Theor Appl Genet (2015) 128:2471–2481
DOI 10.1007/s00122-015-2602-1

ORIGINAL ARTICLE

Potential and limits of whole genome prediction of resistance to Fusarium head blight and Septoria tritici blotch in a vast Central European elite winter wheat population

Vilson Mirdita[1] · Sang He[1] · Yusheng Zhao[1] · Viktor Korzun[2] · Reiner Bothe[2] · Erhard Ebmeyer[2] · Jochen C. Reif[1] · Yong Jiang[1]

Received: 4 May 2015 / Accepted: 28 August 2015 / Published online: 8 September 2015

Abstract
Key message **Fusarium head blight and Septoria tritici blotch resistances are complex traits and can be improved efficiently by genomic selection modeling main and epistatic effects.**
Abstract Enhancing the resistance against Fusarium head blight (FHB) and Septoria tritici blotch (STB) is of central importance for a sustainable wheat production. Our study is based on a large experimental data set of 2325 inbred lines genotyped with 12,642 SNP markers and phenotyped in multi-environmental trials for FHB and STB resistance as well as for plant height. Our objectives were to (1) investigate the impact of plant height on FHB and STB severity, (2) examine the potential of marker-assisted selection, and (3) study the prediction ability of genomic selection modeling main and epistatic effects. We observed low correlations between plant height and FHB ($r = -0.15$; $P < 0.05$) as well as STB severity ($r = -0.17$; $P < 0.05$) suggesting negligible morphological resistances. Cross-validation in combination with association mapping revealed absence of large effect QTL impeding an efficient pyramiding of different resistance loci through marker-assisted selection. The prediction ability of genomic selection was high amounting to 0.6 for FHB and 0.5 for STB resistance. Therefore, genomic selection is a promising tool to improve FHB and STB resistance in wheat.

Communicated by H. Buerstmayr.

V. Mirdita and S. He contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-015-2602-1) contains supplementary material, which is available to authorized users.

✉ Jochen C. Reif
reif@ipk-gatersleben.de

Vilson Mirdita
mirdita@ipk-gatersleben.de

[1] Department of Breeding Research, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstraße 3, Gatersleben, 06466 Stadt Seeland, Germany

[2] KWS Lochow GmbH, Bergen, Germany

Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum*, and Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* (*teleomorph Mycosphaerella graminicola*) severely impact wheat production worldwide. Occurrence of both diseases entails a reduction of grain yield and quality (Buerstmayr et al. 2009; Miedaner et al. 2013). Efforts to control FHB and STB comprise crop rotation, soil tillage, fungicide application, and cultivation of resistant varieties (Yuen and Schoneweis 2007; Kutcher et al. 2011; Willyerd et al. 2012). Fungicide applications against FHB are only operative in a narrow time window (Paul et al. 2010). Moreover, strobilurins, the most widely applied fungicide class against STB, are no longer effective due to mutations in the highly variable pathogen population of *Mycosphaerella graminicola* (Torriani et al. 2009). Consequently, breeding of varieties resistant against FHB and STB is the most sustainable approach to combat both diseases.

Reliable high-throughput phenotyping based on artificial inoculation has been developed to monitor FHB resistance and is routinely deployed in wheat breeding programs (Miedaner and Korzun 2012). In contrast, for STB resistance, artificial inoculation is challenging, because of the strong dependency of the disease pressure on temperature and humidity. In addition, artificial inoculations are for

both diseases labor-intensive necessitating phenotyping in multi-environmental trials (Miedaner et al. 2012).

Marker-assisted selection has been promoted as a promising alternative to phenotypic selection to decrease FHB and STB susceptibility in wheat (Miedaner et al. 2009; Agostinelli et al. 2012). The success of marker-assisted selection for both traits, however, is in Europe so far limited mainly due to absence of major effect loci in elite germplasm and the small to medium population sizes used for QTL detection (Holzapfel et al. 2008; Buerstmayr et al. 2009; Löffler et al. 2009; Miedaner et al. 2011; Miedaner and Korzun 2012). Use of large effect QTL originating from exotic donors is impeded by difficulties to get rid of linkage drag and/or negative pleiotropic effects (Becher et al. 2013).

Genomic selection has been proposed as a powerful tool to enhance prediction accuracy of FHB (Rutkoski et al. 2012) and STB resistance (Miedaner et al. 2013). In genomic selection, the complex genetic architecture is properly handled and genetic relatedness is additionally exploited to predict the performance of non-phenotyped lines (Habier et al. 2007). Recent studies based on a population of nearly 2000 wheat hybrids demonstrated the potential to enhance FHB and STB resistance through genomic selection (Miedaner et al. 2013; Mirdita et al. 2015). Experimental findings for wheat inbred lines are based on small populations ranging from around 200 (Rutkoski et al. 2012) to less than 400 genotypes (Jiang et al. 2015). The magnitude of prediction accuracies is despite the small population sizes encouraging. A large-scale study on the potential and limits of genomic selection to improve FHB and STB resistance, however, is still lacking.

Experimental studies on the accuracy of genomic selection in wheat were often restricted to prediction approaches modeling additive (e.g., Rutkoski et al. 2012; Ornella et al. 2012; Bordes et al. 2014) and dominance effects (Zhao et al. 2013, 2014). Additive and dominance effects are, however, only one component of the genotypic value which also involves epistatic effects (Falconer and Mackay 1996). Genomic selection models have been proposed considering besides main also epistatic effects (Xu 2007; Cai et al. 2011; Wittenburg et al. 2011; Wang et al. 2012). Implementing these approaches is hampered mainly because of the high computational load especially if a large number of markers are available (Jiang and Reif 2015). An attractive solution to reduce the computational load is using an extended genomic best linear unbiased prediction model (EG-BLUP) which is equivalent to a ridge-regression BLUP explicitly modeling epistasis (Jiang and Reif 2015). A further promising alternative is to apply kernel Hilbert space regression (RKHSR; Gianola et al. 2006) which also captures epistatic effects among markers (Gianola and van Kaam 2008; Morota and Gianola 2014; Jiang and Reif 2015).

Our study is based on a large experimental data set of 2325 wheat inbred lines of a commercial breeding program genotyped with 12,642 SNP markers and phenotyped in multi-environmental trials for FHB and STB resistance as well as for plant height. Our objectives were to (1) investigate the impact of plant height on FHB and STB severity, (2) examine the potential of marker-assisted selection, and (3) study the prediction ability of genomic selection modeling main and epistatic effects.

