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Association between line per se and testcross performance for eight agronomic and quality traits in winter rye

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Abstract

Key message   **We investigated associations between line
per se and testcross performance in rye and suggested
that selection for per se performance is valuable for sev-
eral traits in multi-stage selection programs.**

Abstract   Genotypic correlation between line per se and
testcross performance is an important quantitative-genetic
parameter for optimizing hybrid breeding programs. The
main goal of this survey was to study the association of
line per se and testcross performance at the phenotypic
level. We used experimental data from the line per se and
testcross performance of two segregating winter rye pop-
ulations (A, B) with each of 220 progenies tested in six
environments for eight agronomic and quality traits. Geno-
typic variances were considerably larger for per se than for
testcross performance of all investigated traits resulting in
higher heritabilities of the former in most instances. Geno-
typic correlations (r_g) between testcross and line per se per-
formance decreased with increasing complexity of the trait

as shown by the respective heritabilities. They were high-
est ($r_g \geq 0.7$) for plant height and test weight in population
B, and thousand-kernel weight, falling number and starch
content in both populations. A selection of these traits for
line per se performance in early generations will save field
plots in further testing testcross performance and increase
efficiency of hybrid breeding.

Introduction

Hybrid rye (*Secale cereale* L.) has gained a large acreage in
Germany, Austria and Denmark and is of growing impor-
tance in Poland, Sweden, Belorussia, and Russia. Rye
is used for baking, feeding, and bioenergy production. In
most Central European breeding programs hybrid cultivars
are developed by crossing parents from the seed (Petkus)
with the pollen parent pool (Carsten) by cytoplasmic-male
sterility (CMS, Geiger and Miedaner 2009). Main breed-
ing goals are grain or biomass yield, lodging resistance,
plant height, thousand-kernel weight (TKW), test weight,
and falling number as a measure for pre-harvest sprouting.
For the use as baking cereal, the contents of total and solu-
ble pentosan should be high with a moderate to low pro-
tein content. In contrast, high protein and starch contents
are important for feeding, and starch content should be as
high, but protein content as low as possible for bioethanol
production (Miedaner and Hübner 2010).

The main goal of a hybrid breeder is to increase the test-
cross performance of crosses between the genetically con-
trasting heterotic pools. Despite this, evaluation of potential
candidates for line per se performance is often applied in
early stages of selection focusing on traits with high herit-
ability (Tomerius et al. 2008). The advantages of evaluat-
ing line per se instead of testcross performance are lower

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seed production costs and a larger proportion of additive genetic variance exploited in lines compared to hybrids. A prerequisite for an efficient indirect selection is a positive genotypic correlation between the selection criterion, e.g., the line per se and the target criterion, e.g., the testcross performance (Falconer and Mackay 1996). The genotypic correlation between line per se and testcross performance depends on the complexity of the trait under consideration as well as on the predominant type of gene action (Hallauer et al. 2010; Mihaljevic et al. 2005). Intra- and interlocus interaction effects as well as genetic linkage can decrease the magnitude of the genotypic correlation between line per se and testcross performance (Schnell 1961; Smith 1986). Experimental results in maize suggested that genotypic correlation were low for traits with a large magnitude of heterosis, but high for less complex traits exhibiting a low extent of heterosis (for review, see Hallauer et al. 2010).

In hybrid rye, genotypic correlations have been tested in first-generation material for a limited set of traits (Köhler 1986; Wilde 1987; for review see Geiger and Miedaner 1999) with coefficients ranging from 0.4 for grain yield to 0.9 for lodging resistance. Estimates of genotypic correlations for all relevant agronomic and quality traits in actual elite breeding populations in rye are lacking, although they are important to optimize multi-stage selection programs.

Our study is based on two winter rye populations each with 220 progenies derived from biparental crosses among three elite inbred lines. The genotypes were evaluated for line per se and testcross performance for all important traits commonly evaluated for per se performance in rye. The objectives of this survey were to: (1) estimate genetic and non-genetic variances and heritabilities in both material groups and (2) study the association among line per se and testcross performance in order to draw conclusions on the efficiency of line per se selection.

