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Appreciable genetic correlation between inbred lines and testcrosses facilitates breeding for resistance to Fusarium head blight in hybrid rye (*Secale cereale*)

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Abstract

Hybrid breeding is based on selection of inbred lines in early generations. A sufficient genetic correlation between inbred lines and testcrosses is, however, the prerequisite for a higher selection gain in the hybrids. Therefore, we investigated this crucial parameter for Fusarium head blight (FHB) resistance on 60 inbred lines each of the heterotic groups Petkus and Carsten and their corresponding crosses with two testers each at four (inbred lines) or six (testcrosses) environments (location × year combinations). FHB severity measured as percentage of infected spikelets per plot was used as resistance trait, and a correction was made by using flowering time as covariate. Variances for genotype and genotype–environment interaction were high, and the heritability was estimated .84 and .74 for the line per se performance (Petkus and Carsten) and .71 and .78 for the general combining ability (GCA). For both inbred lines and testcrosses, negative correlations with plant height were found ranging from −.20 to −.51. FHB severity was considerably higher for the inbred lines than for the testcrosses. Genetic variance was lower for testcrosses, and dominance effects of the testers and heterosis may be key factors reducing the general infection level of three-way hybrids. The effect of the tester (i.e., difference between testers) was very small and only significant for the Carsten group. The variance for specific combining ability (SCA) was very small, too. This, and high genetic correlations between line per se and GCA ($r = .82$ for Petkus and $r = .72$ for Carsten), does allow or even favour the selection based on line per se performance. Still, in the last generation before the official trials, testcrosses should also be tested for FHB resistance and mycotoxin contents to reliably improve food and feed quality in rye.

KEYWORDS

flowering date, *Fusarium culmorum*, hybrid breeding, plant height, quantitative-genetic parameters, resistance breeding, *Secale cereale*

1 | INTRODUCTION

Hybrid breeding in winter rye was established in the 1970s as successful as earlier for other cross-pollinating crops. At present, 75% of the rye acreage is grown by hybrid cultivars in Germany (BEE, 2020), and they are also available in Austria, Denmark, Estonia, Poland, Belarus, Ukraine, Russian Federation, Canada and USA. Hybrid cultivars outyield the traditional open-pollinated cultivars by 20% in Germany, and the genetic trend for grain yield calculated from the Official Trials 1985–2016 was .773 dt ha $^{-1}$ year $^{-1}$ for hybrid and .237 dt ha $^{-1}$ year $^{-1}$ for population cultivars (Miedaner & Laidig, 2019). The grain yield of the best hybrid cultivars in the Official Trials ranged from 95.5 to 105.1 dt/ha in 2019–2020 illustrating their high production capacity (Bundessortenamt, Hannover, Germany, personal communication). Hybrid cultivars in rye are developed by crossing inbred lines from the seed (Petkus) with the pollen parent pool (Carsten) using a cytoplasmic-male sterility (CMS) system (Miedaner & Laidig, 2019). The CMS source internationally mostly used is the Pampa cytoplasm (Geiger & Schnell, 1970). Restorer genes must lead to the restoration of pollen fertility after crossing (Geiger, 1972).

Hybrid breeding consists of three phases: (1) production and testing of inbred lines, (2) testing of testcrosses for their general and specific combining ability (GCA and SCA, respectively) and (3) composition of the commercial hybrid cultivar. Final aim of hybrid breeding is a maximum selection gain for testcrosses (hybrids) between the genetically contrasting heterotic pools. This could already be achieved by line testing in early selection stages when the genetic correlation between line and testcross performance and the heritability of a given trait are high enough. Generally, this correlation is affected by the complexity of the trait and the predominant type of gene action (Hallauer et al., 2010).

Fusarium head blight (FHB) caused by *Fusarium graminearum*, *F. culmorum* and other *Fusarium* species is a devastating disease of all cereals including rye in the temperate regions (McMullen et al., 1997). The disease results not only in reduced grain yield but also in contamination with mycotoxins of which deoxynivalenol (DON) and zearalenone (ZON) are among the most important ones. They are strictly regulated by the European Union for human consumption (The Commission of the European Communities, 2007). For feeding, orientation values are existing. Although FHB is less harmful in rye than in wheat, it still leads to contamination with mycotoxins (Gaikpa, Lieberherr, et al., 2020). In artificially infected trials, however, the genetic correlations (r_g) between FHB severity and DON concentrations among a total of 218 inbred lines of rye were high enough to rely on the scoring of symptoms given the large populations handled by breeders (r_g = .62, .84, .93, .94, for four populations, respectively; Miedaner et al., 2003).

