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ORIGINAL PAPER

Genomic prediction for rust resistance in diverse wheat landraces

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Abstract
Key message **We have demonstrated that genomic selection in diverse wheat landraces for resistance to leaf, stem and strip rust is possible, as genomic breeding values were moderately accurate. Markers with large effects in the Bayesian analysis confirmed many known genes, while also discovering many previously uncharacterised genome regions associated with rust scores.**
Abstract Genomic selection, where selection decisions are based on genomic estimated breeding values (GEBVs) derived from genome-wide DNA markers, could accelerate genetic progress in plant breeding. In this study, we assessed the accuracy of GEBVs for rust resistance in 206 hexaploid wheat (*Triticum aestivum*) landraces from the Watkins collection of phenotypically diverse wheat genotypes from 32 countries. The landraces were genotyped for 5,568 SNPs using an Illumina iSelect 9 K bead chip assay and phenotyped for field-based leaf rust (Lr), stem rust (Sr) and stripe rust (Yr) responses across multiple years. Genomic Best Linear Unbiased Prediction (GBLUP) and a Bayesian Regression method (BayesR) were used to predict GEBVs. Based on fivefold cross-validation, the accuracy of genomic prediction averaged across years was 0.35, 0.27 and 0.44 for Lr, Sr and Yr using GBLUP and 0.33, 0.38 and 0.30 for Lr, Sr and Yr using BayesR, respectively. Inclusion of PCR-predicted genotypes for known rust resistance genes increased accuracy more substantially when the marker was diagnostic (*Lr34/Sr57/Yr18)* for the presence-absence of the gene rather than just linked (*Sr2*). Investigation of the impact of genetic relatedness between validation and reference lines on accuracy of genomic prediction showed that accuracy will be higher when each validation line had at least one close relationship to the reference lines. Overall, the prediction accuracies achieved in this study are encouraging, and confirm the feasibility of genomic selection in wheat. In several instances, estimated marker effects were confirmed by published literature and results of mapping experiments using Watkins accessions.

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Introduction

Wheat is the world's second most important food crop and is a major source of carbohydrates and protein in the human diet. The three rust diseases; namely leaf rust (Lr), stem rust (Sr) and stripe rust (Yr) are a major threat to wheat production (Murray and Brennan 2009). More than 50 loci for resistance to each of the three rust diseases have been genetically characterised and formally named (McIntosh et al. 2010), however, many of these genes have succumbed to matching virulence in respective pathogens. Identification and characterisation of genetically diverse sources of resistance therefore remains a continuing challenge. The

generation of gene combinations in elite high yielding breeding material is challenging as it is difficult to confirm the definite presence of many genes through bioassays that often rely on linkage (Bariana et al. 2007). Implementing a genomic selection strategy could enable more rapid gains to be made for rust resistance, as all loci underlying variation in rust resistance, including those not yet identified, could be exploited simultaneously. Genomic selection is a two-step procedure, where genome wide DNA markers in linkage disequilibrium (LD) with genetic variants affecting the target trait are used to predict genomic estimated breeding value (GEBV), and then selection decisions are made on the basis of these GEBVs (Meuwissen et al. 2001).

The potential for increased genetic gain using genomic selection has been recognised in wheat (Heffner et al. 2009, 2010) and wheat breeding strategies using genomic selection have been proposed (e.g. Bernardo 2010). Genetic gain from genomic selection is linearly proportional to the accuracy of prediction, and therefore genomic prediction research focuses on the accuracy of GEBV that can be achieved. In wheat, genomic prediction accuracies (based on cross-validation) have been reported for CIMMYT global wheat lines (Crossa et al. 2010), and biparental (Heffner et al. 2011a) and multi-family (Heffner et al. 2011b) populations. All studies to date have achieved moderate to high accuracy (e.g. range 0.3–0.8) with relatively few markers. This is mainly due to the self-pollinating nature of wheat, which leads to a high level of LD. For example, Hao et al. (2011) found moderate LD extended for 5 cM in diverse Chinese wheat lines. Similar estimates of LD were reported by Cavanagh et al. (2013) for the analysis of a worldwide collection of wheat cultivars and landraces. In biparental structures, within family LD, which can stretch for many Mb, can be exploited with genomic selection (Hayes et al. 2009; Heffner et al. 2011a). For across family predictions, the level of LD in the population is important. This depends on the effective population size which is a determinant of the number of independent chromosome segments (Goddard 2009), which in turn is a key parameter driving the accuracy of genomic prediction, along with the number of individuals with genotypes and phenotypes, the trait heritability, and the proportion of the genetic variance captured by the markers (Daetwyler et al. 2008, 2010; Erbe et al. 2013).

