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Improvement of Genomic Prediction in Advanced Wheat Breeding Lines by Including Additive × additive Epistasis

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Abstract

Key message

Including additive-x-additive epistasis in genetic models did not yield an orthogonal partitioning of genetic variances, nevertheless, it improved the predictive ability of grain yield models for advanced wheat breeding lines.

Abstract

Epistasis is the principal non-additive genetic effect in inbred wheat lines and can be used to develop cultivars based on total genetic merit. Correct models for variance components (VCs) estimation are needed to disentangle the genetic architecture of complex traits in wheat. We aimed to i) evaluate the performance of extended genomic best linear unbiased prediction (EG-BLUP) and the natural and orthogonal interactions approach (NOIA) for VCs estimation in a commercial wheat-breeding population, and ii) investigate whether including epistasis in genomic prediction enhance predictive ability (PA) for wheat breeding lines. In total, 2,060 sixth-generation (F6) lines from Nordic Seed A/S breeding company were phenotyped for grain yield over 21-year-x-location combinations in Denmark, and genotyped using 15K Illumina-BeadChip. Four models were used to estimate VCs and heritability at plot level: i) Baseline, ii) Genomic best linear unbiased prediction (G-BLUP), iii) EG-BLUP, and iv) NOIA. Narrow- and broad-sense heritabilities estimated with G-BLUP were 0.15 and 0.31, respectively. EG-BLUP and NOIA failed to achieve orthogonal partition of genetic variances. Even though NOIA removed Hardy-Weinberg equilibrium assumption, both models yielded very similar estimates, indicating that linkage disequilibrium causes the lack of orthogonality. The PA was studied using leave-one-line-out and leave-one-breeding-cycle-out cross-validations. Both EG-BLUP and NOIA increased PA significantly (16.5%) compared to G-BLUP in leave-one-line-out cross-validation. However, the improvement for including epistasis was not observed in the leave-one-breeding-cycle-out cross-validation. We conclude that although the variance partition into orthogonal genetic effects was not possible, epistatic models can be useful to enhance predictions of total genetic merit.

Keywords

Wheat, genomic prediction, epistasis, non-additive effects, genetic interaction.

Introduction

Bread wheat (*Triticum aestivum* L.) production is closely related to food security since it is widespread around the globe and is essential to meet current and future needs for food. In recent years, large scale genotyping technologies have become more accessible, and the availability of high-dimensional genomic data has encouraged researchers and breeders to develop and implement whole-genome regression (WGR) methods. Genomic selection (GS, Meuwissen *et al.* 2001) methods based on WGR have been successfully applied for a variety of quantitative traits of agronomic importance in animals and plants (Crossa *et al.* 2017; Gianola and Rosa 2015; Kristensen *et al.* 2019; Poland *et al.* 2012).

In wheat breeding studies, a distinction is made between the genomic estimated breeding value (estimated additive genetic effects, GEBVs) and the total genetic value (estimated additive plus non-additive genetic effects). Traditionally, wheat breeders have based the selection of lines on phenotypic selection, which can be seen as a measure of total genetic values. The better performance of GS over phenotypic selection has led many wheat breeding programs to implement GS, and base the selection of lines on the prediction of genomic breeding values (GEBVs), which in general are used to select both breeding lines and commercial varieties. However, the non-additive genetic effects can play a relevant role in the determination of complex traits such as grain yield (Carlborg and Haley 2004; Mackay 2014). Separating additive and non-additive genetic effects can be favorable if it contributes to a more accurate estimate of both additive genetic merit as well as total genetic merit. In this context, separating additive and non-additive genetic effects can result in an improved strategy of selection, allowing to select crossing parents based exclusively on the additive effect, and commercial varieties, based on both additive plus non-additive effects.

The non-additive genetic effects are classified into epistasis and dominance (Fisher 1918). Epistasis is defined as the interaction between alleles at different loci, and it can be divided into three pairwise classes: i) additive $\times$ additive, ii) additive $\times$ dominance and iii) dominance $\times$ dominance, and into higher-order classes as three locus interactions (e.g. additive $\times$ additive $\times$ additive, with a total number of 2^3 terms), or higher-order with 2^n number of terms, where n is the number of loci involved in the interaction. In wheat breeding, commercial cultivars are commonly developed by several generations of selfing to create inbred lines. This means that epistatic interactions are fixed in cultivars and can be inherited for future generations used in commercial production.

Modelling additive $\times$ additive epistasis in genomic prediction (GP) can be restrictive due to the high computational load caused by the high number of interactions between markers if all possible interactions are

considered. Under the condition of independent QTL effect and coming from the same normal distribution, a mathematically equivalent alternative to reduce the computational load is to use models including relationship matrices as covariance structure to model the epistatic effects. Several authors have proposed to extend the best linear unbiased prediction (G-BLUP) model (Habier et al. 2007; VanRaden 2008) by adding epistatic terms with a relationship matrix as covariance structure (extended best linear unbiased prediction, EG-BLUP), which can differ in how this matrix is computed (Jiang and Reif 2015; Martini et al. 2016; Su et al. 2012; Xu 2013). Henderson (1985) and Su et al. (2012) proposed to use the Hadamard product of the additive genetic relationship matrix with itself to approximate the additive $\times$ additive epistatic matrix. The resulting relationship matrix captures additive $\times$ additive interactions plus the dominance effect when it is present. Marker-based epistatic relationship matrices are also proposed to estimate the additive $\times$ additive interactions without including the dominance effect (Jiang and Reif 2015; Martini et al. 2016; Xu 2013). The EG-BLUP model ensures orthogonal partition of genetic variance only for populations whose QTL are under Hardy-Weinberg equilibrium (HWE) and in linkage equilibrium (LE) for all combinations of QTL, conditions that are often violated in breeding populations. HWE and LE are required to satisfy the GP assumption of independence between genetic effects (that is, the QTL effects are assumed to be independent from each other). To reach independence among genetic effects, the QTL must be randomly sampled from gametes, but this is not possible when Hardy-Weinberg disequilibrium (HWD) or linkage disequilibrium (LD) are present in the population. HWD and LD introduce dependence within and between loci, respectively, which produce covariances between genetic effects, making the orthogonal partition of genetic variances with the EG-BLUP model impossible. Recently, Vitezica et al. (2017) proposed to use the natural and orthogonal interactions (NOIA) approach (Alvarez-Castro and Carlborg 2007) to model non-additive genetic effects in GP. The NOIA method allows lifting the HWE requirement for an orthogonal partition of genetic variances, assuming only LE, but, to the best of our knowledge, it has not been tested for wheat inbred lines until this study.

The dominance genetic effect has also been investigated in GS for wheat breeding. Dominance is defined as the effects of allelic interaction within loci (Fisher 1918), and it has been particularly relevant for the heterosis effect in hybrid wheat populations (Jiang et al. 2017; Zhao et al. 2015). Jiang et al. (2017) found a heterosis effect for grain yield in a hybrid population of winter wheat derived from crosses among diverse elite parents. In their study, the hybrids outperformed the mid-parents by 10% on average. The relevance of accounting for dominance in prediction models has also been investigated in simulation studies, reporting an increase in the prediction accuracy for populations presenting a dominance effect when dominance is accounted for in prediction models

(Wellmann and Bennewitz 2012). However, for inbred wheat lines, the dominance effects are very low to negligible due to their reduced heterozygosity, and the epistasis is, therefore, the most relevant non-additive genetic effect.

As mentioned above, the assumptions of HWE and LE are strong requirements for genetic models when breeding populations are analyzed. According to Huang and Mackay (2016), when models wrongly assuming HWE or LE are used, the partition of genetic effects into statistical components may not reflect the biological (or functional) genetic architecture of the traits. Nevertheless, several authors have reported that including epistasis in genetic models can be useful to enhance prediction and selection (He et al. 2016; Hu et al. 2011; Martini et al. 2017). Conversely, other studies reported different results, Jarquín et al. (2014) found that including epistasis did not improve PA, and Lorenzana and Bernardo (2009) even found a negative effect of including epistasis in PA. The discrepancies found in the literature regarding the effect of including epistasis in prediction models reflect that more research is required to clarify the aspects influencing the PA when non-additive genetic effects are considered. In addition, other factors that need further investigation are: i) how much is the real contribution of epistasis to the total genetic variance of most relevant wheat complex traits (e.g. grain yield), and ii) how is the predictive performance of GP models that include epistasis when applied in wheat-breeding populations.