Sustainable control of FHB can only be achieved in a joint management system including crop husbandry (crop rotation and soil management), fungicide application and resistance breeding (McMullen et al., 2012). Growing resistant cultivars is the most environmentally friendly and effective measure controlling FHB (Mesterházy, 2020). FHB resistance is also in rye mainly inherited additively by an array of genes with minor and major effects as recently shown by a genome-wide association study on the basis of 465 S_1 inbred lines (Gaikpa, Koch, et al., 2020). Selection of FHB resistance among line populations instead of testcross progenies would lower the costs for seed production because no testcrossing is needed, thus saving time (1 year) and isolation plots. Further, inbred lines exploit a larger proportion of additive genetic variance (Hallauer et al., 2010). This, however, largely depends on the genetic correlation between both material groups. In a previous study on eight agronomic traits, genotypic correlations between inbred lines and testcrosses were high ($r_g \geq .7$) for plant height, thousand-kernel weight, falling number, test weight and starch content but depended also on the tested genetic material (Miedaner et al., 2014). Our main objective was to evaluate this correlation for FHB resistance and the potentially associated traits heading stage, flowering date and plant height in actual elite material of both heterotic groups.

2 | MATERIALS AND METHODS

2.1 | Plant material and field design

Each of 60 inbred lines of winter rye (*Secale cereale* L.) from the Petkus and Carsten gene pool and their corresponding testcrosses with two CMS single cross testers each from the opposite heterotic pool derived from contemporary elite material of KWS LOCHOW GMBH (Bergen, Germany) were used in this study. The lines were evaluated in two locations (Petkus [PET] near Berlin, Federal state of Brandenburg and Wohlde [WOH] near Bergen, Lower Saxony) and two years (2019 and 2020) and thus in four environments (location × year combinations). Testcrosses were also grown in these four environments and additionally at Hohenheim (HOH) close to Stuttgart (Baden-Württemberg) in both years (=6 environments). Entries were mechanically sown in single-row observation plots .8–1.2 m long at a sowing density of 270 kernels/m^2. The used experimental designs were α lattices with two replicates and a block size of 5 or 10 depending on the experiment. The 60 inbred lines of each heterotic group and the respective 120 testcrosses were grown in four experiments adjacent to each other on the same field. To fill up the field designs, check genotypes (inbred lines/hybrids) were used. The experiments were treated with local agronomic practices. To prevent complex interactions between *F. culmorum* and other fungi (Miedaner et al., 2001), fungicides were used in BBCH stages (Meier et al., 2009) 31–33 and 39. Plant growth regulators CCC + Moddus (chlormequat chloride 66% + 250 g/L Trinexapac-ethyl) were applied at BBCH 31 and additionally Camposan (660 g/L Ethephon) at BBCH 37 to prevent preharvest lodging.

2.2 | Inoculation and traits

The highly aggressive single-spore derived isolate of *F. culmorum* FC46 (=IPO 39-01; Snijders & Perkowski, 1990) was used for

inoculum production. Inoculum was prepared on autoclaved wheat kernels as described in detail by Miedaner et al. (1996). Before field inoculation, a spore suspension with a concentration of 1×10^6 spores ml^{-1} was prepared. The plots were then inoculated in the evening. A spore suspension with a dose of about 100 ml m^{-2} was sprayed directly onto the ears with a tractor-driven sprayer. Inoculation took place from the beginning to the end of flowering (BBCH 61-69) and was carried out at least three times at intervals of 2–3 days differently for inbred lines and testcrosses due to the different flowering times. The aim was to inoculate each plot at least once at the timepoint of main flowering (BBCH 65).

FHB severity was visually rated using a scale of 0–100% of infected spikelets per plot starting from the onset of FHB symptoms differentiation (Miedaner et al., 1996). To ensure high differentiation between genotypes and to take temporal differences in infection into account, one to four scorings were carried out. Based on experimental repeatability (calculation as described later), the best scorings were selected resulting in one rating date from PET and WOH and two rating dates from HOH that we combined by calculating a simple mean.

Heading stage was scored once using the BBCH scale (BBCH 51-59). Score 1 corresponded to BBCH stage 51 = *First spikelet is visible, but spike is still in the leaf sheath* and score 9 corresponded to BBCH stage 59 = *Stalk between leaf sheath and spike is at least 10 cm long* (Meier et al., 2009). Scoring was started when the earliest plot reached 9 and the latest was at score 1 to achieve an optimal differentiation. Flowering time was recorded as the date of main flowering (= BBCH 65, i.e., 50% mature anthers are extruded). For this purpose, each plot was observed once a day from the beginning until end of flowering and the date (days after May 1) on which 50% of the plants in a plot reached main flowering was recorded. Plant height was measured once in cm from the ground to the top of the ears excluding awns.

