1   **CV-α: designing validations sets to increase the precision and enable multiple comparison tests**

2   **in genomic prediction**

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9   **Abbreviations:** CV, cross-validation; CV-α, cross-validation alpha-based design; GP, Genomic

10   prediction; RRS, Repeated Random Subsampling; TGV, true genetic value.

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13   **Abstract** Usually, the comparison among genomic prediction models is based on validation schemes

14   as Repeated Random Subsampling (RRS) or K-fold cross-validation. Nevertheless, the design of

15   training and validation sets has a high effect on the way and subjectiveness that we compare models.

16   Those procedures cited above have an overlap across replicates that might cause an overestimated

17   estimate and lack of residuals independence due to resampling issues and might cause less accurate

18   results. Furthermore, posthoc tests, such as ANOVA, are not recommended due to assumption

19   unfulfilled regarding residuals independence. Thus, we propose a new way to sample observations to

20   build training and validation sets based on cross-validation alpha-based design (CV-α). The CV-α was

21   meant to create several scenarios of validation (replicates x folds), regardless of the number of

22   treatments. Using CV-α, the number of genotypes in the same fold across replicates was much lower

23   than K-fold, indicating higher residual independence. Therefore, based on the CV-α results, as proof

24   of concept, via ANOVA, we could compare the proposed methodology to RRS and K-fold, applying

25   four genomic prediction models with a simulated and real dataset. Concerning the predictive ability

26   and bias, all validation methods showed similar performance. However, regarding the mean squared

27   error and coefficient of variation, the CV-α method presented the best performance under the

28   evaluated scenarios. Moreover, as it has no additional cost nor complexity, it is more reliable and

29   allows the use of non-subjective methods to compare models and factors. Therefore, CV-α can be

30   considered a more precise validation methodology for model selection.

31

32   **KEYWORDS:** Repeated random subsampling; K-fold; Cross-validation, model selection

33 **Introduction**

34   Genomic prediction (GP) proposed by Meuwissen et al. (2001) evolved over the years, but it

35 aims to estimate breeding values of unevaluated genotypes. Hence, it is an important tool for plant

36 breeders to shorten the breeding cycle, increase selection accuracy, and assess genetic variation (Heff

37 et al. 2010; Crossa et al. 2017). Usually, to evaluate the prediction accuracy of the genomic prediction

38 models, the data is divided into training and validation sets. The first set is used to fit the genomic

39 prediction model and estimate the marker effects, whereas the validation set is used to validate the

40 effects estimated in the training set and estimate the accuracy of the predictions (Crossa et al. 2011).

41   In the genomic prediction context, several methods and parameters have been proposed for the

42 comparison of prediction models (Blondel et al. 2015). Nevertheless, the predictive ability and the

43 bias of the measures are two of the most commonly utilized to evaluate the superiority and goodness

44 of models and scenarios. The former is estimated by Pearson's correlation between the predicted and

45 true breeding values of individuals contained in the validation set. The latter is obtained by regressing

46 the predicted breeding values over the true ones to obtain the regression coefficient, which indicates

47 the shrinkage (compression) between both (Piepho et al. 2008; Luan et al. 2009).

48   Some studies have shown that the model accuracy is influenced by the training and validation set

49 (Akdemir et al. 2015; Wu et al. 2015; Auinger et al. 2016), being the main schemes to design training

50 and validation sets in GP studies are K-fold cross-validation (Burgueño et al. 2012; Crossa et al. 2014;

51 Fè et al. 2016) and Repeated Random Subsampling (RRS), also called Monte Carlo CV (Würschum

52 et al. 2014; Yu et al. 2016; Zhang et al. 2016). The first consists of splitting the data into k groups

53 (folds) and fit a model using each fold as training and validation sets. Is this sense, if k = 5, the model

54 will be fitted five times. The second consists of randomly split the dataset into training and validation

55 sets. Both schemes are generally repeated *n* times (see Arlot and Celisse, 2010).

56   The accuracy estimate obtained by K-fold might be affected by the number of folds, fold size,

57 and the number of replicates (Wong 2015). Likewise, in cross-validation schemes, the RRS is

58 influenced by the relation between training and validation sets and the number of replicates (Kohavi

59   1995). Furthermore, some factors may lead to biased estimates of predictive ability, such as

60   overlapping between the training and validation set and different relatedness between individuals

61   through sets (Runcie e Cheng 2019). The overlap between training and validation sets over replicates

62   may cause biased results due to the predictions be correlated and non-independent residuals (Amer e

63   Banos 2010). Therefore, neither validation schemes guarantee independence among replicates due to

64   resampling issues. Thus, researchers cannot use standard and non-subjective methods to compare

65   models and factors, such as ANOVA and other multiple comparison tests, due to assumptions

66   unfulfilled regarding residuals independence.

67   It is import point out that as the number of treatments increases, it becomes a challenge to design

68   orthogonal training and validation sets across the replicates without increase substantially the number

69   of replicates. This problem is similar to experimental field designs involving a large number of

70   treatments. However, the balanced incomplete blocks design seeks to maintain homogeneity among

71   blocks and orthogonality across replicates (Yates, 1936). These schemes are widely used to evaluate

72   the quality of models and their selection for field experiments. Moreover, an extension of cross-

73   validation (CV) schemes applying balanced incomplete block design was first proposed by Shao

74   (1993), considering that each fold is treated as "block" and each genotype as a "treatment." The

75   orthogonal distribution of the treatments across the blocks within replicates in the balanced

76   incomplete block designs will guarantee that every pair of treatments appears together according to

77   some rules. Therefore, the CV schemes using the incomplete block design may increase the quality

78   of estimates (Fuchs e Krautenbacher 2016), residuals independence, and may allow further multiple

79   comparison analyses.

80   Based on described above, in this study, we propose a new method to design the training and

81   validation sets for genomic prediction studies based on an alpha-lattice design scheme, called cross-

82   validation alpha-based design (CV-α) and compare its performance to the methods commonly applied

83   in GP studies for model selection. Also, based on the CV-α results, a case of study, via analysis of

84   variance (ANOVA), we could compare the proposed methodology to RRS and K-fold, applying four

85   genomic prediction models with a simulated and real dataset.

86 **Material and Methods**

87   In order to demonstrate the properties of the proposed cross-validation scheme, we aimed to

88 mimic a standard genomic prediction study, for instance, comparing kernels and statistical methods.

