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Comparing genomic selection and marker-assisted selection for Fusarium head blight resistance in wheat (*Triticum aestivum* L.)

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Abstract Genomic selection (GS) and marker-assisted selection (MAS) rely on marker–trait associations and are both routinely used for breeding purposes. Although similar, these two approaches differ in their applications and how markers are used to estimate breeding values. In this study, GS and MAS were compared in their ability to predict six traits associated with resistance to a destructive wheat disease, Fusarium head blight (FHB). A panel consisting in 273 soft red winter wheat lines from the US Midwestern and Eastern regions was used in this study. The statistical models for MAS were built using *Fhb-1*, the best-studied quantitative trait loci (QTL) for FHB resistance, and two sets of QTL: one independently identified by other groups and a newer set identified "*in house*". In contrast, genomic selection models relied on 19,992 SNPs distributed throughout the genome. For the MAS and GS models, marker effects were estimated with ordinary least square and ridge regression best unbiased linear prediction, respectively. Intermediate to high values of prediction accuracy (0.4–0.9) were observed for most GS models, with lower values (<0.3) found for MAS models. Treating QTL as fixed effects in GS models resulted in higher prediction accuracy when compared with a GS model with only random effects, but overestimated accuracies were obtained with *in house* QTL. For the same selection intensity, GS resulted in higher selection differentials than MAS for all traits. Our results indicate that GS is a more appropriate strategy than MAS for FHB resistance.

Keywords Molecular breeding · Genotyping-by-sequencing · *Fusarium graminearum* · Marker-assisted selection · Genomic selection · Deoxynivalenol

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Introduction

Several marker-based strategies are being applied in modern plant breeding with the objective of selecting individuals with superior performance; marker-assisted selection (MAS) and genomic selection (GS) are

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two examples. In MAS, individual lines are selected based on quantitative trait loci (QTL), which are detected through linkage mapping (LM) or genome-wide association studies (GWAS). While LM relies on experimental populations with limited recombination events, GWAS is performed on a panel or collection of lines, taking advantage of all recombination events that occurred throughout the history of the group of lines, usually resulting in higher mapping resolution when compared to LM (Myles et al. 2009). In both LM and GWAS, genomic signals meeting a certain threshold are declared statistically significant, and all the remaining marker–trait associations are excluded from further analysis. Thus, the number of markers per trait used in traditional MAS is generally low. For traits under complex genetic control, with multiple small effect genes contributing to overall phenotypic variation, MAS can be of limited use (Bernardo et al. 2008). In contrast, all available high-quality markers are used in GS for modeling the performance of an individual, regardless of the magnitude of their effect (Meuwissen et al. 2001; Jannink et al. 2010). Given that a marker set has genome-wide coverage, the GS model should theoretically account for all QTL underlying the trait being studied regardless of their effect sizes (Goddard and Hayes 2007). Therefore, for traits with complex inheritance, GS is expected to outperform MAS.

During the last decade, a dramatic reduction in sequencing cost has occurred (Wetterstrand et al. 2016) and numerous next-generation sequencing platforms are now available to the plant research community (Patel et al. 2015). As a result, high-density genome-wide markers are becoming available for most crops, making MAS and GS even more attractive. Assessing how these strategies compare is important for making more rational and efficient use of markers in a breeding pipeline. Heffner et al. (2011) compared GS and MAS for 13 agronomic traits in wheat and reported a 28 % advantage of GS over MAS for prediction accuracy, defined as the correlation between phenotypically estimated breeding values (PEBVs) and genomic estimated breeding values (GEBVs). Wang et al. (2014) found GS to outperform MAS for agronomic and quality traits in inbred and hybrid rye. In the case of grain yield, prediction accuracy increased from 0.12 (MAS) to 0.59 (GS) in one population. However, genomic selection may not always be more advantageous than MAS. For instance, Zhao et al. (2014) compared both methods for heading time and plant height in rye and found the best method to be trait specific. Similarly, Owens et al. (2014) reported that prediction accuracies of provitamin A levels in maize grain using models with markers in the vicinity of a priori biochemical pathway genes were approximately equal to those obtained using models that included genome-wide marker sets.

In GS, several statistical models are available for estimating marker effects, each model with its own assumptions and features (Heslot et al. 2012; Desta and Ortiz 2014). A commonly used method is called ridge regression-best unbiased linear prediction (RR-BLUP), which is based on an infinitesimal model with all markers sharing a common variance, and all effects are shrunken toward zero. When major genes are present, RR-BLUP will underestimate the variance associated with these genes, and in such situations alternate Bayesian GS models can provide higher prediction accuracies (Resende et al. 2012). However, for polygenic traits, this increase in accuracy relative to RR-BLUP is usually small (VanRaden 2008; Moser et al. 2009; Heffner et al. 2011; Resende et al. 2012). Moreover, one important drawback of these Bayesian models is that they are usually very computationally demanding. Thus, an alternative is to treat major genes as fixed effects in the infinitesimal GS model (e.g., RR-BLUP), which would theoretically lead to higher accuracies compared to a situation in which all genes or markers are treated equally (Bernardo 2014; Zhao et al. 2014).