Materials and methods

Plant materials and field trials

A total of 2325 European winter wheat (*Triticum aestivum* L.) lines adapted to Central Europe were used for this study and visually evaluated for FHB and STB resistance in separated plots. Genotypes were advanced breeding lines of the breeding company KWS LOCHOW GmbH (Bergen, Germany) as well as registered varieties. The 2325 lines were evaluated in multi-environmental field trials for FHB resistance in the years 2012 and 2013 in up to five environments (Table S1) with 154 lines tested in both years. The entries were divided into 16 individual trials connected through five common checks (Cubus, KWS Erasmus, Julius, JBAsano, Colonia). The experimental design for each trial was an alpha design with two replications per location with the number of entries per trial ranging from 36 to 308. Plot size was 0.5 m^2 and sowing density was 350 grains m^{-2}. Genotypes were artificially spray-inoculated as described in detail by Kollers et al. (2013). Spray inoculations were performed with 50,000 spores per mL of Fusarium graminearum and Fusarium culmorum isolates (1/3 F.g.:2/3 F.c.) using a water volume of 600 L ha^{-1}. Inoculum production followed established protocols (Miedaner et al. 1996). Briefly, directly before inoculation, wheat kernels were rinsed off with tap water. Spores were counted and diluted for spray inoculation with a common plot sprayer. To compensate for different flowering times of the wheat lines, inoculation was carried out four times starting with the first 10 % of genotypes flowering and an interval of 3–4 days. This procedure should permit the inoculation of each genotype at least once at full flowering (GS65–GS69; Zadoks et al. 1974). FHB infection was visually scored as the percentage of infected ears per plot in an ordinal scale ranging from 0 (fully resistant) to 100 (fully susceptible). For resistance comparison, the arithmetic mean of all individual ratings over the recording dates was used.

The 2325 lines were additionally evaluated in multi-environmental field trials for STB disease severity in up to 11 and plant height in up to nine environments in the years 2012 and 2013 (Table S1). The entries were divided into 16 individual trials connected through five common genotypes (Cubus,

KWS Erasmus, Julius, JBAsano, Colonia). The experimental designs were again alpha designs. In all environments, the disease scoring was based on natural infection. STB disease severity was visually scored plot wise as coverage of flag leaves with lesions bearing pycnidia on a scale from 1 (fully resistant) to 9 (fully susceptible). Plant height was measured in centimeters from soil level to the top of the ear at one time point after heading in each environment referring to the guidelines for official variety testing in Germany.

Phenotypic data analyses

We performed an unweighted two-stage phenotypic data analysis. In the first stage, we analyzed the data for each environment separately using a linear mixed model including genotype, trial, replication, incomplete block, and residual effects. The repeatability in each environment was estimated as $\frac{\sigma_G^2}{\sigma_G^2+\frac{\sigma_e^2}{R}}$, where σ_G^2 refers to the genotypic variance, σ_e^2 is the residual variance and R denotes the number of replications for each genotype. The corresponding variance components were estimated by the restricted maximum likelihood (REML) approach assuming that all effects are random. To estimate the adjusted mean for each genotype in each environment, we fitted an alternative model with fixed genotypic effects. Then, in the second stage, we combined the adjusted means of the genotypes for all environments and fitted again a linear mixed model with genotype, environment and residual effects. The heritability on the line mean basis was estimated as $h^2 = \frac{\sigma_G^2}{\sigma_G^2+\frac{\sigma_e^{-2}}{E}}$, where σ_G^2 refers to the genotypic variance, σ_e^{-2} is the residual variance, E denotes the number of environments tested for each genotype. Since our data was unbalanced, we obtained the expected heritability for subsets of genotypes tested in any specific number of environments. Moreover, we estimated the average heritability setting E as the mean across genotypes. As in the first stage, we first fitted the model with all random effects to estimate the variance components. Then, we fitted another model with fixed genotypic effects to obtain the best linear unbiased estimation (BLUE) of the genotypic value for each genotype across environments. All linear mixed models were implemented using ASReml-R (Gilmour et al. 2009). Note that we used the BLUEs of genotypes in subsequent analyses instead of best linear unbiased prediction (BLUP). BLUEs are in contrast to BLUPs non-shrinked estimates of the genotypic values, which is an important property for further analyses in genomic selection or association mapping.

Genotypic data analyses

The wheat lines in 2012 were genotyped by a 9 k SNP array based on an Illumina Infinium assay (Cavanagh et al. 2013), while the lines in 2013 were genotyped by a 90 k SNP array based on an Illumina Infinium assay (Wang et al. 2014) (Illumina, San Diego, CA, USA). Only 154 lines were tested in both years. We imputed the missing genotypic data, i.e., markers present on the 90 k but absent on the 9 k array using the IMPUTE2 algorithms (Howie et al. 2009; He et al. 2015). After quality control for minor allele frequency above 0.05 following Zhao et al. (2014), 12,642 SNP markers remained as the final genotypic data for all 2325 lines.

We estimated the Rogers' distances (Rogers 1972) for each pair of lines using the imputed marker data. The genetic similarity for a pair of lines was calculated as one minus the Rogers' distance. A hierarchical cluster analysis was performed based on the matrix of pair-wise one minus Rogers' distances.

Genome-wide association mapping and marker-assisted selection

Association mapping was performed using following linear mixed model (Yu et al. 2006):

$$y = \mu + m\alpha + Zg + e$$

where y is the vector of BLUEs for each genotype across environments, μ is the vector of common intercept term, α is the effect of the marker under consideration, m denotes the vector of marker indices, g is the vector of genotype effects, Z is the corresponding design matrix, and e is the residual term. We assumed that the marker effect is fixed and all other effects are random. The population structure was controlled by assuming $g \sim N(0, G\sigma_G^2)$, where σ_G^2 refers to the genotypic variance estimated by a maximum likelihood (REML) approach. G is the genomic relationship matrix estimated based on the marker data following VanRaden (2008) as $G = \frac{WW'}{2\sum_{k=1}^{p} p_k(1-p_k).}$, where $W = (w_{ij})$ is an $n \times p$ matrix with $w_{ij} = x_{ij} - 2p_j$ and x_{ij} is the number of a chosen allele at the j-th locus for the i-th genotype, p_j denotes the allele frequency of the j-th marker. Significance of marker–trait associations was tested based on the Wald F statistic.

The prediction ability of marker-assisted selection was evaluated by fivefold cross-validation with a total of 100 cross-validation runs, using the full data set combining all lines across two years. In each run of cross-validation, the lines were randomly divided into five subsets. Four of the five subsets were used as the estimation set and the remaining one formed the test set. The procedure was repeated 20 times, yielding in total 100 different combinations of estimation and test sets. In practice, 100 runs of cross-validation led to stable estimation of prediction ability and have been applied in a number of previous studies (e.g., Zhao

et al. 2014; Jiang et al. 2015). For each estimation set, we performed association mapping and recorded the detected QTLs under six different significance thresholds. Then, for each threshold, a multiple linear regression model was fitted in the estimation set with detected significant markers. The effects of the significant markers were estimated and then used to predict the genotypic values of the lines in the test set. The cross-validated prediction ability was calculated as the Pearson product–moment correlation between predicted and observed genotypic values of the lines in the test set.