89 Thus, our aim is not comparing genomic matrices or Bayesian and frequentists approaches but simply

90 show that our cross-validation scheme allows multiple comparison tests. For that, we create a

91 simulated population (knowing the true parameters) and also used a well-known real dataset.

92

93 *Simulated dataset*

94   We simulated a population of maize single-crosses from inbred parents to perform genomic

95 prediction studies. For this, we used the *AlphaSimR* package (Gaynor 2019). A founder population of

96 1,000 individuals was simulated with ten chromosomes containing 30,000 segregating loci (SNPs).

97 The individuals were inbred and diploid. Forty-nine individuals were randomly sampled and crossed

98 to compose a partial diallel to obtain 906 hybrids. The phenotypic value (adjusted mean based on

99 heritability) was simulated by randomly sampling 500 QTN from the segregating loci with mean 100

100 and variance 50. The narrow and broad-sense heritabilities were set to be equal to 0.30 and 0.50,

101 respectively. Finally, to understand the effect of the validation methods in the predictive ability and

102 bias of the true genetic (TGV) and phenotypic value, we performed genomics prediction using both

103 metrics. We repeated the simulations 25 times and averaged the estimates above.

104

105 *An empirical case of study: USP maize dataset*

106   We used a dataset of 906 maize single-crosses from a full diallel among 49 tropical inbred

107 lines, according to Griffing's method 4 (Griffing 1956). The experiments were evaluated in two

108 locations, two years, and under two nitrogen levels. The genotypic information from the 49 tropical

109 inbred lines was obtained from Affymetrix® Axiom® Maize Genotyping Array, containing about

110 614,000 SNPs (Unterseer et al. 2014). For more details about the phenotypic and genotypic data, see

111 (Fristche-Neto et al. 2018).

112   The markers with a lower call rate ($< 95\%$), heterozygous loci on at least one individual, and

113   linkage disequilibrium (> 0.90) were removed. The missing markers were imputed using the *Beagle*

114   *4.0* algorithm (Browning e Browning 2009) from the *synbreed* R package (Wimmer et al. 2012).

115   Later, the genotype of each hybrid was built by combining the genotypes of its parental lines and

116   hybrids with minor allele frequency (MAF < 0.05) were removed. After quality control, a total of

117   32,207 SNPs was available for further analysis.

118   To perform the genomic prediction studies, we evaluated the grain yield (GY, Mg ha^{-1}),

119   corrected to 13% moisture, and stand across the eight environments. It was used to estimate the BLUP

120   for hybrids following the model:

121     $$y = Sl + Xb + Wc + Tg + Ui + \varepsilon$$

122   where **y** is the vector of the phenotypic value of hybrids; **l** is the vector of fixed effects of the

123   environment (the combination of site x year x N level); **b** is the vector of fixed effects of blocks within

124   an environment; **c** is the vector of fixed effects of checks; **g** is genotypic values, where $g \sim N(0, I\sigma_g^2)$;

125   **i** is the interaction between environments and checks, where $i \sim N(0, I\sigma_{ge}^2)$; **ε** is the vector of random

126   residuals from checks and hybrid by environments effects, where $\varepsilon \sim N(0, I\sigma_\varepsilon^2)$. σ_ε^2 was jointly

127   estimated based on *e* environments with *r* replicated checks in each site. **S**, **X**, **W**, **T**, and **U** are the

128   incidence matrices for **l**, **b**, **c**, **g**, and **i** (Fristche-Neto et al. 2018).

129

130   *Genomic prediction*

131   To perform the genomic prediction, we used the additive GBLUP model and the Reproducing

132   Kernel Hilbert Spaces regression (RKHS). The following model equation is the general form of these

133   two approaches:

134     $$\hat{g} = 1\mu + Za + \varepsilon$$

135   where $\hat{g}$ is the vector of BLUP; μ is the intercept; **a** is the vector of additive genetic effects

136   with $a \sim N(0, G\sigma_a^2)$; and **ε** is the vector of random residuals with $\varepsilon \sim N(0, I\sigma_\varepsilon^2)$. **1** is the incidence

137   vector of μ, and **Z** is the incidence matrix for a. **G** is the genomic relationship matrix (**G$_a$** – additive

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138   genomic relationship matrix, and $\mathbf{K}$ – for Gaussian kernel), and $\mathbf{I}$ is the identity matrix. σ_a^2 is the

139   additive genetic variance for $\mathbf{G_a}$ or genetic variance for $\mathbf{K}$, and σ_ε^2 is the residual variance. The

140   additive genomic relationship matrix ($\mathbf{G_a}$) was calculated as $\boldsymbol{G_a} = \boldsymbol{WW'}/2\sum_{i=1}^{n} p_i(1 - p_i)$, where

141    $\mathbf{W}$ is the centered matrix of SNPs, and p_i is the frequency of the allele p in locus i (VanRaden 2008).

142   The Gaussian kernel ($\mathbf{K}$) was calculated as $K(\boldsymbol{x_i}, \boldsymbol{x_j}) = \exp(-hd_{ij}^2/q_{0.05})$, where $\mathbf{x_i}$ and $\mathbf{x_j}$ are the

143   marker vectors for the ith and j[th] individuals, respectively, and $q_{0.05}$ is the fifth percentile for the

144   squared Euclidean distance $d_{ij}^2 = \sum_k (x_{ik} - x_{jk})^2$ (Pérez-Elizalbe et al., 2015). The h value was

145   considered equal to 1.

146   We used fitted two prediction models considering two statistical approaches (frequentist and

147   Bayesian Genomic Best Linear Unbiased Predictor - GBLUP), resulting in four scenarios: 1) GBLUP

148   with $\mathbf{G_a}$ kernel (GA_MM); 2) GBLUP with $\mathbf{K}$ kernel (GK_MM); 3) Bayesian GBLUP with $\mathbf{G_a}$ kernel

149   (GA_Bayes), and 4) Bayesian GBLUP with $\mathbf{K}$ kernel (GK_Bayes).

150   The analyses were performed using *ASReml-R* (Butler et al. 2009), and *BGLR* (Pérez and de

151   los Campos, 2014) packages for R. For Bayesian GBLUP models were performed using 10,000

152   iterations, 3,000 burn-in, and 5 thinning values. The convergence checks for Bayesian models are

153   available in the Supplemental Figure S1 and S2.