2.3 | Statistical analyses

For all traits, data analysis of the multi-environment trials was done in a two-stage procedure.

At first stage, genotype means (best linear unbiased estimators, BLUEs) were estimated for the *j*th environment (year-location combination, *yl*) separately by using the following mixed model:

$$Y_{ijkl} = \mu_j + g_{ij} + r_k + b_{kl} + e_{ijkl}. \quad (1)$$

where, μ is the general mean of the phenotypic observation Y_{ijkl} for the *i*th genotype *g*, the *k*th replicate *r* and the *k*th block *b* and with the residual error e_{ijkl}. The genotype was modelled as fixed effect and the effects for block and replicate as random. To correct the (negative) impact of repeated inoculation of the full field trial on FHB for early flowering genotypes, the flowering time was used as additional cofactor in the model for this trait. Outliers were removed in the first stage based on a Bonferroni–Holm method (Bernal-Vasquez et al., 2016) and by cross checking the residual plots of the second stage. The BLUEs from the first stage entred into a model accounting for the different environmental effects:

$$Y_{ijm} = \mu + g_i + yl_j + (g * yl)_{ij} + e_{ijm}, \quad (2)$$

where Y_{ijm} was the environment-specific estimate from the first stage modelled by effects for the *i*th genotype *g* in the *j*th environment *yl* with the genotype-environment interaction (*g*yl*), the general intercept μ and the residual error e_{ijm}. To account for the error (structures) from the first stage in the second stage, we used the reciprocal of the squared standard errors (=diagonal of variance–covariance matrix) as (absolute) weights (method 2, Möhring & Piepho, 2009) and thus constrained the residual variance (units) to one. Independent residual variances were defined for the environment-material combinations in the 'rcov' statement of the asreml-function call (Butler, 2009). All factors, except genotype (and intercept), were fitted as random. To report variance components, the genotype was also fitted as random.

To further split the genotypic means of the testcrosses into the parental (genetic) components, the effect *g* for the *i*th genotype from model 2 was replaced by effects for the tester *T* (GCA female), the inbred lines *L* (GCA male) and the interaction of both $T \times L$ (SCA) as well as the respective environment interactions of each component. The model for the analysis of testcrosses could be written as:

$$Y_{ijm} = \mu + T_i + L_i + (T \times L)_i + yl_j + (T * yl)_{ij} + (L * yl)_{ij} + (T \times L * yl)_{ij} + e_{ijm}. \quad (3)$$

where again, genotype effects were taken as random for variance component estimation and fixed for calculation of adjusted means. To fit a common effect for the environment and thus allow a better comparison between (variances of) testcrosses and inbred lines, all data (inbred lines and tester, both genetic pools) were combined into one data frame and analysed by a single mixed model. To still separate the effects for the testcrosses and inbred lines (models 1 and 2) as well as the different breeding pools, the 'at()' option of the asreml-function was used to define the effects for the respective effects accordingly. A general fixed effect was defined for the material-group combination and the resulting coefficients were used to quantify the differences between breeding pools and inbred lines and testcrosses within breeding pools. To assess the genetic correlation between line per se and testcross performance, we extended this model by additionally allowing for unstructured variance–covariance of the line per se and GCA effect and the interaction thereof (bivariate model). This could be fitted by using a variable specifying the material (line vs. testcrosses) and the option 'us()' and 'corgh()' (=us() but with correlation estimate) in the asreml-function call.

The repeatability in the first stage (BLUEs) was calculated by $H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\overline{v_{BLUE}}}{2}}$, where σ_g^2 was the genetic variance and $\overline{v_{BLUE}}$ the average variance of a difference of the BLUEs (formula 19, Piepho & Möhring, 2007), and the heritability for variance components was calculated by: $H^2 = 1 - \frac{\overline{v_{BLUP}}}{2\sigma_g^2}$, where $\overline{v_{BLUP}}$ was the average variance of a difference of the BLUPs (formula 20, Piepho & Möhring, 2007). To account for the additional genotypes (fillers) in the first stage, $\bar{v}$ was

calculated directly from the standard error of a difference matrix where fillers were removed and the genetic variance was calculated by using two dummy variables, one coding fillers as zero and defining random genotype effects and the second defining the non-fillers as zero. In contrast to most conclusions that were based on the GCA of the testcrosses, (Pearson) correlations between the different traits were based on the individual testcrosses (BLUEs). The mixed models we fitted by using ASReml package version 3 (Butler, 2009) within the statistical software R (R Core Team, 2019).