Materials and methods

Plant material

Two segregating populations, each consisting of 220 F_2 lines were generated by crossing (1) inbred line Lo115-N with inbred line Lo90-N (Pop-A) and (2) inbred line Lo115-N with inbred line Lo117-N (Pop-B). Parents belong to the Petkus gene pool (seed parent) and possessed normal cytoplasm (N). F_2 plants were randomly forwarded to F_3 generation by single-seed descent and selfed again. The 440 randomly taken $F_{3:4}$ lines were crossed to the genetically unrelated cytoplasmic-male sterile (CMS) tester of the Petkus gene pool: B4 (Lo277-16-22-7) × F2 (C222/5 × Lo254). From inbreeding coefficient the parents

of this cross represent S_2 lines and were chosen to achieve a broader tester for combining ability. Crossing was done by open pollination between isolation walls, the pollinating $F_{3:4}$ lines were subjected to self-pollination in the same procedure. Bulked seed from each pollinator representing $F_{3:4}^2$ lines were used to test line per se performance. Lines will, nevertheless, be called $F_{3:4}$ lines throughout the paper for simplicity. Self-fertile rye inbred lines multiplied by open pollination may vary in the proportion of selfing and sibbing (Geiger and Schnell 1970), and consequently, inbreeding coefficient can range between 0.875 and 0.9375 (Becker 2011). All plant materials used in this study were kindly supplied by KWS LOCHOW GMBH, Bergen, Germany.

Field experiments

Field experiments were conducted in each of the years 2010 and 2011 at three locations: (1) Beckedorf (BEK), Germany, N52.5°, E10.3°, 80 m above sea level; (2) Petkus (PET), Germany, N51.6°, E13.2°, 130 m above sea level; and (3) Walewice (WAL), Poland, N52.1°, E19.4°, 184 m above sea level. The location × year combinations were referred to as environments in the following.

Both populations with each of 220 lines were grown in field trials together with their parents (repeated 9 times) and two standard cultivars in two separate but adjacent blocks (lines, testcrosses). Each block was arranged following an incomplete 24 × 10 alpha design with two (testcrosses) or three (lines) replications. Lines were tested in single rows of 1.5 m length with a space between rows of 0.42 m. For the testcrosses, plot size ranged from 5 to 6 m² and seeding rate varied from 150 to 200 kernels m⁻². Details of phenotyping of the testcrosses were described previously in our companion paper (Miedaner et al. 2012). Standard production practices of mineral fertilizer, herbicides and fungicides applications were used following local standards. To avoid lodging, growth regulators were applied three times. Irrigation was applied at PET and WAL by a drip irrigation system when necessary. Inbred lines were subjected to the same procedures with the exception that no growth regulators were applied.

Data were recorded for plant height (cm), 1,000-kernel weight (TKW, g), test weight (kg), falling number (s), total pentosan (%), soluble pentosan (%), protein (%), and starch contents (%). For TKW and falling number, data from one environment (WAL10) was missing. Test weight refers to the weight of 100 l of grain. The traits total pentosan (%), soluble pentosan (%), protein (%), and starch contents (%) were assessed in one replication per environment determined by near-infrared reflectance spectroscopy (NIRS, calibration by KWS SAAT AG, Einbeck) based on two independent samples per plot.

Phenotypic data analysis

Phenotypic data of each material group (lines, testcrosses) were analyzed separately following a two-step procedure. In a first step, ordinary lattice analyses of variance were performed for each environment and population separately (Schutz and Cockerham 1962). In a second step, adjusted means of each genotype were used to estimate variance components across environments for each of the two segregating populations within each material group (lines vs. testcrosses) following Snedecor and Cochran (1980). We assumed in this model that genotype and environment effects were random. Heritability on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance (Knapp et al. 1985). In addition, we set the number of environments and replications to one in order to estimate the heritability on plot-basis according to the formula $\sigma_G^2/\sigma_G^2 + \sigma_{G\times E}^2 + \sigma_{error}^2$, where σ_G^2, σ_{GxE}^2 and σ_{error}^2 are the variance components for genotype, genotype × environment interaction and error, respectively. Test of skewness and kurtosis was performed for each trait (Snedecor and Cochran 1980, pp. 79–81). Coefficients of variation (CV%) were calculated for each trait following Snedecor and Cochran (1980, p. 36) based on the variance component analyses and their F tests:

$$CV\ (\%) = \frac{\sigma}{\mu} \times 100,$$

where σ stands for the standard deviation of the respective variance component and μ for the progeny mean of the respective trait.

