

1     **Genomic Prediction with Genotype by Environment Interaction Analysis for Kernel Zinc**  
2                                   **Concentration in Tropical Maize Germplasm**

3     Edna K. Mageto\*, Jose Crossa<sup>†</sup>, Paulino Pérez-Rodríguez <sup>‡</sup>, Thanda Dhliwayo<sup>†</sup>, Natalia  
4     Palacios-Rojas<sup>†</sup>, Michael Lee\*, Rui Guo<sup>§†</sup>, Félix San Vicente<sup>†</sup>, Xuecai Zhang<sup>†</sup> and Vemuri  
5     Hindu\*\*

6     \*Department of Agronomy, Iowa State University, Ames, IA 50011, USA.

7     <sup>†</sup>International Maize and Wheat Improvement Center (CIMMYT), El Batán, Texcoco CP 56237,  
8     Mexico.

9     <sup>‡</sup>Colegio de Postgraduados, Department of Statistics and Computer Sciences, Montecillos, Edo.  
10    De México 56230, México.

11    <sup>§</sup>College of Agronomy, Shenyang Agricultural University, Shenyang, Liaoning 110866, China.

12    \*\*Asia Regional Maize Program, International Maize and Wheat Improvement Center  
13    (CIMMYT), ICRISAT Campus, Patancheru, Hyderabad, Telangana 502324, India.

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## Genomic prediction for kernel zinc

**KEYWORDS:** *Zea mays L.*, genetics, breeding, zinc, prediction

### Corresponding author:

Dr. Michael Lee

Department of Agronomy, Iowa State University.

1553 Agronomy Hall

Ames, IA 50011-1051

Phone: +1515 294-7951

Email: [mlee@iastate.edu](mailto:mlee@iastate.edu)

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## ABSTRACT

Zinc (Zn) deficiency is a major risk factor for human health, affecting about 30% of the world's population. To study the potential of genomic selection (GS) for maize with increased Zn concentration, an association panel and two doubled haploid (DH) populations were evaluated in three environments. Three genomic prediction models, M (M1: Environment + Line, M2: Environment + Line + Genomic, and M3: Environment + Line + Genomic + Genomic x Environment) incorporating main effects (lines and genomic) and the interaction between genomic and environment (G x E) were assessed to estimate the prediction ability ( $r_{MP}$ ) for each model. Two distinct cross-validation (CV) schemes simulating two genomic prediction breeding scenarios were used. CV1 predicts the performance of newly developed lines, whereas CV2 predicts the performance of lines tested in sparse multi-location trials. Predictions for Zn in CV1 ranged from -0.01 to 0.56 for DH1, 0.04 to 0.50 for DH2 and -0.001 to 0.47 for the association panel. For CV2,  $r_{MP}$  values ranged from 0.67 to 0.71 for DH1, 0.40 to 0.56 for DH2 and 0.64 to 0.72 for the association panel. The genomic prediction model which included G x E had the highest average  $r_{MP}$  for both CV1 (0.39 and 0.44) and CV2 (0.71 and 0.51) for the association panel and DH2 population, respectively. These results suggest that GS has potential to accelerate breeding for enhanced kernel Zn concentration by facilitating selection of superior genotypes.

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## INTRODUCTION

71 Malnutrition arising from zinc (Zn) deficiency is a major risk factor for human health  
72 affecting nearly 30% of the world's population (Bouis and Saltzman 2017; Gannon *et al.* 2017).  
73 The problem is more prevalent in low-and middle income countries (LMICs), and is highly  
74 attributed to lack of access to a balanced diet, reliance on cereal-based diets and ignorance of good

75 nutritional practices (Welch and Graham 2004). Several approaches, such as food fortification,  
76 diversification and supplementation have been tried to reduce Zn deficiency. However, in LMICs,  
77 these methods have not been entirely successful (Misra *et al.* 2004; Stein 2010).

78 Breeding maize for increased Zn concentration may offer some relief. The Zn-enriched  
79 varieties can be widely accessible, will not require continued investment once developed, and they  
80 remain after the initial successful investment and research (Govindan 2011). Recently, maize  
81 varieties with 15-36% more Zn were released in Guatemala and Colombia (Listman 2019).  
82 Nevertheless, increased breeding efforts are required to develop more Zn-enriched varieties for a  
83 diverse range of environments and management practices. Progress toward developing those  
84 varieties has mainly relied upon conventional plant breeding approach that is labor-intensive and  
85 time-consuming. However, with the recent advances in genomics, new methods for plant breeding  
86 such as genomic selection (GS) can be used to identify genotypes with enhanced Zn concentration  
87 more efficiently and rapidly.