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [telemorph: *Gibberella zeae* Schw. (Petch)], is a major wheat disease in most growing areas, including the US Midwestern region where the pathogen can overwinter on maize debris. The pathogen causes significant grain yield reduction (Madden and Paul 2009) and mycotoxin contamination in infected grain. Breeding for FHB resistance has been challenging due to the complex nature of resistance, and labor intensive phenotyping. In addition, MAS and GS for FHB resistance have been hindered due to the delayed availability of high-density, genome-wide markers compared to other economically important crops. One major QTL on the short arm of chromosome 3B, *Fhb-1*, has been identified by independent studies. Additionally, multiple small effect QTL have been detected on nearly all wheat chromosomes (Buerstmayr et al. 2009; Liu et al.

2009; Loffler et al. 2009), which suggests a complex genetic architecture for FHB resistance. Discovered in Chinese spring wheat germplasm, *Fhb-1* has been introduced into spring and winter wheats in North America (Jin et al. 2013) and worldwide. For more than a decade, breeders have extensively used *Fhb-1* and other QTL in their programs, and the levels of FHB resistance in cultivars has improved; however, developing elite, adapted cultivars with high levels of FHB resistance remains difficult given the complex genetic inheritance of resistance, labor intensive phenotyping, and interaction with the environment.

Comparing marker-based strategies for FHB resistance can help breeders decide on how to use markers for selecting more resistant lines. Rutkoski et al. (2012) tested MAS and GS for multiple parameters associated with FHB resistance using a panel of 170 lines from the US cooperative FHB nursery. Working with 2402 Diversity Array Technology (DArT) markers, the authors demonstrated the advantage of GS over MAS. Since their study, marker coverage has improved significantly and genotyping costs have decreased dramatically. Recently, Jiang et al. (2015) used 372 European varieties to compare how different marker sets (782 single sequence repeats—SSRs, and the 9 and 90 k single nucleotide polymorphism—SNP—arrays) and relatedness affected prediction accuracy for FHB index in a MAS and GS context, separately. In our study, we compared MAS and GS models using a panel of 273 breeding lines and SNPs identified from genotyping-by-sequencing (GBS) (Elshire et al. 2011). We also assessed the impact of including markers linked to QTL as fixed effects into GS models on genomic prediction accuracy.

Materials and methods

Plant material and phenotypes

A panel consisting of 273 breeding lines from multiple institutions in the USA was used in this study. Most of the lines originated from the University of Illinois soft red winter wheat breeding program, while the remaining lines came from breeding programs in the US Midwestern and Eastern regions. The panel was phenotyped as described in Arruda et al. (2015). Briefly, six traits associated with FHB resistance were recorded: severity (SEV), incidence (INC), FHB index (FHBdx; [(SEV × INC)/100], Fusarium-damaged kernel (FDK), incidence-severity-kernel index (ISK; [0.3 × INC + 0.3 × SEV + 0.4 × FDK]), and mycotoxin accumulation (DON). SEV was recorded as the percentage of infected spikelets within a wheat head. INC is the percentage of infected heads in an experimental unit. FDK was recorded as the visual estimate of the percentage of Fusarium-damaged kernels in a sample of kernels. DON was recorded using gas chromatography–mass spectrometry at the Department of Plant Pathology, University of Minnesota. Although the trait under consideration is FHB resistance, the six traits described above may not be controlled by the same genes, and thus we use the terms "parameter" and "trait" interchangeably throughout the manuscript. The experimental lines were grown in Urbana, Illinois in 2011, 2013, and 2014. Each year, the experiment was set up as a randomized complete block design (RCBD), with two replications. Because not all lines were planted in 2011, the data were analyzed as an unbalanced design. The experimental unit consisted of 1-m long single rows cultivated in a scab nursery with mist irrigation and grain spawn inoculation. Maize kernels were infected with inoculum produced from isolates collected throughout Illinois over several years, and inoculum was spread in the field at a rate of approximately 287 kg ha^{-1}. For each trait, best linear unbiased predictor (BLUP) was calculated for each line using PROC MIXED SAS version 9.4 (SAS Institute 2013), according to Eq. (1):

$$Y_{ijkl} = \mu + \text{year}_i + \text{block(year)}_{ij} + \text{line}_k + \text{heading}_{ijkl} + (\text{year}x\text{line})_{ik} + \varepsilon_{ijkl} \quad (1)$$

where Y_{ijkl} is the observed phenotype, μ is the overall mean, year_i is the random effect of the ith year, block(year)_{ij} is the random effect of jth block within the ith year, line_k is the random effect of the kth line, heading_{ijk} is a quantitative covariate trait treated as fixed, consisting of the Julian date in which heading was recorded for the lth replicate of the kth line in the jth block within the ith year, year $x\text{line}_{ik}$ is the random effect of the interaction between the ith year and the kth line, and ε_{ijkl} is the random error term. The BLUPs were used as phenotypically estimated breeding values (PEBVs) for model comparisons.