The "Arthur Watkins" collection is a unique potential reference population for genomic predictions of rust resistance. The collection consists of a large number of phenotypically diverse landraces collected from 32 countries in the 1920s to 1930s, which have been assayed for leaf rust, Yr and Sr response variation over multiple years. The aim of this paper was to investigate the accuracy of genomic prediction in a geographically diverse set of wheat landraces from the Watkins collection for field-based resistance to stem rust, leaf rust, Yrin Australia using trait data measured across several years and sites. Genomic Best Linear Unbiased Prediction (GBLUP) and a Bayesian regression method (BayesR) were used in a cross-validation scheme. The effect of including data for PCR markers linked to known rust resistance genes in genomic prediction models was evaluated. The interplay of genomic prediction accuracy and relatedness of the validation and reference populations was also investigated. Finally, the predicted marker effects were cross-validated against published rust resistance loci and quantitative trait loci (QTL). The implications for genomic prediction in breeding wheat cultivars with durable rust resistance are discussed.

Methods

Plant materials

A set of 247 accessions from the Watkins Collection was supplied by Mr Greg Grimes of the Australian Winter Cereals Collection, Tamworth, and they were selected from a total of 838 accessions from field evaluations on the basis of plant type, rust resistance and maturity. Despite some maturity differences, all landraces included in this study flowered by the first week of October. Non *T. aestivum* accessions were removed. The list of accessions included in this study is given in Supplementary Table S1.

Phenotyping

Records on leaf, stem, and Yr resistance were collected in field conditions across several years (2005, 2006, 2007 and 2008). Scores for rust response were consistent across seasons. Field trials were conducted at the Lansdowne and Karalee sites of the University of Sydney, with each accession grown in a single 1 m row. Disease scores were recorded for all rust traits using a scale of 1–9, where 9 was highly susceptible (Bariana et al. 2007). For Yr, multiple records were taken within each year because disease progression could be observed well, and in 2008 disease scores were collected at both field trial sites (Table 1).

Genotyping

The 247 wheat accessions were genotyped using the Infinium iSelect 9 K single nucleotide polymorphism (SNP) assay, the content of which is reported to have minimal ascertainment bias for the analysis of diverse wheat landraces (Cavanagh et al. 2013). Genotyping was performed on the iScan instrument according to the manufacturer's protocols (Illumina). SNP genotype calling was performed using GenomeStudio v2011.1 software (Illumina) and the

Table 1 Number of observations (*N*), number of replicates (nr) and mean rust scores (rust) for leaf rust (Lr), stem rust (Sr) and stripe rust (Yr) across years, sites (K—Karalee, LDN—Lansdowne)

Trait	2005			2006			2007			2008			2008		
	LDN			LDN			LDN			LDN			K		
	N	nr	rust	*N*	nr	rust	*N*	nr	rust	*N*	nr	rust	*N*	nr	rust
Lr	172	1	5.8	201	1	3.9	187	1	4.4	–	–	–	–	–	–
Sr	198	1	5.1	198	1	6.0	193	1	5.6	–	–	–	–	–	–
Yr	197	1	4.2	402	2	4.1	400	2	3.6	443	3	3.5	287	3	4.2

genotype calling algorithm generated by Cavanagh et al. (2013). Monomorphic markers and SNPs with more than 10 % missing data (due to the presence of null alleles or poor genotype call rates) were removed. Similarly, accessions with more than 15 % missing genotypes were removed. Any missing genotypes for the remaining wheat accessions were imputed using a random forest approach with the R package missForests (Rutkoski et al. 2013; Stekhoven 2013). Each wheat accession was also genotyped for DNA markers linked to *Lr34/Sr57/Yr18* and *Sr2*, as described in Lagudah et al. (2006) and Mago et al. (2011), respectively. The final dataset after all edits included 206 *T. aestivum* accessions.

Genomic prediction

A genomic relationship matrix (**G**) was calculated according to Yang et al. (2010). To improve the numerical stability of **G**, loci with an allele frequency <0.01 were removed. Furthermore, duplicate accessions as determined by <50 opposing homozygotes were removed. Principal component analysis was performed on **G** using the R function eigen (R Core Development Team 2010). **G** was sorted by country of origin and the first principal component and visually assessed using a heat map, where increasing colour intensity indicates higher relatedness of accessions.