In this study, we use a large set of winter wheat breeding lines, phenotyped for grain yield in multiple environments and in multiple years. Our study had two specific objectives:

- i) To evaluate the performance of the extended genomic best linear unbiased prediction and the natural and orthogonal interactions approach for variance components estimation in a commercial wheat-breeding population.
- ii) To investigate the effect of including epistasis using the extended genomic best linear unbiased prediction and the natural and orthogonal interactions approach on accuracy of genomic prediction of wheat breeding lines.

Materials and Methods

Experimental data

- The plant material consisted of 2,060 F₆ winter wheat lines (*T. aestivum* L.) developed by the breeding company Nordic Seed A/S. The data was collected from seven breeding cycles from 2013 to 2019, each including around 330 lines evaluated in three locations in Denmark (DK): Odder (Central DK), Holeby (South DK) and Skive (North DK). The F₆ lines of each breeding cycle originated from approximately 60 parental line-crosses, followed by five generations of selfing, including creating single seed descent (SSD) lines in generation F₄. The breeding cycles from 2013 to 2016 were evaluated in two consecutive years (cycle 1: 2013-2014, cycle 2: 2014-2015, cycle 3: 2015-2016, cycle 4: 2016-2017), and the cycles coming from 2017 to 2019 were evaluated in one year only (cycle 5: 2017, cycle 6: 2018, cycle 7: 2019). The field trials consisted of 15 blocks of 46 line plots of 8.25 m² per year × location combination. Each block had two replicates of 21 F₆ lines and two checks randomly assigned. The experimental conditions within the year × location subsets were homogeneous for the trials (e.g. sowing time, application of treatments, assessment time). The quantitative trait analyzed in this study was the yield measured as kg per plot (8.25 m²).

Genotyping

DNA extractions from the plant material were based on a modified CTAB method (Rogers and Bendich 1985). The genotyping was carried out using a 15K Illumina Infinium iSelect HD Custom Genotyping BeadChip technology (Wang et al. 2014). For the quality control, the SNPs with minor allele frequency (MAF) lower than 5 % and missing values higher than 10 % were removed. In total, 10,688 SNPs remained after the quality control.

Statistical models

This study compared four different models. Firstly, a baseline mixed model without genomic information (eq. 1) including fixed and random effect was used as the starting point for the construction of the other models (Cericola et al. 2017; Tsai et al. 2020). Secondly, a G-BLUP (eq. 2) model (Habier et al. 2007; VanRaden 2008) was used to capture the additive genetic effect. The two remaining models EG-BLUP and NOIA (eq. 4 and 5, respectively), extended the previous models by the inclusion of the pairwise additive × additive epistatic term and differ in the method used to build the relationship matrices for the additive and the epistatic effects.

Baseline model

The baseline model (eq. 1) was developed considering the main sources of variability affecting the experimental data and included them as fixed or random effects. A similar model has also been presented in earlier studies working with a set of data from Nordic Seed A/S (Cericola et al. 2017; Tsai et al. 2020). The baseline model can be defined as:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{l} + \mathbf{Z}_2\mathbf{f} + \sum_{i=1}^9 \mathbf{Z}_i\mathbf{s} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is the vector of observed phenotypes; $\mathbf{X}$ is the design matrix for fixed effects; $\mathbf{b}$ is the vector of fixed trial effects nested within year, location and breeding cycle; $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are design matrices of random effects; $\mathbf{l}$ is a vector of line effect with $\mathbf{l} \sim N(0, \mathbf{I}\sigma_l^2)$, where $\mathbf{I}$ is an identity matrix and σ_l^2 is the variance due to uncorrelated line effects; $\mathbf{f}$ is a vector of genotype by environment interaction (lines $\times$ year $\times$ location) with $\mathbf{f} \sim N(0, \mathbf{I}\sigma_f^2)$, where σ_f^2 is the variance due to uncorrelated genotype by environment effects; $\mathbf{s}$ is a vector of spatial effect with $\mathbf{s} \sim N(0, \mathbf{I}\sigma_s^2)$, where σ_s^2 is the spatial effect variance. The spatial effect contains the X and Y coordinate of the target plot and the eight surrounding plots ($n = 9$), for plots located in the border, virtual plots were added to guarantee all plots have $n = 9$ (Supplementary material **Figure 1S**). Therefore, the spatial effect on an individual plot is the sum of effects with the square centered on the plot itself plus the effects of eight surrounding plots with a square centered on those plots; $\mathbf{e}$ is a vector of random residuals with $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, where σ_e^2 is the residual variance. All random components defined are independent and identically distributed.

Note that the genetic term in the baseline model is miss specified since the model assumes all lines to be unrelated. Therefore, it may lead to a biased estimation of the total genetic variance.

G-BLUP model

The G-BLUP model was the second model defined (Habier et al. 2007). The G-BLUP model can be defined as:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{l} + \mathbf{Z}_3\mathbf{g}_1 + \mathbf{Z}_2\mathbf{f} + \sum_{i=1}^9 \mathbf{Z}_i\mathbf{s} + \mathbf{e} \quad (2)$$

where $\mathbf{X}$, $\mathbf{Z}_1$, $\mathbf{b}$, $\mathbf{l}$, $\mathbf{f}$, $\mathbf{s}$, and $\mathbf{e}$ are the same as described in the baseline model (eq. 1); $\mathbf{g}_1$ is a vector of additive genomic breeding values with $\mathbf{g}_1 \sim N(0, \mathbf{G}_{HWE}\sigma_{g_1}^2)$, where $\sigma_{g_1}^2$ is the genomic additive genetic variance, and $\mathbf{G}_{HWE}$ is the genomic relationship matrix (G-matrix) assuming HWE. The G-matrix was constructed based on the first method proposed by VanRaden (2008):

$$\mathbf{G}_{HWE} = \frac{\mathbf{Z}\mathbf{Z}'}{2\sum p_i(1-p_i)} \quad (3)$$

where p_i is the minor allele frequency of the i^{th} SNP; $\mathbf{Z}$ was calculated as $\mathbf{Z} = \mathbf{M} - \mathbf{P}$; $\mathbf{M}$ is a matrix of SNP markers coded 0, 1, 2; and $\mathbf{P}$ is a matrix with the minor allele frequencies of SNP i calculated as $\mathbf{1}(2(p_i - 0.5))$ for column i .

EG-BLUP model

The G-BLUP model (eq. 2) was extended to include the pairwise additive $\times$ additive epistatic interactions term (EG-BLUP). For the EG-BLUP model, we followed Henderson (1985) and Su et al. (2012), who proposed to build an epistatic relationship matrix (hereinafter $\mathbf{H}_{HWE}$) derived directly from the G-matrix. When only second order interactions are considered (i.e. additive $\times$ additive) $\mathbf{H}_{HWE} = \mathbf{G}_{HWE} \odot \mathbf{G}_{HWE}$, where $\odot$ denotes the Hadamard product operation. The EG-BLUP model (eq. 4) is defined as:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{l} + \mathbf{Z}_3\mathbf{g}_1 + \mathbf{Z}_4\mathbf{h}_1 + \mathbf{Z}_2\mathbf{f} + \sum_{i=1}^9 \mathbf{Z}_i\mathbf{s} + \mathbf{e} \quad (4)$$

where $\mathbf{X}$, $\mathbf{Z}_n$, $\mathbf{b}$, $\mathbf{l}$, $\mathbf{f}$, $\mathbf{s}$, $\mathbf{g}_1$, and $\mathbf{e}$ are the same as described in the baseline and G-BLUP models (eq. 1 and 2); $\mathbf{h}_1$ is a vector of epistatic values for the lines with $\mathbf{h}_1 \sim N(0, \mathbf{H}_{HWE}\sigma_{h_1}^2)$, where $\mathbf{H}_{HWE}$ is the epistatic relationship matrix assuming HWE, and $\sigma_{h_1}^2$ is the genomic variance of epistatic effects. Since the $\mathbf{H}_{HWE}$ is computed as the power of the additive relationship matrix, it is also able to capture dominance genetic effects (Martini et al. 2016). However, as we are working with inbred F_6 populations, there is a low level of heterozygosity (2.70 % empirically), and a very low to negligible dominance effect is expected for the analyzed population.

