

1 **CV- $\alpha$ : designing validation sets to increase the precision and enable multiple comparison tests**  
2 **in genomic prediction**

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9 **Abbreviations:** CV, cross-validation; CV- $\alpha$ , cross-validation alpha-based design; GP, Genomic  
10 prediction; RRS, Repeated Random Subsampling; TGV, true genetic value.

11 Received \_\_\_\_\_.

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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 ( $CV-\alpha$ ). The  $CV-\alpha$  was  
21 meant to create several scenarios of validation (replicates x folds), regardless of the number of  
22 treatments. Using  $CV-\alpha$ , 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  $CV-\alpha$  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  $CV-\alpha$  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,  $CV-\alpha$  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. In 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- $\alpha$ ) and compare its performance to the methods commonly applied  
83 in GP studies for model selection. Also, based on the CV- $\alpha$  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<sup>®</sup> Axiom<sup>®</sup> 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<sup>-1</sup>),  
119 corrected to 13% moisture, and stand across the eight environments. It was used to estimate the BLUP  
120 for hybrids following the model:

$$121 \quad \mathbf{y} = \mathbf{S}\mathbf{l} + \mathbf{X}\mathbf{b} + \mathbf{W}\mathbf{c} + \mathbf{T}\mathbf{g} + \mathbf{U}\mathbf{i} + \boldsymbol{\varepsilon}$$

122 where  $\mathbf{y}$  is the vector of the phenotypic value of hybrids;  $\mathbf{l}$  is the vector of fixed effects of the  
123 environment (the combination of site x year x N level);  $\mathbf{b}$  is the vector of fixed effects of blocks within  
124 an environment;  $\mathbf{c}$  is the vector of fixed effects of checks;  $\mathbf{g}$  is genotypic values, where  $\mathbf{g} \sim N(0, \mathbf{I}\sigma_g^2)$ ;  
125  $\mathbf{i}$  is the interaction between environments and checks, where  $\mathbf{i} \sim N(0, \mathbf{I}\sigma_{ge}^2)$ ;  $\boldsymbol{\varepsilon}$  is the vector of random  
126 residuals from checks and hybrid by environments effects, where  $\boldsymbol{\varepsilon} \sim N(0, \mathbf{I}\sigma_\varepsilon^2)$ .  $\sigma_\varepsilon^2$  was jointly  
127 estimated based on  $e$  environments with  $r$  replicated checks in each site.  $\mathbf{S}$ ,  $\mathbf{X}$ ,  $\mathbf{W}$ ,  $\mathbf{T}$ , and  $\mathbf{U}$  are the  
128 incidence matrices for  $\mathbf{l}$ ,  $\mathbf{b}$ ,  $\mathbf{c}$ ,  $\mathbf{g}$ , and  $\mathbf{i}$  (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:

$$134 \quad \hat{\mathbf{g}} = \mathbf{1}\mu + \mathbf{Z}\mathbf{a} + \boldsymbol{\varepsilon}$$

135 where  $\hat{\mathbf{g}}$  is the vector of BLUP;  $\mu$  is the intercept;  $\mathbf{a}$  is the vector of additive genetic effects  
136 with  $\mathbf{a} \sim N(0, \mathbf{G}\sigma_a^2)$ ; and  $\boldsymbol{\varepsilon}$  is the vector of random residuals with  $\boldsymbol{\varepsilon} \sim N(0, \mathbf{I}\sigma_\varepsilon^2)$ .  $\mathbf{1}$  is the incidence  
137 vector of  $\mu$ , and  $\mathbf{Z}$  is the incidence matrix for  $\mathbf{a}$ .  $\mathbf{G}$  is the genomic relationship matrix ( $\mathbf{G}_a$  – additive

138 genomic relationship matrix, and  $\mathbf{K}$  – for Gaussian kernel), and  $\mathbf{I}$  is the identity matrix.  $\sigma_a^2$  is the  
139 additive genetic variance for  $\mathbf{G}_a$  or genetic variance for  $\mathbf{K}$ , and  $\sigma_\varepsilon^2$  is the residual variance. The  
140 additive genomic relationship matrix ( $\mathbf{G}_a$ ) was calculated as  $\mathbf{G}_a = \mathbf{W}\mathbf{W}'/2\sum_{i=1}^n p_i(1 - p_i)$ , 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  $K(\mathbf{x}_i, \mathbf{x}_j) = \exp(-hd_{ij}^2/q_{0.05})$ , where  $\mathbf{x}_i$  and  $\mathbf{x}_j$  are the  
143 marker vectors for the  $i^{\text{th}}$  and  $j^{\text{th}}$  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-Elizalde 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- $\alpha$ ) 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- $\alpha$  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  $\alpha(0,1)$  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  $\alpha(0,1)$ -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- $\alpha$  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- $\alpha$ ) 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- $\alpha$   
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- $\alpha$  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:

$$196 \quad \mathbf{l} = \mathbf{1}\mu + \mathbf{X}_1\mathbf{m} + \mathbf{X}_2\mathbf{n} + \mathbf{X}_3\mathbf{o} + \boldsymbol{\varepsilon}$$

