-P. 1999. Stability analysis using the SAS system. *Agron. J.* 91:154–160. doi:10.2134/agronj1999.00021962009100010024x
- Prasanna, B.M., V. Chaikam, and G. Mahuku. 2012. *Doubled haploid technology in maize breeding: Theory and practice*. CIMMYT, Mexico, DF.
- Prigge, V., and A.E. Melchinger. 2012. Production of haploids and doubled haploids in maize. In: V.M. Loyola-Vargas and N. Ochoa-Alejo, editors, *Plant cell culture protocols*. 3rd ed. Springer, Totowa, NJ. p. 161–172.
- Röber, F.K., G.A. Gordillo, and H.H. Geiger. 2005. In vivo haploid induction in maize – Performance of new inducers and significance of doubled haploid lines in hybrid breeding. *Maydica* 50:275–283.
- Rotarenco, V.A., S.T. Chalyk, A. Jacota, and J. Eder. 2007. Breeding effect of selection at the level of haploid sporophyte in maize. *Bull. Acad. Sci. Moldova* 1:92–98.
- Rotarenco, V.A., G. Dicu, M. Mihailov, and D. State. 2012. Selection and breeding experiments at the haploid level in maize (*Zea mays* L.). *J. Plant Breed. Crop Sci.* 4:72–79.
- SAS Institute. 2006. *User's guide: Statistics*, version 9. SAS Inst., Cary, NC.
- Seitz, G. 1989. Experimentelle und theoretische Untersuchungen zur Beziehung zwischen Linieneigenleistung und allgemeiner Kombinationsfähigkeit bei Silomais. (In German.) PhD diss., University of Hohenheim, Stuttgart, Germany.
- Seitz, G. 2005. The use of doubled haploids in corn breeding. In: Proceedings of the 41st Annual Illinois Corn Breeders' School, Urbana-Champaign, IL. 7–8 Mar. 2005. Dept. of Crop Science, University of Illinois, Urbana, IL. p. 1–7.
- Smith, A.B., B.R. Cullis, and A.R. Gilmour. 2001. Analysing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. *Biometrics* 57:1138–1147. doi:10.1111/j.0006-341X.2001.01138.x
- Smith, O.S. 1986. Covariance between line per se and testcross performance. *Crop Sci.* 26:540–543. doi:10.2135/cropsci1986.0011183X002600030023x
- Utz, H.F. 2009. *PLABSTAT – Software to analyse plant breeding experiments*. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany. https://fsc.uni-hohenheim.de/fileadmin/einrichtungen/plant-breeding/plabstat_manual_eng.pdf (accessed 1 Feb. 2006).
- Wilde, K., H. Burger, V. Prigge, T. Prestler, W. Schmidt, M. Ouzunova, and H.H. Geiger. 2010. Testcross performance of doubled-haploid lines developed from European flint maize landraces. *Plant Breed.* 129:181–185. doi:10.1111/j.1439-0523.2009.01677.x
- Zabirova, E.R., O.A. Shatskaya, and V.S. Shcherbak. 1993. Line 613/2 as a source of a high frequency of spontaneous diploidization in corn. *Maize Genet. Newsl.* 67:67.