Phenotypic (r_p) and genotypic (r_g) correlation were calculated between line per se and testcross performance following Mode and Robinson (1959). All statistical analyses were performed with the PLABSTAT software package (Utz 2011). Relative efficiency (RE) of selection was calculated according to Falconer and Mackay (1996) assuming the same selection intensity for both, line per se and testcross performance:

$$RE = \frac{h_{LP} r_g (LP, TC)}{h_{TC}},$$

where h denotes the square root of heritability and r_g(LP, TC) the genotypic coefficient of correlation between line per se (LP) and testcross (TC) performance.

Results

Inbred parents of population A differed considerably in plant height, falling number, test weight, and total pentosan content, while those of population B were rather similar except for falling number (Table 1). Means of the testcrossed parents were also similar with the exception of plant height and thousand-kernel weight in population B. Testcross progenies of both populations showed higher

Table 1 Means and standard error of means of eight traits for parental lines (P1, P2) and each of 220 entries of Pop-A (Lo115 × Lo90) and Pop-B (Lo115 × Lo117) for line per se (LP) and testcross (TC) performance evaluated in six environments

Entry	Plant height(cm)	TKW (g)[a]	Falling number (s)[a]	Test weight (kg)	Total pentosan (%)	Soluble pentosan (%)	Protein (%)	Starch (%)
Pop-A								
P1 (Lo115)	88.3 ± 0.3	32.6 ± 0.1	146.7 ± 0.9	64.2 ± 0.2	9.99 ± 0.1	2.70 ± 0.02	13.16 ± 0.08	55.50 ± 0.07
P2 (Lo90)	94.7 ± 0.4	33.8 ± 0.2	94.0 ± 0.7	58.2 ± 0.6	7.74 ± 0.3	2.97 ± 0.02	13.58 ± 0.05	53.56 ± 0.19
LP	95.0 ± 0.3	33.8 ± 0.1	110.2 ± 1.7	63.2 ± 0.1	9.99 ± 0.05	2.80 ± 0.01	13.81 ± 0.04	54.02 ± 0.08
T × P1 (Lo115)	115.8 ± 0.6	35.5 ± 0.1	165.0 ± 1.7	68.9 ± 0.1	10.10 ± 0.03	2.29 ± 0.02	9.55 ± 0.04	61.53 ± 0.05
T × P2 (Lo90)	114.1 ± 0.3	35.1 ± 0.1	171.8 ± 2.0	69.0 ± 0.05	9.89 ± 0.03	2.25 ± 0.02	9.53 ± 0.04	61.61 ± 0.06
TC	114.6 ± 0.2	35.1 ± 0.1	164.2 ± 0.8	69.3 ± 0.05	10.04 ± 0.02	2.29 ± 0.01	9.51 ± 0.01	61.62 ± 0.04
Pop-B								
P1 (Lo115)	88.2 ± 0.3	32.6 ± 0.1	147.9 ± 1.6	63.4 ± 0.2	9.50 ± 0.1	2.52 ± 0.02	13.18 ± 0.1	55.65 ± 0.2
P2 (Lo117)	87.4 ± 0.3	30.9 ± 0.1	95.7 ± 0.7	63.7 ± 0.4	9.20 ± 0.1	2.60 ± 0.03	14.14 ± 0.1	55.80 ± 0.1
LP	92.2 ± 0.3	32.3 ± 0.1	114.1 ± 1.0	63.5 ± 0.1	8.81 ± 0.06	2.52 ± 0.01	13.80 ± 0.04	54.94 ± 0.08
T × P1 (Lo115)	113.9 ± 0.3	35.4 ± 0.2	190.4 ± 1.7	69.6 ± 0.1	10.32 ± 0.03	2.33 ± 0.02	9.70 ± 0.05	61.40 ± 0.08
T × P2 (Lo117)	109.0 ± 0.3	34.1 ± 0.1	174.0 ± 1.8	71.9 ± 0.1	10.35 ± 0.05	2.18 ± 0.02	10.00 ± 0.04	61.45 ± 0.06
TC	112.3 ± 0.2	34.4 ± 0.1	185.7 ± 0.7	70.8 ± 0.1	10.30 ± 0.02	2.22 ± 0.01	9.81 ± 0.02	61.53 ± 0.05

[a] Five environments

means and medians than line progenies for all traits except for soluble pentosan and protein contents (Table 1; Fig. 1).