3 | RESULTS

The inoculations resulted in appreciable FHB severities at all individual environments (Table 1). Repeatabilities were high for the inbred lines and moderate to high for the testcrosses. Petkus lines were, on average, 12.9 percentage points more susceptible than Carsten lines, and the testcrosses did only slightly differ between breeding pools (Table 2). On average, inbred lines had 23.0 (Petkus) and 11.5 (Carsten) percentage points higher FHB severity than the corresponding testcrosses. The range of FHB severities was larger among the inbred lines compared with the testcrosses, and the smaller range of testcrosses was also found for other agronomic traits (Table 3). Moreover, inbred lines and testcrosses from the Petkus breeding pool were on average 5.3 and 6.2 cm ($P < .05$) shorter and slightly earlier heading than those from the Carsten breeding pool. On average, inbred lines were shorter (19.1 and 20.0 cm for Petkus and Carsten breeding pool, respectively) than testcrosses; however, some inbred lines of both breeding pools were very tall. Differences between inbred lines and testcrosses in mean heading stage were negligible; however, testcrosses had a more narrow period of heading. Similarly, the flowering date showed, on average, small differences, but the flowering phase among testcrosses was, on average, 2.7 days, among inbred lines 6.2 and 8.5 days for both gene pools, respectively. Heritability estimates were high for these agronomic traits in both inbred lines and testcrosses ranging from .75 to .95 (Table 3).

TABLE 1 Means and repeatabilities (in brackets) for Fusarium head blight severity of 60 inbred lines and their corresponding testcrosses in the individual environments and across environments as well as population means for each of the two testcross populations (TC) in the heterotic pools Petkus and Carsten

	Inbred lines		Testcrosses	
Parameter	**Petkus**	**Carsten**	**Petkus**	**Carsten**
Individual environments:				
HOH-19	NA	NA	30.8 (.62)	31.6 (.62)
PET-19	69.8 (.92)	52.6 (.94)	44.6 (.47)	35.0 (.84)
WOH-19	66.8 (.87)	67.9 (.86)	47.8 (.49)	41.2 (.54)
HOH-20	NA	NA	30.5 (.62)	38.2 (.64)
PET-20	33.4 (.96)	26.1 (.96)	21.8 (.90)	26.0 (.90)
WOH-20	63.5 (.88)	43.2 (.84)	38.9 (.63)	39.2 (.63)
Across environments:				
Mean	59.1	48.22	36.0	34.7
Range	9.9–85.5	17.7–78.6	18.8–46.7	22.3–51.4
$LSD_{5\%}$	18.0	19.7	7.9	8.0
TC 1	NA	NA	34.8[a]	32.0[a]
TC 2	NA	NA	37.1[a]	37.3[b]

Note: If testcross1 and testcross 2 share the same letter for a column the (fixed) effect of both means was not significantly different ($P \leq .05$) by Wald-test of the mixed model.

Abbreviations: HOH, Hohenheim; $LSD_{5\%}$, least significant differences at $P \leq .05$; NA, not assessed; PET, Petkus; WOH, Wohlde, 19 and 20, test years 2019 and 2020, respectively.

TABLE 2 Estimated model coefficients for Fusarium head blight severity with standard errors (in brackets) across four (inbred lines) and six (testcrosses) environments

Intercept			
46.1 (5.3)			
Petkus		**Carsten**	
Inbred lines	Testcrosses	Inbred lines[a]	Testcrosses
12.9 (2.8)	−10.1 (2.3)	0 (0)	−11.5 (3.4)

[a]Carsten inbred lines were defined as the first factor level and thus estimated to be zero.

For the inbred lines, variances were high for both the genotype and the genotype-environment interaction (Table 4). In the Petkus breeding pool, the genetic variance was larger in comparison to the genotype-environment interaction and in the Carsten breeding pool vice versa. The genotype effect for the testcrosses was split into a tester (GCA female), a line (GCA male) and a tester–line interaction effect (SCA). In both breeding pools, variances for tester and SCA were very small and largest for the line and the line–, tester– and SCA–environment interactions. The small tester and SCA variances were additionally proven by a significant correlation between the two testers for FHB severity in each gene pool ($r = .69$ and .78, $P < .001$, for Petkus and Carsten, respectively; Table 5). The heritability for the inbred lines was .74 and .84 (Carsten and Petkus) and .78 and .71 for the testcrosses (Table 4). The heritability of the testcrosses was increased by two additional test environments. If only (the overlapping) four environments were considered in the analysis, heritability dropped to .76 and .61 (Table S1). Additional environments did, however, only marginally influence the sizes and ratios of the genetics-related variances. To deduce implications on the gene action (additive vs. dominant), we calculated a linear regression of testcross performance (per se and GCA) on the line per se performance (Figure 1). In case of only additive gene action at single and across genes, we would expect a slope of .5 because in the testcrosses one allele will be substituted by the tester allele. In the Petkus breeding pool, the slope was estimated to be .21 and .24 (Testers 518 and 618) and in the Carsten breeding pool .29 and .30 (Testers 218 and 318) indicating dominant effects of the testers. Similar results could be obtained on (randomly modelled) genotype basis by dividing the (genetic) covariance by the variance for the inbred lines resulting in .26 (Petkus) and .36 (Carsten).