Genomic best linear unbiased prediction (GBLUP) was run in ASReml 3.0 (Gilmour et al. 2009) using the following Restricted Maximum Likelihood (REML) model:

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Xb} + \mathbf{Zg} + \mathbf{e} \quad (1)$$

where **y** a vector of rust phenotypes, **1** is a vector of ones, μ is the mean, **X** and **Z** are design matrices, **b** is a vector of fixed effects, **g** is a vector of random additive genetic effects distributed as $N\left(0,\ \sigma_g^2\mathbf{G}\right)$, σ_g^2 is the genetic variance captured by the markers, **e** is a random residual term with $N\left(0,\ \sigma_e^2\mathbf{I}\right)$, and σ_e^2 is the error variance. Multiple records per accession were weighted equally in GBLUP analyses. Genomic heritabilities were estimated as $h^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_e^2}$. Fixed effects included year in all models as well as site when available (Table 1). PCR marker tests for the presence-absence of *Sr2* and *Lr34/Sr57/Yr18* were included as fixed effects for these genes in additional GBLUP analyses for Sr, Lr, and Yr.

Best linear unbiased estimates

Best linear unbiased estimates (BLUE) of rust scores were calculated using the raw phenotype data (Henderson 1984), to account for fixed effects such as year and location. These estimates were then used as "phenotypes" for BayesR and also for calculating the accuracy of genomic prediction, free of year and location effects. The following model was fitted in ASReml (Gilmour et al. 2009): $\mathbf{y} = \mathbf{1}\mu + \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}^*$, where **u** is a vector of random accession effects distributed as $N\left(0,\ \sigma_{\mathrm{ID}}^2\mathbf{I}\right)$, σ_{ID}^2 is the variance due to accessions, e* is a vector of random residuals distributed as $N\left(0,\ \sigma_{e*}^2\mathbf{I}\right)$, and σ_{e*}^2 is the error variance. Fixed effects included year and site (Table 1). Multiple records per accession were weighted equally in BLUE analyses. A multi-variate analysis where each year or environment is considered a separate trait may be another useful way to treat this data. Genotype by environment interactions (G × E) are known to affect plant genomic predictions (e.g. Heslot et al. 2014). Two interactions, accession by site and accession by year, were fitted for the Yr trait as random terms in the BLUE models to determine the proportion of the error and accession variance they explain.

The BLUEs were used to implement the Bayesian genomic prediction method, BayesR (Erbe et al. 2012). The difference between GBLUP and BayesR is that BayesR assumes a priori that some SNPs will have no associated effect on the trait, and that some SNPs can have a moderate to large effect. This is achieved using a mixture of four normal distributions with increasing variance to model marker effects. In contrast, the prior assumption on GBLUP is that all SNPs have small effects that come from the same normal distribution. BayesR applied the model:

$$\hat{\boldsymbol{u}} = \mathbf{1}\mu^* + \mathbf{Wm} + \mathbf{e}^{**} \quad (2)$$

where $\hat{\boldsymbol{u}}$ is a vector of BLUE solutions, μ^* is the BayesR analysis mean, **W** is a design matrix relating $\hat{\boldsymbol{u}}$ to random marker effects (**m**), and e** is the BayesR error. BayesR is more fully described in Erbe et al. (2012). The main difference of BayesR to other Bayesian genomic prediction

methods is its modeling of four marker variance distributions, where each distribution explains gradually more of the genetic variance but contains fewer markers. Thus, the variance attributed to a marker in the four distributions was: $\sigma_1^2 = 0, \sigma_2^2 = 0.0001\sigma_g^2, \sigma_3^2 = 0.001\sigma_g^2$, or $\sigma_4^2 = 0.01\sigma_g^2$, and σ_g^2 was estimated with GBLUP model 1 using REML. A Dirichlet distribution was used as the source of priors for the proportion of markers in each distribution. All priors were identical to those used by Erbe et al. (2012). Ten parallel chains of 100,000 iterations (20,000 burn-in) were run for each subset and marker effects were calculated as the mean of all parallel non-burn-in iterations. In GBLUP fixed effects were fitted simultaneously with **G** (Eq. 1), whereas in the BayesR implementation BLUEs were first calculated and then fitted. Fitting BLUEs in GBLUP resulted in very similar accuracy to simultaneous fitting of fixed effects. PCR marker tests were not included as fixed effects in BayesR analyses. However, it is expected that including them would affect analyses similarly in BayesR as in GBLUP.