We also calculated the effective number of significant markers as proposed by Jiang et al. (2015). This parameter was estimated as following: we first performed principal component analysis with the significant markers among the lines (in the estimation set), and then extracted the minimal number of principal components needed to portray 95 % of the total variation. Calculations were done with the statistical software R (R Core Team 2014) and the R package GAPIT (Lipka et al. 2012).

Genomic selection

Four genomic selection models, including two additive and two epistatic models, were applied to evaluate the prediction accuracy. The ridge-regression best linear unbiased prediction (RR-BLUP; Whittaker et al. 2000; Meuwissen et al. 2001) and Bayes-Cπ (Habier et al. 2011) only consider additive effects of markers. While the reproducing kernel Hilbert space regression (RKHS; Gianola and van Kaam 2008) and extended genomic best linear unbiased prediction (EG-BLUP; Jiang and Reif 2015) exploit both the additive and additive × additive epistatic effects among markers.

Let n be the number of genotypes, p be the number of markers and l be the number of environments. The RR-BLUP model has the form $y = 1_n\mu + X\alpha + e$, where y is the vector of BLUEs of genotypic values obtained in the phenotypic data analyses, 1_n denotes the vector of 1′s, μ is the overall mean, α is the vector of additive effects of markers, $X = (x_{ij})$ is the $n \times p$ matrix of markers with x_{ij} being the number of a chosen allele at the j-th locus for the i-th genotype, and e is the residual. In the model, we assumed that marker and residual effects are random and $\alpha \sim N\left(0, \sigma_\alpha^2\right)$, $e \sim N(0, \sigma_e^2)$, where $\sigma_\alpha^2 = {}^{\sigma_G^2}/_{p}$ and $\sigma_e^2 = {}^{\sigma_R^2}/_{l}$. Note that σ_G^2 and σ_R^2 are the estimated genotypic and residual variance components in the phenotypic data analyses. The estimation of α is given by the mixed model equations (Henderson 1975).

The Bayes-Cπ model shares the basic setting $y = 1_n\mu + X\alpha + e$ with RR-BLUP. An additional random variable π, whose prior distribution is uniform on the interval $[0, 1]$, is introduced to the model. The marker effect α is assumed to be zero with probability π and $\alpha \sim N\left(0, \sigma_\alpha^2\right)$ with probability $(1–\pi)$. The variance σ_α^2 has a scaled inverse Chi-squared prior distribution. The prior distribution of the residual is $e \sim N(0, \sigma_e^2)$ and σ_e^2 also has a scaled inverse Chi-squared prior distribution. The parameters in the model were inferred via a Gibbs sampler algorithm, which was run for 10,000 cycles. The first 1000 cycles were discarded as burn in and the samples of α from all later cycles were averaged to obtain estimates of the marker effects.

The RKHS model is of the form $y = 1_n\mu + Zg + e$, where y, 1_n, μ and e are the same as in the RR-BLUP model, g is the vector of genotypic values and Z is a design matrix allocating phenotypic records to genotypes. We assumed that $g \sim N(0, K\sigma_g^2)$, where $K = (k(x_i, x_j))$ is an $n \times n$ semi-positive definite matrix whose entries are functions of marker profiles of pairs of genotypes. In this study, we chose the Gaussian kernel matrix K, i.e., $k(x_i, x_j) = \exp\left[-\frac{x_i - x_j}{h}\right]$. We used the kernel averaging method to optimize the value of h and the Bayesian approach to estimate the parameters in the model (de los Campos et al. 2010).

The EG-BLUP model has the form $y = 1_n\mu + Zg_1 + Zg_2 + e$, where y, 1_n, μ, e and Z are the same as in the RKHS model. g_1 denotes the vector of additive genotypic values and g_2 is the vector of additive × additive epistatic genotypic values. We assume that $g_1 \sim N(0, G\sigma_{g_1}^2)$ and $g_2 \sim N(0, H\sigma_{g_2}^2)$. G denotes the $n \times n$ genomic relationship matrix among all genotypes calculated as in VanRaden (2008), which is the same as in the association mapping. H is the epistatic relationship matrix defined as $G\#G$ (Henderson 1985), where # denotes the Hadamard (element-wise) product of matrices. Parameters were estimated using the Bayesian approach with the multi-kernel method (Pérez and de los Campos et al. 2014).

The prediction abilities of the four models for each trait were evaluated in a fivefold cross-validation scheme as in the marker-assisted selection. The ability of prediction was defined as the correlation between observed and predicted genotypic values of the lines in the test set: $r_{GS} = cor\left(y_{\text{pred}}, y_{\text{obs}}\right)$.

The RR-BLUP and the Bayes Cπ model were implemented using R (R Core Team 2014). The RKHS and EG-BLUP model were implemented using the R package BGLR (Pérez and de los Campos 2014).

Results

Genetic diversity of the panel of 2325 elite winter wheat lines

The 2325 wheat lines were fingerprinted using 12,642 polymorphic SNP markers. The minor allele frequencies

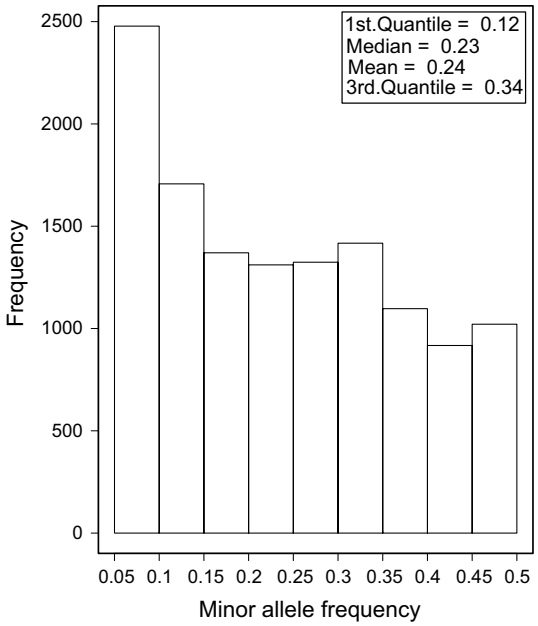

Fig. 1 Distribution of the minor allele frequency of the 2325 winter wheat lines genotyped with 12,642 SNPs

averaged 0.24 with a 1st quantile of 0.12 and a 3rd quantile of 0.34 (Fig. 1). The heat plot of the genetic similarities among the 2325 lines revealed an absence of a clear subpopulation structure (Fig. 2) with a wide range of values of one minus Rogers' distances approximating a normal distribution (Fig. S1). This was further supported by a PCoA analysis which revealed that the first principal coordinates explained only small proportions of the total molecular variation (Fig. S4).