TABLE 3 Summary statistics for agronomic traits of 60 inbred lines and their corresponding testcrosses with two testers in each of the heterotic pools Petkus and Carsten and means for both testcross (TC) populations across four (inbred lines) and six (testcrosses) environments

	Plant height (cm)		Heading stage (BBCH)[a]		Flowering date[b]	
Parameter	Petkus	Carsten	Petkus	Carsten	Petkus	Carsten
Inbred lines:						
Mean	103.5	108.8	55.9	55.4	27.7	28.7
Range	90.6–123.6	93.0–134.0	52.4–58.3	52.2–59.1	22.9–31.4	25.0–31.2
$LSD_{5\%}$	6.00	6.40	1.53	1.61	1.02	1.21
H^2	.92	.90	.75	.81	.95	.91
Testcrosses:						
Mean	122.6	128.8	55.4	54.9	26.4	25.6
Range	114.6–132.7	118.8–144.8	53.7–56.6	53.6–57.0	25.2–28.0	24.0–26.7
$LSD_{5\%}$	2.82	3.05	.73	.48	.59	.67
H^2	.94	.93	.77	.83	.84	.79
TC 1	125.77[a]	129.56[a]	55.24[a]	55.17[a]	26.48[a]	25.82[a]
TC 2	119.44[b]	128.00[a]	55.50[b]	54.63[b]	26.41[a]	25.47[b]

Note: If testcross1 and testcross 2 share the same letter for a single trait (column) the (fixed) effect for the tester was not significant ($P \leq .05$) by Wald-test of the mixed model, thus both means were not significantly different.
Abbreviations: H^2, entry-mean heritability; $LSD_{5\%}$, least significant differences at $P \leq .05$.
[a]Without WOH 2020.
[b]Days starting from May 1.

TABLE 4 Variance and covariance components with standard errors (SE) plus heritability estimates (H^2) for Fusarium head blight severity of 60 inbred lines and their testcrosses with two testers for each of the heterotic pools Petkus and Carsten across four (inbred lines) and six (testcrosses) environments

	Petkus		Carsten	
Parameter	Estimate	SE	Estimate	SE
Environment (E)[a]	147.0	94.5	147.0	94.5
Inbred lines:				
Genotype (G)	222.1	48.8	132.7	33.8
G × E	147.6	18.4	175.9	21.3
H^2	.84		.74	
Testcrosses:				
Tester (T, GCA female)	.4	3.9	3.5	20.7
Line (L, GCA male)	22.4	6.0	32.7	7.8
T × L (SCA)	1.8	2.2	1.5	2.0
T × E	12.4	7.4	63.5	30.5
L × E	20.9	4.7	24.6	4.6
T × L × E	44.1	4.6	39.1	4.1
$H^2_{GCA\ male}$	.71		.78	
Covariance (correlation):				
G (lines - GCA male)	57.8 (.82)	14.3	47.3 (.72)	13.3
G × E (lines - GCA male)	10.5 (.19)	6.5	20.8 (.32)	7.3

Abbreviations: GCA, general combining ability; SCA, specific combining ability.
Note: All estimates were based on a (bivariate) mixed model defining a single environmental effect for both inbred lines and testcrosses and unrelated (diagonal variance structure) effects for testcross-specific components (Tester, Tester × Line) and unstructured variance–covariance effects for the genotype (line per se = G, and GCA) and the genotype-environment interactions resulting in both variance and covariance (correlation) estimates. The residual-error variance was constrained to one due to the use of a two-stage analysis and fitting errors from the first stage via weights in the second stage.
[a]$N_{\text{inbred lines}} = 4$; $N_{\text{testcrosses}} = 6$; $N_{\text{inbred lines}} \cap N_{\text{testcrosses}} = 4$.

TABLE 5 Phenotypic correlations (Pearson) between inbred lines versus testcrosses and the testcross progenies 1 versus 2 for each of 60 entries across four (inbred lines) and six (testcrosses) environments

Trait combination	Heading stage[a]	Flowering time	Plant height	FHB severity
Inbred lines—Testcrosses:				
Petkus	.77	.68	.77	.59
Carsten	.74	.70	.65	.58
Testcrosses Tester 1—Tester 2:				
Petkus	.60	.70	.88	.69
Carsten	.79	.68	.86	.78

Note: All coefficients of correlation are significantly different from zero at $P = .001$.
[a]Without WOH 2020.