Accuracies for genomic prediction were calculated in a cross-validation design that was repeated five times and each replicate divided the data into five random folds of accessions, where all records of an accession were contained within onefold to avoid prediction of an accession from its own phenotypes. For each cross-validation fold, the genomic prediction accuracy was calculated by correlating the GEBVs with the BLUEs [e.g. r (GEBV, $\hat{\boldsymbol{u}}$)]. When PCR marker test genotypes for *Sr2* and *Lr34/Sr57/Yr18* were fitted as fixed effects, the correlation was also calculated as r (GEBV + PCReffect, $\hat{\boldsymbol{u}}$). The mean and standard deviation across all 25-folds was reported. This cross-validation method was used in both the GBLUP and BayesR analyses. The accuracy of an individual's BLUE ($\hat{\boldsymbol{u}}$) would be higher than $\sqrt{h^2}$, due to repeated observations. Thus, to approximate the accuracy of a true breeding value, the correlations were divided by the mean accuracy of validation accessions calculated from the prediction error variance (PEV) of GBLUP as mean(rPEV) $= \frac{1}{N}\sum_{i=1}^{N}(1 - \text{PEV}_i)/\hat{\sigma}_g^2$, where N is the number of accessions with observations.

Finally, a BayesR run with exactly the same priors and number of iterations but using all accessions was performed to estimate marker effects for comparison with the effects of known loci. These were scaled by the adjusted phenotypic standard deviation of the analysed trait to allow for comparisons across traits.

It has been shown in livestock populations that the relatedness of the validation to the reference populations has a substantial influence on genomic prediction accuracy (Clark et al. 2012; Daetwyler et al. 2012; Habier et al. 2010; Pszczola et al. 2012). Two main genomic relationship measures were calculated using the genomic relationship matrix (**G**). First, the mean genomic relationship (Gmean) of each validation line was calculated as the mean of all **G** off-diagonals shared with reference lines. Second, the mean of a certain number (X) of largest relationships (GtopX) of a validation line was calculated by collecting the X largest **G** off-diagonals shared with reference lines and calculating their mean. The parameter X was varied from 1, 5, 10, 20, 30, 40, to 50, to investigate the number of high genomic relationships that were most associated with genomic prediction accuracy. Gtop10 had been shown to be more indicative of genomic prediction accuracy than Gmean (Clark et al. 2012; Pszczola et al. 2012). These relationship measures were calculated in each cross-validation fold (five per cross-validation replicate). Subsequently, within a cross-validation replicate, the GEBVs and relationship measures for all accessions were collected. The mean for each relationship measure was calculated across the 206 lines and it was divided in two groups: more related (>mean) and less related (<mean). Prediction accuracy [r (GEBV, $\hat{u}$)] was then calculated within high and low groups and regressed onto the corresponding mean group (high or low relatedness) relationship measure to investigate which relationship measure explained more of the accuracy of genomic prediction. The significance (P values) and goodness-of-fit values (R^2) were then used to determine the relationship measure most associated with prediction accuracy.

Results

Genetic relatedness among wheat accessions

A total of 7,364 SNPs were successfully genotyped across the 247 wheat accessions using the 9 K iSelect SNP bead chip assay. After removing monomorphic markers and SNPs with more than 10 % missing data, a total of 5,568 SNPs remained. Further quality filtering (see Materials and methods), reduced the population size for subsequent analyses to 206 *T. aestivum* accessions.

Principal component analysis showed no clear relationships among the 206 Watkins accessions (Fig. 1). The amount of genetic variation explained by the first and second principal components was 10.3 and 5.5 %, respectively. No clear clustering by country of origin was observed. Regional labelling revealed that the first principal component tended to loosely cluster European, Australian and North African lines, however, Asian and Middle Eastern accessions showed no such trend.

A heat map of the **G** matrix (Fig. 2) revealed a wide spectrum of low to moderate relatedness between accessions within countries. In each country, there tended to be a small number of accessions that were moderately related. Some pairs of accessions in different countries were also moderately related. However, overall, within country relatedness did not differ markedly to across country relatedness.

Genomic prediction for rust resistance traits

Genomic prediction accuracies for the rust resistance traits using the basic model (without PCR marker tests for *Sr2* and *Lr34/Sr57/Yr18* fitted as fixed effects) ranged from 0.27 (Sr) to 0.48 (Yr) for GBLUP (mean across traits 0.42) and from 0.30 (Yr) to 0.38 (Sr) for BayesR (mean 0.34, Table 2). Fitting the genotypes predictive of *Lr34/Sr57/Yr18* and *Sr2* increased the accuracy slightly for all traits (Table 2). More substantial increases were observed for the *Lr34/Sr57/Yr18* than for *Sr2*, which could be expected as the *Lr34/Sr57/Yr18* marker is diagnostic for the presence-absence of the gene. The inclusion of *Sr2* in the Sr model only had a minimal effect on prediction accuracy. *Sr2* is an introgression from Yarosalv emmer (McIntosh et al. 1995), and is therefore unlikely to be present in common wheat landraces. Furthermore, the *Sr2* marker is not diagnostic and may not be perfectly linked to the causative Sr gene, therefore its presence does not necessarily mean that the causative mutation is present in the Watkins accessions. Hence, it was anticipated the *Sr2* marker would be less beneficial than the *Lr34/Sr57/Yr18* marker for genomic prediction.