Fusarium head blight and Septoria tritici blotch severity

The evaluation of the single environments revealed high repeatability values for FHB severity with a range from 0.74 to 0.95 and moderate to high repeatability values for STB severity ranging from 0.56 to 0.92 (Fig. S2). Repeatability values for PH ranged from 0.8 to 0.9. The genetic variance was significantly ($P < 0.01$) larger than zero for all three traits. Moreover, we observed a wide variation of BLUEs (Fig. 3). The shape of the distribution for FHB and STB severity approximated a normal distribution, which is typical for a quantitative disease resistance. The Pearson moment correlation of the BLUEs estimated across environments between FHB and for STB disease severity was low and amounted to 0.12. Correlations between FHB and STB disease severity and plant height were negative and low and amounted to −0.15 and −0.17, respectively.

Marker-assisted and genomic selection

The prediction ability of marker-assisted selection increased monotonically with relaxed significance thresholds up to an optimum P value of 0.01 for all three traits (Fig. 4).

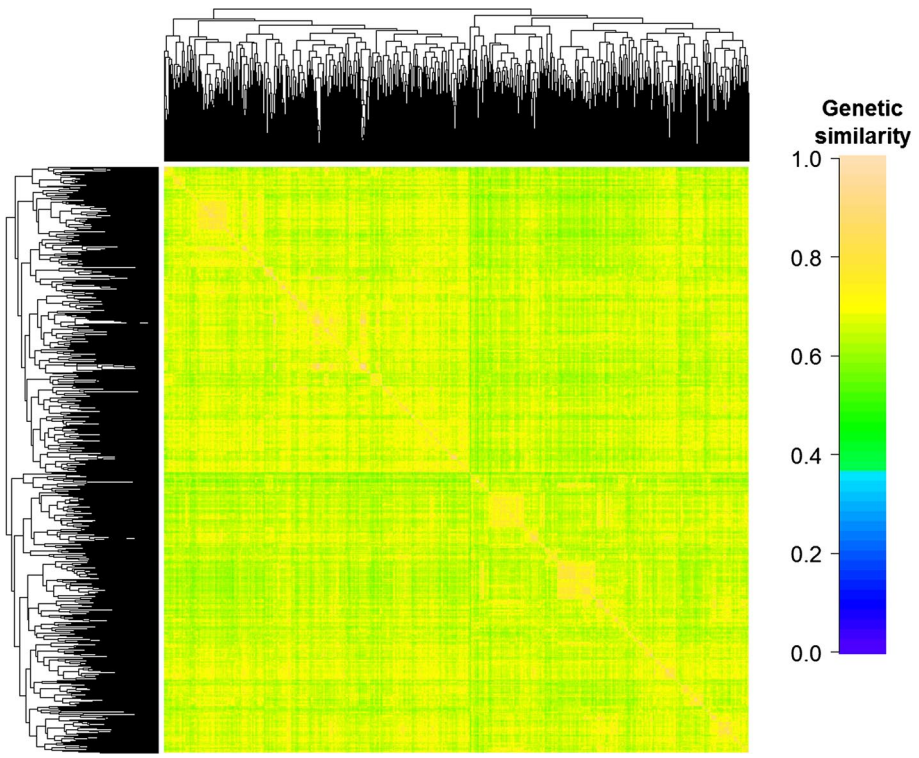

Fig. 2 Pairwise genetic similarities defined as one minus the Rogers' distances estimated for the 2325 winter wheat inbred lines. Average linkage clustering was used for ordering the individual lines

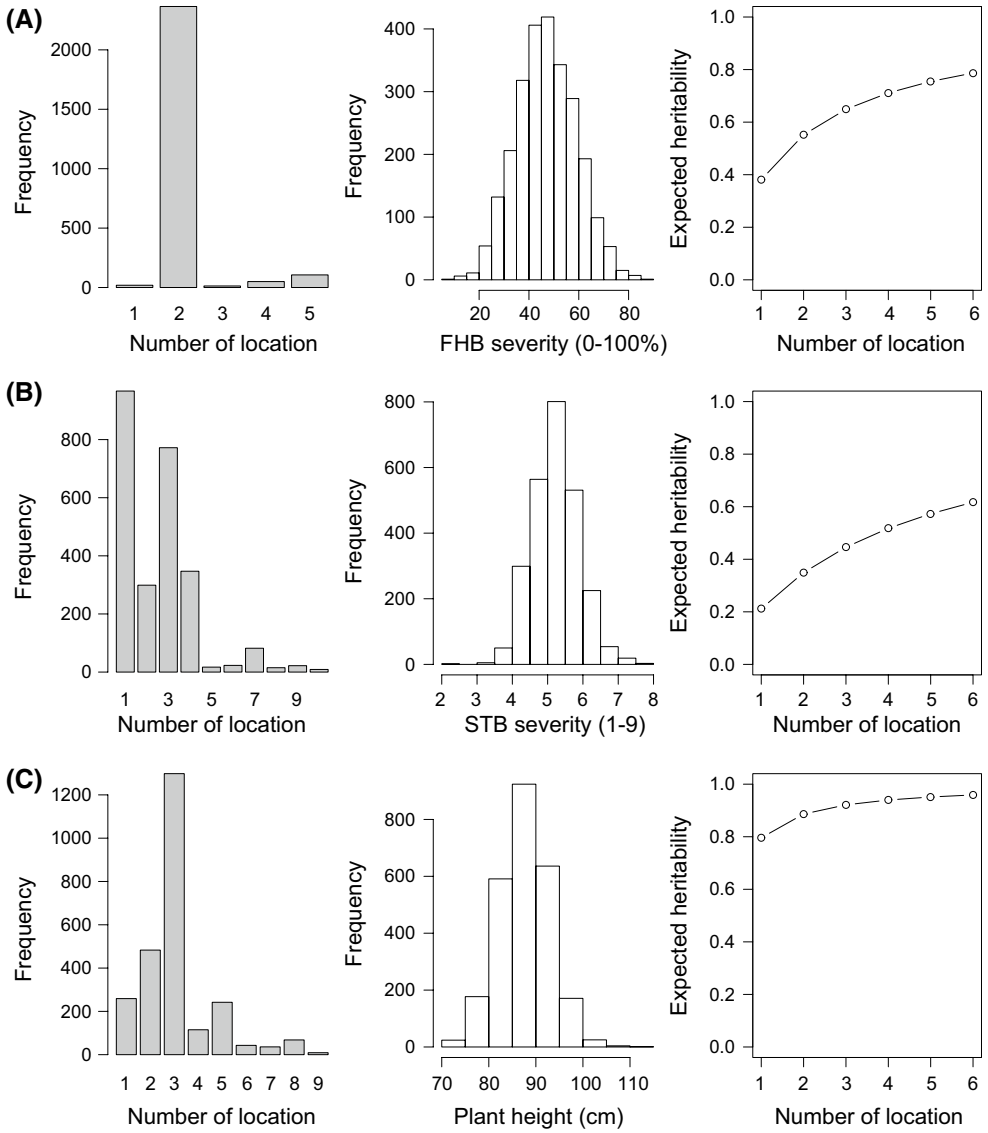

Fig. 3 Number of environments in which the 2325 lines have been evaluated, distribution of their best linear unbiased estimates (BLUE), and heritability in dependency on the number of environments for **a** Fusarium head blight (FHB) and **b** Septoria tritici blotch (STB) severity, as well as **c** plant height