The G-BLUP (eq. 2) and EG-BLUP (eq. 4) models defined so far work under the assumptions of HWE and LD, which has been the most common assumption in GS. When HWD and LD are present, the contrasts used to quantify and separate genetic effects and build the relationship matrices do not achieve orthogonality among genetic effects.

NOIA approach

For our last model, we based on the NOIA approach proposed by Alvarez-Castro and Carlborg (2007) and later introduced in GS by Vitezica et al. (2017). The NOIA approach still works under the assumption of LE among QTL but allows for relaxing the HWE assumption for an orthogonal estimation of genetic variances. The fourth model (hereinafter NOIA model) is defined as:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{l} + \mathbf{Z}_3\mathbf{g}_2 + \mathbf{Z}_4\mathbf{h}_2 + \mathbf{Z}_2\mathbf{f} + \sum_{i=1}^9 \mathbf{Z}_i\mathbf{s} + \mathbf{e} \quad (5)$$

where $\mathbf{X}$, $\mathbf{Z}_n$, $\mathbf{b}$, $\mathbf{l}$, $\mathbf{f}$, $\mathbf{s}$, and $\mathbf{e}$ are the same as described in the baseline model (eq. 1); $\mathbf{g}_2$ is a vector of genomic breeding values with $\mathbf{g}_2 \sim N(0, \mathbf{G}_{NOIA}\sigma_{g_2}^2)$, where $\mathbf{G}_{NOIA}$ is the additive relationship matrix and $\sigma_{g_2}^2$ is the genomic additive variance; $\mathbf{h}_2$ is a vector of epistatic genomic values for the lines with $\mathbf{h}_2 \sim N(0, \mathbf{H}_{NOIA}\sigma_{h_2}^2)$, where $\mathbf{H}_{NOIA}$ is the epistatic relationship matrix and $\sigma_{h_2}^2$ is the genomic epistatic variance. The relationship matrices $\mathbf{G}_{NOIA}$ and $\mathbf{H}_{NOIA}$ were constructed based on Vitezica et al. (2017):

$$\mathbf{G}_{NOIA} = \frac{\mathbf{H}_a \mathbf{H}_a'}{\text{tr}(\mathbf{H}_a \mathbf{H}_a')/n} \quad (6)$$

$$\mathbf{H}_{NOIA} = \frac{\mathbf{G}_{NOIA} \odot \mathbf{G}_{NOIA}}{\text{tr}(\mathbf{G}_{NOIA} \odot \mathbf{G}_{NOIA})/n} \quad (7)$$

where $\mathbf{H}_a$ is a n rows (number of lines) $\times$ m columns (number of markers) matrix containing the additive coefficients, as:

$$\mathbf{H}_a = \begin{pmatrix} \mathbf{h}_{a_1} \\ \vdots \\ \mathbf{h}_{a_n} \end{pmatrix}$$

where $\mathbf{h}_{a_i}$ is a row vector for the i^{th} individual with m columns. For individual 1 with marker $j = 1, \dots, m$, the element $\mathbf{h}_{a_1j}$ is equal to:

$$\mathbf{h}_{a_1j} = \begin{cases} -(p_{Aa} - 2p_{aa}) \\ -(1 - p_{Aa} - 2p_{aa}) \\ -(2 - p_{Aa} - 2p_{aa}) \end{cases} \text{ for genotypes } \begin{cases} AA \\ Aa \\ aa \end{cases}$$

where p_{Aa} and p_{aa} are the genotypic frequencies for the genotypes Aa and aa in locus A . The terms $\text{tr}(\mathbf{H}_a \mathbf{H}_a')/n$ and $\text{tr}(\mathbf{G}_{NOIA} \odot \mathbf{G}_{NOIA})/n$ are the traces for $\mathbf{H}_a \mathbf{H}_a'$ and $\mathbf{G}_{NOIA} \odot \mathbf{G}_{NOIA}$ matrices, which standardize $\mathbf{G}_{NOIA}$ and $\mathbf{H}_{NOIA}$ to a variance equal to 1.

Variance components and heritability

The estimation of VCs was performed using the Average Information Restricted Maximum Likelihood (AI-REML) algorithm in the DMU software (Madsen and Jensen 2013). The phenotypic variance of the plot (σ_p^2) for the G-BLUP model (eq. 2) was calculated as:

$$\hat{\sigma}_p^2 = \hat{\sigma}_l^2 + d(\mathbf{G}_{HWE})\hat{\sigma}_{g_1}^2 + \hat{\sigma}_f^2 + 9\hat{\sigma}_s^2 + \hat{\sigma}_e^2 \quad (8)$$

where $\hat{\sigma}_l^2$ is the estimated variance of the line that cannot be attributed to the markers; $d(\mathbf{G}_{HWE})$ is the mean of the diagonal elements of the $\mathbf{G}_{HWE}$; $\hat{\sigma}_{g_1}^2$ is the genomic estimated additive variance; $\hat{\sigma}_f^2$ is the genotype by

environmental estimated variance; $9\hat{\sigma}_s^2$ is the estimated spatial variance for an individual plot ($\hat{\sigma}_s^2$) multiplied by nine, which is the total number of plots considered as random effect for each observation; $\hat{\sigma}_e^2$ is the estimated variance of residuals. Narrow (eq. 9) and broad-sense (eq. 10) plot heritability for the G-BLUP model (eq. 2) were estimated as:

$$\hat{h}^2 = d(G_{HWE})\hat{\sigma}_{g_1}^2 / \hat{\sigma}_p^2 \quad (9)$$

$$\hat{H}^2 = (\hat{\sigma}_l^2 + d(G_{HWE})\hat{\sigma}_{g_1}^2) / \hat{\sigma}_p^2 \quad (10)$$

Additionally, for the EG-BLUP (eq. 4) and NOIA (eq. 5) models, the estimated epistatic variances ($\hat{\sigma}_{h_1}^2$ and $\hat{\sigma}_{h_2}^2$ for EG-BLUP and NOIA models, respectively) multiplied by their diagonals ($d(\mathbf{H}_{HWE})$ and $d(\mathbf{H}_{NOIA})$) were considered in the calculation of broad-sense heritability and total phenotypic variance ($\hat{\sigma}_p^2$) for those models. For the baseline model, only the broad-sense heritability was calculated.