The genomic heritabilities estimated using the REML model (Eq. 1) were 0.49, 0.29, 0.52 for Lr, Sr, and Yr, respectively (Table 2). In general, the level of genomic prediction accuracy for each rust trait reflected the level of heritability. This was expected since the accuracy of genomic prediction is governed by the heritability, number of accessions, and number of independent chromosome segments (Daetwyler et al. 2010; Goddard 2009; Hayes et al. 2009). It should be noted that other factors also affect the accuracy of genomic prediction. For example, marker density is important if collectively the markers are not dense enough to capture all causative variants (Daetwyler 2009; Erbe et al. 2013).

Regressions of adjusted phenotypes on GEBVs were calculated to check for possible bias in the evaluation.

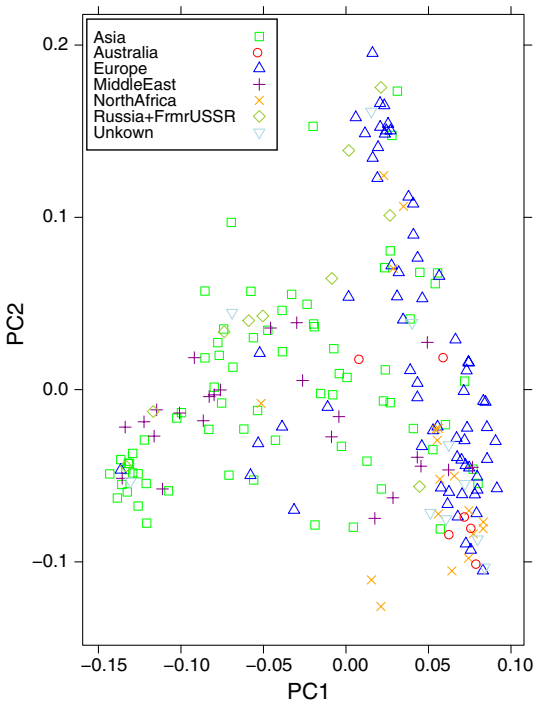

Fig. 1 Plot of the first and second principal components (PC). Accessions are labelled by geographical region of origin

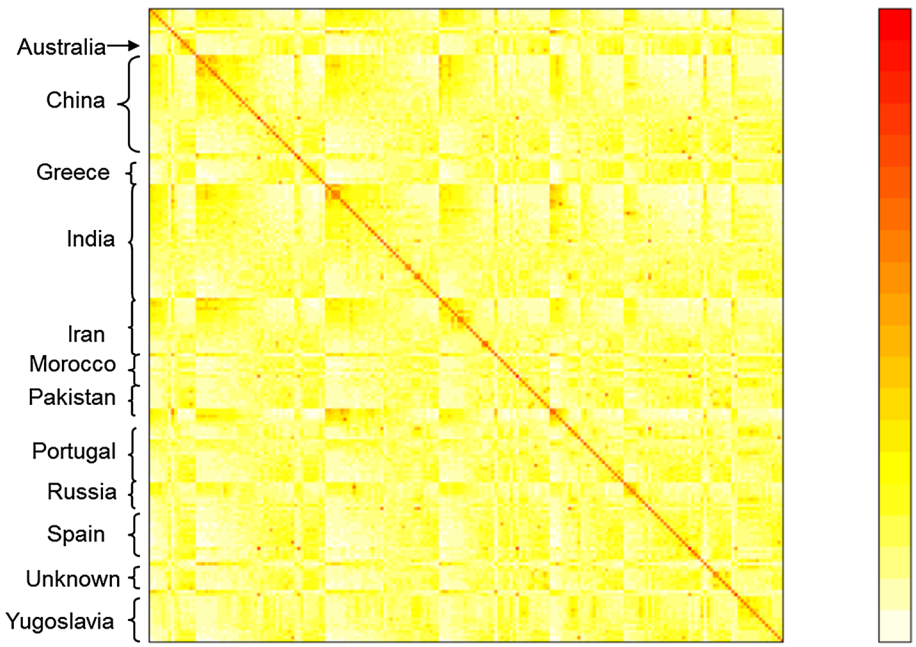

Fig. 2 Heat map of genomic relationship matrix. Accessions are sorted by country of origin and principal component 1. Colours indicate extent of relatedness, with white indicating least related and red more related. Not all countries are labelled, a complete list of country of origin is available in Table S1