197 where  $\mathbf{l}$  is the vector of Pearson correlation transformed by Fisher z-transformation using the  
198 R package *DescTools* (Signorell et al., 2019);  $\mu$  is the overall mean;  $\mathbf{m}$  is the vector of statistical  
199 approach effect;  $\mathbf{n}$  is the vector of relationship kernel;  $\mathbf{o}$  is the vector of interaction between statistical  
200 approach and kernel; and  $\boldsymbol{\varepsilon}$  is the vector of residuals.  $\mathbf{X}_1$ ,  $\mathbf{X}_2$  e  $\mathbf{X}_3$  are incidence matrices for  $\mathbf{m}$ ,  $\mathbf{n}$ , and  
201  $\mathbf{o}$ , respectively. Quadratic components were estimated by the method of moments based on mean  
202 square expectation.

203 **Results**

204 *CV- $\alpha$*

205 We performed several analyses to evaluate cross-validation alpha-based design (CV- $\alpha$ )  
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- $\alpha$  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-  $\alpha$ . 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- $\alpha$  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- $\alpha$  presented similar performance and were lower than RRS (Table 2).

230 For mean squared error and coefficient of variation,  $CV-\alpha$  presented lower values than K-fold and  
231 RRS. The coefficient of variation for K-fold was 34.70% and 10% higher than  $CV-\alpha$  for PA and bias,  
232 respectively (Table 2).

233 We applied the  $CV-\alpha$  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 significant effects for types of the kernel  
236 for predictive ability and bias. Gaussian kernel (**K**) presented higher PA (0.44) than **G<sub>a</sub>** (0.42) and  
237 lower bias (1.00 and 0.98, for **K** and **G<sub>a</sub>**, 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.  $CV-\alpha$  presented lower proportions of residual variances and higher variance due to the kernel  
244 and statistical approaches effects.

245

## 246 Discussion

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

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

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

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

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

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 (Miształ et al. 2014; Miształ 2016). Based on  
274 this,  $CV-\alpha$  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  $CV-\alpha$  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,  $CV-\alpha$  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.  $CV-\alpha$  reveals the lesser  
284 occurrence of pairs of genotypes in the same fold across replicates, causing a more precise estimative.  
285 The  $CV-\alpha$  methodology consists of applying  $\alpha(0,1)$  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  $CV-\alpha$  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  $CV-\alpha$  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  $CV-\alpha$  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 **G<sub>a</sub>**.  
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- $\alpha$  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- $\alpha$  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- $\alpha$  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- $\alpha$  is that it does not require any additional cost regarding computing  
317 demand or complexity. Furthermore, CV- $\alpha$  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.