Relaxing the significance threshold further led to a decrease in the prediction ability. The maximum prediction ability for the three traits amounted to 0.41, 0.36, and 0.42 for FHB and STB severity as well as plant height, respectively. Interestingly, we observed only marginal differences in prediction ability between the two complex diseases and plant height despite the presence of large effect genes for the latter trait. In line with this finding, we observed absence of a strong signal for marker–trait associations for plant height on chromosome 4B and 4D (Fig. S3), where the Rht-B1 and Rht-D1 genes are located. The 2325 lines were not fingerprinted for functional markers for Rht-B1 and Rht-D1 genes in our study. Thus, a likely explanation for the absence of large effect QTL on 4B and 4D is the lack of SNPs in the used array, which is in tight linkage disequilibrium with Rht-B1 and Rht-D1. The prediction ability for all three traits increased by 8–11 % by applying genomic instead of marker-assisted selection (Fig. 5). The choice of the genomic selection model had a crucial impact on the prediction ability. Using EG-BLUP and RKHS yielded substantially higher prediction ability compared to BayesCπ and RR-BLUP with an average superiority of 10 %.

Discussion

We examined the potential and limits of marker-assisted and genomic selection using extensive molecular and

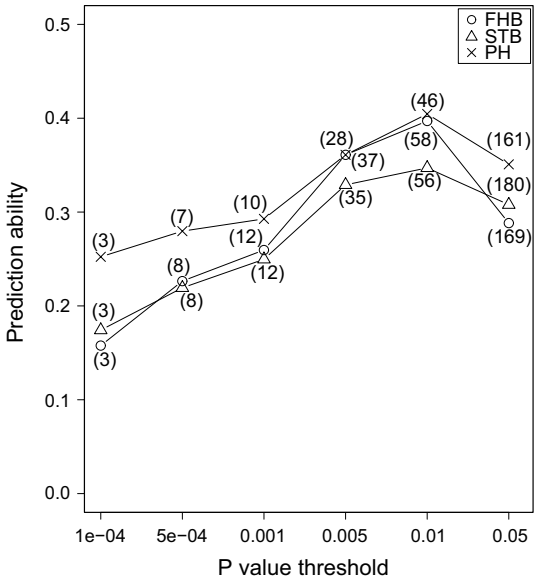

Fig. 4 Prediction ability of the individuals in the test sets based on marker–trait associations detected in estimation sets for Fusarium head blight (FHB) and Septoria tritici blotch (STB) severity, as well as plant height (PH) with 90 k SNP array data at different levels of significance. *Numbers in brackets* indicate the effective number of QTL detected in the estimation set based on 100 cross-validations

phenotypic data from a commercial wheat breeding program. Following the optimal allocation of resources in multi-stage selection (Utz 1969), the structure of the phenotypic data is very unbalanced (Table S1; Fig. 3). We applied an unweighted two-step analysis of the phenotypic data ameliorating experimental design effects and, thus, simplifying further analyses. This two-step approach is not expected to lead to a substantial decrease in the power of the phenotypic data analysis (Möhring and Piepho 2009) nor of the genomic selection results (Schulz-Streeck et al. 2013) as compared to a one-step analysis.

Disease severity is only marginally associated with plant height

Previous studies revealed a large influence of plant height as morphological resistant mechanism against FHB infection (Mesterházy 1995; Miedaner and Voss 2008). In contrast to the previous findings, we observed only a marginal correlation of -0.15 ($P < 0.05$) for FHB despite the large variation in plant height ranging from 71 to 113 cm. The low correlation can on one hand be explained by the use of parents for wheat breeding, which have been improved intensively in the past decade for FHB resistance and for which negative correlations among traits have been successfully broken. On the other hand, the low correlation can also be explained by the high frequency of Rht-B1 or Rht-D1 genes in Central European germplasm.

Plant height was also described, along with other traits, as disease-escape mechanism for STB (Arraiano et al. 2009). The low correlation observed between plant height and STB severity cannot be explained with intensive resistance breeding during the past decade (O'Driscoll et al. 2014) but rather points to the low relevance of morphological resistance against STB. Consequently, ideotypes exhibiting high harvest index combined with pronounced FHB and STB resistance are not impeded by strong negative trait correlations.

Balance between power of QTL detection and false positive rate

Optimal choice of significance threshold is crucial for a successful implementation of marker-assisted selection (Knapp 1998; Moreau et al. 1998; Schön et al. 2004). The optimum choice should reflect a balance between a high QTL detection power and tolerable frequency of false positives (Utz and Melchinger 1994; Beavis 1998). We observed the highest prediction accuracies at significance level of 0.01 (Fig. 4). At this P level, we detected for all three traits around 50 effective QTL covering large parts of the genome (Fig. S3). Relaxing the significance threshold further yielded a substantially larger number of effective QTL but led to a decrease in prediction abilities. This clearly suggests a substantial increase in the number of false positives.

Gowda et al. (2014) applied association mapping in a population of wheat hybrids with gradual differences in relatedness and concluded that prediction ability of marker-assisted selection is not only influenced by knowledge on QTL but also exploits relatedness. If relatedness was the main driving factor in our study, one would expect a monotonical increase in the prediction accuracies. This was not the case (Fig. 4) indicating that relatedness only marginally impacted the accuracy of marker-assisted selection. Our findings are in contrast to a recent association mapping study for FHB resistance based on a diverse panel of 371 wheat inbred lines reporting relevance of relatedness (Jiang et al. 2015). The observed discrepancy can be explained by the six times larger population size in our study as compared to the survey of Jiang et al. (2015) leading to a substantial larger power of QTL detection.