Cross-validation schemes and model validation

The PA ($r_{\hat{g},p}$) of the models was evaluated using two cross-validation (CV) schemes: i) leave-one-line-out (LOO), and ii) leave-one-breeding-cycle-out (LSO) CV. The LOO CV scheme was used to get the PA with the largest reference population possible and investigate the potential performance of the genetic models on PA. The LOO strategy was performed by masking the phenotype of a single line and using the remaining lines to predict the GEBV and the Genomic Estimated Epistatic Value (GEEV) of the masked line. This methodology was repeated n-times (n = no. of lines = 2,060) until all lines were predicted. The LSO CV was used to measure the PA of genetic models in conditions closer to those observed in wheat breeding programs. For LSO the phenotypes from a breeding cycle were masked, and the information from the remaining breeding cycles was used to predict the genetic values. This process was repeated n-times (n = no. of breeding cycles = 7) until all breeding cycles were predicted. The PA was calculated as the Pearson correlation between the lines averages after correcting by fixed effects and the predicted values, being the additive predicted values (predicted GEBVs) for the G-BLUP model, and the additive (predicted GEBVs) and epistatic (predicted GEEVs) values for the EG-BLUP and NOIA models. The lines averages corrected by fixed effects were computed first subtracting the fixed effects from each corresponding plot observation, and then averaging the values of the lines without fixed effect across replications. To contrast the PA for models in the LOO CV scenario, an ordinary nonparametric bootstrap with replacement based on a sample size equal to n = 2,060 (full sample size), and 10,000 replicates was performed. In each bootstrap replication the PA was recorded until reach 10,000 bootstrap based PAs, and the standard error of PAs was obtained. The bootstrap procedure was performed for G-BLUP and for both epistatic models, and a two-tailed

paired *t*-test was used to contrast the bootstrap PAs from different models (*P*-value: 0.01). The relative difference (RD) in PA between prediction for the additive genetic effect using G-BLUP (GEBVs) and total genetic effect using EG-BLUP (GEBVs + GEEVs) was estimated as: $RD = \frac{EGBLUPr_{\hat{g},p} - GBLUPr_{\hat{g},p}}{GBLUPr_{\hat{g},p}}$.

The statistics for bias in prediction of genetic values (μ_{wp}), and a test for variance inflation, measured as the regression coefficient between observed on predicted values ($b_{w,p}$), were estimated according to the LR method (Legarra and Reverter 2018). The μ_{wp} was calculated as $\mu_{wp} = E(\widehat{u}_p - \widehat{u}_w)$; where $\widehat{u}_p$ represented the mean of the genomic estimated values with “partial” (subscript *p*) information (estimations when their own phenotypes were masked) and $\widehat{u}_w$ represented the mean of the genomic estimated values with “whole” (subscript *w*) information (estimations with the complete phenotypes), respectively. The statistics μ_{wp} has an expected value of 0 when the estimations are unbiased. The $b_{w,p}$ was calculated as the regression of estimated values obtained with whole information (subscript *w*) on the estimated with partial information (subscript *p*), $b_{w,p} = \frac{cov(\widehat{u}_w, \widehat{u}_p)}{var(\widehat{u}_p)}$. The statistic $b_{w,p}$ has an expectation $E(b_{w,p}) = 1$ when there is no under or over dispersion of the predictions. Additionally, the Pearson correlation was used to compare predictions between models, where the correlation between the estimated values with whole information for the G-BLUP and EG-BLUP models ($\rho_{G\ BLUP_{GEBV}, EG\ BLUP_{GEBV}}$ and $\rho_{G\ BLUP_{GEBV}, EG\ BLUP_{GEEV}}$) were calculated.

Results

Phenotyping and genotyping

The descriptive statistics for grain yield are presented in **Table 1**. The average yield was 8.71 kg of grain for an 8.25 m² plot, ranging from 3.85 to 12.35 kg/8.25m², and the coefficient of variation was 11.27 % when using the simple SD with all observations.

A total of 10,688 SNPs passed the quality control filters and were used to build the genomic relationship matrices. According to the heat map and the principal component analysis of the G-matrix (**Figure 1**), there was no clear separation of breeding cycles. However, there was a trend that lines coming from the first four breeding cycles were more separated by the first principal component from lines coming from last three breeding cycles. The first and second principal components together explained 52.8 % of the total variance (40.4 and 12.4 % of the variance for first and second principal component, respectively) showing that there are strong relationships between the lines included in the study. The observed level of heterozygosity of the lines had an average value of 2.70 % as expected after five generations of selfing.

Variance components and heritability

Four models differing in how the genetic components were treated (eq. 1, 2, 4 and, 5) were used to estimate VCs and the narrow-sense and broad-sense plot heritabilities (**Table 2**). The estimates for total phenotypic ($\hat{\sigma}_p^2$) and error variance ($\hat{\sigma}_e^2$) were equivalent for all models. The highest variance was attributed to the genotype by environment interaction, which explained around 40 % of the total variability. The estimated total genetic variance ($\hat{\sigma}_G^2$) was largest when the G-BLUP model was used, followed by the models including epistasis (eq. 4 and 5) with a slightly lower value, and the baseline model (eq. 1) with the lowest value. The models using genomic information captured around 15 % more $\hat{\sigma}_G^2$ compared to the baseline model.

The proportions of total genetic variance $\hat{\sigma}_G^2$ estimated by the different models are shown in **Figure 2**. For the G-BLUP model, the estimated additive variance ($\hat{\sigma}_{g1}^2$) was approximately half of the total genetic variance $\hat{\sigma}_G^2$ (48.8 %). The partition of the estimated variances for the EG-BLUP and NOIA models changed considerably compared to the G-BLUP model. The estimate of additive genetic variances ($\hat{\sigma}_{g1}^2$ and $\hat{\sigma}_{g2}^2$ for EG-BLUP and NOIA models, respectively) were reduced to approximately 20 %, and the estimated epistatic variances ($\hat{\sigma}_{h1}^2$ and $\hat{\sigma}_{h2}^2$) represented 65.4 % of the total genetic variance $\hat{\sigma}_G^2$. Note, that the inclusion of an epistatic term in the models captured much of what had previously been part of the estimated line and additive variances in the G-BLUP model,

these results were similar for both epistatic models (see *EG-BLUP and NOIA equivalences* section). For the interpretation of these results, it cannot be ignored that all models tested in this work assume LE between QTL at different loci for an orthogonal estimation of genetic variances, but the LE requirement is not met by the current wheat-breeding population (Cericola et al. 2017). Further explanations of the effect of departures from LE on the estimation of genetic variances are presented later in the discussion section “*Effect of linkage disequilibrium on genetic variances*”.

The G-BLUP model had the highest H^2 estimate, which was 0.31. The models including epistasis (EG-BLUP and NOIA) had a slightly lower H^2 than the G-BLUP, with an H^2 of 0.30. The narrow-sense heritability estimated (h^2) for the G-BLUP model on the plot level had a value of 0.15. For the EG-BLUP and the NOIA models, h^2 was not estimated because the estimation of additive variance may be especially biased due to the lack of orthogonality among genetic effects when the epistatic genetic effect is considered in the model (see section *Effect of linkage disequilibrium on genetic variances* for more details).

EG-BLUP and NOIA equivalence

As demonstrated by Joshi et al. (2020), for populations where the dominance effect is not significant, the EG-BLUP and NOIA methods are equivalent. When the frequency of heterozygotes is low (and therefore there is no dominance effect expected for the population), the allelic frequencies of the markers used to build G_{HWE} and the genotypic frequency used to build G_{NOIA} have similar values. Therefore, the G_{HWE} and G_{NOIA} relationship matrices differ only in the scale parameter (denominator of the formula), which resulted in similar VCs estimates and predictive performance.

Genomic prediction

The PA between the lines averages after correcting for fixed effects and the predicted genetic values ($r_{\hat{g},p}$) was evaluated for the proposed models using LOO and LSO CV schemes (**Figure 3**). In order to simplify the reading and as the EG-BLUP and NOIA models were equivalent, only results from GBLUP and EG-BLUP are displayed in this section. The Baseline model was not included because such a model has no PA in CVs due to the model assumptions of independence between lines.

In the LOO CV, the highest PA was observed for prediction of total genetic merit (additive plus epistatic genetic effects), combining the predictions for the additive effect (GEBVs) plus the predictions for the epistatic effect (GEEVs) from the EG-BLUP model. The theoretical maximum PA was also the highest for the EG-BLUP model combining additive plus epistatic predictions (green bars in **Figure 3**). The PA of the EG-BLUP for total

genetic merit was contrasted to the PA of G-BLUP for the additive effect, and it was significantly different in a two-tailed paired *t*-test (*P*-value: 0.01), showing an increase of 16.5 % in PA for the EG-BLUP model. The GEBVs and GEEVs from EG-BLUP model used separately showed a lower PA than the combined (GEBVs + GEEVs). The highest PA for the additive value of the lines was achieved using the GEBVs from the G-BLUP model. Conversely, for the LSO CV scheme, the highest PA between predicted genetic values and corrected phenotypes was reached when the GEBVs from G-BLUP were used (PA = 0.20), and the PA using the GEBVs plus GEEVs from EG-BLUP (PA = 0.19) represent a decrease of 3.9 % compared to the G-BLUP.