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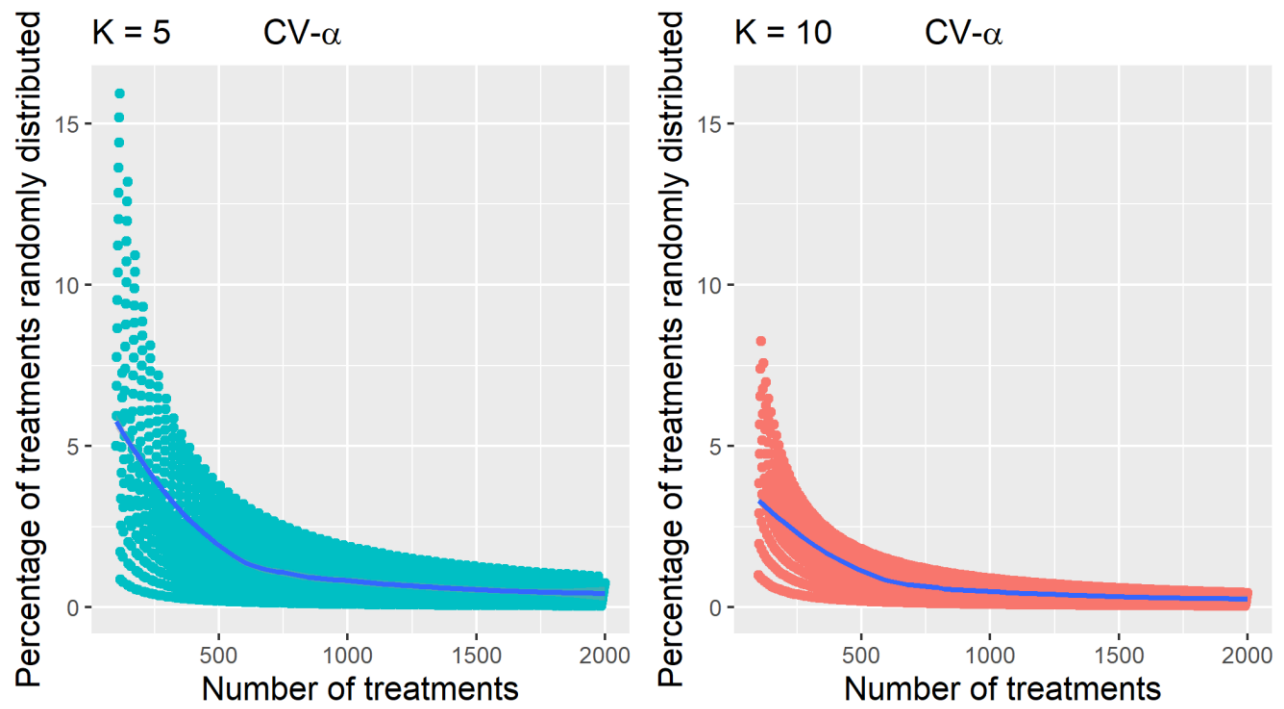
323 **Conflict of interest:** The authors declare no conflict of interest.