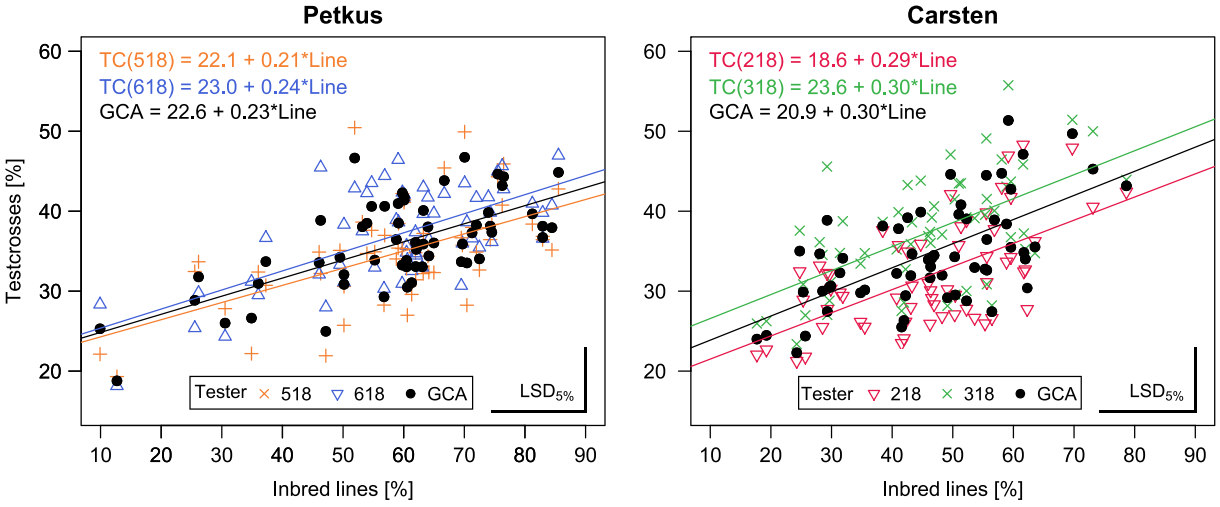

FIGURE 1 Correlation of Fusarium head blight severity (percentage of infected ear) between 60 inbred lines and their testcrosses with each of two testers and general combining ability (GCA = mean, different colour and symbols) for the heterotic pools Petkus (left, Tester 1 = 518; Tester 2 = 618) and Carsten (right, Tester 1 = 218; Tester 2 = 318) across four (inbred lines) and six (testcrosses) environments combined in a single model with common environmental effect. A linear regression was estimated from the best linear unbiased estimates (BLUEs), and the resulting formula and regression line is displayed in the plot. The bars in the right corner have the length of the least significant difference at $P \leq .05$ ($LSD_{5\%}$) [Color figure can be viewed at wileyonlinelibrary.com]

Another approach to test for additive gene action would be based on variance components directly. For solely additive variance σ^2_A, we expect $\sigma^2_{per\,se} = 2\sigma^2_A$ and $\sigma^2_{GCA} = 0.5\sigma^2_A$ so that $\sigma^2_{per\,se} = 4\sigma^2_{GCA}$, with $\sigma^2_{per\,se}$ being the variance component for the inbred lines and σ^2_{GCA} being the variance component for GCA variance. For the Petkus breeding pool, we calculated $\sigma^2_{per\,se} = 9.9\sigma^2_{GCA}$ and for the Carsten breeding pool $\sigma^2_{per\,se} = 4.1\sigma^2_{GCA}$. The genetic correlation between inbred lines and GCA males that can also be calculated from covariance estimates was .82 and .72 for the Petkus and Carsten breeding pool, respectively (Table 4).

For the agronomic traits heading stage, flowering date and plant height, BLUEs of inbred lines and testcrosses were correlated and the coefficients ranged between .65 and .77 ($P < .001$) in both heterotic groups (Table 5). The correlation of FHB severity with heading stage and flowering date was negligible due to the correction of the resistance trait by using flowering date as covariate (Table 6). The correlation of FHB severity with plant height was negative ranging from −.20 (Carsten inbred lines) to −.51 (Petkus inbred lines). Taller entries were generally less susceptible (Figure S1).

4 | DISCUSSION

Resistance to FHB severity in hybrid rye is clearly a quantitatively inherited trait as shown previously (Gaikpa, Koch, et al., 2020). Typical is a significant genetic variation ranging from low to high FHB severity with no genotype being noninfected. It should be noted that rye is generally harder to score than wheat caused by a blue-green colour and a distinct glaucosity of the heads, both reducing the optical difference between prematurely bleached and healthy spikelets.