Model validation

The regression coefficient ($b_{w,p}$), used as a test of variance inflation, was measured as the slope of the regression between observed and predicted values (**Figure 4**). In the LOO CV, the $b_{w,p}$ did not present significant under- or over-dispersion since it had values around 1 for both models (**Figure 4, a-c**). The GEBVs from G-BLUP and EG-BLUP models had both a $b_{w,p}$ value of 0.99, while the GEEVs presented a value of 1.04. The $b_{w,p}$ was also estimated for the combination of EG-BLUP predictions (GEBVs + GEEVs, data not displayed in the plot), which took an intermediate value of 1.02. In the LSO CV, the $b_{w,p}$ statistic indicates over-dispersion (inflation) for predicted values since it had values below 1 (**Figure 4, d-f**). The GEBVs from G-BLUP and EG-BLUP models had $b_{w,p}$ values of 0.85 and 0.91, respectively, while the GEEVs from the EG-BLUP model had a lower $b_{w,p}$ value of 0.70. The $b_{w,p}$ for the combination of EG-BLUP (GEBVs + GEEVs, data not displayed in the plot) predictions took an intermediate value of 0.78.

The bias in prediction of genetic values (μ_{wp}) was analyzed following the LR method (**Table 3**). For both LOO and LSO CVs, all the predictions showed a μ_{wp} close to 0 for G-BLUP and EG-BLUP models, which reflects unbiased estimation for all cases.

Correlation between G-BLUP and EG-BLUP estimates

The additive and epistatic estimates using complete phenotypic information for the G-BLUP and EG-BLUP models were compared using Pearson's correlation ($\rho_{G-BLUP_w EG-BLUP_w}$). The correlation for GEBVs between G-BLUP and EG-BLUP models had a high value of 0.94, while the correlation between GEBVs from G-BLUP and GEEVs from EG-BLUP, had a lower value of 0.65. It was also reflected in the ranking of the best lines for the different genetic effects, where 7 of the 10 lines with highest GEBVs were common for G-BLUP and EG-BLUP predictions, but when GEBV from G-BLUP and GEEV from EG-BLUP were compared, only 3 of 10

lines were common. The Supplementary material (**Figure S2**) shows the squared estimated genomic values for the additive and epistatic effects. As it can be observed, the differences in the magnitude of the correlations between the genetic estimated effects are translated into more similar (e.g. **Figure S2a** and **S2b**) or different (e.g. **Figure S2a** and **S2c**, or **S2b** and **S2c**) squared estimated genomic values.

Discussion

In this study, we investigated the performance of the EG-BLUP and NOIA models in the estimation of VCs for a set of advanced wheat breeding lines from the commercial breeding company Nordic Seed A/S. The EG-BLUP and NOIA models were not able to achieve an orthogonal estimation of genetic variance components, and both epistatic models yielded similar variance estimates and predictive performance in the analyzed population. We also investigate the PA ability for the developed models in two CVs schemes: i) leave-one-line-out and ii) leave-one-breeding-cycle-out. We observed a significant increase of 16.5 % (P -value < 0.01) in the PA for the LOO CV when EG-BLUP and NOIA models were used to predict total genetic merit compared to G-BLUP predictions. However, the improvement for including epistasis was not observed in the LSO CV, where smaller differences between PA from G-BLUP and both epistatic models were observed.

Variance components

The partition of genetic variance through EG-BLUP and NOIA models led to problems of non-orthogonality of genetic effects. The clearest signal of lack of orthogonality was observed in the difference of the estimated additive variance between G-BLUP and both epistatic models. When the epistatic effect was present in the models, it caused a considerable reduction in the additive variance (58.4% of reduction) compared to the G-BLUP model estimation. The causes of non-orthogonal partition of genetic variances are attributed to the use of models that wrongly assume HWE and LE (Hill and Mäki - Tanila 2015; Vitezica et al. 2017). Most natural and breeding populations violate HWE and LE assumptions for several reasons. For example, inbreeding in wheat lines will strongly deviate genic proportions from HWE. The NOIA approach relaxed the HWE assumption by constructing the relationship matrices based on G_{NOIA} (Vitezica et al. 2017) relationship matrix. Therefore, if the QTL of the population are in LE (and the marker information is in complete LD with QTL), the additive and additive $\times$ additive terms of NOIA model are orthogonal by definition, and the additive and epistatic genetic effect are independent (Cockerham 1954). However, this is a theoretical scenario, and the LE assumption may never be valid for GS since QTL are in LD due to population evolution and selection, and there is incomplete LD among markers and QTL.

The EG-BLUP and NOIA models yielded very similar estimates of genetic variances. We found that in this population with a low level of heterozygosity, and therefore, low dominance effect, the allelic frequency used to build G_{HWE} and the genotypic frequency used to build G_{NOIA} had similar values. Therefore, the genomic relationship matrices from both epistatic models differ only in the scaling parameter, resulting in similar estimates

of VCs. This result agrees with what recently demonstrated by Joshi et al. (2020), where for a low dominance Tilapia population, both models yielded similar estimates of genetic variances for different traits. In our study, using HWE assumption did not affect the orthogonality of genetic variance for a wheat-breeding population, which can be evidenced in the same negative sampling correlation (-0.36) among additive and additive $\times$ additive genetic effects for both epistatic models. Our results suggest that both approaches failed in the VCs estimation when linkage disequilibrium (LD) is present. These results have also been consistent with the simulation study performed by Vitezica et al. (2017), where they tested the performance of EG-BLUP and NOIA models in an LD simulated population, and concluded that VCs were wrongly estimated with both approaches.

Narrow and Broad-sense Heritability

The interpretation of the h^2 is strongly related to the orthogonality of the estimated genetic variances. When additive and non-additive effects are considered in the genetic models, and forces like HWD or LD operate in the population, the estimation of h^2 is affected due to bias in the estimates of additive genetic variance. The analyzed population of advanced wheat breeding lines (and generally most advances breeding populations), has been under strong selection pressure, and therefore, a significant level of LD among QTL is expected due to co-selection. Despite the possible bias in the estimation of the additive variances due to LD, the interpretation of the h^2 for models including only additive genomic effects as the G-BLUP, has proven to be useful in plant and animal breeding over the years. The h^2 estimated using the G-BLUP model was 0.15, representing around half of the total genetic variation, and H^2 was 0.31 to 0.30 for G-BLUP and E-GBLUP models, respectively. The genetic variance captured by the line is composed by the additive variance that are not captured by markers and by non-additive variance. Therefore, the difference between H^2 and h^2 may suggest a relevant non-additive effect for wheat grain yield in the analyzed population, which also agrees with the prior expert-knowledge from the breeding company.

Effect of linkage disequilibrium on genetic variances

In this section, we first mention the partition of genetic variance under HWE and LE, which is the optimal scenario for the orthogonal partition of genetic variances. Then we explain how the genetic variances are affected by the LD among QTL. The partition of total genetic variance under HWE and LE (Falconer and Mackay, 1996) and assuming only pairwise epistatic interactions can be defined as: $\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_{AA}^2 + \sigma_{AD}^2 + \sigma_{DD}^2$. For inbred lines, the dominance effect is expected to be very low to negligible, therefore, the total genetic variance can be reduced to the sum of additive and additive $\times$ additive components: $\sigma_G^2 = \sigma_A^2 + \sigma_{AA}^2$. In HWE and LE, the additive genetic variance is defined as: $\sigma_A^2: 2 \sum_{r=1}^m p_r(1 - p_r)a_r^2$ and the additive $\times$ additive variance is σ_{AA}^2 :

$4 \sum_{r < s} p_r p_s (1 - p_r)(1 - p_s)(aa)_{rs}^2$, where r is a QTL = 1, 2 ... m , with m the number of QTL and two alleles A_r and a_r , p_r denote the frequency of allele A_r at locus r , and a and aa the additive and additive $\times$ additive genetic effects at QTL r , respectively.