A wide genotypic variation of the segregating progenies was found resulting in coefficients of genotypic variation (CV_G) significantly larger than zero ($P < 0.01$) for all traits in the two populations for both line per se and testcross performance (Table 2). These estimates were, however, for all traits substantially larger for line per se than for testcross performance. Coefficients of variation for genotype × environment interaction ($CV_{G×E}$) and error (CV_e), however, were also larger for line per se than for testcross performance for all traits except for plant height in population B where $CV_{G×E}$ was similar. This resulted in larger or similar entry-mean heritabilities for the line per se compared to testcross performance in most instances. On plot basis, estimates of heritability were substantially larger for line per se than testcross performance. Accordingly, ranges of entry-mean values were larger for the lines than for the testcrosses (Fig. 1). This was most obvious for the pentosan, protein, and starch contents where the ranges of the testcrosses was very small in both populations. Comparing the traits, largest variation was found for TKW and falling number.

Coefficients of genotypic correlation among traits tended to be higher for line per se than for testcross performance (Table 3). Generally, a positive correlation of plant height to thousand-kernel weight was found in both material groups. Other correlations were restricted to quality traits. For line per se performance, moderate correlations were detected among falling number, test weight, and total pentosan content. Across both populations and material groups only two correlations were moderate to high: a positive correlation between test weight and starch content and a negative correlation between protein and starch content.

The phenotypic correlations (r_p) between line per se and testcross performance ranged from 0.25 for soluble pentosan content to 0.74 for starch content estimated in population B (Table 4). As expected, the genotypic correlations (r_g) between line per se and testcross performance were for all traits higher than the phenotypic correlations.

Discussion

Superiority of testcrosses

All traits in our study revealed a significant ($P < 0.01$) genotypic variation for line per se and testcross progenies (Table 2), thus giving a reliable basis for estimating relevant quantitative-genetic parameters. For plant height, thousand-kernel weight, test weight, falling number, and starch content, testcrosses clearly showed a superiority over the lines in both populations. As a consequence, we

used in the following the coefficients of variation (CV%) instead of variance components to allow for a direct comparison among traits and material groups. The superiority observed in our survey was caused by improved vegetative growth of the testcrosses leading to taller and more vital plant stands and larger and plumper kernels as illustrated by differences in plant height, thousand-kernel and test weight (Table 1). From earlier studies it is known that heterosis for grain yield in rye is mainly due to higher thousand-kernel weight and larger number of kernels per ear, both yield components multiplicatively contributing to higher grain yields (Geiger and Wahle 1978). As a consequence, starch content located within the endosperm is higher in the testcross progenies compared to the lines. On the contrary, protein contents were considerably lower in both testcross populations than in the lines due to a dilution effect by the larger kernels as previously described (Falke et al. 2010). The differences between lines and testcrosses might have been even larger when the lines would have been grown in large-drilled plots and not in one-row plots surrounded by an empty row on each side, thus providing much more space for the plants for development. This was, however, not possible due to the severe inbreeding depression in rye that makes it not feasible to produce large amounts of seed for such a vast segregating population.