Genotyping data

The procedures for single nucleotide polymorphism (SNP) identification are described in Arruda et al. (2016), and libraries were prepared for sequencing based on the protocol of Poland et al. (2012). In short, genotyping-by-sequencing (GBS) was employed on the 273 breeding lines using three two-enzyme combinations, where a rare cutter (*PstI*-HF) was combined with three common cutters (*MspI*, *HinP1I*, and *BfaI*) for genomic complexity reduction. TASSEL GBS version 4 was used against a pseudo-reference genome developed from the Chinese Spring chromosome survey sequence. The following SNP filtering criteria were applied: (1) maximum per-marker missing data level of 50 %; (2) minimum allele frequency of 5 %; (3) maximum heterozygosity level of 10 %; iv) SNPs mapped to single chromosomes; v) after imputing the missing data with the expectation maximization method (Rutkoski et al. 2013), redundant, non-informative SNPs were removed from the analyses using the LD tagSNP selection option ($r^2 > 0.8$) in JMP Genomics 7 (SAS Institute 2015; Carlson et al. 2004). At the end, 19,992 SNPs were identified.

Quantitative trait loci (QTL) information

Marker-assisted selection and GS models were built using different sets of QTL: *Fhb-1*; QTL identified by independent groups, hereafter referred to as "*independent*" QTL; and a set of "*in house*" QTL, identified in our own panel consisting of the 273 breeding lines described above. The *independent* group included three QTL on chromosomes 3B, one of them being *Fhb-1*, one QTL on chromosome 5A, and the plant height *RhtD1* gene on chromosome 4D (Table 1). These loci are associated with multiple traits corresponding to FHB resistance. In addition, they are routinely used by wheat breeders participating in the US Cooperative FHB nurseries supported by the US Wheat and Barley Scab Initiative (www.scabusa.org). The *in house* QTL were identified in a GWAS (Arruda et al. 2016) using a compressed unified mixed linear model to assess marker–trait associations (Zhang et al. 2010; Yu et al. 2006) in the Genome Association and Prediction Integrated Tool package (Lipka et al. 2012). This model included principal components (Price et al. 2006) and a kinship matrix (VanRaden 2008) calculated from the 19,992 SNPs to account for population structure and familial relatedness, respectively. The Benjamini and Hochberg (1995) procedure was used to control for the multiple testing problem at a false discovery rate (FDR) of 10 %. The number of significant *in house* QTL varied with the trait under consideration (Table 2), with five being the highest number of significant QTL for one trait (INC). When building the MAS and GS models, the top five markers with the lowest FDR-adjusted *P* values were selected for each trait so that all traits would have the same number of markers in their statistical models.

Comparison of models

Three models representing MAS (called MAS1, MAS2, and MAS3) and five models representing GS (called GS1 to GS5) were compared. The MAS models were built with the following marker information: only *Fhb-1* for MAS1; *independent* QTL for MAS2; and *in house* QTL for MAS3. Ordinary least square (OLS) regression was used to estimate marker effects for the MAS models. The GS models were based on RR-BLUP under five different scenarios: all 19,992 SNPs treated as random effects in GS1; all SNPs as random effects and five randomly selected SNPs treated as fixed effects in GS2; all SNPs as random effects and the signals corresponding to *Fhb-1* treated as fixed effect in GS3; all SNPs as random effects and *independent* QTL treated as fixed effects in GS4; and lastly, all SNPs as random effects and *in house* QTL treated as fixed effects in GS5. Both MAS3 and GS5 were specifically built to assess the magnitude of the "inside trading" effect, which has been said to occur when prediction accuracies are assessed using QTL that were identified in the same group of lines, potentially resulting in inflated prediction accuracies.