88 In a GS breeding scheme, genome-wide DNA markers are used to predict which  
89 individuals in a breeding population are most valuable as parents of the next generation (cycle)  
90 of offspring (Meuwissen *et al.* 2001; de los Campos *et al.* 2009; Pérez-Rodríguez *et al.* 2012).  
91 Kernel Zn concentration is determined at the end of a plant's life cycle, so GS can enable  
92 selection of promising genotypes earlier in the life cycle. This reduces the time and cost of  
93 phenotypic evaluation and may increase the genetic gain per unit time and cost (Heslot *et al.*  
94 2015; Manickavelu *et al.* 2017; Arojju *et al.* 2019).

95 The utility and effectiveness of GS has been examined for many different crop species,  
96 marker densities, traits and statistical models and varying levels of prediction accuracy have been  
97 achieved (de los Campos *et al.* 2009, 2013; Crossa *et al.* 2010, 2013, 2014; Jarquín *et al.* 2014;

98 Pérez-Rodríguez *et al.* 2015; Zhang *et al.* 2015; Velu *et al.* 2016). Although the number of  
99 markers needed for accurate prediction of genotypic values depends on the extent of linkage  
100 disequilibrium between markers and QTL (Meuwissen *et al.* 2001), a higher marker density can  
101 improve the proportion of genetic variation explained by markers and thus result in higher  
102 prediction accuracy (Albrecht *et al.* 2011; Zhao *et al.* 2012; Combs and Bernardo 2013; Liu *et al.*  
103 2018). Importantly, higher prediction accuracies have been obtained when genotypes of a  
104 population are closely related than when genetically unrelated (Pszczola *et al.* 2012; Combs and  
105 Bernardo 2013; Spindel and McCouch 2016).

106         Initially, GS models and methods were developed for single-environment analyses and  
107 they did not consider correlated environmental structures due to genotype by environment (G x E)  
108 interactions (Crossa *et al.* 2014). The differential response of genotypes in different environments  
109 is a major challenge for breeders and can affect heritability and genotype ranking over  
110 environments (Monteverde *et al.* 2018). Multi-environment analysis can model G x E using genetic  
111 and residual covariance functions (Burgueño *et al.* 2012), markers and environmental covariates  
112 (Jarquín *et al.* 2014), or marker by environment (M x E) interactions (Lopez-Cruz *et al.* 2015).  
113 This approach to GS can successfully be used for biofortification breeding of maize because multi-  
114 environment testing is routinely used in the development and release of varieties.

115         Modelling covariance matrices to account for G x E allows the use of information from  
116 correlated environments (Burgueño *et al.* 2012). Mixed models that allow the incorporation of a  
117 genetic covariance matrix calculated from marker data, rather than assuming independence among  
118 genotypes improves the estimation of genetic effects (VanRaden 2008). The benefit of using  
119 genetic covariance matrices in G x E mixed models is that the model relates genotypes across  
120 locations even when the lines are not present in all locations (Monteverde *et al.* 2018). GS models

121 capable of accounting for multi-environment data have extensively been studied in different crops  
122 (Zhang *et al.* 2015; Cuevas *et al.* 2016, 2017; Velu *et al.* 2016; Jarquín *et al.* 2017; Sukumaran *et*  
123 *al.* 2017a; Monteverde *et al.* 2018; Roorkiwal *et al.* 2018). In those studies, incorporating G x E  
124 demonstrated a substantial increase in prediction accuracy relative to single-environment analyses.

125 Kernel Zn has been investigated in several quantitative trait loci (QTL) analyses in maize  
126 and each study has reported that Zn concentration is under the control of several loci. The  
127 phenotypic variation explained by those loci ranges from 5.9 to 48.8% (Zhou *et al.* 2010; Qin *et*  
128 *al.* 2012; Šimić *et al.* 2012; Baxter *et al.* 2013; Jin *et al.* 2013; Zhang *et al.* 2017a; Hindu *et al.*  
129 2018). A Meta-QTL analysis across several of those studies identified regions on chromosome 2  
130 that might be important for kernel Zn concentration (Jin *et al.* 2013). Additionally, genomic  
131 regions associated with Zn concentration were recently reported in a genome-wide association  
132 study of maize inbreds adapted to the tropics (Hindu *et al.* 2018). Whereas some of the regions  
133 were novel, four of the twenty identified were located in already reported QTL intervals. Taken  
134 together, the QTLs may be used in a breeding program through marker-assisted selection (MAS)  
135 or GS.

