

Article

Variance Components and Correlations between Doubled Haploid Lines from Two European Flint Landraces and Their Corresponding Testcrosses for Gibberella Ear Rot Resistance, Silking Time, and Plant Height in Maize

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Abstract: Predicting the resistance of hybrids from lines is a relevant approach for accelerating the improvement of disease resistance in hybrid breeding. In this study, genetic variation and covariation among 76 DH lines from two flint landraces, Kemater (KE) and Petkuser (PE), and their corresponding testcrosses (TC) were estimated for the first time for this material for Gibberella ear rot (GER), days to silking (DS), and plant height (PHT). Lines and TC were evaluated in four and two environments, respectively, under artificial infection with GER. TC were, on average, 42% less GER infected than their lines. TC matured 3–4 days earlier and were about 110 cm taller than the lines. GER resistance was 10% higher in KE lines and TC than PE lines and TC. Significant ($p < 0.001$) genotypic and genotype-by-environment interaction variances were found for all traits. Genotypic variances were generally smaller among TC than lines. Broad-sense heritability estimates were moderate to high for GER severity (0.56–0.82) and high for DS (0.78–0.88) and PHT (0.86–0.94) with higher values always observed in lines. Significant, moderate correlations between TC and line per se performance were found for GER resistance in both KE and PE ($r = 0.37$ and 0.55 , respectively). For the two agronomic traits, correlations were higher ($r = 0.59$ – 0.76) than for GER resistance. Genomic prediction accuracies were moderate to high for GER resistance ($r = 0.49$ – 0.63) and generally higher for DS and PHT. In conclusion, a pre-selection of DH lines for GER resistance should be feasible; however, TC should be additionally tested on a later selection stage to aim for GER-resistant hybrid cultivars.

Keywords: Gibberella ear rot resistance; *Fusarium graminearum*; correlations; genomic prediction accuracy; testcrosses; line per se



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1. Introduction

Maize (*Zea mays* L.) is the most important cereal crop after wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) and is grown for food and feed across the world [1–3]. It is considered a major food source with a high contribution to food and nutrition security in diverse regions such as Africa, where the consumption ranges from 52 to 328 g/person/day [1,4]. In industrial countries, maize is often used for feeding livestock in Europe and ethanol production in the United States [2].

Unfortunately, persisting yield gaps were found in maize production across regions [5,6]. Projections in maize production demonstrated the necessity for intensive improvement efforts to close the existing yield gaps in order to satisfy food and feed demands by the growing human populations by 2050 [5,6]. Producers are experiencing several constraints, including high disease infections that cause huge grain losses from field to storage, resulting in up to 30% of yield loss [7]. Toxicogenic ear rots are major components

thereof [8], causing a serious threat to food and feed safety because of their ability to produce a wide range of mycotoxins [9–12]. Different types of toxigenic ear rots caused by *Fusarium* spp. occur depending on the geographical location and prevailing climate or weather [13]. In cooler regions, such as Europe, northern United States, Canada and some higher altitudes in Africa, *Gibberella* ear rot (GER) and *Fusarium* ear rots (FER) are the major types that infect greater proportions of maize. *Gibberella* ear rot is caused by *Fusarium graminearum* or its sexual stage known as *Gibberella zeae*, which reduces yield, affects grain quality, and contaminates the grains with mycotoxins, such as deoxynivalenol (DON) and zearalenone (ZON) [14,15]. *Fusarium* ear rot is caused by *F. verticillioides* (teleomorph *G. fujikoroï*) and some other fungi. In Germany, *Gibberella* and *Fusarium* ear rot infections were recently reported as the most dominant disease in maize with their relative occurrence depending on temperature and humidity in the respective year [16].