Prediction accuracy

A four-fold cross-validation scheme was used to calculate prediction accuracy. Initially, the 273 lines were divided into four groups, three groups comprising the training population (TP) and one group consisting of the validation population (VP). Prediction accuracy was calculated according to Dekkers (2007) and Albrecht et al. (2011):

Table 1 Quantitative trait loci (QTL) and reduced height gene associated with Fusarium head blight resistance identified by independent groups

Chromosome	Gene/QTL	Markers	References
3B	*Fhb1*	*umn10* and *gwm533*	Liu et al. (2008) and Zhou et al. (2002)
3B	*3B-Massey*	*IWA6105 and IWA8137*	Xiong (2013)
5A	*Qfhs.ifa-5A*	*gwm304*	Liu et al. (2007)
3B	*Fhb-3Bc*	*KASP-Fhb-3Bc-6105*	Unpublished
4D	*RhtD1*	*wMAS000002*	http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/kasp_download.php?URL

Table 2 Chromosomal position, marker information, and coefficient of determination (r^2) of the top five markers associated with Fusarium head blight (FHB) resistance detected in a genome-wide association study (Arruda et al. 2016)

Trait	C[a]	P[b]	SNP	r^2
SEV	3B	18.32[c]	IWGSC_CSS_3B_ scaff _10676713_7175	0.08
	3B	10.19[c]	IWGSC_CSS_3B_ scaff _10352272_5482	0.05
	3B	6.86	IWGSC_CSS_3B_ scaff _10698462_2332	0.04
	3B	3.37[c]	IWGSC_CSS_3B_ scaff _10699215_3620	0.04
	4B	80.97	IWGSC_CSS_4BL_ scaff _7034084_1682*	0.04
INC	7D	70.84	IWGSC_CSS_7DS_ scaff _3876750_2023	0.16
	6A	134.15	IWGSC_CSS_6AL_ scaff _5780077_12152	0.06
	4D	0	IWGSC_CSS_4DS_ scaff _2300354_4482	0.06
	4A	78.35	IWGSC_CSS_4AL_ scaff _7146617_11335	0.06
	7A	22.82	IWGSC_CSS_7AS_ scaff _4132011_1400	0.05
FHBdx	3B	18.32[c]	IWGSC_CSS_3B_ scaff _10676713_7175	0.07
	6B	46.64	IWGSC_CSS_6BS_ scaff _2977132_3529*	0.05
	7D	70.84	IWGSC_CSS_7DS_ scaff _3876750_2023*	0.04
	3B	6.83	IWGSC_CSS_3B_ scaff _10698462_2332*	0.04
	3B	10.19[c]	IWGSC_CSS_3B_ scaff _10352272_5482*	0.04
FDK	1A	27.24	IWGSC_CSS_1AS_ scaff _3314747_2298*	0.05
	3A	131.87	IWGSC_CSS_3AL_ scaff _4427089_5739*	0.04
	4A	0	IWGSC_CSS_4AS_ scaff _5938251_8590*	0.04
	7B	77.11	IWGSC_CSS_7BS_ scaff _3109240_581*	0.04
	4A	78.35	IWGSC_CSS_4AL_ scaff _7110688_882*	0.04
ISK	3B	18.32[c]	IWGSC_CSS_3B_ scaff _10676713_7175	0.07
	7D	70.84	IWGSC_CSS_7DS_ scaff _3876750_2023	0.16
	4A	78.35	IWGSC_CSS_4AL_ scaff _7110688_882*	0.04
	3B	18.32	IWGSC_CSS_3B_scaff_10665035_6913*	0.03
	5A	10.56	IWGSC_CSS_5AL_ scaff _2731838_4049*	0.04
DON	3B	73.67	IWGSC_CSS_3B_ scaff _10413672_4839	0.07
	3B	18.32[c]	IWGSC_CSS_3B_ scaff _10676713_7175	0.07
	1D	19.04	IWGSC_CSS_1DS_ scaff _1879930_3352	0.06
	3B	73.67	IWGSC_CSS_3B_ scaff _10764618_2168	0.06

Table 2 continued

Trait	C[a]	P[b]	SNP	r^2
	2A	16.45	IWGSC_CSS_2AS_ scaff _5273750_3562*	0.05

SEV Severity, *INC* Incidence, *FHBdx* FHB index, *FDK* Fusarium-damaged kernel, *ISK* Incidence-severity-kernel, index, *DON* deoxynivalenol concentration

* Not statistically significant (FDR-adjusted *P* value ≥0.10) in the genome-wide association study by Arruda et al. (2016)

[a] C = Chromosome

[b] P = genetic position in centimorgans (cM)