136 A wide array of maize genetic studies has reported considerable effects of G x E  
137 interactions for kernel Zn concentration (Oikeh *et al.* 2003, 2004; Long *et al.* 2004; Chakraborti *et*  
138 *al.* 2009; Prasanna *et al.* 2011; Agrawal *et al.* 2012; Guleria *et al.* 2013). However, genotypes with  
139 high-Zn concentration have been identified in both tropical and temperate germplasm (Ahmadi *et*  
140 *al.* 1993; Bänziger and Long 2000; Brkic *et al.* 2004; Menkir 2008; Chakraborti *et al.* 2011;  
141 Prasanna *et al.* 2011; Hindu *et al.* 2018). Additionally, evaluation procedures for kernel Zn are  
142 labor-intensive, expensive and time-consuming (Palacios-Rojas 2018). To the best of our  
143 knowledge, no study has examined the predictive ability of GS methods that incorporate G x E for

144 Zn concentration in maize. Within the framework of the reaction norm model (Jarquín *et al.* 2014),  
145 the potential of GS for Zn using maize inbreds adapted to tropical environments were assessed.  
146 The objectives of this study were; (i) to evaluate the prediction ability for Zn using an association  
147 mapping panel and two bi-parental populations evaluated in three tropical environments, (ii) to  
148 assess and compare the predictive ability of different GS models, and (iii) to examine the effects  
149 of incorporating G x E on prediction accuracy for Zn.

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## MATERIALS AND METHODS

### **Zinc association mapping (ZAM) panel**

154 The ZAM panel consists of 923 inbreds from maize breeding programs of the  
155 International Maize and Wheat Improvement Center (CIMMYT). The panel represents wide  
156 genetic diversity for kernel Zn concentration (Hindu *et al.* 2018).

157

### **Bi-parental DH populations**

159 From the ZAM panel, four inbreds with contrasting Zn concentration were selected and  
160 used to form two bi-parental (doubled haploid [DH]) populations (Table 1). DH1 was derived  
161 from the F1 generation of a mating between CML503, a high-Zn inbred (31.21 µg/g) with  
162 CLWN201, a low-Zn inbred (22.62 µg/g). DH2 was derived from the F1 generation of a mating  
163 between CML465, another high-Zn inbred (31.55 µg/g) with CML451, a moderate-Zn inbred  
164 (27.88 µg/g). DH1 and DH2 were comprised of 112 and 143 inbreds, respectively.

165

166 **Experimental design and phenotypic evaluation**

167 **Zinc association mapping (ZAM) panel**

168 The ZAM panel was grown at CIMMYT research stations in Mexico, during the months  
169 of June through September and November through March at Agua Fria in 2012 and 2013, and  
170 Celaya in 2012. Plot sizes and the experimental designs (Hindu *et al.* 2018).

171

172 **Bi-parental DH populations**

173 The DH populations were grown at CIMMYT research stations in Mexico; Celaya in  
174 2014 and Tlaltizapan (18°41'N, 99° 07' W; 962.5 m asl) in 2015 and 2017. In 2014 and 2015,  
175 both populations were evaluated in single-replication trials (Hindu *et al.* 2018). In 2017, a  
176 randomized complete block design (RCBD) with two replications was used. The rows were 2.5  
177 m long and 75 cm apart and each genotype was grown in a single row plot. All plots were  
178 managed according to the recommended agronomic practices for each environment.

179 From the ZAM panel and each DH population, four to six plants in each plot were self-  
180 pollinated, hand-harvested at physiological maturity, hand-shelled and dried to a moisture  
181 content of 12.5%. The bulked kernels from each plot are considered a representative sample and  
182 were used in subsequent Zn analyses as described (Hindu *et al.* 2018).