Mycotoxin contamination is a strong impetus for breeding *Fusarium* resistance. Their quantification, however, is costly and not achievable in large breeding populations with thousands of entries. Inoculation with an aggressive *F. graminearum* isolate led to strong phenotypic correlations between GER symptoms and DON concentrations amounting to $r = 0.95$ for inbred lines and $r = 0.88$ – 0.91 for testcrosses (TC) as reported by Bolduan et al. [17]. Accordingly, the phenotypic correlation between GER and DON was $r = 0.93$ and between GER and ZON $r = 0.91$ in another set of elite TC [18]. Correlations between GER severity and DON or ZON concentrations were also very strong in a larger line population ($n = 182$, $r = 0.97$ and 0.92 , respectively) [19]. Thus, it is not necessary to invest in the costly and time-consuming mycotoxin analyses as long as artificial infections with an aggressive isolate are performed.

Fungicides are currently not released for this purpose in Germany as they are not fully efficient in the control of *Fusarium* species. They are also harmful to health and the environment [20]. Moreover, the development of high-yielding varieties with improved disease resistance was reported as the most appropriate approach to effectively reduce ear rot damages in maize [14,21–23]. The identification of resistance sources and use of appropriate breeding methods are major steps forward in developing highly ear-rot-resistant maize varieties. European maize landraces encompass several QTLs controlling GER severity that can be introgressed in high-yielding maize varieties [24].

The importance and accuracy of using the performance of parental lines as predictors of hybrid performance were analysed previously for ear rot resistance in maize [18] and *Fusarium* head blight in wheat [25] but only in European materials. For agronomic traits in maize, these correlations have been established already [26,27]. Predicting hybrid resistance from line per se is relevant for reducing selection cycle length, facilitating early breeding stage selection, and maximising gains from selection [28]. Generally, lines display a greater genetic variation and are selected as a first step before using TC for selecting general combining ability (GCA). Thus, a pre-selection of inbred lines on GER resistance would allow one to integrate only the more resistant fraction into the resource-demanding GCA tests. The availability of DH lines should allow for a more accurate prediction because the DH lines are fully inbred and masking effects are avoided. The effective and accurate prediction of hybrid performance requires significant associations between performances of the hybrids and lines for the traits of interest. Because this parameter is highly dependent on the maize materials used, we estimated for the first time the variances and covariances for GER severity from two European flint landraces. In particular, we aimed to analyse: (i) the genetic variation of *Gibberella* ear rot resistance, silking time, and plant height among the double haploid (DH) lines from two European flint landraces and their corresponding TC, and (ii) the accuracy of using line performance as a predictor of hybrid performance for *Gibberella* ear rot resistance. We hypothesise that: (i) genotypic variances are higher in DH lines than the TC and that (ii) DH line performance is a good predictor of TC for GER resistance.

2. Materials and Methods

2.1. Plant Materials

In this study, we used 40 and 36 double haploid (DH) lines from two European flint maize landraces, “Kemater Landmais Gelb” (KE, from Austria) and “Petkuser Ferdinand Rot” (PE, from Germany), respectively, and their corresponding 76 testcrosses (TC), using the French dent line “F353” provided by INRAE as tester [29]. The lines were chosen for testcrossing based on their agronomic appearance in 2018 out of a total set of 500 DH lines described in detail by Gaikpa et al. [24]. The respective crosses were made in an off-season program in Chile.

2.2. Field Experiments

Field experiments were conducted in Hohenheim (near Stuttgart) and Gondelsheim (near Karlsruhe) in Germany. The 76 DH lines were inoculated in 2018 and 2019 in each of the two locations, while the 76 corresponding TC were evaluated in 2019 at the same two locations. The experiments were conducted using two alpha-lattice designs with two replicates each grown adjacent to each other. The line experiment was reported very recently in detail [24]. Therefore, we give here for brevity the most important points only. Each plot consisted of 20 plants in a single row of 3 m length with a distance between rows of 0.75 m and within rows of 0.15 m. Both DH lines and TC were inoculated using the aggressive *Fusarium graminearum* isolate IFA66 described by Bolduan et al. [17] and generously shared by Prof. Dr. M. Lemmens, IFA Tulln, Austria. The ears of 8–10 plants per plot were inoculated at 4–6 days after 50% silk emergence, leaving out the border plants. Approximately 2 mL and 3 mL of the inoculum (concentration 1.5×10^4 spores mL⁻¹) for lines and TC, respectively, were injected into each ear through the silk channel [30]. Data were recorded on Gibberella ear rot (GER) severity (%), days to silking (DS), and plant height (PHT, cm). GER severity was visually recorded at physiological maturity of 8–10 plants per plot as the percentage of symptomatic kernels per ear on a quantitative scale from 0–100%, where 0% = no *Fusarium* mould visible and 100% = entire ear covered with *Fusarium* mould [31].