[c] SNPs in linkage disequilibrium with the SSRs markers *umn10* and *gwm533*, used to detect *Fhb-1*

$$\frac{r(\text{GEBV}:\text{PEBV})}{\sqrt{H^2}} \quad (2)$$

where r is the Pearson's correlation between the GEBVs and the PEBVs in the validation population, and H^2 is the broad-sense heritability, estimated by Arruda et al. (2016). For each model and trait, 75 TPs were randomly obtained, resulting in 300 values of accuracy (the four-fold cross-validation resulted in four values of accuracy for each randomly selected TP). The mean prediction accuracy of the eight models was compared in SAS PROC GLM using the Ryan-Einot-Gabriel-Welch test at $\infty = 0.05$ level. We also calculated the mean prediction accuracy of each model relative to GS1. Overestimation of prediction accuracy can be obtained when closely related lines such as full-sibs and/or half-sibs are in the TP and VP. In order to avoid such a situation, the 273 breeding lines were grouped in 58 clusters of genetically similar lines. Clusters were obtained in JMP Pro 12 (SAS Institute 2015b) using the *k*-means clustering algorithm (Hartigan and Wong 1979) on marker data (Ly et al. 2013; Arruda et al. 2015). This procedure grouped closely related lines in clusters. Then, folds for cross-validation were created using the cluster numbering (1–58). In other words, instead of assigning breeding lines into TP and VP, the clusters were used for such division, with all breeding lines from the same cluster ending up in either TP or VP. Prediction accuracies were calculated in *R* (R Development Core Team 2013) using the "lm()" function for the MAS models and the "mixed.solve()" function of the rrBLUP package (Endelmann 2011) for the GS models. The same folds were used to compare the eight models.

Selection differential

The selection differential (S) is defined as the difference of mean values between the selected and unselected group, and it is directly related to response to selection (R): $R = h^2S$, where h^2 is the narrow-sense heritability. Because we were not able to estimate h^2, the selection differential was obtained in order to have an indication of R. Initially, GEBVs were calculated for the breeding lines using the four-fold cross-validation scheme described above. More specifically, the genotypic matrix was multiplied by the mean SNP effect across the 300 runs to obtain the GEBVs. Then, for each trait, the 273 lines were ranked based on their GEBVs (provided by MAS1, MAS2, MAS3, GS1, GS2, GS3, GS4, and GS5). In other words, eight different ranks were obtained for each trait. The selection differential was then calculated as the difference between the mean PEBV of the top 5, 10, 15, 20, and 25 % best lines and the mean PEBV of the reference, unselected group of 273 lines.

Results

Cross-validated prediction accuracies for six traits corresponding to FHB resistance are presented in Fig. 1. Overall, intermediate to high values (0.4–0.9) of accuracy were observed for most GS models, with lower values (<0.3) found for MAS models. FDK showed the highest values of prediction accuracy, with the mean across all models equal to 0.63. Conversely, the lowest mean value was observed for SEV (0.40). In nearly all situations, the models with the highest and lowest accuracies were obtained with GS5 and MAS1,