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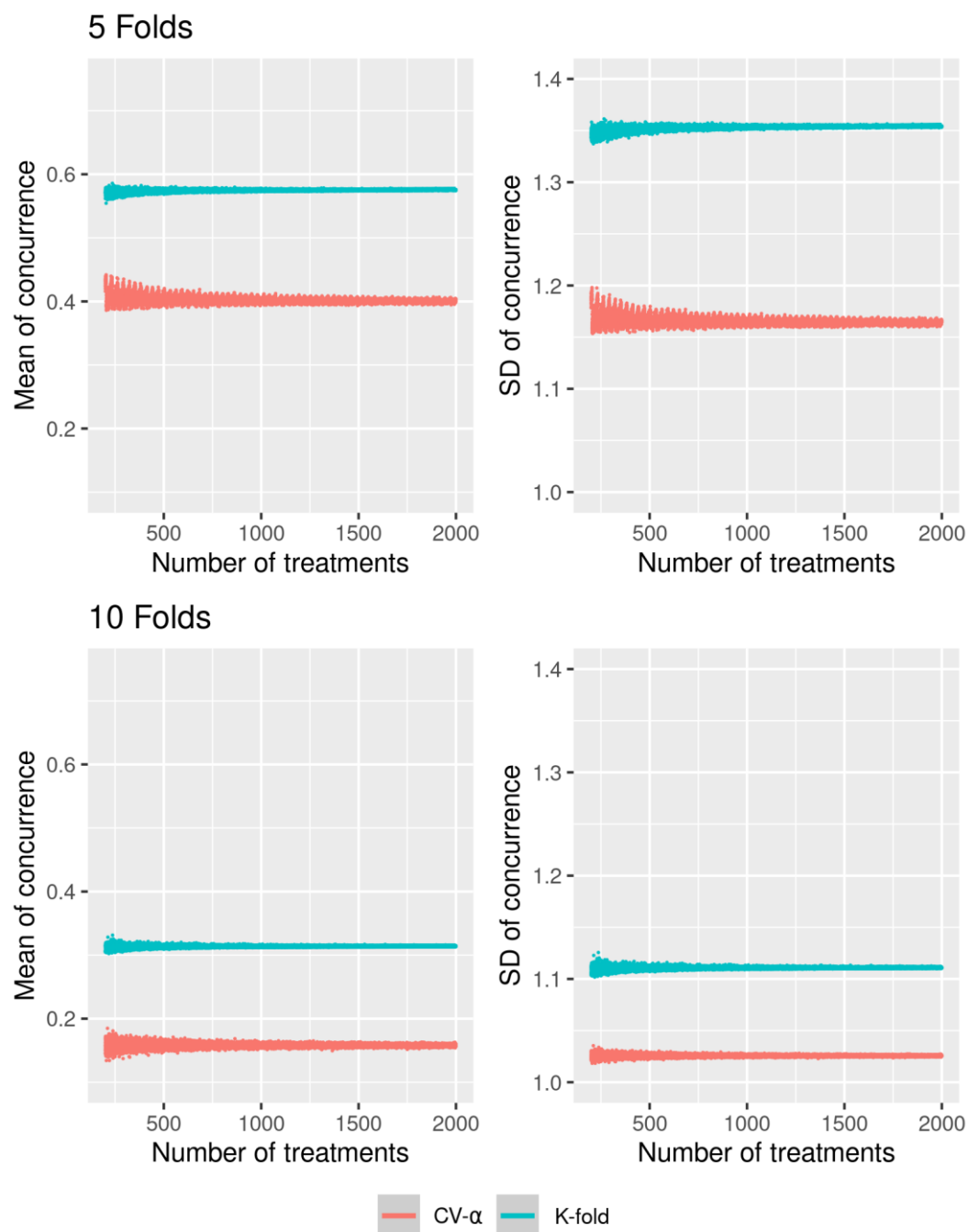
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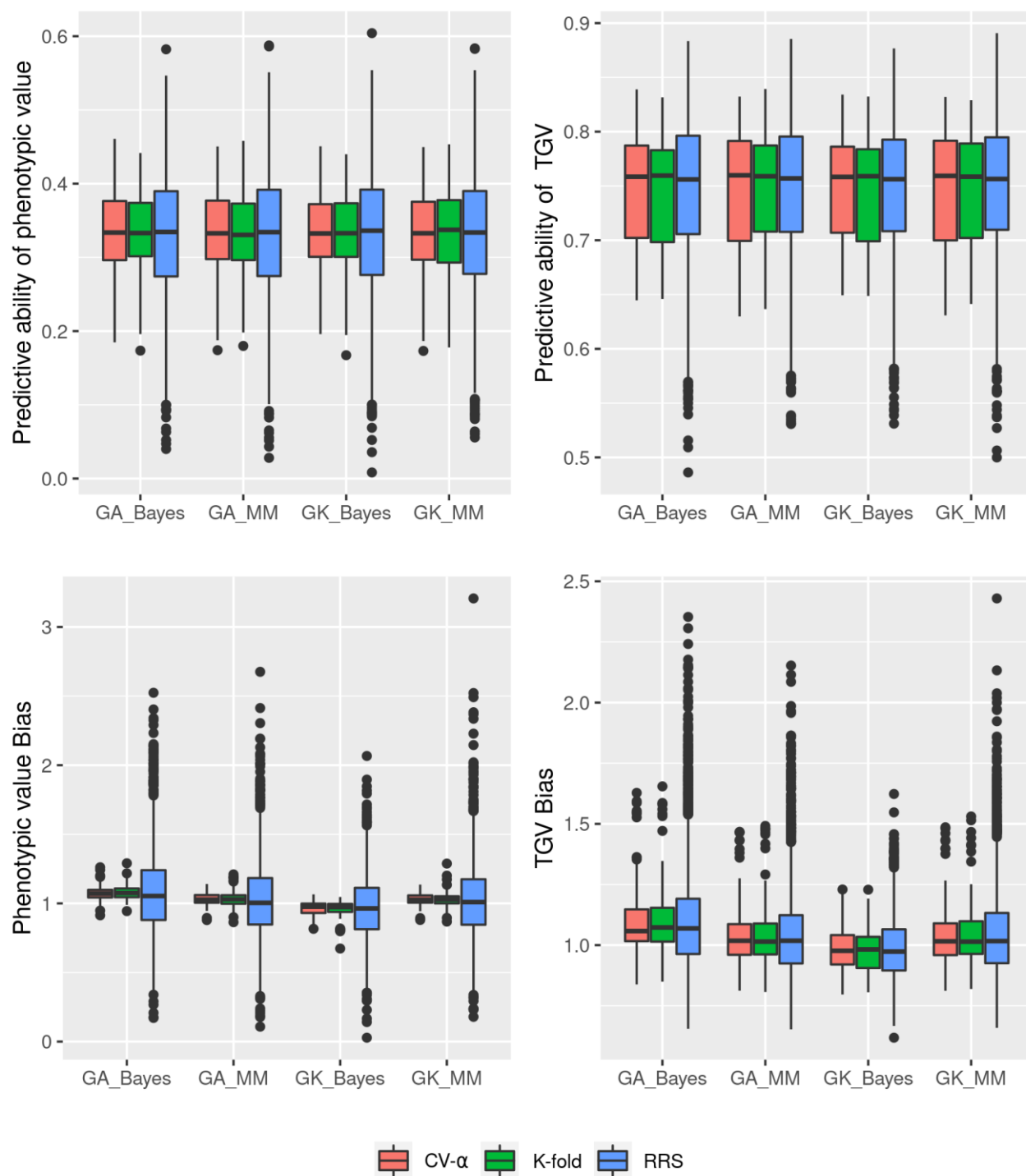
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**Figure 1.** The proportion of treatments randomly distributed into folds to attend the alpha-design presupposition using CV- $\alpha$  with 5-folds with four replicates (a), and 10-folds with two replicates (b), based on simulated data.