4.1 | FHB resistance in rye is affected by flowering date and plant height

The dependance of FHB severity on heading stage or flowering date could be removed in this dataset by using the flowering date as covariate. As result, the respective correlations were not significant anymore (Table 6). This is an important feature, because covariation

TABLE 6 Phenotypic correlations (Pearson) between Fusarium head blight (FHB) severity with heading stage (equal to BBCH stages), flowering time (in days after May 1) and plant height (cm) for each of 60 entries in the heterotic pools Petkus and Carsten and for the respective line and testcross (TC) populations across four (inbred lines) and six (testcrosses) environments

	Correlation of FHB[a] severity with …		
Plant material	**Heading stage[b]**	**Flowering time**	**Plant height**
Inbred lines			
Petkus	−.27*	.04	−.51***
Carsten	−.10	−.02	−.20
Testcrosses			
Petkus TC 1	.10	−.01	−.25
Petkus TC 2	−.16	.13	−.28*
Carsten TC 1	−.08	−.16	−.42***
Carsten TC 2	.12	−.21	−.44***

[a]Best linear unbiased estimates (BLUEs) of FHB severity were corrected by the variable flowering time to reduce the (negative) impact on early flowering genotypes.
[b]Without WOH 2020.
*Significant at $P \leq .05$.
***Significant at $P \leq .001$.

between both traits would underestimate the FHB severity of late genotypes. The correlation between FHB severity and plant height is known from wheat (e.g., Mao et al., 2010; Yan et al., 2011) where reduced height (*Rht*) genes occur extensively. Dominant dwarfing genes were not used in the rye material analysed in this paper to the best of our knowledge (J. Eifler, personal communication). Despite this, a negative correlation also occurred in this study though the inoculum was applied by spray inoculation on top of the plants ($r = -.20$ to $-.51$; Table 6). For both, inbred lines and testcrosses tall plants tended to be more resistant which raises the question of the causes. One factor could be the plant architecture, taller plants might have a better microclimate at the height of the ear (more wind, lower moisture) and this could also contribute to the higher resistance of testcrosses compared to the inbred lines. Yan et al. (2011) mainly found an effect of plant height in wheat on type 1 FHB resistance (=resistance to infection). Another cause could be a randomly occurring colocalization of QTLs for FHB severity and plant height because both quantitative traits are inherited by many QTLs scattered across the whole genome (Gaikpa, Koch, et al., 2020; Miedaner et al., 2011). This has been frequently detected in wheat independently from reduced height (*Rht*) genes (Holzapfel et al., 2008; Mao et al., 2010), and in a rye GWAS, two QTLs for plant height were found being associated with FHB-resistance QTLs with moderate effects (Gaikpa, Koch, et al., 2020). For practical breeding, this means that FHB resistance should not be selected without considering plant height, because gradual culm elongation by resistance selection is not tolerable in the already long-strawed rye crop. On the other hand, the moderate correlation allows to select for shorter, more resistant entries although higher population sizes are then needed (Figure S1). Moreno-Amores et al. (2020) recently demonstrated that both plant height and heading date can successfully be incorporated into a genomic selection model.

4.2 | Inbred lines are more susceptible than their corresponding testcrosses

The inbred lines had, on average, a considerably higher FHB severity than the testcrosses. This may be caused by genetic or biological reasons. Genetically, the effect of the testers reduced the line per se performance by factor .2 to .3 as estimated by linear regression of testcrosses on inbred lines. We would expect a reduction by .5 from only additive gene action. The simplest explanation for not fulfilling our expectations would be the presence of dominant alleles for FHB severity in tester genotypes. In a mapping approach for FHB in rye (Gaikpa, Koch, et al., 2020), several QTLs were found, of which a few were reported to be dominant but generally they had low effect sizes. The preponderance of additive gene action would be supported by almost equal sizes of large GCA female and GCA male variances found in experiments based on a factorial mating design (Miedaner & Geiger, 1996). However, as those studies were done decades ago, it may be reasonable that ongoing selection resulted in selection on dominant effects, which are today fixed in breeding pools (and testers) and thus reducing the general FHB level of genotypes by testcrossing. Because breeding of restorer lines (Carsten pool) is generally less challenging than maintainer lines, the Carsten tester used for Petkus lines may have even more (dominant) genes resulting in an even lower slope of the tester regression. Diallel or factorial mating designs are generally better suited to investigate the gene action of the tested breeding material. By only using two testers, the GCA estimate of the testers (female) will not be representative, the GCA of the inbred lines (males) will always be the mean of those two testcrosses and the SCA estimate of each inbred line for both line-tester combinations will be of equal size, just with opposite sign. Consequently, neither the GCA female variance nor the SCA variance could be used to deduce information about additive or dominant effects.