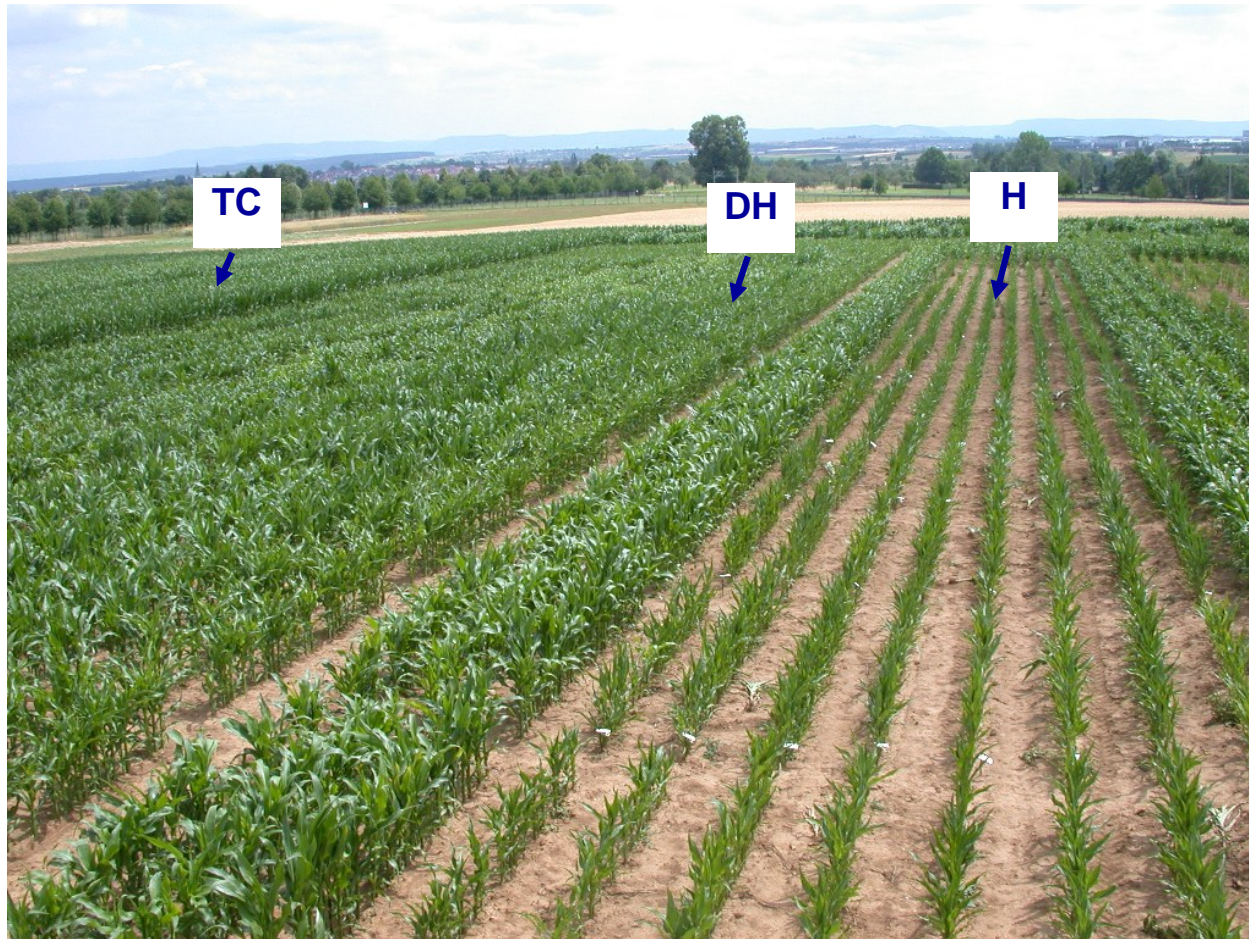


Figure S1. Overview of the field experiment at Hohenheim depicting the blocks of haploid lines (H), doubled haploid lines (DH) and testcrosses (TC); photo taken on the 17th of July 2004



Figure S2. Haploid lines at flowering time. Note the uniformity and distinctness of the lines; photo taken at Hohenheim on the 26th of July 2004



Figure S3. Haploid line with well developed silks, photo taken at Hohenheim on the 26th of July 2004



Figure S4. Main tassel branch of a haploid plant with a few extruded pollen-shedding anthers; photo taken at Hohenheim on the 9th August 2004

Table S1. Geographic and climatic description of the test sites used in the present study

Descriptor	Test site					
	Hohenheim	Eckartsweier	Lichtenau	Frankendorf	Künzing	Bernburg
Abbreviation	HOH	EWE	LIC	FRA	KÜN	BER
Nearby city	Stuttgart	Offenburg	Baden-Baden	Freising	Passau	Halle
State	Baden-Württ.	Baden-Württ.	Baden-Württ.	Bavaria	Bavaria	Saxony-Anhalt
Coordinates	48.71N/9.19E	48.52N/7.85E	48.72N/8.01E	48.34N/11.97E	48.66N/13.07E	51.80N/11.74E
Elevation [m above MSL]	400	124	125	460	310	85
Average temp. [°C]						
April to October						
Long-time	13.6	15.2	14.7	13.0	13.2	13.8
2004	14.7	15.6	15.5	14.1	14.4	14.2
2005	15.0	15.5	15.8	14.3	14.5	14.7
Precipitation [l m ⁻²]						
April to October						
Long-time	477	565	562	567	576	310
2004	433	456	548	459	567	408
2005	462	446	517	539	836	364
Operated by	Univ. of Hohenheim	Univ. of Hohenheim	SWS [†]	Bavaria State Inst. For Agronomy	Monsanto Agrar Germany GmbH	KWS Saat AG

[†] Now Dow AgroSciences GmbH

Table S2. A. Means of doubled haploid (DH) lines, their haploid (H) versions, and testcrosses of the DH lines to two opposite tester lines (TC1, TC2) evaluated in field trials at four locations (Hohenheim (HOH), Eckartsweier (EWE), Frankendorf (FRA), and Bernburg (BER)) in 2004 and 2005 for various quantitative traits, **KWS** set of genetic materials

[illegible]

Table S2. B. Means of doubled haploid (DH) lines, their haploid (H) versions, and testcrosses of the DH lines to two opposite tester lines (TC1, TC2) evaluated in field trials at four locations (Hohenheim (HOH), Eckartsweier (EWE), Frankendorf (FRA), and Lichtenau (LIC) (BER)) in 2004 and 2005 (in 2004 only TC1) for various quantitative traits, **SWS** set of genetic materials

[illegible]

Table S2. C. Means of doubled haploid (DH) lines, their haploid (H) versions, and testcrosses of the DH lines to one opposite tester line (TC1) evaluated in field trials at four locations (Hohenheim (HOH), Eckartsweier (EWE), Frankendorf (FRA), and Künzing (KÜN)) in 2004 and 2005 for various quantitative traits, **MON** set of genetic materials

[illegible]