Cross-validation is not severely biased by absence of clear subpopulations

Cross-validations have been used extensively for QTL mapping (Utz et al. 2000) as well as for genomic selection (Heslot et al. 2014) and have been recently also suggested for association mapping studies (Zhao et al. 2013; Würschum and Kraft 2014). Previous cross-validation studies in

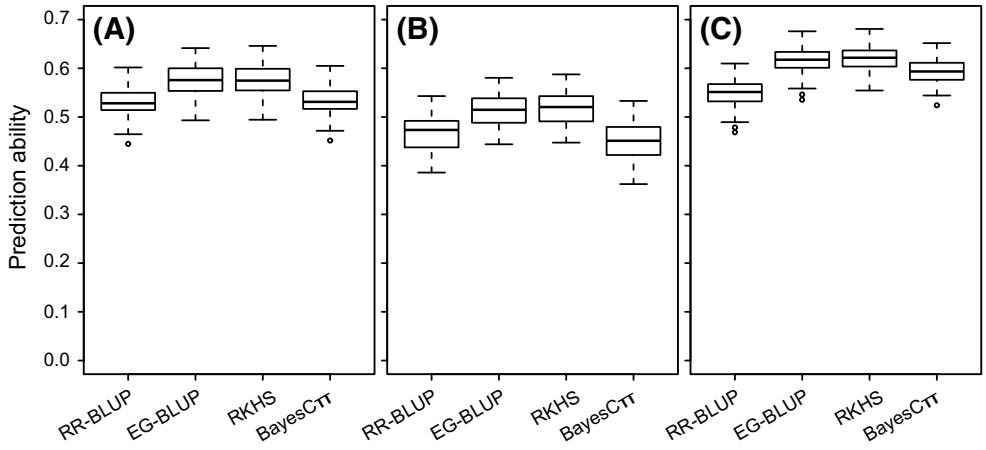

Fig. 5 *Box*-and-*whisker* plots of prediction ability of **a** Fusarium head blight (FHB) and **b** Septoria tritici blotch (STB) severity, as well as **c** plant height for the four genomic selection methods RR-BLUP, EG-BLUP, RKHS, and BayesCπ

maize revealed that population structure largely impacts the results of prediction accuracies (Windhausen et al. 2012; Zhao et al. 2012; Riedelsheimer et al. 2013). Therefore, cross-validations were often performed within biparental families (e.g., Lian et al. 2014). The analyses of the population structure of our data revealed presence of some family structure but absence of large biparental families and clearly defined subpopulation (Fig. 2), which is in accordance to a previous study in wheat (Isidro et al. 2015). Consequently, cross-validations can be performed across all 2325 inbred lines not being strongly biased by pronounced population stratification effects.

Association mapping revealed absence of QTL with large effects

We observed in our cross-validation study at a stringent significance threshold only low prediction abilities for FHB and STB severity with values not passing 0.2 (Fig. 4). Our findings are in accordance with those of a previous study on the genetic architecture of FHB and STB severity in a Central European hybrid wheat population pointing towards the absence of large effect QTL in Central European wheat lines (Mirdita et al. 2015). The observed lack can be explained by the absence of the major QTL Fhb1 located on chromosome 3BS and Fhb2 located on chromosome 6BS originating from the exotic donor Sumai-3 (Waldron et al. 1999; Buerstmayr et al. 2009). In addition, large effect QTL detected for plant height such as Rht-B1 (Handa et al. 2008), Rht-D1 (Draeger et al. 2007), and Ppd-D1 (Beales et al. 2007) are due to low trait correlations ($r = -0.15$; $P < 0.05$) also of minor relevance for FHB resistance. For STB severity, the Stb6 (Arraiano et al. 2001) and Stb3 (Adhikari et al. 2004) resistance genes both located on chromosome 6 were previously suggested as candidates substantially contributing to the resistance in European winter wheat. In contrast, we have not detected any SNPs located on chromosome 6 exhibiting small *P* values (Fig. S3).

Presence of small effect QTL hampers an efficient pyramiding strategy for FHB and STB disease severity. Consequently, marker-assisted selection has only limited potential. Genomic selection tackles the complex genetic architecture more properly and is therefore an attractive alternative solution.

Modeling epistasis in genomic selection substantially increases prediction abilities

We used two genomic selection approaches, EG-BLUP and RKHS, modeling besides main also epistatic effects (Jiang and Reif 2015). For all three traits, the genomic selection approaches including epistasis (EG-BLUP, RKHS) outperformed those focusing exclusively on main effects (RR-BLUP, BayesCπ; Fig. 5). The superiority of RKHS has been previously observed for the selfing species wheat and barley (Heslot et al. 2012; Pérez-Rodríguez et al. 2012) and is in contrast to findings in the outcrossing species maize (Jiang and Reif 2015). The observed differences depending on the mating system are in line with a study reporting a major impact of additive by additive epistasis on the genetic architecture of heterosis for rice but not for maize (Garcia et al. 2008). Consequently, for wheat breeding, it seems beneficial to routinely implement genomic selection approaches modeling main and epistatic effects leading to substantially improved prediction accuracies.

A recent simulation study proposed fast track breeding strategies exclusively based on genomic selection if prediction accuracies surpassed 0.65 (Longin et al. 2015). We observed prediction abilities, i.e., correlations between

observed and predicted genotypic values not standardized with the square root of heritability, of around 0.6 for FHB and of 0.5 for STB severity (Fig. 5). Taking the heritability estimates into account (Fig. 3) led to the conclusion that fast track genomic selection strategies are spurring rapid advances in breeding for FHB and STB resistance in wheat.

Author contribution statement JCR, EE, and RB conceived the design of this study. VK coordinated the SNP genotyping. EE and RB coordinated the experiments including the phenotypic trait measurements of the plant materials. VM, YJ, SH, and JCR made the concept and wrote the manuscript. YZ and SH performed the genomic selection analyses. YJ and SH performed the phenotypic data analyses and the association mapping analyses. All authors have read and approved the final manuscript.

Compliance with ethical standards

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

References

Adhikari TB, Wallwork H, Goodwin SB (2004) Microsatellite markers linked to the Stb2 and Stb3 genes for resistance to Septoria tritici blotch in wheat. Crop Sci 44:1403–1411

Agostinelli AM, Clark AJ, Brown-Guedira G, Van Sanford DA (2012) Optimizing phenotypic and genotypic selection for Fusarium head blight resistance in wheat. Euphytica 186:115–126

Arraiano LS, Worland AJ, Ellerbrook C, Brown JKM (2001) Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6x'. Theor Appl Genet 103:758–764

Arraiano LS, Balaam N, Fenwick PM, Chapman C, Feuerhelm D, Howell P, Smith SJ, Widdowson JP, Brown JKM (2009) Contributions of disease resistance and escape to the control of Septoria tritici blotch of wheat. Plant Pathol 58:910–922

Beales J, Turner A, Griffiths S, Snape J, Laurie DA (2007) A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (*Triticum aestivum* L.). Theor Appl Genet 115:721–733

Beavis WD (1998) QTL analyses: power, precision, and accuracy. In: Paterson AH (ed) Molecular dissection of complex traits. CRC Press, New York, pp 145–162