Large genotypic variances in inbred populations

This study was performed with two segregating populations within the Petkus heterotic group. Contrary to earlier papers, we used elite second-cycle material that has gone several times through the process of selfing. In the last 20 years line per se performance has increased dramatically and, therefore, the data are valuable for modern hybrid germplasm. With two segregating populations with randomly drawn lines, we should cover about half the additive variance that is available within the total gene pool. Data are valuable for improving this gene pool via reciprocal recurrent selection. We calculated coefficients of variation (CV) instead of variances for (1) accounting for the heterotic increase of several traits and (2) to allow comparisons among traits with different dimensions, e.g., falling number was ranging between 62 and 200 s, but soluble pentosan content between 2.2 and 2.8 %. With CV, a direct comparison of traits and materials groups is possible. Significant and high estimates of CV_G and entry-mean heritabilities showed large genotypic differences for all traits and effective selection is possible among the 440 inbred lines. The relationship of CV_G between line per se and testcross performance was 1.3 times (plant height) to 4.1 times (total pentosan) larger for lines than for testcrosses. This is in agreement with experimental results in
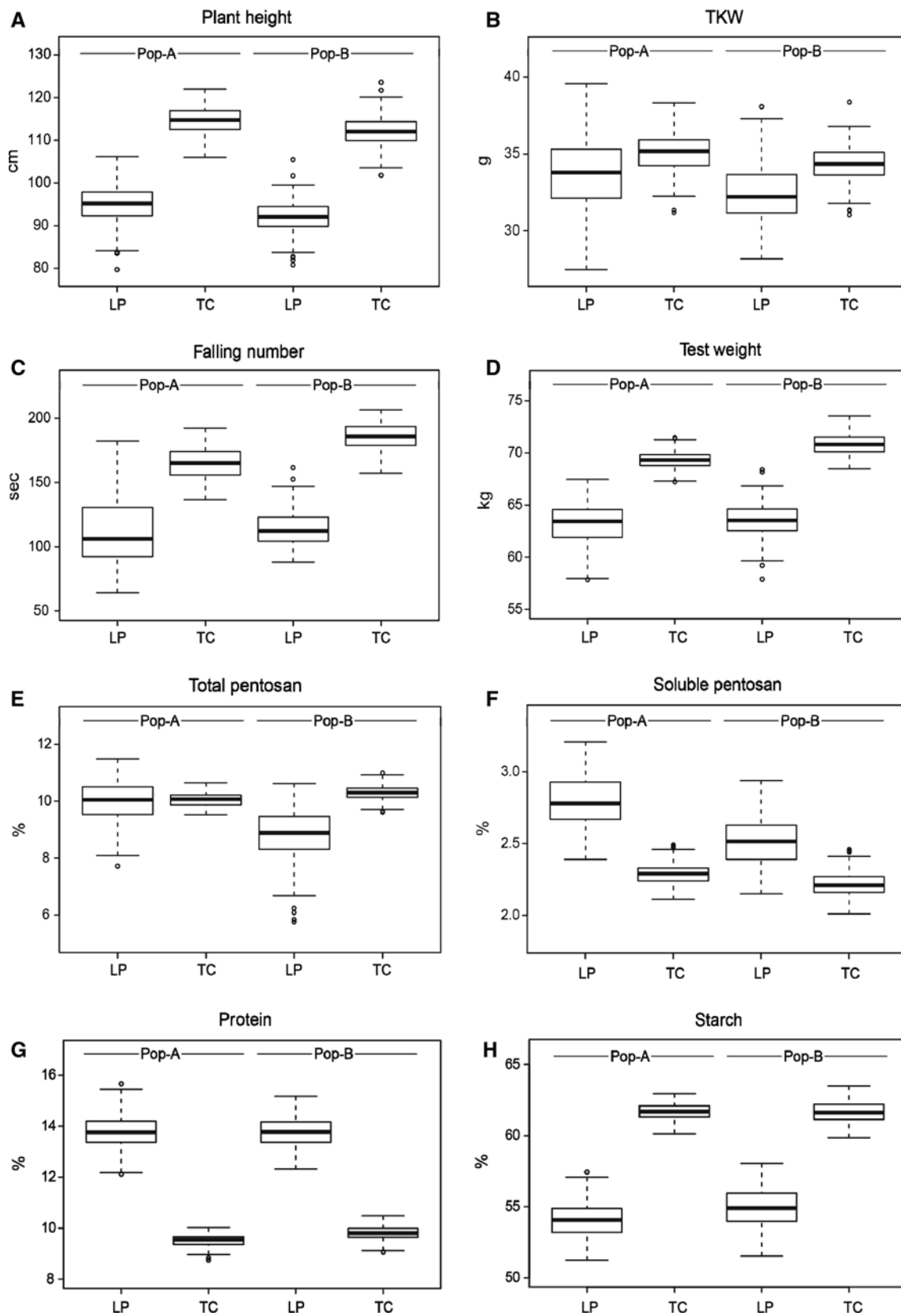
Fig. 1 Box-whisker plots for line per se (*LP*) and testcross performance (*TC*) of eight traits estimated from each of 220 entries of Pop-A (Lo115 × Lo90) and Pop-B (Lo115 × Lo117) across six environments (TKW and falling number only five environments)