2.3. Statistical Analysis

Separately for lines and TC, trait values were used to calculate the best linear unbiased estimates (BLUEs) and variance components using the ASReml-R 3.0 package [32] following the mixed linear model described by Gaikpa et al. [24]. The broad-sense (H^2) heritability was estimated following the standard procedure described by Hallauer et al. [33].

Pearson’s product–moment correlation test was performed for GER severity, days to silking (DS), and plant height (PHT) to investigate association patterns between TC and line per se performance of the two DH populations (KE and PE) using the function “cor.test” in R software version 4.0.3 [34]. Moreover, correlations between GER severity and the two agronomic traits were determined. Cross-validation genomic prediction (GP) accuracies were determined using DH lines as the training set and the corresponding testcrosses as the validation set for the two populations separately using the R package “rrBLUP” [35,36]. We used the high-density Affymetrix® Axiom® Maize Genotyping Array optimised for temperate maize [37] with 388,999 SNP markers, as described previously in detail by Gaikpa et al. [24].

3. Results

3.1. Testcrosses and DH Line Performances across Environments

DH lines showed a large variation for GER severity and the two agronomic traits (Table 1). The differences from the line performance in four vs. two environments were rather small, and the reactions of KE and PE lines were similar. GER severity was, on average, 10% higher in DH lines and TC of the PE population than DH lines and TC of the KE population in all environments (Table 1). TC were much less infected with GER than

the lines and their range was smaller. TC were, on average, 4.04 days and 3.15 days earlier and 109.86 cm and 117.25 cm taller than the DH lines for KE and PE, resp. (Table 1).

Table 1. Means and ranges of Gibberella ear rot (GER) severity, days to silking (DS), and plant height (PHT) for DH lines and testcrosses (TC) from “Kemater Landmais Gelb” (KE) and “Petkuser Ferdinand Rot” (PE) across four and two environments (Env.), respectively.

| Parameter | DH Lines–4 Env | | DH Lines–2 Env | | TC–2 Env | |
|-----------|----------------|--------|----------------|--------|----------|--------|
| | KE | PE | KE | PE | KE | PE |
| GER (%) | | | | | | |
| Minimum | 16.49 | 11.44 | 25.11 | 33.09 | 0.74 | 3.48 |
| Maximum | 86.82 | 91.90 | 93.60 | 94.54 | 50.76 | 54.20 |
| Mean | 42.13 | 55.04 | 54.98 | 63.92 | 11.82 | 22.33 |
| DS (days) | | | | | | |
| Minimum | 73.71 | 70.27 | 75.76 | 74.27 | 75.77 | 75.00 |
| Maximum | 86.37 | 84.73 | 92.21 | 86.99 | 82.19 | 80.99 |
| Mean | 79.54 | 78.98 | 82.57 | 81.07 | 78.53 | 77.92 |
| PHT (cm) | | | | | | |
| Minimum | 96.32 | 70.51 | 96.53 | 72.66 | 217.56 | 201.23 |
| Maximum | 189.79 | 145.55 | 203.80 | 149.69 | 297.52 | 268.95 |
| Mean | 129.30 | 110.98 | 133.84 | 114.24 | 243.70 | 231.49 |

Large differences were observed between DH lines and TC in GER severity for both landraces with Petkuser always being more susceptible (Figure 1).