154

155   *Cross-validation alpha-based design*

156   The cross-validation alpha-based design (CV-α) is an extension of the methodology presented

157   by (Shao 1993) and consists of assigning treatments to folds in each replication by applying the alpha-

158   lattice sorting premises. The CV-α was intended to create scenarios with two, three, or four replicates,

159   regardless of the number of treatments. Each replicate is split into folds, and the number of folds will

160   determine the percentage of training and validation sets. Each fold across replicates is based on the

161   α(0,1) lattice design aiming to reduce the concurrences of any two treatments in the same fold (block)

162   across the replicates (Patterson e Williams 1976).

163   However, the α(0,1)-lattice design assumptions involve the number of blocks (*s*) and block

164   size (k) (number of the folds and fold size, in our context) to determine the number of treatments

165   (Patterson e Williams 1976). As the number of treatments is variable in a real scenario, we compute

166   the nearest smallest number to attend the assumptions above, and the remaining treatments are

167   randomly allocated into the folds. The alpha lattice design was created using the *agricolae* package

168   (Mendiburu 2019), and the scripts are available at Github (https://github.com/allogamous/CV-Alpha).

169   In order to compare CV-α with the other two benchmarks schemes, we simulated two

170   scenarios: 5-folds with four replicates and 10-folds with two replicates. First, we simulated a scenario

171   with the number of treatments varying from 200 to 2,000 and computed the percentage of remaining

172   treatments that were randomly assigned into folds for each scenario. After, we compared the same

173   two benchmarks schemes according to the mean and standard deviation of the concurrence of any

174   two genotypes, i.e., the number of folds containing both genotypes. The simulations were replicated

175   ten times.

176

177   *Model comparison*

178   To evaluate the cross-validation alpha-based design (CV-α) performance, we compared it to

179   benchmark validation schemes: repeated random subsampling (RRS) and K-fold using real and

180   simulated datasets for genomic prediction. For RRS, we used 100 replicates, each with 80% of the

181   data for the training set, and the remaining 20% of the data for the validation set, whereas for CV-α

182   and K-fold were used five-folds and four replicates. The number of replicates or folds for each method

183   considers the most common values for genome prediction studies using Bayesian and frequentist

184   approaches (Zhao et al. 2013; Zhang et al. 2015, 2016; Yu et al. 2016).

185   From those, we obtained the predictive ability of each statistical model for the different CV

186   methods. The predictive ability was estimated as Pearson's correlations between the predicted and

187   observed phenotypes. For each CV method, we estimated the slope coefficient for the regression of

188   the predicted values of the validation sets on its phenotypes. For this, the regression coefficient

189   between predicted and genetic value was considered the prediction bias, measuring the degree of

190   inflation/deflation of prediction genomics. Nonbiased models are expected to have a regression

191   coefficient equal to 1. For CV-α and K-fold, the level of averaging considered was at replicates.

192   Although RRS and K-fold schemes do not have independence between replicates, ANOVA have been

193   used to compare the predictive abilities from different models, even breaking the independence

194   assumption. To verify how variance components of models are affected by these methods, we perform

195   the ANOVA test considering the following model:

196    $$l = \mathbf{1}\mu + X_1 m + X_2 n + X_3 o + \varepsilon$$

197   where l is the vector of Pearson correlation transformed by Fisher z-transformation using the

198   R package *DescTools* (Signorell et al., 2019); μ is the overall mean; m is the vector of statistical

199   approach effect; n is the vector of relationship kernel; o is the vector of interaction between statistical

200   approach and kernel; and ε is the vector of residuals. X_1, X_2 e X_3 are incidence matrices for m, n, and

201   o, respectively. Quadratic components were estimated by the method of moments based on mean

202   square expectation.

203 **Results**

204 *CV-α*

205   We performed several analyses to evaluate cross-validation alpha-based design (CV-α)

206 performance. For this, we computed the number of treatments that were randomly assigned among

207 folds and the concurrence between pairs of treatments in the same fold across replicates (Figure 1).

208 The results reveal that the proportion of treatments randomly assigned among folds reduces as the

209 number of treatments increases and tends to converge to 0.38% and 0.27% for five-folds with four

210 replicates and ten folds with two replicates, respectively (Figure 1). Considering the concurrence

211 between pairs of treatments in the same fold across replicates, the CV-α reveals lower mean and

212 standard deviation in both evaluated scenarios when compared with the K-fold CV (Figure 2).

213

214 *Genomic prediction (simulated dataset)*

215   To understand the effects of validation schemes on genomic prediction, we simulated

216 populations to obtain true genetic values (TGV) and phenotypic values. The validation methods did

217 not significantly influence the average prediction ability of TGV and phenotypic values.

218 Nevertheless, the RRS has several "extreme" values when compared to K-fold and CV- α. Besides,

219 RRS showed a more substantial variation for bias, with several values overtaking 0.5 and 1.5 for

220 phenotypic and TGV. (Figure 3).

221   For PA and bias, TGV, and phenotypic value, in terms of mean and standard deviation, the

222 three validation methods do not differ among them (Table 1), except for phenotypic bias for RRS. On

223 the other hand, when we considered mean squared error (MSE) and coefficient of variation (CV),

224 CV-α showed the lowest CV for all scenarios evaluated, when compared with RRS and K-fold.

225

226

227 *Proof of concept*

228   For the maize dataset, PA and bias showed similar mean values for all validation methods. In

229 terms of SD, K-fold, and CV-α presented similar performance and were lower than RRS (Table 2).

230   For mean squared error and coefficient of variation, CV-α presented lower values than K-fold and

231   RRS. The coefficient of variation for K-fold was 34.70% and 10% higher than CV-α for PA and bias,

232   respectively (Table 2).

233   We applied the CV-α to validate two statistical approaches (Bayesian and Mixed models) and

234   two types of kernels (Additive and Gaussian kernel) for genomic prediction models (Table 3). For

235   this, we applied a two-way ANOVA, and it was observed significative effects for types of the kernel

236   for predictive ability and bias. Gaussian kernel (**K**) presented higher PA (0.44) than $\mathbf{G_a}$ (0.42) and

237   lower bias (1.00 and 0.98, for **K** and $\mathbf{G_a}$, respectively). For the type of two statistical approaches, the

238   Bayesian reveals a more biased estimation (0.98) when compared with GBLUP (1.01) (Table 3).

239   We can note that the proportion of phenotypic variance explained variation by each source of

240   variation vary across validation schemes (Figure 3). PA and bias had similar performance across CV

241   schemes for residual variance but vary for other variances. The RRS presented higher residual

242   variance and lower variances due to model effects. For the interaction, K-fold showed higher values

243   for PA. CV-α presented lower proportions of residual variances and higher variance due to the kernel

244   and statistical approaches effects.