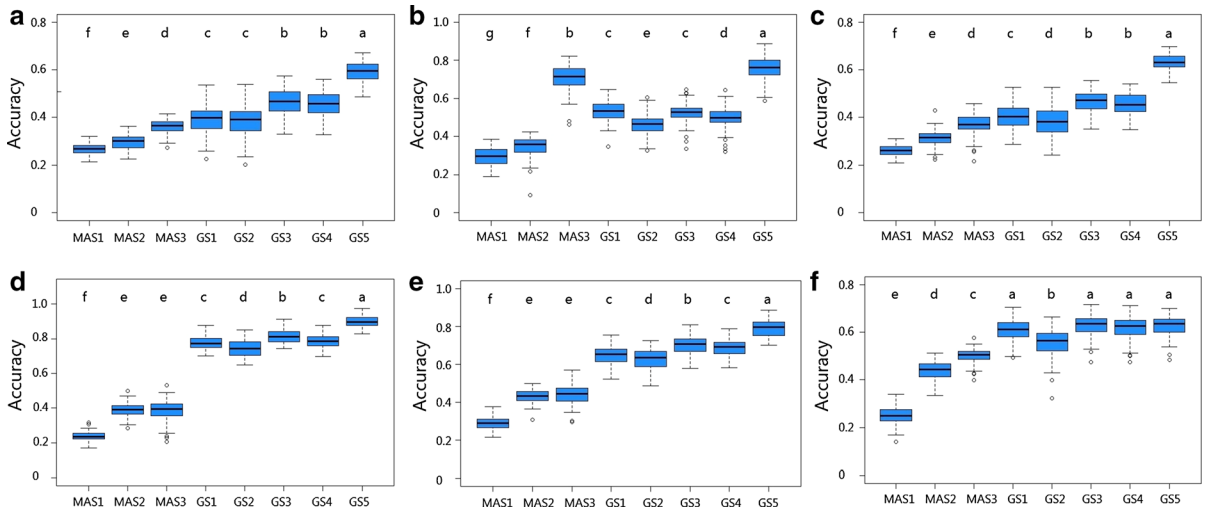

Fig. 1 Cross-validated prediction accuracies for six traits corresponding to Fusarium head blight (FHB) resistance: severity (**a**), incidence (**b**), FHB index (**c**), Fusarium-damaged kernel (**d**), incidence-severity-kernel index (**e**), and DON concentration (**f**). *MAS1* Marker-assisted selection with *Fhb-1* using linear regression. *MAS2* Marker-assisted selection with "*independent*" QTL using multiple linear regression. *MAS3* Marker-assisted selection with "*in house*" QTL using multiple linear regression. *GS1* genomic selection with 19,992 SNPs. *GS2* genomic selection with 19,992 SNPs + 5 randomly selected SNPs treated as fixed effects. *GS3* genomic selection with 19,992 SNPs + *Fhb-1* treated as fixed effect. *GS4* genomic selection with 19,992 SNPs + "*independent*" QTL treated as fixed effects. *GS5* genomic selection with 19,992 SNPs + "*in house*" QTL treated as fixed effects. All marker estimates were obtained using a four-fold cross-validation scheme, 75 randomly selected training populations (TP) of size 205 and validation population (VP) of size 68. GS models were based on ridge regression-best linear unbiased predictor (RR-BLUP), and MAS models were based on ordinary least square (OLS) regression. Treatments with the *same letter* are not significantly different according to the Ryan–Einot–Gabriel–Welch q test at $\alpha = 0.05$ level. Graph produced using *R* (R Development Core Team 2013)

Table 3 Prediction accuracy for traits corresponding to Fusarium head blight (FHB) resistance from marker-assisted selection (MAS) and genomic selection (GS) models relative to GS1 (100)

Trait	MAS1	MAS2	MAS3	GS1	GS2	GS3	GS4	GS5
SEV	68	76	92	100	97	119	117	151
INC	54	65	132	100	87	98	93	142
FHBdx	64	78	91	100	95	114	113	156
FDK	31	50	50	100	96	105	102	116
ISK	45	67	68	100	97	108	107	123
DON	40	73	82	100	91	103	102	103
Average	50	68	86	100	94	108	105	132

The mean prediction accuracy of each model was transformed having the mean prediction of GS1 = 100

SEV Severity, *INC* Incidence, *FHBdx* FHB index, *FDK* Fusarium-damaged kernel, *ISK* Incidence-severity-kernel, index, *DON* Deoxynivalenol concentration

respectively. In fact, a striking advantage of GS5 over other models was observed for some traits such as SEV and FHBdx, possibly reflecting the "inside trading" effect. Averaged across all traits, GS5 showed a 32 % advantage relative to GS1. At the same time, GS3 and GS4 were 8 and 5 % superior to GS1 (Table 3).

For all traits except SEV, a significant difference was detected between GS1 and GS2, with an advantage for GS1 (Fig. 1). In other words, by treating some

randomly selected SNPs as fixed effect (GS2), a reduction in accuracy was observed. In addition, no difference was found between GS3 and GS4 for SEV, FHBdx, and DON. For the other traits, GS3 resulted in higher prediction accuracy. The difference between GS3 and GS4 is inclusion of QTL identified by independent groups on chromosomes 3B (*3B-Massey* and *Fhb-3Bc*), 5A (*Qfhs.ifa-5A*), and the *RhtD1* gene on chromosome 4D, which are included in as fixed effect in the GS4 model and not in the GS3 model. These loci were not associated with FHB resistance in our panel, possibly explaining the neutral or reducing effect on accuracy in the genomic selection context; however, in marker-assisted selection, having these four markers added to *Fhb-1* (MAS2) was more beneficial than *Fhb1* by itself (MAS1). A model with one single marker is an over simplification of the complex genetic architecture of FHB resistance, but it reflects a breeding strategy that has been used by breeders in the past and that is why it was included in this study. By adding four extra markers, even if they were loosely associated with FHB resistance in our panel, an increase in accuracy was observed for all traits. It is possible that these four markers are helping to explain relatedness among the breeding lines, thus, having a positive impact on accuracy. In genomic selection, they may also be beneficial if treated as random, but many thousands of SNPs are already present. The best two models for INC, MAS3 and GS5, both included the five significant *in house* QTL. This is the only situation in which a MAS model was superior to a GS model. The results for FDK and ISK were similar to each other, with higher values of accuracy observed for FDK. This similarity could possibly be explained by the higher weight assigned to FDK when calculating ISK (40 %), relative to the other traits (30 % for SEV and 30 % for INC). A striking difference was observed between the MAS models and GS models for these two traits (Fig. 1d, e), especially FDK. For instance, average MAS accuracies