Table 2 Coefficients of variation (CV_G genotype, $CV_{G\times E}$ genotype $\times$ environment interaction, CV_e error), heritabilities (H^2) on entry-mean and plot basis and their confidence intervals (CI) of eight traits estimated from each of 220 entries of Pop-A (Lo115 $\times$ Lo90) and Pop-B (Lo115 $\times$ Lo117) for line per se (LP) and testcross (TC) performance across six environments

Parameter	Plant height (cm)	TKW (g)[a]	Falling number (s)[a]	Test weight (kg)	Total pentosan (%)	Soluble pentosan (%)	Protein (%)	Starch (%)
Pop-A								
CV_G								
LP	4.35**	6.13**	20.72**	2.82**	5.15**	5.05**	3.97**	1.94**
TC	1.78**	3.27**	5.66**	1.09**	1.73**	1.95**	1.49**	0.78**
$CV_{G\times E}$								
LP	2.52**	3.02**	20.75**	3.14[b]	11.33[b]	8.75[b]	4.69[b]	2.13[b]
TC	2.05**	2.50**	7.52**	0.79**	2.82**	4.37**	3.15**	0.78**
CV_{error}								
LP	4.92	4.67	10.87	_[b]	_[b]	_[b]	_[b]	_[b]
TC	4.02	4.28	8.77	1.04	2.82	6.18	3.64	0.95
H^2								
LP	0.89	0.92	0.85	0.83	0.55	0.66	0.81	0.83
CI_{LP}	0.86–0.91	0.90–0.93	0.81–0.87	0.79–0.86	0.45–0.63	0.58–0.72	0.77–0.85	0.79–0.86
TC	0.61	0.81	0.63	0.86	0.65	0.33	0.48	0.77
CI_{TC}	0.69–0.80	0.76–0.84	0.54–0.70	0.83–0.88	0.56–0.71	0.17–0.45	0.35–0.57	0.72–0.81
$H^2_{plot\ basis}$								
LP	0.38	0.55	0.44	0.44	0.17	0.25	0.42	0.45
TC	0.13	0.30	0.19	0.41	0.16	0.06	0.09	0.29
Pop-B								
CV_G								
LP	3.73**	5.49**	10.62**	2.11**	7.95**	5.61**	3.77**	2.12**
TC	2.89**	2.95**	4.35**	1.40**	1.94**	2.47**	2.50**	1.08**
$CV_{G\times E}$								
LP	1.90**	2.94**	13.60**	3.50[b]	15.02[b]	9.72[b]	4.35[b]	2.04[b]
TC	1.91**	2.40**	4.62**	0.76**	2.91**	4.50**	2.88**	0.78
CV_{error}								
LP	5.11	4.80	9.79	_[b]	_[b]	_[b]	_[b]	_[b]
TC	3.17	3.75	7.81	0.98	2.75	6.18	3.06	0.86
H^2								
LP	0.87	0.90	0.76	0.69	0.63	0.67	0.82	0.87
CI_{LP}	0.84–0.89	0.87–0.92	0.70–0.80	0.61–0.74	0.54–0.69	0.59–0.73	0.77–0.85	0.84–0.89
TC	0.85	0.80	0.65	0.92	0.63	0.46	0.74	0.88
CI_{TC}	0.82–0.88	0.76–0.84	0.56–0.71	0.90–0.93	0.54–0.70	0.33–0.55	0.68–0.79	0.85–0.90
$H^2_{plot\ basis}$								
LP	0.32	0.49	0.28	0.27	0.22	0.25	0.43	0.52
TC	0.38	0.31	0.19	0.50	0.19	0.09	0.26	0.46

[a] Five environments

[b] $CV_{G\times E}$ and CV_e combined because only one replication was available

maize (Mihaljevic et al. 2005; Grieder et al. 2012) and quantitative-genetic theory, because under the assumption of additive gene action, CV_G among fully inbred lines is expected up to be four times larger than among testcrosses (Smith 1986).