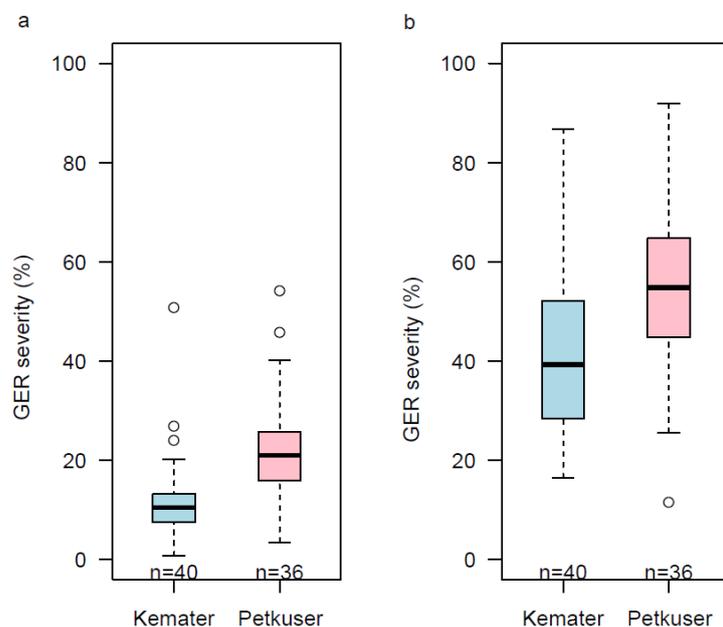


Figure 1. Box plots showing Gibberella ear rot (GER) severity for (a) testcrosses (TC), and (b) DH lines of “Kemater Landmais Gelb” and “Petkuser Ferdinand Rot” (2 environments). The thick horizontal lines in the boxes represent the median values; n = number of entries.

3.2. Variance Components and Heritability Estimates in Testcrosses and DH Lines

Significant ($p < 0.01$) genotypic and genotype-by-environment ($G \times E$) interaction variances were found for all traits among both TC and DH lines (Table 2). Genotypic variances were higher for DH lines than for testcrosses in all traits, as expected. Broad-sense heritability (H^2) was moderate to high, depending on the trait and population. Genotypic variances (σ_g^2) for GER severity and PHT of the DH lines were higher in KE than PE landraces, while the opposite was found for DS. On the other hand, in TC, the

genotypic variances (σ_g^2) of GER and DS were higher for PE than KE, resulting in higher H^2 in PE than KE for both traits.

Table 2. Variance components and broad-sense heritabilities (H^2) for *Gibberella* ear rot severity (GER), days to silking (DS), and plant height (PHT) for DH lines and TC performance from “Kemater Landmais Gelb” (KE) and “Petkuser Ferdinand Rot” (PE).

| Population | Parameter | GER (%) | DS (days) | PHT (cm) |
|------------|------------------------|---------|-----------|----------|
| DH lines | | | | |
| KE | σ_g^2 | 259.81 | 8.87 | 416.66 |
| | σ_{ge}^2 | 78.82 | 3.28 | 60.04 |
| | σ_ε^2 | 308.70 | 3.58 | 86.26 |
| | H^2 | 0.82 | 0.88 | 0.94 |
| PE | σ_g^2 | 211.48 | 11.16 | 207.73 |
| | σ_{ge}^2 | 114.31 | 2.40 | 55.56 |
| | σ_ε^2 | 308.70 | 3.58 | 86.26 |
| | H^2 | 0.76 | 0.91 | 0.89 |
| TC | | | | |
| KE | σ_g^2 | 39.48 | 1.36 | 207.33 |
| | σ_{gl}^2 | 31.49 | 0.26 | 28.65 |
| | σ_ε^2 | 63.13 | 1.02 | 68.72 |
| | H^2 | 0.56 | 0.78 | 0.87 |
| PE | σ_g^2 | 67.26 | 2.35 | 191.90 |
| | σ_{gl}^2 | 28.14 | 0.16 | 26.79 |
| | σ_ε^2 | 63.13 | 1.02 | 68.72 |
| | H^2 | 0.69 | 0.88 | 0.86 |

Furthermore, $G \times E$ interaction variances represented 6.81–79.76% of the genotypic variances (σ_g^2) for all traits in the two populations (Table 2). In the KE population, the relative importance of $G \times E$ interaction variance (σ_{ge}^2) was higher among TC (79.76% of σ_g^2) than among DH lines (30.33% of σ_g^2) for GER severity. On the contrary, the relative proportion of $G \times E$ interaction variance was relatively higher in DH lines than TC lines for DS and similar in both lines for PHT. In the PE population, $G \times E$ interaction variances were smaller in TC lines than DH lines in comparison to the corresponding genotypic variances for all traits.