183

184 **Genotypic data**

185 Genomic DNA was extracted from leaf tissues of all inbred lines (ZAM panel and DH  
186 populations) using the standard CIMMYT laboratory protocol (CIMMYT, 2005). The samples  
187 were genotyped using the genotyping by sequencing (GBS) method at the Institute for Genomic  
188 Diversity, Cornell University, USA (Elshire *et al.* 2011; Crossa *et al.* 2013). The restriction

189 enzyme ApeK1 was used to digest DNA, GBS libraries were constructed in 96-plex and  
190 sequenced on a single lane of Illumina HiSeq2000 flow cell (Elshire *et al.* 2011). To increase the  
191 genome coverage and read depth for SNP discovery, raw read data from the sequencing samples  
192 were analyzed together with an additional ~30, 000 global maize collections (Zhang *et al.* 2015).

193 SNP identification was performed using TASSEL 5.0 GBS Discovery Pipeline with B73  
194 (RefGen\_v2) as the reference genome (Elshire *et al.* 2011; Glaubitz *et al.* 2014). The source code  
195 and the TASSEL GBS discovery pipeline are available at <https://www.maizegenetics.net> and the  
196 SourceForge Tassel project <https://sourceforge.net/projects/tassel>. For each inbred, the pipeline  
197 yielded 955, 690 SNPs which were distributed on the 10 maize chromosomes. After filtering  
198 using a minor allele frequency of 0.05 and removing SNPs with more than 10% missing data,  
199 181,889 (ZAM panel) and 170, 798 (bi-parental) SNPs were used for genomic prediction.

200

## 201 **Phenotypic data analysis**

202 For the ZAM panel, broad-sense heritability ( $H^2$ ) across environments was estimated as:

203

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2/l + \sigma_e^2/lr}$$

204 where  $\sigma_G^2$  is the variance due to genotype,  $\sigma_{GE}^2$  is variance due to genotype x environment,  $\sigma_e^2$  is  
205 the error variance,  $l$  is the number of environments and  $r$  is the number of replications using  
206 multi-environment trial analysis with R (META-R) (Alvarado *et al.* 2016). For the DH  
207 populations, variance components based on the genomic relationship matrix were computed  
208 using BGLR package as implemented in GBLUP (Pérez and de los Campos 2014). An estimate  
209 of narrow-sense heritability ( $\hat{h}^2$ ) for each DH population was calculated as:

210 
$$\hat{h}^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_e^2}$$

211 where  $\hat{\sigma}_g^2$  is an estimate of the additive genetic variance and  $\hat{\sigma}_e^2$  is an estimate of the residual  
212 variance.

213 Correlation coefficients between Zn and environments, descriptive statistics and  
214 phenotypic data distribution using boxplots were generated in R (core Team 2018). Line means  
215 (genotypic values) for the ZAM panel were estimated as Best Linear Unbiased Estimators  
216 (BLUEs) with a random effect for replications nested within each environment. Raw data  
217 (values) were used for the DH populations.

218

## 219 **Statistical models**

220 Genomic models used in this study were based on the reaction norm model which models  
221 the markers (genomic) by environment interaction (Jarquín *et al.* 2014). This model is an  
222 extension of the Genomic Best Linear Unbiased Predictor (GBLUP) random effect model, where  
223 the main effects of lines (genotypes), genomic, environments and their interactions are modelled  
224 using covariance structures that are functions of marker genotypes and environmental covariates.  
225 In this study, environment is the combination of site and year (site-by-year). A brief description  
226 of the models is given below.

227

### 228 **M0. Phenotypic baseline model**

229 The phenotypes  $y_{ij}$  are modelled as:

230 
$$y_{ij} = \mu + E_i + L_j + EL_{ij} + e_{ij},$$

231 this linear model represents the response of the  $j^{\text{th}}$  ( $j=1, \dots, J$ ) genotype/line tested in the  $i^{\text{th}}$   
232 ( $i=1, \dots, I$ ) environment  $[\{y\}_{ij}]$  as the sum of an overall mean  $\mu$  plus random environmental main  
233 effect  $[E_i \stackrel{iid}{\sim} N(0, \sigma_E^2)]$ , the random genotype main effect  $[L_j \stackrel{iid}{\sim} N(0, \sigma_L^2)]$ , the random interaction  
234 between the  $j^{\text{th}}$  genotype and the  $i^{\text{th}}$  environment  $[EL_{ij} \stackrel{iid}{\sim} N(0, \sigma_{EL}^2)]$  and a random error term  
235  $[e_{ij} \stackrel{iid}{\sim} N(0, \sigma_e^2)]$ . From this linear model,  $N(.,.)$  denotes a normal random variable, *iid* stands for  
236 independent and identically distributed responses and  $\sigma_E^2$ ,  $\sigma_L^2$ ,  $\sigma_{EL}^2$ ,  $\sigma_e^2$  are the variances for  
237 environment, genotype, genotype by environment and residual error, respectively. The baseline  
238 model does not allow borrowing of information among genotypes because the genotypes were  
239 treated as independent outcomes. Thus, models used in this study were derived from the baseline  
240 model by subtracting terms or modifying assumptions and/or incorporating genomics/marker  
241 information.