245

Discussion

The main advantages of considering the α-design instead of the balanced incomplete block design (BICV) are the flexibility regarding the number of treatments and folds (Singh e Bhatia 2017), reduce the concurrence between pairs of treatments, increase the quality of estimates (Fuchs e Krautenbacher 2016) and residuals independence, allowing further multiple comparison analyses. The α-design is widely used in plant breeding experiments as well as its ANOVA (Alam et al. 2017; Ta et al. 2018; Galic et al. 2019). Based on this, in the context of genomic prediction, the flexibility of the CV-α is a good alternative to compare genomic selection models.

Our results reveal that CV-α reduces the concurrence between pairs of treatments (genotypes) in the same fold across replicates and its standard deviation when compared with the K-fold scheme (Figure 2). The concurrence of any two treatments causes dependence among folds, and comparative tests become less precise. Thus, the CV-α designs fold and replicates with few or non-concurrence across folds, generating a more independent and better scheme for composing training and validation sets in a genomic prediction context.

Comparison between CV-α, K-fold, and RRS must be pondered since they have a different level of averaging and different numbers of replicates compared with RRS, although CV-α and K-fold are equivalent (Wong 2015). RRS showed a higher number of outliers, probability as results of the different levels of average. However, it is an internal procedure for the method. The strategy to divide folds and replicates according to the alpha-lattice design, as we suggest into CV-α, permits we consider as replicate level mean, similar to replicate the effect in the alpha-lattice design.

Moreover, the RRS showed a large variation in the estimates for PA and, especially, for prediction bias. We expected values for bias around 1.0. However, the RRS showed several values overtaking 0.5 and 1.5, which shows a considerable inflation/deflation on the estimates. These results indicate that RRS is a less accurate method, mainly when we use few replicates.

Estimates more accurate combined with few replicates to run a CV scheme is desirable, especially when we consider a large number of genotypes, which is common in plant and animal

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272 breeding. In these cases, to compute the inverse matrix, the genomic relationship matrix is a

273 challenge, and several studies have been aiming this (Misztal et al. 2014; Misztal 2016). Based on

274 this, CV- α is a good alternative to design CV schemes and has a more precise estimative in a case

275 where the number of replicates is a limitation.

276 The simulated and real datasets reveal that CV-α had a similar performance to K-fold and

277 RRS when compared in terms of mean and standard deviation for predictive ability and bias. On the

278 other hand, when we consider in terms of MSE and coefficient of variation, CV-α has better

279 performance due to higher independence across replicates.

280 Traditionally, in genomic prediction studies, model comparison and selection are based on

281 subjective methods such as mean and standard deviation without a comparative test. Some studies

282 also considered ANOVA and other statistical tests. Although due to assumption unfulfilled regarding

283 residuals independence and our results, this is not be recommended. CV-α reveals the lesser

284 occurrence of pairs of genotypes in the same fold across replicates, causing a more precise estimative.

285 The CV-α methodology consists of applying α(0,1) lattice design to design the folds across replicates,

286 and because of this, it allows post hoc test to model comparison.

287 The results above indicate that CV-α had a more precise estimative trough the reduction of

288 coefficient of variation, and the variance components were better discriminated across the factors in

289 the two-way ANOVA. It reveals how the impact of folds design across each replicate shift the

290 proportion of the total variation explained by each model factor reducing the residual variance.

291 Furthermore, the ANOVA test using RRS and K-fold to compare the performance of different models

292 can produce mistake conclusions, since the estimative of variance components load bias. Therefore,

293 CV-α allows determining how much variation each model factor has and compares different genomic

294 selection models based on the ANOVA test and posthoc test. Furthermore, the use of CV-α does not

295 imply any additional computer cost or complexity in the validation process of model selection.

296 As proof of concepts, we applied the proposed methodology to exemplify model selection.

297 For the simulated and maize dataset, both do not show considerable differences across approaches

298 (GBLUP and Bayesian) and kernel type (Additive genomic and Gaussian kernel) for predictive

299 ability. Although, for the maize dataset, the use of **K** kernel showed higher predictive ability than $\mathbf{G_a}$.

300 This result is expected since the **K** kernel captures additive and non-additive effects (Heslot et al.

301 2012). For bias, mixed models showed less biased results. Although, comparison among these models

302 is not the focus of these studies since they have already been extensively studied (Chen et al. 2014;

303 Gota e Gianola 2014; Cuevas et al. 2017).

304   In the context of genomic prediction studies, there are other ways to design training and

305 validation sets. The CV-α may be expanded for these cases to better designing training and test sets

306 across replicates and environments, such as CV1 and CV2 schemes (Burgueño et al. 2012) and other

307 multi-environment and multi-trait studies. Also, the CV-α may be applied in any other cross-

308 validation studies to select models and verify as the model factors behave according to the different

309 sources of variation.

310 **Conclusion**

311   This study showed that the CV-α method is a good alternative to design cross-validations folds

312 and replicates, mainly when researchers want to compare genomic prediction models, increasing

313 precision in the model estimative, and to unravel the model factors impact in the total variation. Even

314 though there were no differences in the mean and standard deviation for predictive ability and bias,

315 our proposal was more accurate in terms of the mean squared error and coefficient of variation.

316 Another advantage of CV-α is that it does not require any additional cost regarding computing

317 demand or complexity. Furthermore, CV-α allows using the non-subjective methods to compare

318 models and factors, through ANOVA and other multiple comparison tests, such as Tukey and Scott-

319 Knott.

323   **Conflict of interest:** The authors declare no conflict of interest.