3.3. Correlations and Genomic Prediction Accuracies between Testcrosses and DH Line Performances under *Gibberella* Ear Rot Infection

Positive significant correlations were detected between DH lines and testcrosses for both KE and PE populations (Figure 2a). The correlation for GER severity was moderate between KE lines and TC ($r = 0.37$) and also for PE lines and TC ($r = 0.55$). However, the correlation observed for GER severity was lower compared to that of days to silking and plant height, which revealed high positive correlations between line per se and testcrosses for both KE and PE populations (Figure 2). In addition, correlation coefficients were lower in the KE population for GER severity and days to silking but not for plant height. In addition, pairwise correlations between GER severity, days to silking, and plant height were weak and non-significant for both DH and TC lines in the two populations (Figure S1).

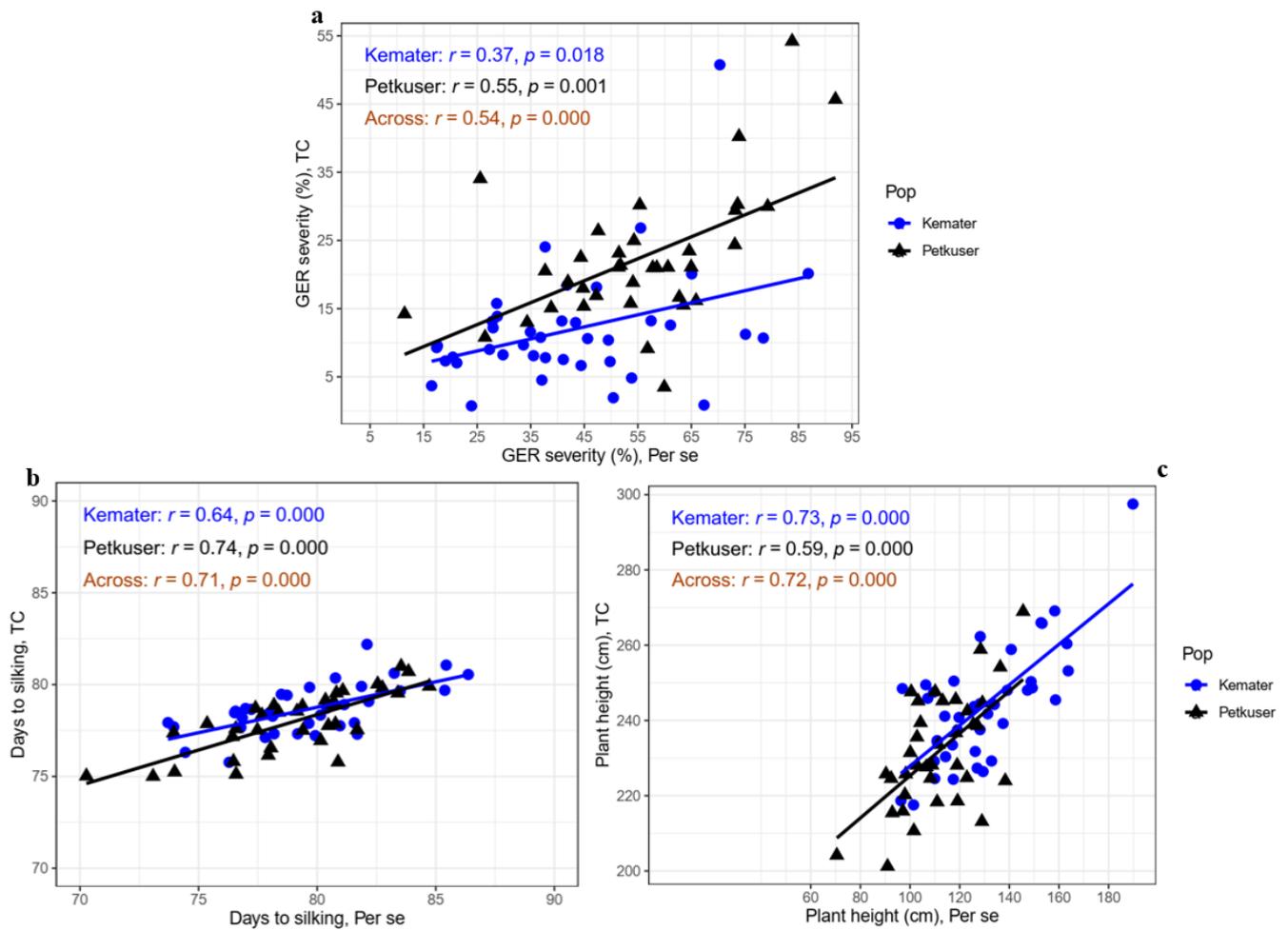


Figure 2. Scatter plots showing correlations (r) between testcross (TC) and line per se performance of two landrace populations (Pop) for (a) Gibberella ear rot (GER) severity, (b) days to silking, and (c) plant height.