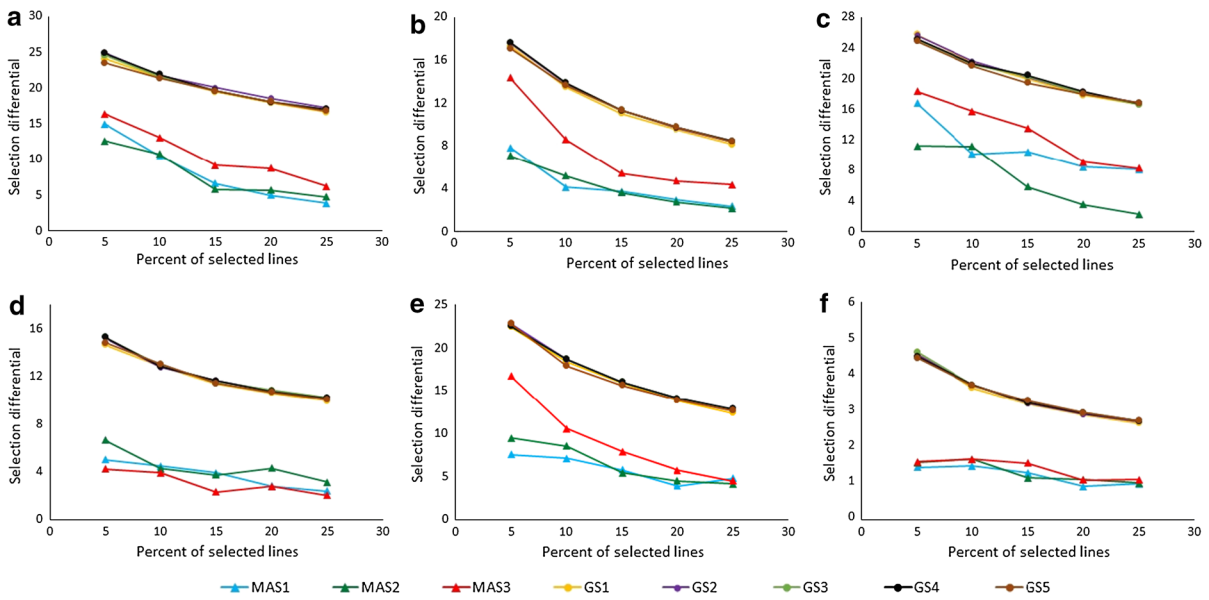

Fig. 2 Selection differential for six traits corresponding to Fusarium head blight (FHB) resistance: severity (**a**), incidence (**b**), FHB index (**c**), Fusarium-damaged kernel (**d**), incidence-severity-kernel index (**e**), and deoxynivalenol concentration (**f**). The numbers on the *x-axis* represent the percentage of selected lines after ranking 273 breeding lines according to GEBVs estimated from five different models: *MAS1* Marker-assisted selection with *Fhb-1* using linear regression. *MAS2* Marker-assisted selection with "*independent*" QTL using multiple linear regression. *MAS3* Marker-assisted selection with "*in house*" QTL using multiple linear regression. *GS1* genomic selection with 19,992 SNPs. *GS2* genomic selection with 19,992 SNPs + 5 randomly selected SNPs treated as fixed effects. *GS3* genomic selection with 19,992 SNPs + *Fhb-1* treated as fixed effect. *GS4* genomic selection with 19,992 SNPs + "*independent*" QTL treated as fixed effects. *GS5* genomic selection with 19,992 SNPs + "*in house*" QTL treated as fixed effects. Graph produced using JMP (SAS 2015b)

for FDK varied from 0.24 (MAS1) to 0.39 (MAS2 and MAS3), whereas average GS accuracies varied from 0.74 (GS2) to 0.90 (GS5). The GS models showed high accuracies for DON, but there were no significant differences among GS1, GS3, GS4, and GS5.

The selection differential varied greatly between MAS and GS models (Fig. 2). Compared to the other traits, the change in the selection differential for DON and FDK was less pronounced with the increase in selection intensity when the MAS models were applied, indicating these models were not appropriate for ranking the breeding lines for these traits. This could be due to the fact that other genomic regions may contribute to variation in these two traits. In fact, none of the SNPs used in MAS3 for FDK were significant (Table 2). In the case of DON, the favorable alleles for the SNPs used in MAS3 are already in high frequency (Arruda et al. 2016). Thus, little change on the mean DON is expected when selecting for these loci. For the other traits, the decrease in the selection differential was more pronounced as more lines were selected.

Discussion

Breeding for FHB resistance remains a major challenge among wheat breeders for several reasons. Since the introgression of *Fhb*-1 from Sumai3 and its derivatives into adapted germplasm in North America (Jin et al. 2013), significant emphasis has been put on using *Fhb-1* and a few other QTL as targets for MAS. Breeders have had success in selecting for FHB resistance, with the level of FHB resistance in commercial cultivars improving over the last two decades for some breeding groups; however, many factors including the complex genetic architecture of FHB resistance, difficulties in precision phenotyping, and delayed availability of high-density genome-wide molecular markers in wheat have hindered progress in breeding for resistance to this important disease.