Becher R, Miedaner T, Wirsel SG (2013) 8 Biology, diversity, and management of FHB causing Fusarium species in small-grain cereals. In: Frank K (ed) Agricultural applications. Springer, Heidelberg, pp 199–241

Bordes J, Goudemand E, Duchalais L, Chevarin L, Oury FX, Heumez E, Lapierre A, Perretant MR, Rolland B, Beghin D, Laurent V, Le Gouis J, Storlie E, Robert O, Charmet G (2014) Genome-wide association mapping of three important traits using bread wheat elite breeding populations. Mol Breed 33:755–768

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed 128:1–26

Cai X, Huang A, Xu S (2011) Fast empirical Bayesian LASSO for multiple quantitative trait locus mapping. BMC Bioinform 12:211

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S et al (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci 110:8057–8062

de Los Campos G, Gianola D, Rosa GJ, Weigel KA, Crossa J (2010) Semi-parametric genomic-enabled prediction of genetic values using reproducing kernel Hilbert spaces methods. Genet Res 92:295–308

Draeger R, Gosman N, Steed A, Chandler E, Thomsett M, Srinivasachary N, Schondelmaier J, Buerstmayr H, Lemmens M, Schmolke M, Mesterhazy A, Nicholson P (2007) Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor Appl Genet 115:617–625

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. Longman, London

Garcia AA, Wang FS, Melchinger AE, Zeng ZB (2008) Quantitative trait loci mapping and the genetic basis of heterosis in maize and rice. Genetics 180:1707–1724

Gianola D, van Kaam JB (2008) Reproducing kernel Hilbert spaces regression methods for genomic assisted prediction of quantitative traits. Genetics 178:2289–2303

Gianola D, Fernando RL, Stella A (2006) Genomic-assisted prediction of genetic value with semiparametric procedures. Genetics 173:1761–1776

Gilmour AR, Gogel B, Cullis BR, Thompson R (2009) ASReml user guide release 3.0. VSN International Ltd, Hemel Hempstead

Gowda M, Zhao Y, Würschum T, Longin CFH, Miedaner T, Ebmeyer E, Schachschneider R, Kazman E, Schacht J, Mette MF, Reif JC (2014) Relatedness severely impacts accuracy of marker-assisted selection for disease resistance in hybrid wheat. Heredity 112:552–561

Habier D, Fernando RL, Dekkers JCM (2007) The impact of genetic relationship information on genome-assisted breeding values. Genetics 177:2389–2397

Habier D, Fernando RL, Kizilkaya K, Garrick DJ (2011) Extension of the Bayesian alphabet for genomic selection. BMC Bioinform 12:186

Handa H, Namiki N, Xu D, Ban T (2008) Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. Mol Breed 27:71–84

He S, Zhao Y, Mette MF, Bothe R, Ebmeyer E, Sharbel TF, Reif JC, Jiang Y (2015) Prospects and limits of marker imputation in quantitative genetic studies in European elite wheat (*Triticum aestivum* L.). BMC Gen 16:168

Henderson CR (1975) Best linear unbiased estimation and prediction under a selection model. Biometrics 31:423–447

Henderson CR (1985) Best linear unbiased prediction of non-additive genetic merits. J Anim Sci 60:111–117

Heslot N, Yang HP, Sorrells ME, Jannink JL (2012) Genomic selection in plant breeding: a comparison of models. Crop Sci 52:146–160

Heslot N, Jannink JL, Sorrells ME (2014) Perspectives for genomic selection applications and research in plants. Crop Sci 55:105–126

Holzapfel J, Voss HH, Miedaner T, Korzun V, Häberle J, Schweizer G, Mohler V, Zimmermannn G, Hartl L (2008) Inheritance of resistance to Fusarium head blight in three European winter wheat populations. Theor Appl Genet 117:1119–1128

Howie BN, Donnelly P, Marchini J (2009) A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 5:e1000529

Isidro J, Jannink JL, Akdemir D, Poland J, Heslot N, Sorrells ME (2015) Training set optimization under population structure in genomic selection. Theor Appl Genet 128:145–158

Jiang Y, Reif JC (2015) Modelling epistasis in genomic selection. Genetics. doi:10.1534/genetics.115.177907

Jiang Y, Zhao Y, Rodemann B, Plieske J, Kollers S, Korzun V, Ebmeyer E, Argillier O, Hinze M, Ling J, Röder MS, Ganal MW, Mette MF, Reif JC (2015) Potential and limits to unravel the genetic architecture and predict the variation of Fusarium head blight resistance in European winter wheat (*Triticum aestivum* L.). Heredity 114:318–326

Knapp SJ (1998) Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci 38:1164–1174

Kollers S, Rodemann B, Ling J, Korzun V, Ebmeyer E, Argillier O, Hinze H, Plieske J, Kulosa D, Ganal MW, Röder MS (2013) Whole genome association mapping of Fusarium head blight resistance in European winter wheat (*Triticum aestivum* L.). PLOS One 8(2):e575500

Kutcher HR, Johnston AM, Bailey KL, Malhi SS (2011) Managing crop losses from plant diseases with foliar fungicides, rotation and tillage on a Black chernozem in Saskatchewan, Canada. Field Crop Res 124:205–212

Lian L, Jacobson A, Zhong S, Bernardo R (2014) Genomewide prediction accuracy within 969 maize biparental populations. Crop Sci 54:1514–1522

Lipka AE, Tian F, Wang Q et al (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397–2399

Löffler M, Schön CC, Miedaner T (2009) Revealing the genetic architecture of FHB resistance in hexaploid wheat (*Triticum aestivum* L.) by QTL meta-analysis. Mol Breed 23:473–488

Longin CFH, Mi X, Würschum T (2015) Genomic selection in wheat: optimum allocation of test resources and comparison of breeding strategies for line and hybrid breeding. Theor Appl Genet. doi:10.1007/s00122-015-2505-1

Mesterházy A (1995) Types and components of resistance to Fusarium head blight of wheat. Plant Breed 114:377–386

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829

Miedaner T, Korzun V (2012) Marker-assisted selection for disease resistance in wheat and barley breeding. Phytopathology 102:560–566

Miedaner T, Voss HH (2008) Effect of dwarfing Rht genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Sci 48:2115–2122

Miedaner T, Gang G, Geiger HH (1996) Quantitative-genetic basis of aggressiveness of 42 isolates of *Fusarium culmorum* for winter rye head blight. Plant Dis 80:500–504

Miedaner T, Wilde F, Korzun V, Ebmeyer E, Schmolke M, Hartl L, Schön CC (2009) Marker selection for Fusarium head blight resistance based on quantitative trait loci (QTL) from two European sources compared to phenotypic selection in winter wheat. Euphytica 166:219–227

Miedaner T, Würschum T, Maurer HP, Korzun V, Ebmeyer E, Reif JC (2011) Association mapping for Fusarium head blight resistance in European soft winter wheat. Mol Breed 28:647–655