The genetic variances when LD among QTL is present, were first defined by Wang and Zeng (2006): $\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_{AA}^2 + \sigma_{AD}^2 + \sigma_{DD}^2 + cov(A, D) + cov(A, AA) + cov(A, AD) + cov(A, DD) + cov(D, AA) + cov(D, AD) + cov(D, DD) + cov(AA, AD) + cov(AA, DD) + cov(AD, DD)$, and for the case of wheat inbred lines, assuming no dominance effect, the total genetic variance is: $\sigma_G^2 = \sigma_A^2 + \sigma_{AA}^2 + cov(A, AA)$. The additive variance under LD is redefined as:

$$\sigma_A^2 = 2 \sum_{r=1}^m p_r (1 - p_r) a_r^2 + 2 \sum_{r \neq s} a_r a_s D_{rs}$$

where D_{rs} is the coefficient of digenic disequilibrium (ignoring trigenic and higher-order linkage disequilibria): $D_{rs} = p_{rs} - p_r p_s$, and the additive $\times$ additive variance is:

$$\begin{aligned} \sigma_{AA}^2 = & 2 \sum_{r < s} (aa)_{rs}^2 [(1 - 2p_r)(1 - 2p_s)D_{rs} + 2p_r(1 - p_r)p_s(1 - p_s)] \\ & + 2 \sum_{r \neq s \neq s'} (aa)_{rs}(aa)_{rs'} [(1 - 2p_r)D_{rss'} + 2p_r(1 - p_r)D_{ss'}] \\ & + \frac{1}{2} \sum_{r \neq s \neq r' \neq s'} (aa)_{rs}(aa)_{r's'} (D_{rr'ss'} + D_{rs'}D_{r's} + D_{rr'}D_{ss'} - D_{rs}D_{r's'}) \end{aligned}$$

In addition, under LD a new term $cov(A, AA)$ representing the covariance between additive and additive $\times$ additive epistatic effect is also influencing the total genetic variance. The $cov(A, AA)$ term is defined as:

$$cov(A, AA) = 2 \sum_{r < s} (aa)_{rs} D_{rs} [(1 - 2p_r)a_r + (1 - 2p_s)a_s] + \sum_{r \neq s \neq r'} a_{r'}(aa)_{rs} D_{r'rs}$$

For more details on the derivation of the additive and additive $\times$ additive variances and their covariances under LD see Wang and Zeng (2006). Note that under LD, the additive and additive $\times$ additive variances do not specifically quantify the variances depending on the additive and additive $\times$ additive gene action, but it also is influenced by variances depending on the coefficient of digenic disequilibrium (D_{rs}) among QTL and covariances between genetic effects. It leads to non-independence among genetic effect, which can also be evidenced in the high negative estimates of sampling correlations among additive and additive $\times$ additive genetic effects (Vitezica et al. 2017). In the wheat data analyzed a high negative correlation among additive and additive $\times$ additive genetic

effects of -0.36 was observed for both epistatic models. For an orthogonal partition of variance into genetic components, the estimates of sampling genetic correlation between additive and additive $\times$ additive effects is expected to be 0 (correlation of zero indicates independence between model effects).

Genomic predictive ability

The PA estimated as the correlation between the lines averages after correcting for fixed effects and the predicted values of the G-BLUP and both epistatic models was estimated for the LOO and LSO CV schemes. In the LOO CV, the PA for epistatic models outperformed the G-BLUP with a significant (*P-value*: 0.01) increase of 16.5 %. The increase of PA found in our study confirms a substantial level of additive $\times$ additive epistasis for grain yield in the analyzed population. However, the improvement in PA for including epistasis was not observed when the LSO was used. The differences in the performance of models in the LOO and LSO CVs indicates a strong influence of relationships among individuals from the reference and validation population over the PA, as full sibs are excluded in the LSO scenario. In the literature, the effect of including epistasis in GP has been population dependent and has varied among studies. While in some studies the PA increased (He et al. 2016; Heslot et al. 2012), in others it changed very little (Jarquín et al. 2014) or even decreased (Lorenzana and Bernardo 2009). Increases in PA ranging from 4 to 25 % has been found when shifting from additive to additive plus epistatic models and using random folding CV (*five-fold* or *10-fold*) in wheat (Crossa et al. 2010; He et al. 2016; Heslot et al. 2012; Jiang and Reif 2015), which agrees with the range of improvement found in our study for the LOO CV. The highest PA achieved using epistatic models for the LOO CV also indicates that the statistical advantage of modelling epistasis is still valid under the LD scenario, which has also been shown by Jiang and Reif (2015) using simulations. Contrasting these results, Lorenzana and Bernardo (2009) using a *five-fold* CV found a poorer performance for predictions when the model accounted for additivity and epistasis in comparison with a model accounting only for additivity. The discrepancies among the results found may be explained by differences in the level of additive $\times$ additive epistasis among the evaluated populations. According to Forneris et al. (2017), including an epistatic term in the models when there was no epistatic effects present, led to lower accuracies. Therefore, the knowledge about the genetic architecture of the trait may be a relevant factor to determine the potential of including epistasis in GS.

The PA for the additive effect (breeding values) were compared between G-BLUP and both epistatic models. For both LOO and LSO CVs, higher PAs were observed for the predicted GEBV from G-BLUP than for the predicted GEBV from the EG-BLUP or NOIA models. It indicates that considering epistasis in the GS models

did not improve the prediction of additive effects. Hence, our results suggested that the G-BLUP model remained as the best choice to predict the additive genetic values.

Inflation of variance and bias

The test for variance inflation led to regression coefficients close to 1 for the LOO CV, which means that none of the proposed models had a significant under- or over-dispersion in their predictions. Note that the LOO CV represent an optimal scenario due to the use of the largest possible reference population for predictions, and therefore, under- or over- dispersion in predictions of genetic values is in general not observed. In the LSO, values of $b_{w,p}$ lower than 1 were observed for predictions of both genetic effects (GEBVs and GEEVs), indicating over-dispersion of genomic predicted values. Particularly, predictions of epistatic values (GEEVs) for EG-BLUP and NOIA models had the lowest $b_{w,p}$ value ($b_{w,p}=0.70$), suggesting that the epistatic predictions were more sensitive to the lack of information in the reference population. The bias (μ_{wp}) of predictions had coefficients close to 0 for GEBVs and GEEVs in both CVs utilized; it indicates that unbiased genomic values were reached for all proposed models.

Correlation between G-BLUP and EG-BLUP estimates

We found that Pearson's correlation between GEBVs from G-BLUP and epistatic models were high (0.94) compared to the correlation between GEBVs and genomic estimated epistatic values (0.65). Accordingly, differences were also evidenced in a change of ranking between lines with superior additive value (based on GEBVs) and lines with superior total genetic value (based on GEBVs plus genomic estimated epistatic values), indicating that the use of epistatic models to predict lines with higher total genetic value led to a different selection of candidate lines than using the G-BLUP model.

The current study first helps to elucidate the contribution of epistasis to the genetic architecture of grain yield in advanced wheat breeding lines. As reflected by the LOO CV, this study evidences the potential of increasing the PA for total genetic merit by including epistasis in genomic selection models. Further studies are required to investigate the influence of genetic relationship on the performance of epistatic predictions and to develop CV methods capable to successfully exploit epistatic interactions in wheat breeding. In addition, the development of a GS models that lift the LE requirement should be an area for future research.