The genomic prediction analysis using the line per se data as the training set and the TC as the validation set revealed a relatively high cross-validation prediction accuracy (0.49 for KE and 0.63 for PE) for GER severity (Figure 3) compared to what was reported previously within KE and PE DH lines [24]. Genomic prediction accuracies were relatively higher (0.49 to 0.85) compared to the phenotypic correlations for all traits. However, the prediction accuracy for GER severity remained lower compared to that of days to silking and plant height in both the KE and PE populations. Likewise, the prediction accuracies in the KE population were lower compared to those of the PE population for all traits.

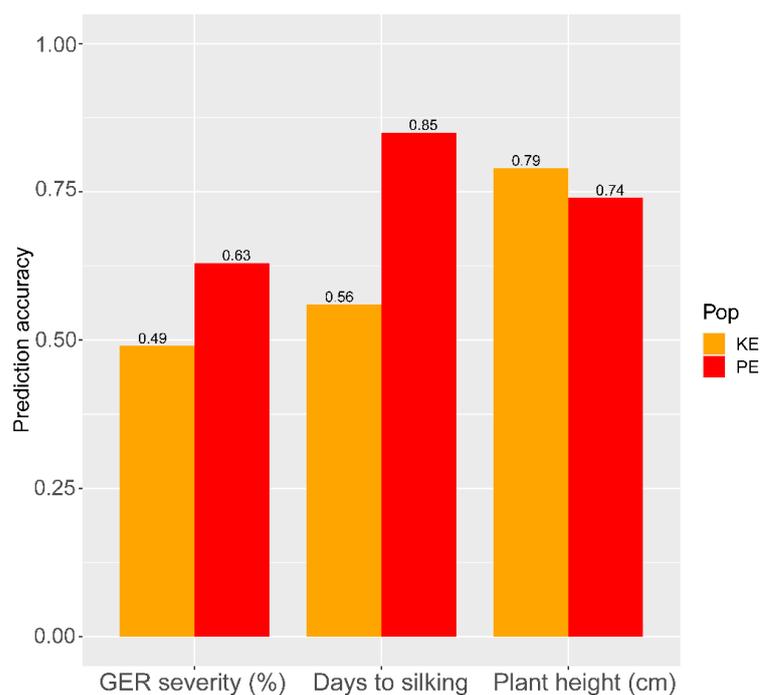


Figure 3. Cross-validated prediction accuracies for *Gibberella ear rot* (GER) severity, days to silking, and plant height using the line per se data as the training set and the corresponding testcrosses as the validation set for “Kemater Landmais Gelb” (KE) and “Petkuser Ferdinand Rot” (PE).

4. Discussion

Understanding association patterns between DH lines and TC with regard to key traits of interest is of paramount importance for the accurate selection of parents in hybrid breeding schemes. This study was conducted to investigate for the first time the GER resistance among TC and DH lines from two old European landraces, Kemater (KE) and Petkuser (PE), and to compare the correlations with the agronomic traits days to silking and plant height.