Miedaner T, Risser P, Paillard S, Schnurrbusch T, Keller B, Hartl L, Holzapfel J, Korzun V, Ebmeyer E, Utz HF (2012) Broad-spectrum resistance loci for three quantitatively inherited diseases in two winter wheat populations. Mol Breed 29:731–742

Miedaner T, Zhao Y, Gowda M, Longin CFH, Korzun V, Ebmeyer E, Kazman E, Schachschneider R, Reif JC (2013) Genetic architecture of resistance to Septoria tritici blotch in European wheat. BMC Gen 14:858

Mirdita V, Liu G, Zhao Miedaner T, Longin CFH, Gowda M, Mette MF, Reif JC (2015) Genetic architecture is more complex for resistance to Septoria tritici blotch than to Fusarium head blight in Central European winter wheat. BMC Gen 16:430

Möhring J, Piepho HP (2009) Comparison of weighting in two-stage analyses of series of experiments. Crop Sci 49:1977–1988

Moreau L, Charcosset A, Hospital F, Gallais A (1998) Marker-assisted selection efficiency in populations of finite size. Genetics 148:1353–1365

Morota G, Gianola D (2014) Kernel-based whole-genome prediction of complex traits: a review. Front Gen 5:363

O'Driscoll A, Kildea S, Doohan F, Spink J, Mullins E (2014) The wheat–Septoria conflict: a new front opening up? Trends Plant Sci 19:602–610

Ornella L, Singh S, Perez P, Burgueño J, Singh R, Tapia E, Bhavani S, Dreisigacker S, Braun HJ, Mathews K, Crossa J (2012) Genomic prediction of genetic values for resistance to wheat rusts. Plant Gen 5:136–148

Paul PA, McMullen MP, Hershman DE, Madden LV (2010) Meta-analysis of the effects of triazole-based fungicides on wheat yield and test weight as influenced by Fusarium head blight intensity. Phytopathology 100:160–171

Pérez P, de los Campos G (2014) Genome-wide regression & prediction with the BGLR statistical package. Genetics. doi:10.1534/genetics.114.164442

Pérez-Rodríguez P, Gianola G, González-Camacho JM, Crossa J, Manès J, Dreisigacker S (2012) Comparison between linear and non-parametric regression models for genome-enabled prediction in wheat. G3 Genes Gen Genet 12:1595–1605

R Core Team (2014) R: A language and environment for statistical computing. R foundation for statistical computing, Vienna. http://www.R-project.org/. Accessed 2 Sept 2015

Riedelsheimer C, Endelman JB, Stange M, Sorrells ME, Jannink JL, Melchinger AE (2013) Genomic predictability of interconnected biparental maize populations. Genetics 194:493–503

Rogers JS (1972) Measures of genetic similarity and genetic distance. Stud Genet 7:145–153

Rutkoski J, Benson J, Jia Y, Brown-Guedira G, Jannink JL, Sorrells M (2012) Evaluation of genomic prediction methods for fusarium head blight resistance in wheat. Plant Gen 5:51–61

Schön CC, Utz HF, Groh S, Truberg B, Openshaw S, Melchinger AE (2004) Quantitative trait locus mapping based on resampling in a vast maize testcross experiment and its relevance to quantitative genetics for complex traits. Genetics 167:485–498

Schulz-Streeck T, Ogutu JO, Piepho HP (2013) Comparisons of single-stage and two-stage approaches to genomic Selection. Theor Appl Genet 126:69–82

Torriani SFF, Brunner PC, McDonald BA, Sierotzki H (2009) QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. Pest Manag Sci 65:155–162

Utz HF (1969) Mehrstufenselektion in der Pflanzenzüchtung. Verlag Eugen Ulmer, Germany

Utz HF, Melchinger AE (1994) Comparison of different approaches to interval mapping of quantitative trait loci. In: Biometrics in plant breeding: applications of molecular markers. Proceedings of the ninth meeting of the EUCARPIA section biometrics in plant breeding pp 6–8

Utz HF, Melchinger AE, Schön CC (2000) Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics 154:1839–1849

VanRaden PM (2008) Efficient methods to compute genomic predictions. J Dairy Sci 91:4414–4423

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP mapping of QTL for fusarium head blight resistance in wheat. Crop Sci 39:805–811

Wang D, El-Basyoni IS, Baenziger PS, Crossa J, Eskridge KM, Dweikat I (2012) Prediction of genetic values of quantitative traits

with epistatic effects in plant breeding populations. Heredity 109:313–319

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE et al (2014) Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. Plant Biotech J 12:787–796

Whittaker JC, Thompson R, Denham MC (2000) Marker-assisted selection using ridge regression. Genet Res 75:249–252

Willyerd KT, Li C, Madden L, Bradley V, Bergstrom CA, Sweets GC, McMullen LE et al (2012) Efficacy and stability of integrating fungicide and cultivar resistance to manage Fusarium head blight and deoxynivalenol in wheat. Plant Dis 96:957–967

Windhausen VS, Atlin GN, Hickey JM, Crossa J, Jannink JL, Sorrells ME, Raman B, Cairns JE, Tarekegne A, Semagn K (2012) Effectiveness of genomic prediction of maize hybrid performance in different breeding populations and environments. G3 Genes Gen Genet 2:1427–1436

Wittenburg D, Melzer N, Reinsch N (2011) Including non-additive genetic effects in Bayesian methods for the prediction of genetic values based on genome-wide markers. BMC Genet 12:74

Würschum T, Kraft T (2014) Cross-validation in association mapping and its relevance for the estimation of QTL parameters of complex traits. Heredity 112:463–468

Xu S (2007) An empirical Bayes method for estimating epistatic effects of quantitative trait loci. Biometrics 63:513–521

Yu J, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208

Yuen GY, Schoneweis SD (2007) Strategies for managing Fusarium head blight and deoxynivalenol accumulation in wheat. Int J Food Microbiol 119:126–130

Zadoks MC, Chang TT, Konzak F (1974) A decimal code for the growth stages of cereals. Weed Res 15:415–421

Zhao Y, Gowda M, Liu W, Würschum T, Maurer HP, Longin CFH, Ranc N, Reif JC (2012) Accuracy of genomic selection in European maize elite breeding populations. Theor Appl Genet 124:769–776

Zhao Y, Gowda M, Würschum T, Longin CFH, Korzun V, Kollers S, Schachschneider R, Zeng J, Fernando R, Dubcovsky J (2013) Dissecting the genetic architecture of frost tolerance in Central European winter wheat. J Exp Bot 64:4453–4460

Zhao Y, Mette MF, Gowda M, Longin CFH, Reif JC (2014) Bridging the gap between marker-assisted and genomic selection of heading time and plant height in hybrid wheat. Heredity 112:638–645