Conclusions

In this research, we found that the extended genomic best linear unbiased prediction (EG-BLUP) and the natural and orthogonal interactions approach (NOIA) models did not provide an orthogonal partition of genetic variances into additive and epistatic effects, and both models yielded very similar variances estimates for a population of advanced wheat breeding lines. The lack of orthogonality among genetic effects was attributed to the population linkage disequilibrium. Nevertheless, including additive $\times$ additive epistasis in genetic models increased PA for total genetic merit significantly (16.5%) compared to G-BLUP for a leave-one-line-out cross-validation. These result implied that substantial additive $\times$ additive epistatic variance for grain yield is present in the analyzed wheat population. The advantage of including epistasis in PA was not observed for a leave-one-breeding-cycle-out cross-validation. Further studies are required to: i) investigate and develop CV methods capable to successfully exploit epistatic interaction in wheat breeding, and ii) develop a genomic selection model capable of removing the linkage equilibrium requirement and guarantee an orthogonal partition of genetic variances for breeding populations.

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Conflict of interest

On behalf of all authors, the corresponding authors states that there is no conflict of interest.

Availability of data and material

The datasets analysed during the current study are available in the Harvard dataverse public repository at the following link: <https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/ULGTGT>.

Author contribution statement

Conceptualization: JJ, MR, PS, JRA.

Data Curation: MR, JRA, PS, JO.

Formal Analysis: MR.

Funding acquisition: JJ, MR, JRA, JO, AJ.

Investigation: MR, JJ.

Methodology: MR, JJ, XG.

Project administration: MR, JJ.

Resources: JJ, PS.

Software: MR.

Supervision: JJ, PS.

Validation: MR, HL.

Visualization: MR.

Writing - original draft: MR

Writing - review and editing: JJ, PS, HL, XG, JRA, JO, AJ.

Code availability

Not applicable.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publications

Not applicable.

Abbreviations

ADD: predicted additive values

CV: cross-validation

DK: Denmark

EG-BLUP: extended genomic best linear unbiased prediction

EPI: predicted epistatic values

G-BLUP: genomic best linear unbiased prediction

GEBV: genomic estimated breeding value

GEEV: genomic estimated epistatic value

GP: genomic prediction

GS: genomic selection

HWE: Hardy-Weinberg equilibrium

LD: linkage disequilibrium

LE: linkage equilibrium

LOO: leave-one-line-out

LSO: leave-one-breeding-cycle-out

MAF: Minor allele frequency

NOIA: natural and orthogonal interactions approach

PA: predictive ability

RD: relative difference

SNP: single nucleotide polymorphism

VC: variance components

WGR: whole genome regression

References

- Alvarez-Castro JM, Carlborg O (2007) A unified model for functional and statistical epistasis and its application in quantitative trait Loci analysis *Genetics* 176:1151-1167 doi:10.1534/genetics.106.067348
- Carlborg Ö, Haley CS (2004) Epistasis: too often neglected in complex trait studies? *Nature Reviews Genetics* 5:618-625
- Cericola F, Jahoor A, Orabi J, Andersen JR, Janss LL, Jensen J (2017) Optimizing Training Population Size and Genotyping Strategy for Genomic Prediction Using Association Study Results and Pedigree Information. A Case of Study in Advanced Wheat Breeding Lines *PLoS One* 12:e0169606 doi:10.1371/journal.pone.0169606
- Cockerham CC (1954) An extension of the concept of partitioning hereditary variance for analysis of covariances among relatives when epistasis is present *Genetics* 39:859
- Crossa J et al. (2010) Prediction of genetic values of quantitative traits in plant breeding using pedigree and molecular markers *Genetics* 186:713-724 doi:10.1534/genetics.110.118521
- Crossa J et al. (2017) Genomic Selection in Plant Breeding: Methods, Models, and Perspectives *Trends Plant Sci* 22:961-975 doi:10.1016/j.tplants.2017.08.011
- Fisher RA (1918) 009: The Correlation Between Relatives on the Supposition of Mendelian Inheritance
- Forneris NS, Vitezica ZG, Legarra A, Perez-Enciso M (2017) Influence of epistasis on response to genomic selection using complete sequence data *Genet Sel Evol* 49:66 doi:10.1186/s12711-017-0340-3
- Gianola D, Rosa GJM (2015) One Hundred Years of Statistical Developments in Animal Breeding *Annual Review of Animal Biosciences* 3:19-56 doi:10.1146/annurev-animal-022114-110733
- Habier D, Fernando RL, Dekkers JC (2007) The impact of genetic relationship information on genome-assisted breeding values *Genetics* 177:2389-2397
- He S et al. (2016) Genomic selection in a commercial winter wheat population *Theor Appl Genet* 129:641-651 doi:10.1007/s00122-015-2655-1
- Henderson C (1985) Best linear unbiased prediction of nonadditive genetic merits in noninbred populations *Journal of animal science* 60:111-117
- Heslot N, Yang H-P, Sorrells ME, Jannink J-L (2012) Genomic Selection in Plant Breeding: A Comparison of Models *Crop Science* 52:146-160 doi:10.2135/cropsci2011.06.0297
- Hill W, Mäki - Tanila A (2015) Expected influence of linkage disequilibrium on genetic variance caused by dominance and epistasis on quantitative traits *Journal of animal breeding and genetics* 132:176-186

- Hu Z, Li Y, Song X, Han Y, Cai X, Xu S, Li W (2011) Genomic value prediction for quantitative traits under the epistatic model *BMC genetics* 12:15
- Huang W, Mackay TF (2016) The Genetic Architecture of Quantitative Traits Cannot Be Inferred from Variance Component Analysis *PLoS Genet* 12:e1006421 doi:10.1371/journal.pgen.1006421
- Jarquín D, Kocak K, Posadas L, Hyma K, Jedlicka J, Graef G, Lorenz A (2014) Genotyping by sequencing for genomic prediction in a soybean breeding population *BMC genomics* 15:1-10
- Jiang Y, Reif JC (2015) Modeling Epistasis in Genomic Selection *Genetics* 201:759-768 doi:10.1534/genetics.115.177907
- Jiang Y, Schmidt RH, Zhao Y, Reif JC (2017) A quantitative genetic framework highlights the role of epistatic effects for grain-yield heterosis in bread wheat *Nature Genetics* 49:1741-1746 doi:10.1038/ng.3974
- Joshi R, Meuwissen THE, Woolliams JA, Gjoen HM (2020) Genomic dissection of maternal, additive and non-additive genetic effects for growth and carcass traits in Nile tilapia *Genet Sel Evol* 52:1 doi:10.1186/s12711-019-0522-2
- Kristensen PS, ed Jahoor A, Andersen JR, ad Orabi J, Janss L, Jensen J (2019) Multi-trait and trait-assisted genomic prediction of winter wheat quality traits using advanced lines from four breeding cycles *Crop Breeding, Genetics and Genomics* 1
- Legarra A, Reverter A (2018) Semi-parametric estimates of population accuracy and bias of predictions of breeding values and future phenotypes using the LR method *Genet Sel Evol* 50:53 doi:10.1186/s12711-018-0426-6
- Lorenzana RE, Bernardo R (2009) Accuracy of genotypic value predictions for marker-based selection in biparental plant populations *Theor Appl Genet* 120:151-161 doi:10.1007/s00122-009-1166-3
- Mackay TF (2014) Epistasis and quantitative traits: using model organisms to study gene-gene interactions *Nat Rev Genet* 15:22-33 doi:10.1038/nrg3627
- Madsen P, Jensen J (2013) An User's Guide to DMU, Version 6, Release 5.1. Center for Quantitative Genetics and Genomics. In: Dept. of Molecular Biology and Genetics, University of Aarhus. Research Centre Foulum Tjele, Denmark,
- Martini JW, Gao N, Cardoso DF, Wimmer V, Erbe M, Cantet RJ, Simianer H (2017) Genomic prediction with epistasis models: on the marker-coding-dependent performance of the extended GBLUP and properties of the categorical epistasis model (CE) *BMC Bioinformatics* 18:3 doi:10.1186/s12859-016-1439-1