A biological explanation could be that the inbred lines are more prone to *Fusarium* infection due to their inbreeding depression that reduces vitality and the speed of basic physiological processes. As *Fusarium* species are known to benefit from host weakness, the fungus could potentially move faster within the heads of inbred lines compared to testcrosses, thus reaching the embryo earlier and producing a relative higher proportion of infection. Additionally, the inbred lines have considerably shorter heads what also might favour fungal infection because a higher proportion of spikelets per head gets infected compared to the much longer ears of testcrosses in the same period of time. It has been recently shown in maize that considerable parts of the genome get randomly lost during inbreeding (Roessler et al., 2019). So there could be an interaction between inbreeding depression and FHB severity that varies among the inbred lines but, on average, lead to a higher susceptibility of inbred lines versus testcrosses. Inbred lines are also shorter in plant stature what might favour infections considering the negative correlation between FHB severity and plant height.

4.3 | High genetic variance and genetic correlation allows an efficient (pre-)selection of inbred lines

Both inbred lines and testcrosses in both heterotic groups showed a high amount of genetic variance, thus giving a reliable basis for breeding of FHB resistant cultivars. Despite the variance of testcrosses may be smaller than expected from pure additive effects, genetic correlations between line per se and GCA (male) performance of .82 and .72 for the Petkus and Carsten gene pool, respectively, could be considered high enough to select on a line per se basis. Still, the genotype–environment interaction that was also high must be considered and inbred lines must be tested in a reasonable number of environments. The genetic correlation refers only to GCA effects. If we wanted to select also on SCA in the testcrosses, we propose to select genotypes with FHB performance below the regression line (Figure 1) as they may constitute dominant genes that may not be present in the tester genotypes. However, also here, the interaction with the environment must be considered (Table 4). For FHB resistance, it was previously shown that the correlation was affected by selection. In a recurrent selection programme for FHB resistance, the material selected over several cycles showed no correlation between line and testcross performance ($r = .09$), while integrating a non-selected population resulted in a similar phenotypic correlation like in this study ($r = .68$; Miedaner & Wilde, 2019). In our study, no pre-selection for FHB resistance has been taken place, so the 60 inbred lines can be classified as representative for the respective heterotic group. For most disease resistances in rye (powdery mildew, foot rot, stem rust and leaf rust), a high correlation was found that allows an effective line selection (Miedaner & Wilde, 2019). Generally, some selection on inbred lines is always reasonable, irrespective of the testcross performance because inbred lines have to show at least a moderate FHB resistance as they are the basis of the hybrid seed production and FHB infection leads to a reduced amount of seed and, even worse, a reduced seed germination and vitality.

5 | CONCLUSIONS

The genetic correlations between inbred lines and testcrosses of .82 and .72 for the Petkus and Carsten gene pool, respectively, greatly facilitate resistance selection because it is much easier to select phenotypically among inbred lines than among testcrosses for several reasons: (1) A larger part of additive-genetic variance among inbred lines can be exploited, (2) heritabilities are higher (i.e., less locations are needed) and (3) the production of testcross seed is not necessary in this early generation that is usually used in commercial breeding schemes for greatly reducing population size before the costly testcrossing phase starts (Wilde & Miedaner, 2021). At an early selfing stage, also, a genomic prediction for FHB resistance could be integrated into the breeding scheme when training populations from previous cycles exist (Gaikpa, Koch, et al., 2020; Miedaner et al., 2020). Hence, selection on line basis is especially valuable in practical breeding, because selection intensity can be much higher among inbred lines than among testcrosses with the same monetary budget. Selection of FHB resistance based on line performance should, therefore, be effective for improving testcross performance. It can be recommended that at a later stage of line development, also, the best testcrosses are inoculated with *Fusarium* in parallel to the ultimate testing for grain yield, in order to register only the best candidates for the Official Trials. This would enhance the security, because considerable deviations from the regression by individual genotypes are always possible resulting in testcrosses that are more susceptible than expected from the respective parental lines. This would also allow to measure the DON and ZON concentrations in this smaller selected elite population to ensure less mycotoxin contamination in food and feed.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

TM conceived the manuscript and designed and supervised the experiment. MR and FO performed the trait recording in Hohenheim in 2019 and 2020, respectively, and a pre-calculation of all data. SK technically prepared the experiment in Hohenheim in both years and did the preliminary calculations, whereas PG reanalysed the whole dataset and additionally edited the manuscript. JE shared the genetic material and supervised the trait recording in Petkus and Wohlde. All authors read and edited the manuscript.

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DATA AVAILABILITY STATEMENT

Data are available in the article and the supporting material.

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