References

Akdemir D, Sanchez JI, Jannink JL (2015) Optimization of genomic selection training populations with a genetic algorithm. Genet Sel Evol 47:1–10 . doi: 10.1186/s12711-015-0116-6

Alam MA, Seetharam K, Zaidi PH, et al (2017) Dissecting heat stress tolerance in tropical maize (Zea mays L.). F Crop Res 204:110–119 . doi: 10.1016/j.fcr.2017.01.006

Amer PR, Banos G (2010) Implications of avoiding overlap between training and testing data sets when evaluating genomic predictions of genetic merit. J Dairy Sci 93:3320–3330 . doi: 10.3168/jds.2009-2845

Arlot S, Celisse A (2010) A survey of cross-validation procedures for model selection. Stat Surv 4:40–79 . doi: 10.1214/09-SS054

Auinger HJ, Schönleben M, Lehermeier C, et al (2016) Model training across multiple breeding cycles significantly improves genomic prediction accuracy in rye (Secale cereale L.). Theor Appl Genet 129:2043–2053 . doi: 10.1007/s00122-016-2756-5

Blondel M, Onogi A, Iwata H, Ueda N (2015) A Ranking Approach to Genomic Selection. PLoS One 10:e0128570 . doi: 10.1371/journal.pone.0128570

Browning BL, Browning SR (2009) A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. Am J Hum Genet 84:210–223 . doi: 10.1016/j.ajhg.2009.01.005

Burgueño J, de los Campos G, Weigel K, Crossa J (2012) Genomic prediction of breeding values when modeling genotype × environment interaction using pedigree and dense molecular markers. Crop Sci 52:707–719 . doi: 10.2135/cropsci2011.06.0299

Butler DG, Cullis BR, Gilmour AR, Gogel BJ (2009) ASReml User Guide Release 3.0. 398

Chen L, Li C, Sargolzaei M, Schenkel F (2014) Impact of genotype imputation on the performance of GBLUP and Bayesian methods for genomic prediction. PLoS One 9:1–7 . doi: 10.1371/journal.pone.0101544

Crossa J, Pérez-Rodríguez P, Cuevas 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

Crossa J, Pérez P, de los Campos G, et al (2011) Genomic selection and prediction in plant breeding. J Crop Improv 25:239–261 . doi: 10.1080/15427528.2011.558767

Crossa J, Pérez P, Hickey J, et al (2014) Genomic prediction in CIMMYT maize and wheat breeding programs. Heredity (Edinb) 112:48–60 . doi: 10.1038/hdy.2013.16

Cuevas J, Crossa J, Montesinos-López OA, et al (2017) Bayesian genomic prediction with genotype × environment interaction kernel models. G3 Genes, Genomes, Genet 7:41–53 . doi: 10.1534/g3.116.035584

Fè D, Ashraf BH, Pedersen MG, et al (2016) Accuracy of genomic prediction in a commercial perennial ryegrass breeding program. Plant Genome 9: . doi: 10.3835/plantgenome2015.11.0110

361 Fristche-Neto R, Akdemir D, Jannink JL (2018) Accuracy of genomic selection to predict maize
362   single-crosses obtained through different mating designs. Theor Appl Genet 131:1153–1162 .
363   doi: 10.1007/s00122-018-3068-8

364 Fuchs M, Krautenbacher N (2016) Minimization and estimation of the variance of prediction errors
365   for cross-validation designs. J Stat Theory Pract 10:420–443 . doi:
366   10.1080/15598608.2016.1158675

367 Galic V, Franic M, Jambrovic A, et al (2019) Genetic correlations between photosynthetic and yield
368   performance in maize are different under two heat scenarios during flowering. Front Plant Sci
369   10:1–11 . doi: 10.3389/fpls.2019.00566

370 Gaynor C (2019) AlphaSimR: Breeding Program Simulations

371 Gota M, Gianola D (2014) Kernel-based whole-genome prediction of complex traits: A review.
372   Front Genet 5:1–13 . doi: 10.3389/fgene.2014.00363

373 Griffing B (1956) Concept of general and specific combining ability in relation to diallel crossing
374   systems. Aust J Biol Sci 9:463–493

375 Heff EL, Lorenz AJ, Jannink J, Sorrells ME (2010) Plant Breeding with Genomic Selection : Gain
376   per Unit Time and Cost. Crop Sci 50:1681–1690 . doi: 10.2135/cropsci2009.11.0662

377 Heslot N, Yang HP, Sorrells ME, Jannink JL (2012) Genomic selection in plant breeding: A
378   comparison of models. Crop Sci 52:146–160 . doi: 10.2135/cropsci2011.06.0297

379 Kohavi R (1995) A Study of Cross-Validation and Bootstrap for Accuracy Estimation and Model
380   Selection. Int Jt Conf Artif Intell

381 Luan T, Woolliams JA, Lien S, et al (2009) The Accuracy of Genomic Selection in Norwegian Red
382   Cattle Assessed by Cross-Validation. Genetics 1126:1119–1126 . doi:
383   10.1534/genetics.109.107391

384 Mendiburu F (2019) agricolae: Statistical Procedures for Agricultural Research

385 Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of Total Genetic Value Using Genome-
386   Wide Dense Marker Maps

387 Misztal I (2016) Inexpensive computation of the inverse of the genomic relationship matrix in
388   populations with small effective population size. Genetics 202:401–409 . doi:
389   10.1534/genetics.115.182089

390 Misztal I, Legarra A, Aguilar I (2014) Using recursion to compute the inverse of the genomic
391   relationship matrix. J Dairy Sci 97:3943–3952 . doi: 10.3168/jds.2013-7752

392 Patterson HD, Williams ER (1976) A new class of resolvable incomplete block designs. Biometrika
393   63:83–92 . doi: 10.1093/biomet/63.1.83

394 Pérez P, de los Campos G (2014) Genome-Wide Regression and Prediction with the BGLR
395   Statistical Package. Genetics 2:483–495

396 Piepho HP, Möhring J, Melchinger AE, Büchse A (2008) BLUP for phenotypic selection in plant
397   breeding and variety testing. Euphytica 161:209–228 . doi: 10.1007/s10681-007-9449-8

Runcie D, Cheng H (2019) Pitfalls and Remedies for Cross Validation with Multi-trait Genomic Prediction Methods. G3 Genes, Genomes, Genet g3.400598.2019 . doi: 10.1534/g3.119.400598

Shao J (1993) Linear model selection by cross-validation. J Am Stat Assoc 88:486–494 . doi: 10.1016/j.jspi.2003.10.004

Signorell A (2019) DescTools: Tools for Descriptive Statistics

Singh P, Bhatia D (2017) Incomplete block designs for plant breeding experiments. Agric Res J 54:607–611 . doi: 10.5958/2395-146x.2017.00119.3

Ta KN, Khong NG, Ha TL, et al (2018) A genome-wide association study using a Vietnamese landrace panel of rice (Oryza sativa) reveals new QTLs controlling panicle morphological traits. BMC Plant Biol 18:1–15 . doi: 10.1186/s12870-018-1504-1

Unterseer S, Bauer E, Haberer G, et al (2014) A powerful tool for genome analysis in maize: Development and evaluation of the high density 600 k SNP genotyping array. BMC Genomics 15:1–15 . doi: 10.1186/1471-2164-15-823

VanRaden PM (2008) Efficient methods to compute genomic predictions. J Dairy Sci 91:4414–4423 . doi: 10.3168/jds.2007-0980