Table 2 Genomic heritability (h^2) and prediction accuracy (SD) for leaf (Lr), stem (Sr), and stripe (Yr) rust from Genomic Best Linear Unbiased Prediction (GBLUP) and Bayesian Regression (BayesR), *SD* standard deviations

Trait	h^2	GBLUP			BayesR
		Fivefold (SD)[a]	Fivefold (SD)[b] *Lr34/Sr57/Yr18*	Fivefold (SD)[b] *Sr2*	Fivefold (SD)[a]
Lr	0.486	0.351 (0.096)	0.477 (0.135)	–	0.328 (0.119)
Sr	0.294	0.270 (0.122)	0.314 (0.143)	0.284 (0.132)	0.381 (0.078)
Yr	0.515	0.437 (0.103)	0.461 (0.112)	–	0.300 (0.180)

[a] Basic model

[b] Basic model including PCR marker predicted genotypes fitted as fixed effects

Table 3 Slope of the regression (SD) of adjusted phenotypes on genomic breeding values which is used to measure evaluation bias

Trait	GBLUP			BayesR
	Fivefold[a]	Fivefold[b] *Lr34/Sr57/Yr18*	Fivefold[b] *Sr2*	Fivefold[a]
Lr	0.417 (0.144)	0.519 (0.220)	–	1.157 (0.468)
Sr	0.321 (0.223)	0.366 (0.264)	0.302 (0.200)	1.052 (0.847)
Yr	0.740 (0.200)	0.695 (0.208)	–	1.358 (0.406)

SD standard deviations

[a] Basic model

[b] Basic model including PCR marker predicted genotypes fitted as fixed effects

The slope of this regression has an expectation of 1. Slopes of <1 or >1 (i.e. bias) mean that GEBVs over or under predict the phenotype. Slopes that deviate considerably from expectations are important when GEBVs are combined with or compared to non-genomic measures of genetic merit (such as pedigree information), as their scales might differ considerably leading to a bias towards or against selecting individuals with a GEBV. The intercepts were close to zero for all rust traits (data not shown) and the slopes ranged from 0.3 to 1.4 (Table 3), with a mean of 0.7. The most extreme values were observed for Sr, where fitting with *Sr2* as a fixed effect led to a large downward bias (0.3). As discussed above, *Sr2* was not expected to be present in the Watkins accessions and thus fitting it resulted in an inappropriate model and significant downward bias. Generally, when the inclusion of the trait-linked marker increased accuracy and it also reduced bias.

Relationship between genomic prediction accuracy and relatedness of the validation and reference populations

The genomic relationship of validation to reference lines has been shown to be an important factor in the accuracy of genomic prediction (Clark et al. 2012; Habier et al. 2007, 2010; Pszczola et al. 2012) and it is, therefore, an important component of reference population design. However, it is not clear what relationship measure should be used to design optimal reference populations for genomic prediction. Two genomic measures of relatedness of validation accessions to the reference population were investigated: mean genomic relationship (Gmean) and mean of top *X* relationships (GtopX). The number of high genomic relationships (*X*) most associated with genomic prediction accuracy were investigated using several values (1, 5, 10, 20, 30, 40, 50). Gtop1 (i.e. the highest relationship of validation to reference lines) was most associated with genomic prediction accuracy in all three rust traits (Range *P* value 3×10^{-6} to 2×10^{-4}, R^2 0.83–0.94, Supplementary Table S3). In the following we therefore concentrate on the Gmean and Gtop1 relationship measures.

The connection between genetic relatedness and genomic prediction accuracy was investigated by separating the validation accessions into two groups that were more and less related to the reference population than the mean of the respective relationship measure. The mean genomic relationship (Gmean) was close to zero (−0.010), as expected because the Yang et al. (2010) algorithm effectively sets the mean relationship to zero (Supplementary Table S4) (negative relationships simply mean that pairs of individuals are less related than pairs of individuals in the artificially set base). Genomic prediction accuracy was also not associated with Gmean (R^2 0.04, Fig. 3). In contrast, the mean (across validation accessions) of the highest relationship (Gtop1) (0.988) was much higher, also as expected. The Gtop1 measure was also more highly associated with prediction accuracy (R^2 0.60, Fig. 3). These results suggest that Gtop1 is a good indicator of genomic prediction accuracy and that accuracy will be high if at least one close relationship exists between validation and reference.

Genotype by environment interactions

The focus of this paper was on the genomic prediction accuracy that could be achieved without explicitly considering G × E (year and site effects were of course fitted as fixed

effects in our models). However, we did investigate the extent of G × E in Yr by fitting random accession-by-year (ID × Year) and accession-by-site (ID × Site) effects in the BLUE models. Both interaction terms explained a significant proportion of the phenotypic variation, as determined by differences in the log likelihood of the models (Supplementary Table S5). The ID × Site effect explain a greater proportion of the variance than the ID × Year effect. Both interactions terms, when fitted, reduced the error variance and also the variance due to accessions. The most significant model included a random accession effect, ID × Site and ID × Year. Further improvements in genomic prediction accuracy may be possible by including G × E effects.