242

### 243 **M1. Environment + Line**

244 This model is obtained by retaining the first three components from the baseline model  
245 (overall mean, random environment main effect and random line main effect) while their  
246 underlying assumptions remain unchanged.

$$247 \quad y_{ij} = \mu + E_i + L_j + e_{ij}. \quad [1]$$

248 Here environments were considered as site-by-year combinations.

249

### 250 **M2. Environment + Line + Genomic**

251 Another representation of the random main effect of line  $L_j$  in the previous model is  
252 considering a linear combination between markers and their correspondent marker effects,  $g_j =$   
253  $\sum_{m=1}^p x_{jm} b_m$ , such that

$$254 \quad y_{ij} = \mu + E_i + L_j + g_j + e_{ij} \quad [2]$$

255 where  $b_m \stackrel{iid}{\sim} N(0, \sigma_b^2)$  represents the random effect of the  $m^{\text{th}}$  ( $m=1, \dots, p$ ) marker,  $x_{jm}$  is the  
256 genotype of the  $j^{\text{th}}$  line at the  $m^{\text{th}}$  marker and  $\sigma_b^2$  its correspondent variance component.

257 Therefore,  $\mathbf{g} = (g_1, \dots, g_J)'$ , is the vector of genetic effects, and follows a normal density with  
258 mean zero, and a co-variance matrix  $Cov(\mathbf{g}) = \mathbf{G}\sigma_g^2$  with  $\mathbf{G} = \frac{\mathbf{XX}'}{p}$  being the genomic relationship  
259 matrix (Lopez-Cruz *et al.* 2015) that describes genetic similarities among pairs of individuals. In  
260 this model, the line effect  $L_j$  is retained to account for imperfect information and model mis-  
261 specification because of potential imperfect linkage disequilibrium between markers and  
262 quantitative trait loci (QTLs).

263

### 264 **M3. Environment + Line + Genomic + Genomic $\times$ Environment**

265 This model accounts for the effects of lines  $L_j$ , of markers (genomic)  $g_j$ , of environments ( $E_i$ )  
266 and the interaction between markers (genomic) and the environment ( $Eg_{ij}$ ). The model includes  
267 the interaction between markers (genomics) and the environment via co-variance structure  
268 (Jarquín *et al.* 2014). The model is as follows:

$$269 \quad y_{ij} = \mu + E_i + L_j + g_j + Eg_{ij} + e_{ij} \quad [3]$$

270 Where  $Eg_{ij}$  is the interaction between the genetic value of the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  environment  
271 and  $\mathbf{Eg} = \{Eg_{ij}\} \sim N(\mathbf{0}, (\mathbf{Z}_g \mathbf{G} \mathbf{Z}_g') \# (\mathbf{Z}_E \mathbf{Z}_E') \sigma_{Eg}^2)$ , where  $\mathbf{Z}_g$  and  $\mathbf{Z}_E$  are the correspondent  
272 incidence matrices for the effects of genetic values of genotypes and environments, respectively,

273  $\sigma_{Eg}^2$  is the variance component of  $Eg$  and # denotes the Hadamard product (element-to-element  
274 product) between two matrices.

275

## 276 **Prediction accuracy assessment using cross-validation**

277 Two distinct cross-validation schemes that mimic prediction problems that breeders may face  
278 when performing genomic prediction were used (Burgueño *et al.* 2012). One random cross-  
279 validation (CV1) evaluates the prediction ability of models when a set of lines have not been  
280 evaluated in any environment (prediction of newly developed lines). In CV1, predictions are  
281 entirely based on phenotypic records of genetically related lines. The second cross-validation  
282 (CV2) is related to incomplete field trials also known as sparse testing, in which some lines are  
283 observed in some environments but not in others. In CV2, the goal is to predict the performance  
284 of lines in environments where they have not yet been observed. Thus, information from related  
285 lines and the correlated environments is used, and prediction assessment can benefit from  
286 borrowing information between lines within an environment, between lines across environments  
287 and among correlated environments.