Wimmer V, Albrecht T, Auinger HJ, Schön CC (2012) Synbreed: A framework for the analysis of genomic prediction data using R. Bioinformatics 28:2086–2087 . doi: 10.1093/bioinformatics/bts335

Wong TT (2015) Performance evaluation of classification algorithms by k-fold and leave-one-out cross validation. Pattern Recognit 48:2839–2846 . doi: 10.1016/j.patcog.2015.03.009

Wu X, Lund MS, Sun D, et al (2015) Impact of relationships between test and training animals and among training animals on reliability of genomic prediction. J Anim Breed Genet 132:366–375 . doi: 10.1111/jbg.12165

Würschum T, Abel S, Zhao Y (2014) Potential of genomic selection in rapeseed (Brassica napus L.) breeding. Plant Breed 133:45–51 . doi: 10.1111/pbr.12137

Yates F (1936) Incomplete Randomized Blocks. Ann Eugen 7:121–140 . doi: 10.1111/j.1469-1809.1936.tb02134.x

Yu X, Li X, Guo T, et al (2016) Genomic prediction contributing to a promising global strategy to turbocharge gene banks. Nat Plants 2: . doi: 10.1038/nplants.2016.150

Zhang X, Pérez-Rodríguez P, Semagn K, et al (2015) Genomic prediction in biparental tropical maize populations in water-stressed and well-watered environments using low-density and GBS SNPs. Heredity (Edinb) 114:291–299 . doi: 10.1038/hdy.2014.99

Zhang X, Sallam A, Gao L, et al (2016) Establishment and optimization of genomic selection to accelerate the domestication and improvement of intermediate wheatgrass. Plant Genome 9:1–18 . doi: 10.3835/plantgenome2015.07.0059