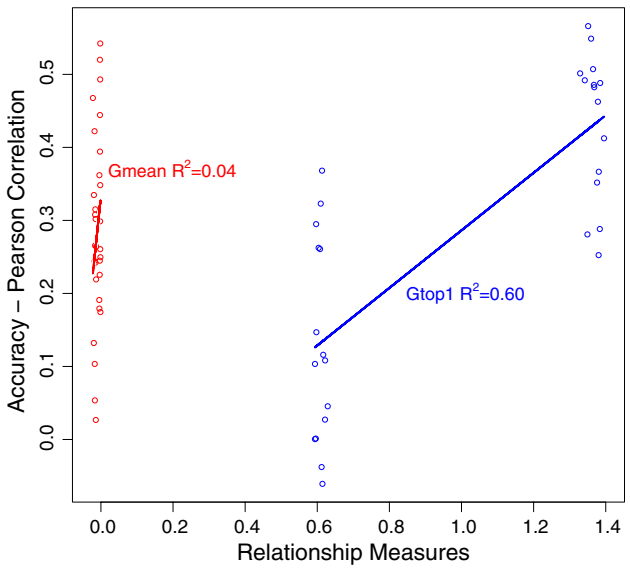

Fig. 3 Linear regression of genomic prediction accuracy (Pearson correlations) on two relationship measures of validation individuals to the reference population: mean genomic relationship (Gmean) and mean top 1 genomic relationship (Gtop1). Each point represents an accuracy in leaf, stem and stripe rust in less or more related categories from five repeated random cross-validations each with five folds (30 points per relationship measure)

Marker effects for rust resistance traits

In an effort to determine if markers of large to moderate effect were contributing to our accuracy of genomic prediction, and to further investigate the genetic basis for rust resistance, we investigated whether the largest BayesR marker effects were concordant with the location of known rust resistance loci. The BayesR SNP effects were first scaled by the adjusted phenotypic standard deviation and positioned on a consensus genetic SNP map (Cavanagh et al. 2013). For the three rust resistance traits, no very large marker effects were identified (Fig. 4, Supplementary Table S2), suggesting that many loci contributed towards trait variation. In general, the magnitude of marker effects reflected the heritability of the traits. On average, marker effects for Sr were lower than Lr and Yr, consistent with lower Sr heritability (Table 2; Fig. 4). The improved resolution in marker effects observed for Yr (Fig. 4), compared to Lr and Sr, likely reflected the availability of more (about threefold) phenotypic records for this trait (Table 1).

Genomic regions for rust resistance

We compared genetic map positions (as reported in Cavanagh et al. 2013) of the first 40 predicted SNPs with largest marker effects for each trait (Supplementary Table S2) with the chromosomal location of published and unpublished rust resistance loci. By comparing trait-linked SNPs identified by genetic mapping of rust resistance loci in crosses conducted with some of the Watkins accessions included in this study (Bansal and Bariana, unpublished results), we were able to validate in five instances the association of the SNP allele identified by genomic prediction with a specific rust resistance locus. For example, SNPs predicted for Yr and Lr resistance on chromosomes 4A, 5B, 4D and

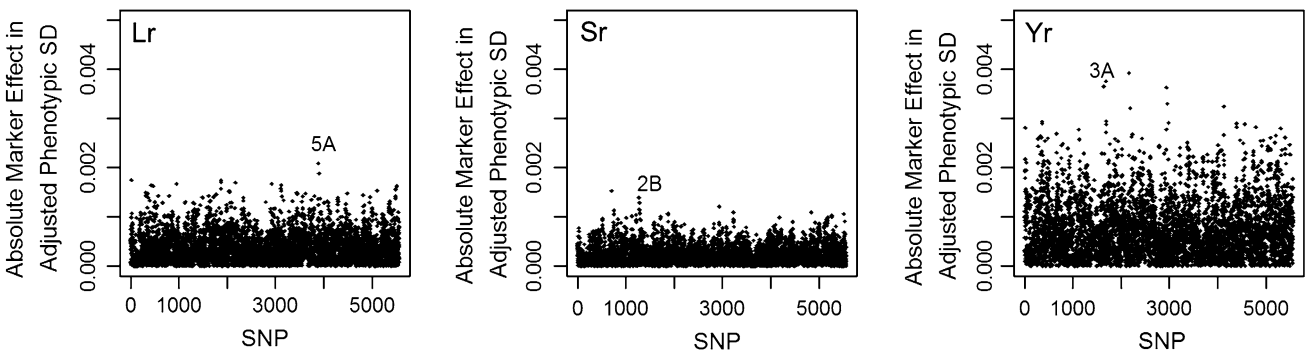

Fig. 4 Mapped marker effects in phenotypic standard deviations (SD) for leaf (Lr), stem (Sr), and stripe (Yr) rust SNPs are ordered (left to right) by position on a consensus SNP genetic map (Cavanagh et al. 2013). Chromosome labels refer to closest peak