$CV_{G\times E}$ were largely higher for line than for test-cross performance in most instances (see Table 2). This difference was more pronounced for falling number, test weight, total and soluble pentosan, and starch contents illustrating a lower phenotypic stability of inbred lines compared to testcrosses. This is a direct consequence of inbreeding depression and was observed for grain yield and yield components among each of eight inbred lines and single-cross rye hybrids in a previous study (Wahle

Table 3 Coefficients of genotypic correlation between eight traits tested as line per se and testcross performance for each of 220 entries of Pop-A (Lo115 × Lo90, above diagonal) and Pop-B (Lo115 × Lo117, below diagonal) across six environments

	PLH	TKW[a]	FN[a]	TW	TP	SP	PROT	STAR
Line per se performance								
PLH		0.43++	0.36++	0.36++	0.48++	−0.40++	−0.19++	0.12+
TKW[a]	0.23++		0.11+	0.15++	0.27++	−0.27++	−0.22++	0
FN[a]	0.21++	−0.06		0.57++	0.75++	−0.60++	−0.04	0.24++
TW	0.23++	−0.20++	0.45++		0.61++	−0.62++	−0.17++	0.62++
TP	0.38++	0.02	0.51++	0.65++		−0.40++	0.04	0.31++
SP	−0.03	0.06	−0.19++	0	0.21++		0.44++	−0.53++
PROT	−0.03	0.08+	−0.15+	0.01	0.17+	0.66++		−0.58++
STAR	0.05	−0.46++	0.19++	0.58++	0.18++	−0.42++	−0.59++	
Testcross performance								
PLH		0.45++	−0.17+	−0.34++	0.12+	0	0.02	−0.39++
TKW[a]	0.47++		−0.33++	−0.19++	0.07	−0.41++	−0.17+	−0.24++
FN[a]	0.17+	−0.18+		0.04	0.15+	0.18+	0	0.05
TW	−0.08+	−0.05	−0.04		−0.43++	−0.44++	−0.06	0.61++
TP	0.30++	0.44++	0.09	−0.04			0.10	−0.40++
SP	−0.35++	−0.55++	−0.02	0.19+	−0.06		0.09	−0.31++
PROT	0	0.42++	−0.35++	0.22++	0.47++	−0.25++		−0.64++
STAR	−0.35++	−0.63++	0.19++	0.24++	−0.65++	0.41++	−0.77++	

PLH plant height, *TKW* thousand-kernel weight, *FN* falling number, *TW* test weight, *TP* total pentosan content, *SP* soluble pentosan content, *PROT* protein content, *STAR* starch content

+, ++ Estimate greater than once and twice its standard error, respectively

[a] Five environments

Table 4 Estimates of phenotypic (r_p) and genotypic (r_g) coefficients of correlation between line per se (LP) and testcross (TC) performance and relative efficiency (RE) of indirect selection on LP to improve TC for eight traits estimated from each of 220 entries of Pop-A (Lo115 × Lo90) and Pop-B (Lo115 × Lo117) across six environments

Population	Parameter	Plant height	TKW[a]	Falling no.[a]	Test weight	Total pentosan	Soluble pentosan	Protein	Starch
Pop-A	r_p	0.40**	0.59**	0.69**	0.44**	0.30**	0.28**	0.37**	0.54**
	r_g	0.48++	0.68++	0.94++	0.51++	0.50++	0.51++	0.58++	0.66++
	RE	0.70	0.77	1.26	0.49	0.42	1.02	0.98	0.71
Pop-B	r_p	0.70**	0.57**	0.52**	0.57**	0.25**	0.25**	0.49**	0.74**
	r_g	0.80++	0.67++	0.73++	0.72++	0.37++	0.42++	0.62++	0.84++
	RE	0.82	0.75	0.85	0.54	0.37	0.28	0.69	0.83

[a] Five environments

and Geiger 1978). Interestingly, thousand-kernel weight had a similar CV of genotype × environment interaction for inbred lines and testcrosses. This is contrary to the data of Wahle and Geiger (1978) indicating that modern inbred lines have a much higher phenotypic stability than the first-generation lines caused by subsequent selection by the breeders. Accordingly, the line means for thousand-kernel weight were in our study almost doubled compared to the results reported by Geiger and Wahle (1978).