- Martini JW, Wimmer V, Erbe M, Simianer H (2016) Epistasis and covariance: how gene interaction translates into genomic relationship *Theor Appl Genet* 129:963-976 doi:10.1007/s00122-016-2675-5
- Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps *Genetics* 157:1819-1829
- Poland J et al. (2012) Genomic selection in wheat breeding using genotyping - by - sequencing *The Plant Genome* 5
- Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues *Plant molecular biology* 5:69-76
- Su G, Christensen OF, Ostersen T, Henryon M, Lund MS (2012) Estimating additive and non-additive genetic variances and predicting genetic merits using genome-wide dense single nucleotide polymorphism markers *PLoS One* 7:e45293 doi:10.1371/journal.pone.0045293
- Tsai HY et al. (2020) Use of multiple traits genomic prediction, genotype by environment interactions and spatial effect to improve prediction accuracy in yield data *PLoS One* 15:e0232665 doi:10.1371/journal.pone.0232665
- VanRaden PM (2008) Efficient methods to compute genomic predictions *Journal of dairy science* 91:4414-4423
- Vitezica ZG, Legarra A, Toro MA, Varona L (2017) Orthogonal Estimates of Variances for Additive, Dominance, and Epistatic Effects in Populations *Genetics* 206:1297-1307 doi:10.1534/genetics.116.199406
- Wang S et al. (2014) Characterization of polyploid wheat genomic diversity using a high - density 90 000 single nucleotide polymorphism array *Plant biotechnology journal* 12:787-796
- Wang T, Zeng ZB (2006) Models and partition of variance for quantitative trait loci with epistasis and linkage disequilibrium *BMC Genet* 7:9 doi:10.1186/1471-2156-7-9
- Wellmann R, Bennewitz J (2012) Bayesian models with dominance effects for genomic evaluation of quantitative traits *Genetics research* 94:21-37
- Xu S (2013) Mapping quantitative trait loci by controlling polygenic background effects *Genetics* 195:1209-1222 doi:10.1534/genetics.113.157032
- Zhao Y et al. (2015) Genome-based establishment of a high-yielding heterotic pattern for hybrid wheat breeding *Proceedings of the National Academy of Sciences* 112:15624-15629 doi:10.1073/pnas.1514547112

Figures

Figure. 1. Genomic relationship between the 2060 F6 lines from 2013 to 2019 breeding cycles. **(a)** Heat map of G-matrix, red colors represents more related individuals and yellow colors less related. **(b)** Principal Component Analysis (PCA) of G-matrix. The colors of the PCA represent the different breeding cycles to which the lines correspond. The variances explained by PCA1 and PCA2 are 40.4 and 12.4 %, respectively.

Figure 2. Percentage of genetic variances (blue: σ^2_{Line} , green: $\sigma^2_{Additive}$, yellow: $\sigma^2_{Epistatic}$) captured by the different models. **(a)** Genetic variance estimated for the baseline model; the variance of the line (σ^2_{Line}) represents a combination of additive plus non-additive variances. **(b)** Genetic variances estimated for genomic best linear unbiased prediction model; σ^2_{Line} represents the non-additive variance plus the additive variance not captured by SNPs, $\sigma^2_{Additive}$ represents the additive variance captured by SNPs. **(c)** and **(d)** shown the genetic variances estimated for extended genomic best linear unbiased prediction and natural and orthogonal interactions approach models, respectively. Under an orthogonal partition of variances into genetic components, σ^2_{Line} is expected to reflect the additive and non-additive variance that was not captured by SNPs, and $\sigma^2_{Additive}$ and $\sigma^2_{Epistatic}$ are expected to represent the additive and the pairwise additive $\times$ additive epistatic variance captured by SNPs, respectively.

Figure 3. Barplot of genomic best linear unbiased prediction (G-BLUP) and extended genomic best linear unbiased prediction (EG-BLUP) predictive abilities for leave-one-line-out (LOO) and leave-one-breeding-cycle-out (LSO) cross-validations based on bootstrap distribution, $r=10,000$. ADD: predicted additive values (GEBVs), EPI: predicted epistatic values (GEEVs), ADD + EPI: sum of ADD and EPI. Green lines are the theoretical maximum predictive ability (PA). The maximum PA for the G-BLUP model and for the ADD of the epistatic models was calculated as: $\sqrt{nh^2/(1 + (n - 1)h^2)}$, where n is the average number of lines repetitions; the maximum PA for ADD + EPI and EPI of epistatic models were calculated using the proportion of total variance explained by additive plus epistatic effects (for the case ADD + EPI), and the proportion of variance explained for the epistatic component (for EPI) instead of h^2 .

Figure 4: Slope of regression ($b_{w,p}$) among observed and predicted genetic values for genomic best linear unbiased prediction and extended genomic best linear unbiased prediction models in leave-one-line-out cross-validation (**a, b, c**) and leave-one-breeding-cycle-out cross-validation (**d, e, f**). The yellow lines represent the line for regression of observed on predicted genetic values. The blue lines represent a reference regression line with intercept 0 and slope 1. represent the ADD: predicted additive values (GEBVs), EPI: predicted epistatic values (GEEVs). The numeric values into each plot represent the coefficient of regression ($b_{w,p}$) for each case.

Tables

Table 1. Descriptive statistics for the yield of F6 wheat breeding lines.

Trait	No. of Lines	No. of Plots	Units	Average (SD)	Min. Value	Max. Value	Coefficient of variation (%)
Yield	2,060	18,525	kg grain/8.25m ²	8.71 (0.98)	3.85	12.35	11.27

No.: number; SD: standard deviation; Min: Minimum; Max: Maximum.

Table 2. Estimation of variance component, narrow-sense, and broad-sense plot heritabilities.

Models	σ_{Line}^2	$\sigma_{Additive}^2$	$\sigma_{Epistatic}^2$	$\sigma_{Spatial}^2$	σ_{GxE}^2	σ_{error}^2	<i>Plot heritabilities</i>	
							h^{2*}	H^2
Baseline	0.089 (0.004)	-	-	0.043 (0.002)	0.131 (0.003)	0.057 (0.001)	-	0.28
G-BLUP	0.053 (0.004)	0.051 (0.007)	-	0.044 (0.002)	0.131 (0.003)	0.057 (0.001)	0.15	0.31
EG-BLUP	0.014 (0.005)	0.020 (0.006)	0.064 (0.008)	0.044 (0.002)	0.131 (0.003)	0.057 (0.001)	-	0.30
NOIA model	0.014 (0.005)	0.020 (0.006)	0.064 (0.008)	0.044 (0.002)	0.131 (0.003)	0.057 (0.001)	-	0.30

*the narrow-sense heritability (h^2) was estimated only for the genomic best linear unbiased prediction model, due to by the lack of orthogonality of genetic components, h^2 was not representative for the extended genomic best linear unbiased prediction (EG-BLUP) and the natural and orthogonal interactions approach (NOIA) models. σ_{Line}^2 : variance not captured by markers; $\sigma_{Additive}^2$: additive variance; $\sigma_{Epistatic}^2$: epistatic variance; $\sigma_{Spatial}^2$: spatial variance; σ_{GxE}^2 : genotype by environment interaction variance; σ_{error}^2 : error variance; H^2 : broad-sense heritability . The values between parentheses are the standard errors (SE) of the estimates.

Table 3. Estimated bias (μ_{wp}) for G-BLUP and EG-BLUP predictions.

Model	Model	Genetic effect	bias (μ_{wp})
Leave-one-line-out cross-validation	G-BLUP	ADD	0.0005
	EG-BLUP	ADD	0.0004
		EPI	0.0013
Leave-one- breeding-cycle-out cross-validation	G-BLUP	ADD	-0.0108
	EG-BLUP	ADD	-0.0045
		EPI	-0.0123

ADD: predicted additive values (GEBV), EPI: predicted epistatic values (GEEV).

Figures

Figure 1

Genomic relationship between the 2060 F6 lines from 2013 to 2019 breeding cycles. (see Manuscript file for full figure caption)

Figure 2

Percentage of genetic variances captured by the different models. (see Manuscript file for full figure caption)

Figure 3

Barplot of genomic best linear unbiased prediction. (see Manuscript file for full figure caption)

Figure 4

Slope of regression. (see Manuscript file for full figure caption)

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