In this study, we used 19,992 GBS-SNPs and QTL information to build and compare marker-based models for multiple traits corresponding to FHB resistance. Genomic selection and MAS models were designed in such a way that they would reflect breeding strategies currently in use by breeders or have potential to be used in the near future. Our results showed that MAS can lead to poor accuracy for traits associated with FHB resistance, especially when using only *Fhb-1* (MAS1). The poor performance of this model could be associated with the low frequency of *Fhb-1* in our panel (5.5 %). At the same time, GS models greatly outperformed MAS models for all traits, particularly for FDK, which seemed to not be controlled by large effect QTL. For one trait, INC, MAS3 was able to provide accuracy values higher than some GS models. It is possible that INC is under a simpler genetic control when compared to the other traits. This advantage could also be attributed to the "inside trading" effect. Also, the *in house* QTL marker IWGS_CSS_7DS_scaff_3876750_2023 had the highest r^2 of any marker tested (Table 2). Zhao et al. (2014) found MAS outperforming GS for heading time in rye, but not for plant height. In their study, the photoperiod insensitivity gene *Ppd-D1* was found to be associated with a single SNP, whereas plant height was associated with 16 SNPs. A similar conclusion was reached by Spindel et al. (2015), who found MAS to outperform GS for flowering time in rice, controlled by a large effect QTL, whereas RR-BLUP outperformed MAS and other GS models for grain yield, which is known to be quantitatively inherited in rice.

An important assumption of RR-BLUP is that all marker effects share a common variance, no matter how important a particular marker may be for explaining the variation of the trait. This is an unrealistic assumption in the breeding context. By treating QTL as fixed effects in GS models, the QTL effect estimate is not forced to have the same variance as those of the genome-wide markers, which could lead to increased prediction accuracy. In this study, setting QTL as fixed effects improved prediction accuracy for all traits except DON. In fact, neither *Fhb-1* (GS3) nor QTL (GS4 and GS5) treated as fixed effects resulted in higher accuracy for this trait. Rutkoski et al. (2012) compared several GS models and MAS for FHB resistance and concluded that GS always outperformed MAS, but treating QTL as fixed effects did not improve accuracy. However, in a recent study with resistance to stem rust in wheat (caused by *Puccinia graminis* f.sp. *tritici*), Rutkoski et al. (2014) showed that treating major genes as fixed effects can lead to higher GS prediction accuracy. We demonstrated a 32 % advantage of GS model with *in house* QTL treated as fixed, relative to a model with all SNPs treated as random effects. At the same time, more

modest advantage was observed when one (*Fhb-1*) or multiple *independent* QTL were treated as fixed effect. These results suggest the "inside trading" effect can lead to upward biased genomic prediction accuracy. In other words, by using the same panel of lines for QTL identification and prediction analyses with those same QTL, our results indicate that inflated estimation of prediction accuracy can be obtained. To our knowledge, this is the first study to quantify the "inside trading" effect in a plant breeding program context. Further analyses involving independent sets of breeding lines and environments would help understanding this effect in a broader context.

If treating QTL as fixed effect in the genomic selection increases prediction accuracy, we wondered what would have happened to accuracies if randomly selected markers would have been chosen. Our results showed that accuracy can be reduced by treating random SNPs as fixed effect. For a marker not associated with the trait being tested, it is preferable having this marker with effect estimate close to zero than allowing it to have a larger influence on the model (larger effect). In a simulation study, Bernardo (2014) observed that having a single gene treated as fixed in GS using RR-BLUP was never disadvantageous, except when the variability explained by the major gene was lower than 10 %. In our panel, all 19,992 SNPs but one (IWGS_CSS_7DS_scaff_3876750_2023) explained less than 10 % of the variability (Arruda et al. 2016).

Although the GS models differed in terms of prediction accuracy, they performed equally well for selection differential. One possible explanation is that the differences in prediction from these GS models were not enough to substantially change the order of the breeding lines when they are ranked for a specific trait. The same cannot be said for the MAS models, with MAS3 resulting in higher selection differential than MAS1 and MAS2 in nearly all scenarios. This advantage of MAS3, however, is most likely due to the "inside trading" effect. Initially present at 5.5 % of the breeding lines, *Fhb-1* by itself (MAS1) was never the best option, which is in accordance with the complex genetic architecture of FHB resistance.

Conclusion

GS models greatly outperformed MAS models in both prediction accuracy and selection differential for parameters associated with FHB resistance. Treating significant QTL as fixed effects in GS resulted in even higher accuracies; however, accuracies can be inflated by the "inside trading" effect. Although some GS models were more accurate than others, the differences resulted in minimal change in the order of GEBV ranks. These results indicate that GS is a more appropriate marker-based strategy when breeding for a complex trait such as FHB resistance.

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