Correlation among traits

Only a few, moderate correlations were consistently found among both populations and both material groups. Plant height was moderately associated with thousand-kernel weight in most instances ($r_g \sim 0.5$), again indicating that straw length is an important fitness parameter in rye (Miedaner et al. 2011) contributing indirectly to grain yield in both inbred lines and testcrosses. High starch content was consistently associated with low protein content ($r_g \sim −0.6$

to −0.8), making selection for high ethanol yields easy (Rode 2008) because exactly this relationship is needed. This might be indirectly caused via kernel architecture as outlined above. Accordingly, high test weight is correlated with high starch content. Among the inbred lines high falling numbers (corresponding to low pre-harvest sprouting) were associated with high total and soluble pentosan contents, indicating that these are key traits for baking quality.

Correlation between per se and testcross performance

Phenotypic and genotypic correlations between per se and testcross performance vary considerably depending on the crop and the trait investigated (e.g., Hallauer et al. 2010; Mihaljevic et al. 2005; Schwegler et al. 2013; Grieder et al. 2012). In our study, moderate to high estimates were found for all traits in at least one of the populations (Table 4) with the highest coefficients for plant height and test weight in Pop-B, and thousand-kernel weight, starch content and falling number (r_g = 0.7–0.9) in both populations. Thus, we suppose mainly additive genetic effects for these traits. This is supported by the high accordance between parental means compared to their progeny means among lines and testcrosses. The results of our study are in good accordance with earlier experimental rye data reporting r_g of 0.8–0.9 for plant height and 0.7 for thousand-grain weight as the only two overlapping traits between previous studies (Köhler 1986; Wilde 1987; Tomerius et al. 2008). For total and soluble pentosan and protein contents, we observed moderate correlations. Obviously, these traits also had a small phenotypic range in the testcrosses only (Fig. 1). We attributed this to the fact that elite lines have been used as parents underlying a strong pre-selection in previous breeding cycles to high baking quality where pentosan contents must be high and protein contents should be low. Alternatively, a tester with a high frequency of favorable dominant alleles results in a reduced correlation between per se and testcross performance (Smith, 1986). As we used a single-cross tester in this study, however, this effect should be reduced (Miedaner et al. 2012).

Efficiency of selection based on per se performance in early generations of multi-stage selection programs

Genotypic coefficients of variation were considerably larger for per se than for testcross performance of all investigated traits (Table 2) as expected from quantitative-genetic theory (Hallauer et al. 2010). This illustrates that it is much easier to select phenotypically among lines than among testcrosses. Accordingly, estimates of entry-mean heritability were larger for per se than testcross performance. The only exceptions were test weight and total pentosan content where the heritability estimate was similar or even lower for line per se than for testcross performance. Consequently, the increased genotypic variance was compensated for these traits by the likewise increased genotype × environment interaction variance. For selecting for low total and soluble pentosan and high protein contents for feeding purposes, genetic variation should be broadened by introgressing specific rye materials with these features.

A pre-selection in the line stage is only valuable if it results in a high response for testcross performance. The effectiveness of this pre-selection can be measured by the relative efficiency of selection (RE, see Table 4). It should be noted, however, that testcross performance might be overestimated in this calculation, because the tester genotype used in this study is most probably not identical with the future seed parent of a commercial hybrid. Nevertheless, a ratio >1 means that indirect response to selection on line per se performance is superior over selection of the testcross progenies directly. RE ratios were different among both populations. For Pop-A indirect selection would be effective for falling number, soluble pentosan and protein contents, because in these traits r_g (LP, TC) is rather high and/or the difference in heritability between line per se and testcross performance is large. Caused by a low r_g (LP, TC), RE of indirect selection for improving testcross performance was lowest in test weight and total pentosan content in both populations and for soluble pentosan content in Pop-B. We assumed a similar selection intensity for this calculation. Having in mind, however, that selection intensity can be much higher on the line stage than for testcrosses caused by the larger number of genotypes that can be tested, also plant height, thousand-kernel weight and starch content should reveal a high indirect selection response (i.e., RE > 1) and should, therefore, be effective for improving testcross performance.

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

Ethical standards The experiments reported in this study comply with the current laws of Germany.

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