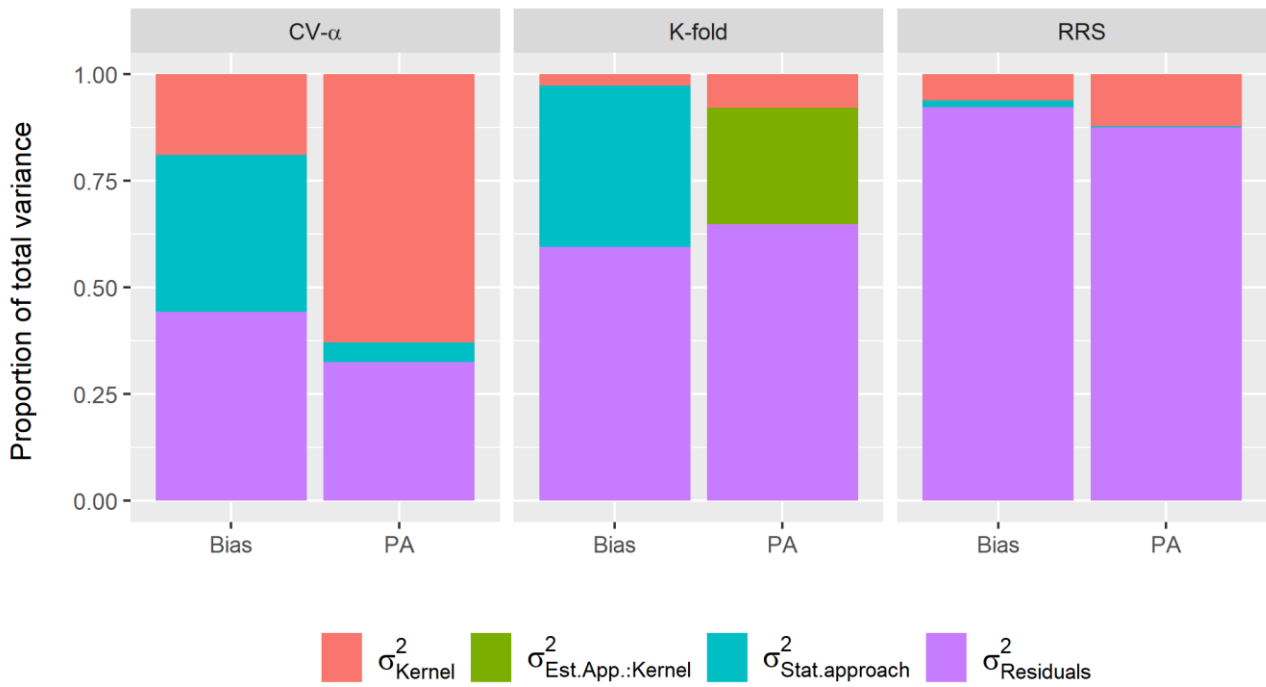


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**Figure 2.** Concurrence (number of times that a pair of treatments appear together in the same fold) mean and standard deviation between treatments pairs in the same fold across replicates using CV- $\alpha$  and K-fold with 5 and 10 folds with 4 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- $\alpha$ , K-fold, and RRS) and four genomic prediction models (scenarios).



450

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

Scheme	Parameter	Mean	SD	MSE	CV (%)
CV- $\alpha$	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- $\alpha$ , K-fold, and RRS) for predictive ability (PA) and bias

Model	CV- $\alpha$				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				
<b>CV (%)</b>		2.16	2.70		2.91	2.97		14.61	18.38				
<b>Mean</b>		0.433	0.99		0.440	1.02		0.433	1.00				
<b>SD</b>		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

	<b>PA</b>			
	<b>G<sub>a</sub></b>	<b>K</b>	<b>Marginal Means</b>	
Bayesian	0.424	0.436	0.430	
Mixed models	0.426	0.445	0.436	
Marginal Means	0.425	b	0.441	a

	<b>Bias</b>			
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

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.