288 In CV1 and CV2, a fivefold cross-validation scheme was used to generate the training and  
289 validation sets to assess the prediction ability for Zn within the ZAM panel and each DH  
290 population. The data were randomly divided into five subsets, with 80% of the lines assigned to  
291 the training set and 20% assigned to the validation set. Four subsets were combined to form the  
292 training set, and the remaining subset was used as the validation set. Permutation of five subsets  
293 taken one at a time led to five training and validation data sets. The procedure was repeated 20  
294 times and a total of 100 runs were performed in each population. The average value of the  
295 correlations between the phenotype and the genomic estimated breeding values (GEBVs) from

296 100 runs was calculated for the ZAM panel, and each DH population for Zn in each environment  
297 and was defined as the prediction ability ( $r_{MP}$ ).

298

### 299 **Data availability**

300 All models were fitted in R (core Team 2018) using the BGLR package (Pérez and de los  
301 Campos 2014). All phenotypic and genomic data can be downloaded from the link:

302 <http://hdl.handle.net/11529/10548331>

303

## 304 **RESULTS**

### 305 **Descriptive statistics**

306 Mean values of kernel Zn concentration were estimated for each environment and across  
307 environments (Tables 2A and 2B). For the ZAM panel, kernel Zn ranged from 14.76 to 39.80  
308  $\mu\text{g/g}$  in Celaya 2012, 15.16 to 42.52  $\mu\text{g/g}$  and 17.05 to 46.52  $\mu\text{g/g}$  in Agua Fria 2012 and 2013,  
309 respectively (Figure 1). The highest mean (29.53  $\mu\text{g/g}$ ) for Zn was observed in Agua Fria 2013.  
310 DH1 had Zn values ranging from 16.00 to 48.00  $\mu\text{g/g}$  in Celaya 2012, 16.00 to 35.00  $\mu\text{g/g}$  in  
311 Tlaltizapan 2015 and 15.50 to 39.00  $\mu\text{g/g}$  in Tlaltizapan 2017, while the respective values for DH  
312 2 were 17.70 to 43.14  $\mu\text{g/g}$ , 15.60 to 37.80  $\mu\text{g/g}$  and 14.70 to 37.60  $\mu\text{g/g}$  (Figures 2A and 2B).  
313 The highest means for Zn were observed in Celaya 2014 (25.38  $\mu\text{g/g}$ ) and 2017 (27.96  $\mu\text{g/g}$ ) for  
314 DH1 and DH2, respectively (Table 2B). Across environments, heritability ( $H^2/\widehat{h}^2$ ) estimates  
315 were 0.85, 0.83 and 0.76 for the ZAM panel, DH1 and DH2, respectively (Tables 2A and 2B).  
316 There were significant positive correlations between environments for Zn (Table 3), accounting  
317 for the moderate to high heritability estimates.

318 Principal component analysis for the ZAM panel suggested presence of a relatively  
319 diverse set of lines, and 452 principal components (PCs) were needed to explain 80% of the  
320 genotypes' variance (Figures 3A and 3B). The first two principal components explained 3.85%  
321 of the total variance. For the DH populations first two eigenvectors separated them two groups  
322 (DH1 and DH2) and 56 principal components were needed to explain 80% of the genotypes'  
323 variance (Figures 3C and 3D). The first two principal components explained 27.50% of the total  
324 variation for the DH populations.

325

### 326 **Prediction ability in different populations**

327 Cross-validated  $r_{MP}$  values for kernel Zn were estimated for the ZAM panel and DH  
328 populations (Tables 4, 5 and 6). The average  $r_{MP}$  values in CV1 were consistently lower than  
329 those in CV2, suggesting the importance of using information from correlated environments  
330 when predicting performance of inbred lines. The mean  $r_{MP}$  values in CV1 and CV2 for the  
331 ZAM panel were 0.39 and 0.71, respectively (Table 4). For the DH populations, average  $r_{MP}$   
332 values were 0.53 for DH1-CV1, 0.44 for DH2-CV1 (Table 5), 0.70 for DH1-CV2 and 0.51 for  
333 DH2-CV2 (Table 6).

334 In the ZAM panel, the highest values in CV1 (0.47) and CV2 (0.72) were obtained in  
335 Celaya and Agua Fria 2012 (Table 4). For the bi-parental populations, both under CV1 and CV2,  
336 higher  $r_{MP}$  values were observed for DH1 compared to DH2. The highest values in CV1 (0.56)  
337 and CV2 (0.71) were observed in Tlaltizapan 2017 and 2015, all for DH1 (Tables 5 and 6). The  
338 consistently higher  $r_{MP}$  values in CV1 and CV2 of DH1 could be attributed to the higher (0.58 to  
339 0.62) correlation values between environments (Table 3).