4.1. DH Lines Are Considerably More Susceptible Than Their Testcrosses

The DH lines revealed a much higher GER severity than that of their corresponding TC for both landraces. This difference is governed by (1) the resistance of the tester, (2) the inheritance of GER resistance, and (3) the effect of inbreeding depression. The choice of the tester is an important feature in hybrid breeding. A good tester should discriminate the lines and rank them correctly for their general combining ability for the trait [38]. In our case, the tester may have been very resistant, and/or the resistance could have been due, at least partly, to dominant alleles. GER resistance was reported to be quantitatively inherited with both additive and dominance effects according to Butrón et al. [39] and Martin et al. [15]. For the KE population, the presence of additive gene effects was recently shown by Gaikpa et al. [24] using GWAS and genomic prediction approaches. Plant height did perfectly follow the expectations of a two times higher line per se variance than testcross variance under the assumption of an additive gene action. The real cause of the difference between line and TC performance can, however, not be determined, because the tester line was unfortunately not included in the experiment. However, in the following year, the tester line F353 proved to be moderately susceptible to GER (25–57%, Bettina Kessel, pers. commun.). The large effect of the tester on GER resistance was shown previously by Löffler et al. [18], who used two highly susceptible flint testers with 74% and 89% GER severity, respectively, with the outcome that the inbred lines were more resistant than the TC. In contrast, Bolduan et al. [17] used a resistant and a highly susceptible tester (26% vs. 97% GER resistance), and the TC were similarly susceptible or resistant (30% vs. 73%).

Another cause for the high mean susceptibility of the lines could be inbreeding depression by uncovering recessive deleterious variants [40]. These DH lines were directly drawn from the landraces by female pathogenesis and are unselected samples being fully inbred in one step; i.e., they have never been selected for inbreeding tolerance before. Thus, inbreeding depression is expected to be high, and, indeed, many signs of inbreeding defects were visible among the line populations, such as low emergence rate, poor growth rate, lodging, poor seed set, and high tillering. Additionally, unwanted traits—such as high leaf chlorosis; tillering; and extreme susceptibility to common smut (*Ustilago maydis*), common rust (*Puccinia sorghi*), and several viruses—were reported [24,41,42]. This is the reason why about 70% of DH lines from such landraces could not be used in practical breeding [41]. Because *F. graminearum* is a fungus that could exploit the physiological weakness of hosts to accelerate the infection process, inbreeding depression might have favoured GER severity [19]. Although all DH lines have the same maximal inbreeding coefficient, they can still suffer differently from inbreeding depression according to their genomic makeup, and it has recently been shown that considerable parts of the genome are randomly lost during inbreeding [40]. Therefore, there could be an interaction between the suffering from inbreeding depression and GER severity that varies among the lines but, on average, leads to a much higher susceptibility of lines vs. TC. On the other hand, inbreeding depression also indicates heterosis for GER resistance in TC. Accordingly, Bolduan et al. [17] found a low-to-moderate mid-parent heterosis for GER resistance within elite flint lines of 9 percentage points for the susceptible tester and 34 percentage points for the more resistant one. In our study, the TC were, on average, three to four days earlier and about 110 cm taller than the DH lines, reflecting the known high heterosis of these agronomic traits.

Our results revealed that DH lines of the KE population were, on average, more resistant to Gibberella ear rot (GER) than DH lines of the PE population. This is in accordance with the results of a larger study on the same landraces with 250 DH lines per landrace. Similarly, TC developed from the KE population were more resistant than those from the PE population.

Good hybrid performance for GER resistance is also stressed by Mesterhazy et al. [22] with different *Fusarium* spp. ear rots, indicating that hybrid breeding could considerably help to better manage ear rot diseases and achieve higher food safety when both parents of the hybrid are under selection.

4.2. Variance Components Show Large Differences between Lines and Testcrosses

Genotypic variances for DH lines were very high for GER severity. Accordingly, broad-sense heritability (H^2) estimates of GER resistance in KE and PE populations were, respectively, 0.82 and 0.76 in lines, and 0.56 and 0.69 in derived TC. These estimates were higher than values reported by Wen et al. [21]. However, the two agronomic traits showed even higher heritability estimates throughout, especially for the TC.