genetically mapped to the resistance loci formally named as *Yr51*, *Yr47*, and *Lr67*, respectively. Similarly, markers effects predicted on chromosomes 1A, 2B, 3B and 2B, 5A, 7B genetically mapped to yet unnamed resistance loci for Yr and Sr, respectively. (Supplementary Table S2). Typically, between one-third and one half of the largest 40 marker effects mapped to chromosomal regions that contained published resistance loci and QTL (Supplementary Table S2).

Discussion

We have investigated the accuracy for genomic prediction of rust resistance traits in a diverse sample of wheat landraces. The general level of accuracy was moderate with an average accuracy across traits of 0.38, which is comparable to other published studies in wheat and maize (Crossa et al. 2010; Heffner et al. 2011b). The inclusion of genotypes (predicted using PCR markers) for known rust resistance genes as fixed effects in the genomic prediction models generally yielded increases in prediction accuracy. Prediction accuracy increased more dramatically (e.g. Lr from 0.35 to 0.48) when including the *Lr34/Sr57/Yr18* genotypes than when using the *Sr2* marker. However, *Sr2* is not expected in wheat landraces because it is an introgression. Furthermore, because the marker for *Sr2* is not a gene-based marker, its presence in an accession may not necessarily mean *Sr2* is present. In contrast, the *Lr34/Sr57/Yr18* markers are diagnostic, which further explains their respective predictive abilities.

The accuracy of genomic prediction for a complex trait, where genetic variation is the result of mutations at a large number of loci, is mainly influenced by the trait heritability, the proportion of the genetic variance captured by the markers (i.e. marker density), reference population size, and effective population size. The relatedness of the reference and validation populations is implicitly contained in the term effective population size and, thus, it is a key determinant of genomic prediction accuracy. We are not aware of an estimate for effective population size for our dataset. Therefore, to put our results in context with other studies, we investigated the extent of relatedness using the genomic relationship matrix (Fig. 2). The genomic relationship of half-sibs in outbred populations is expected to be ~0.25 in the Yang et al. (2010) genomic relationship matrix. In livestock, such high relatedness in the data yields high accuracy (e.g. Habier et al. 2010). In our study of a diverse set of inbred wheat lines, the Gtop1 measure was most associated with accuracy and values substantially higher than 0.25 were observed, even in the less related group (Supplementary Table S4). Thus, it is not surprising that relatively high accuracies are achieved with 206 accessions. While our dataset was globally diverse, it seems that it also included a sufficiently large number of close relatives to enable good genomic prediction (Table S3, S4). Our dataset was too small to further separate the validation sets into even less related groups, as this would have raised the sampling variance of our correlations (Fisher 1915) to an unacceptably high level making inference impossible. However, it is expected that the prediction accuracy is low for accessions without close relatives in the reference. The implications for reference populations are clear. They need to be sufficiently diverse to predict a wide variety of lines, but, ideally, they should also contain at least one line that is highly related to a selection candidate to achieve accurate genomic prediction.

BayesR mapping revealed few markers of large effect (Fig. 4), suggesting that many trait loci contributed to genetic variation for leaf rust, Sr and Yr resistance. Nevertheless, it was possible to validate many of the marker effects predicted across the three traits using genetic mapping populations involving the Watkin accessions used in this study, and by comparing the genetic map position for predicted marker effects with the known location of published rust resistance loci and QTL. Our results shows the potential utility of genomic prediction for breeding wheat varieties with more durable rust disease resistance. The use of GEBVs to choose parents for the next generation of crossing, combined with introgression of specific desireable alleles, can be used to accelerate the rate of genetic gain in breeding programs by combining favourable alleles for rust resistance. Similarly, the selection of accessions that possess SNP alleles with large predicted marker effects not associated with known rust resistance loci could be used to further characterise potentially new sources of resistance. We are currently performing genetic analysis in the remaining landraces that were predicted to carry potential new sources of resistance, both to confirm the genomic predictions and increase genetic diversity for rust resistance in Australian wheat breeding programs.

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

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