Zhao Y, Zeng J, Fernando R, Reif JC (2013) Genomic prediction of hybrid wheat performance. Crop Sci 53:802–810 . doi: 10.2135/cropsci2012.08.0463
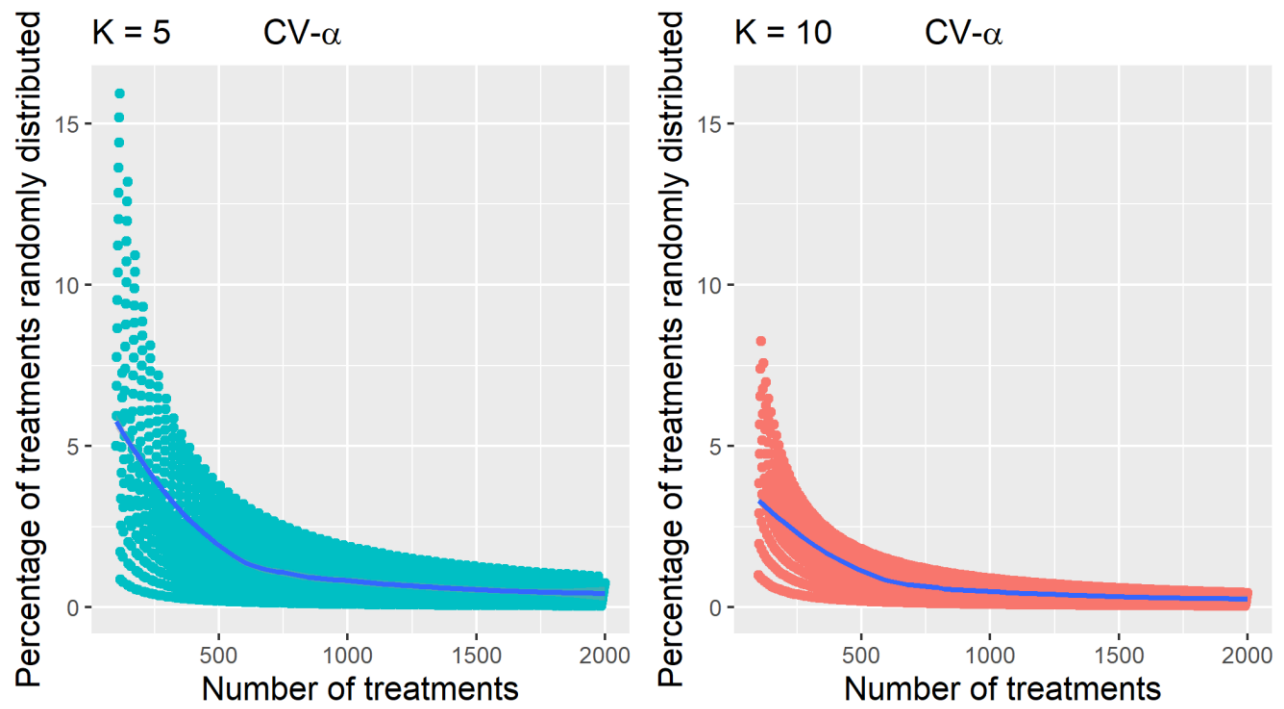
Figure 1. The proportion of treatments randomly distributed into folds to attend the alpha-design presupposition using CV-α with 5-folds with four replicates (a), and 10-folds with two replicates (b), based on simulated data.
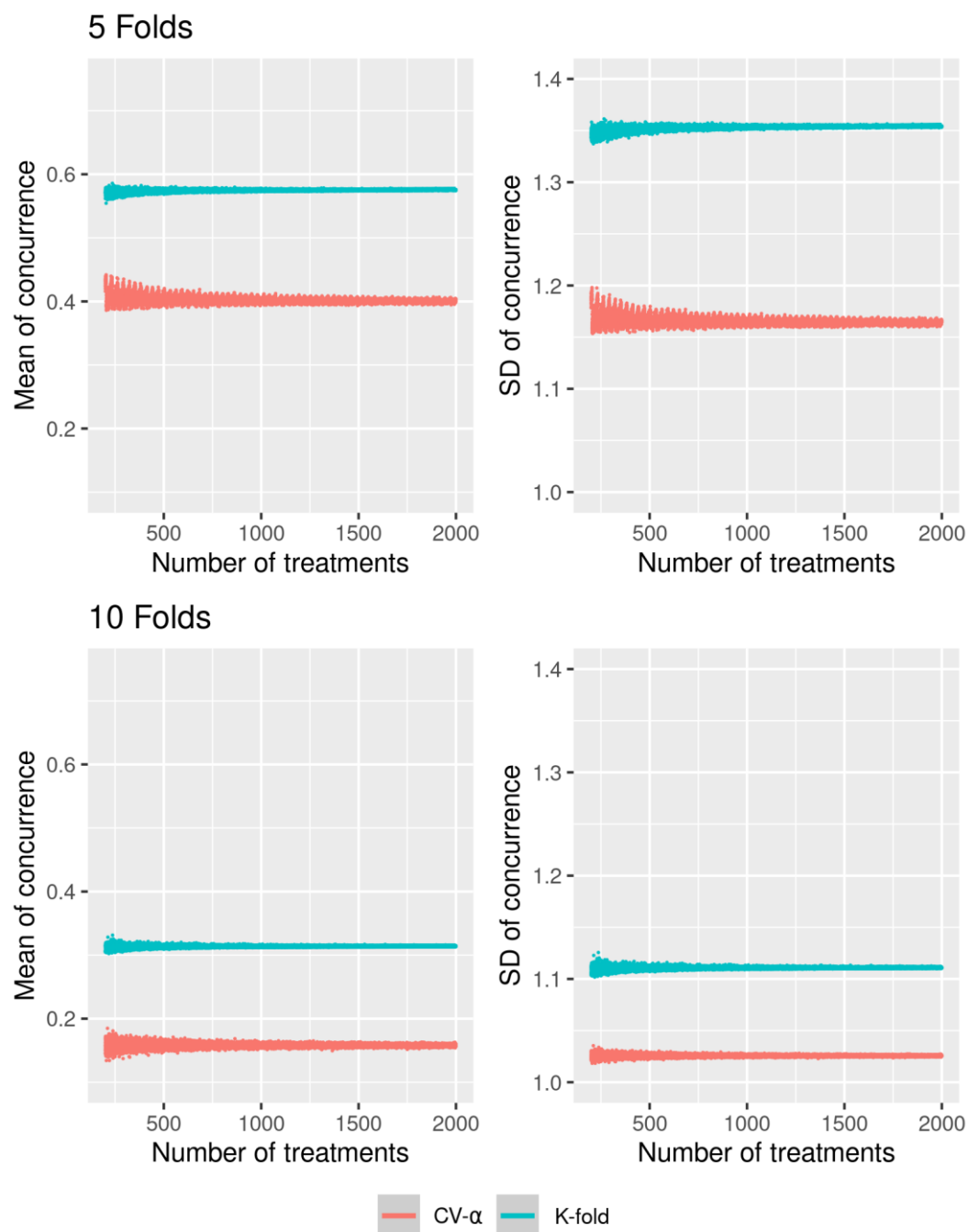
Figure 2. Concurrence (number of times that a pair of treatments appear together in the same fold) mean and standard deviation between treatments pairs in the same fold across replicates using CV-α and K-fold with 5 and 10 folds with 4 and 2 replicates, respectively, based on simulated data.
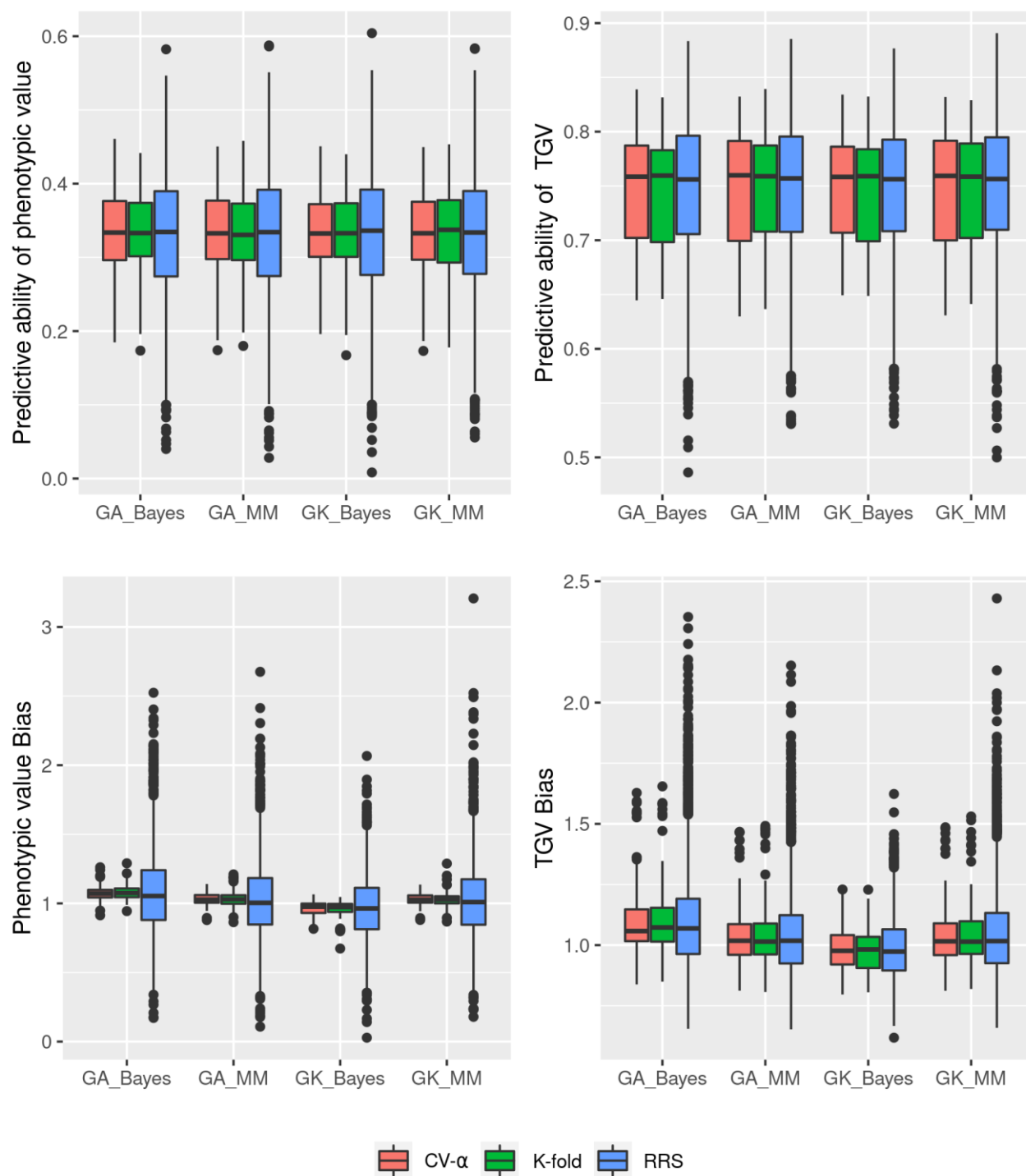
Figure 3. Predictive ability (PA) and bias for TGV and phenotypic value for three validation schemes (CV-α, K-fold, and RRS) and four genomic prediction models (scenarios).