For all traits and populations analysed, the genotypic variances and heritability estimates for DH lines were higher than for TC following quantitative–genetic expectations [17,43]. However, although we used a single line as tester, the variances among lines were much higher than expected. Again, this may be due to random inbreeding effects among lines that also inflate the variances. Additionally, the presence of non-additive gene effects, particularly with GER severity and days to silking, could be a cause. Of course, the differences in genotypic variances also reflect the high differences in means between DH lines and TC.

4.3. Moderate Associations and Genomic Prediction Accuracies between Line and Testcross Performance

Positive moderate associations between TC and DH lines were found for GER severity ($r \sim 0.5$) averaged over both landraces. This is consistent with previous studies that found similar association patterns between TC and line per se for GER rating [17] and reduced mycotoxins concentrations [18] in European elite maize. The existence of significant associations could facilitate the prediction of hybrid performance from line per se as

reported by Ertiro et al. [26] for fodder quality traits in maize. However, associations for days to silking and plant height were considerably higher when calculated across populations ($r \sim 0.7$), indicating a preponderance of additive inheritance for these traits and reflecting the higher H^2 . Accordingly, the prediction of days to silking and plant height of maize hybrids by their line per se performance is already routine in large-scale breeding programs [27,44]. For GER resistance, however, the moderate estimates of the correlation between DH lines and TC, which are confirmed by the literature, will result in only a moderately correlated indirect selection response for TC [17]. Therefore, there should only be a mild selection for line performance followed by selection among TC in a subsequent step. In any event, TC are produced for selecting the combining ability for yield. When selecting the 10% best KE lines, these were also among the 10% best TC; however, for PE this was not the case. Furthermore, relatively high prediction accuracies were found for GER resistance over both landraces ($r \sim 0.6$), indicating the relevance of genomic selection in predicting GER resistance of hybrids using line per se performance [13]. Our genomic prediction should be considered as preliminary due to the restricted number of entries and environments; however, it is a first promising approach that should definitely be pursued further.

The pre-selection on the basis of line performance has the enormous advantage that the full variance of the lines is under selection, although the total variation might be triggered by inbreeding defects. However, the most susceptible lines should nonetheless be discarded, as they could lead to seed quality issues in line multiplication and commercial hybrid production. Lines should at least have a basic resistance to GER to avoid bad seed quality and low emergence rates.

5. Conclusions

The development of high-yielding varieties with improved disease resistance is an appropriate approach to effectively reduce Gibberella ear rot (GER) damage in maize. Significant genetic variation is available in DH lines from the Kemater (KE) and Petkuser (PE) landraces to effectively develop high GER-resistant maize hybrids. Large genetic variation was found among lines for GER severity, days to silking, and plant height. TC were considerably more resistant to GER than DH lines. Moderate correlations were found between TC and DH lines for GER resistance, indicating the possibility of a pre-selection of large DH populations by discarding the most susceptible lines in a first selection stage before a second more rigid selection on a TC basis to develop GER-resistant maize hybrids. Considering the presence of large genotype-by-environment interaction variances and the complexity of quantitative traits, genomic approaches, such as genome-wide association (GWAS) and genomic selection (GS), could be used as a complement of phenotyping for more effective prediction of GER resistance of hybrids from line performance.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11061039/s1>, Figure S1: Scatter plots showing correlations (R) between GER severity and the two agronomic traits. (a) GER severity vs. days to silking for line per se, (b) GER severity vs. plant height for line per se, (c) GER severity vs. days to silking for TC, and (d) GER severity vs. plant height for TC.

Author Contributions: Conceptualisation, T.M. and D.S.G.; methodology, T.M.; software, F.A. and D.S.G.; validation, F.A. and D.S.G.; formal analysis, F.A. and D.S.G.; investigation, D.S.G.; resources, B.K., T.P.; data curation, F.A. and B.K.; writing—original draft preparation, F.A.; writing—review and editing, T.M., D.S.G.; visualisation, F.A.; supervision, T.M.; project administration, T.M. and T.P.; funding acquisition, F.A. and D.S.G. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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