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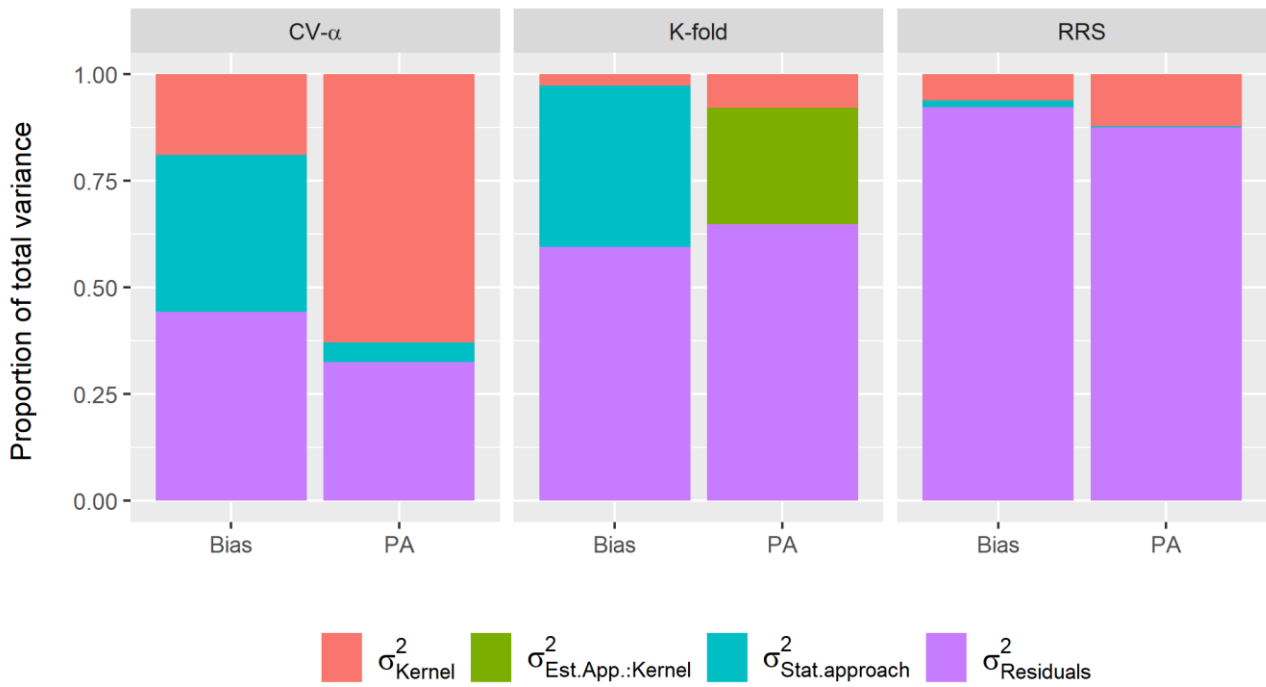
Figure 4. The proportion of total variance decomposed into effects of the kernel, statistical approach, the interaction between the kernel and statistical approach, and residual for bias and predictive ability (PA) applied in three cross-validation schemes (CV-α, K-fold, RRS).

454

455

456 **Table 1.** Averaged of 25 simulated datasets for mean, standard deviation (SD), mean squared error
457 (MSE), and coefficient of variation (CV) for predictive ability (PA) and bias for three CV schemes
458 (CV-α, K-fold, and RRS)

Scheme	Parameter	Mean	SD	MSE	CV (%)
CV-α	**PA of phenotypic value**	0.331	0.063	0.00017	3.78
	PA of TGV	0.748	0.053	0.00022	1.50
	Phenotypic Bias	1.024	0.064	0.00217	4.26
	TGV Bias	1.049	0.149	0.00040	1.68
K-Fold	**PA of phenotypic value**	0.331	0.062	0.00016	3.84
	PA of TGV	0.748	0.053	0.00023	1.52
	Phenotypic Bias	1.027	0.072	0.00251	4.36
	TGV Bias	1.050	0.151	0.00049	1.80
RRS	**PA of phenotypic value**	0.331	0.084	0.00414	19.41
	PA of TGV	0.748	0.062	0.00616	8.10
	Phenotypic Bias	1.024	0.274	0.07283	25.47
	TGV Bias	1.050	0.192	0.01366	10.26

459

460

461 **Table 2.** Summary of ANOVA, mean, standard deviation (SD), and coefficient of variation (CV) for
462 three validation schemes (CV-α, K-fold, and RRS) for predictive ability (PA) and bias

Model	CV-α				K-fold				RRS			
	Df	PA	Bias		Df	PA	Bias		Df	PA	Bias	
		MS				MS				MS		
St.Approaches	1	0.0002	0.0049	*	1	0.0002	0.0048	*	1	0.0035	0.1278	.
Kernel	1	0.0016 **	0.0025	.	1	0.0007	. 0.0005		1	0.1292 **	0.4748	**
St.Approaches:Kernel	1	0.0001	0.0001		1	0.0005	0.0002		1	0.0002	0.0123	
Residuals	12	0.0001	0.0007		12	0.0002	0.0009		396	0.0046	0.0340	
CV (%)		2.16	2.70			2.91	2.97			14.61	18.38	
Mean		0.433	0.99			0.440	1.02			0.433	1.00	
SD		0.014	0.033			0.016	0.033			0.070	0.188	

463 **, *, ., ns: Significant at 1%, 5% , 10% and non-significant of error probability by F- test.

464 Statistical approaches (St. Approaches), Degrees of freedom (Df), Predictive ability (PA), Mean Squared (MS)

465

466 **Table 3.** Means, marginal means, and Tukey's test for the type of kernels and statistical approaches
467 for predictive ability (PA) and bias

	G_a		K		Marginal Means	
PA						
Bayesian	0.424		0.436		0.430	
Mixed models	0.426		0.445		0.436	
Marginal Means	0.425	b	0.441	a		
Bias						
Bayesian	0.963		0.992		0.977	B
Mixed models	1.002		1.023		1.012	A
Marginal Means	0.982	b*	1.008	a	0.995	

468 *Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ by
469 the Tukey test at 5% and 10%* probability.