340

## 341 Prediction ability of different models

342 Comparing the  $r_{MP}$  values obtained from each model, M1 had the lowest (-0.001, -0.03  
343 and 0.04) accuracies in CV1 for the ZAM panel and DH populations (Tables 4 and 5). Those  
344 values were improved in CV2 because the predictions benefited from previous records (collected  
345 from other environments) of lines whose Zn values were being predicted. When M1 was  
346 expanded to M2 by adding the main effects of markers, the  $r_{MP}$  values at each environment and  
347 across environments were increased. For example, in CV1, M2, >100-fold increase in  $r_{MP}$  values  
348 were observed for the ZAM panel and DH populations, and in CV2, M2, average  $r_{MP}$  values  
349 increased by 2.98%, 2.94% and 11.11% for the ZAM panel, DH1 and DH2, respectively (Tables  
350 4, 5 and 6).

351 The multi-environment model (M3), which includes the interaction between markers  
352 (genomic) and the environment ( $Eg_{ij}$ ) gave higher prediction accuracy than single-environment  
353 models (M1 and M2). In CV1, mean  $r_{MP}$  values increased from 0.37 (M2) to 0.39 (M3) for the  
354 ZAM panel and from 0.43 (M2) to 0.44 for DH2 (Tables 4 and 5). Similar trends were observed  
355 in CV2 for the ZAM panel and DH2 (Tables 4 and 6). However, in both CV1 and CV2 of DH1,  
356 incorporating  $Eg_{ij}$  did not improve  $r_{MP}$  values for Zn (Tables 5 and 6). For CV1, M3,  $r_{MP}$  values  
357 for Zn in individual environments ranged from 0.34 to 0.47 for the ZAM panel (Table 4), 0.51 to  
358 0.55 for DH1 and 0.35 to 0.50 for DH2 (Table 5). For CV2, M3, those values ranged from 0.69  
359 to 0.72 for the ZAM panel, 0.68 to 0.70 for DH1 and 0.43 to 0.56 for DH2 (Tables 4, 5 and 6).

360

361

## DISCUSSION

362 Overall, moderate to high prediction ability values for kernel Zn were observed for the  
363 ZAM panel and DH populations. This could be attributed to the heritabilities observed for kernel  
364 Zn (Tables 2A and 2B). Similar observations were reported for Zn concentration in wheat (Velu  
365 *et al.* 2016; Manickavelu *et al.* 2017). High quality predictions with high accuracy for GS  
366 programs are expected for traits with moderate to higher heritability estimates (Combs and  
367 Bernardo 2013; Lian *et al.* 2014; Muranty *et al.* 2015; Saint Pierre *et al.* 2016; Manickavelu *et*  
368 *al.* 2017; Zhang *et al.* 2017b, 2019; Arojju *et al.* 2019). Consistent with a study on Zn and iron  
369 (Fe) concentration in spring wheat, the prediction accuracies in this study are sufficient to  
370 discard at least 50% of the inbreds with low-Zn concentration (Velu *et al.* 2016).

371 Data from both bi-parental populations and diverse collection of inbreds have been used  
372 for GS and cross-validation (CV) experiments have shown that prediction accuracies could also  
373 be affected by the relatedness between training and prediction sets (Habier *et al.* 2007; de Roos  
374 *et al.* 2009; Asoro *et al.* 2011; Daetwyler *et al.* 2013; Cericola *et al.* 2017; Crossa *et al.* 2017). In  
375 this study, average predicted accuracies were higher for CV1 of the bi-parental populations (0.53  
376 for DH1 and 0.44 for DH2) compared to the ZAM panel (0.39). Higher predicted values in CV1  
377 of the DH populations could be attributed to the closer relationship between DH lines in the  
378 training and prediction sets, maximum linkage disequilibrium (LD) between a marker and a  
379 QTL, and controlled population structure (Bernardo and Yu 2007; Albrecht *et al.* 2011; Zhang *et*  
380 *al.* 2015). In collections of diverse inbreds, prediction accuracy may depend on the ancestral  
381 relationships between the lines. So, in experiments using such collections of lines, prediction  
382 accuracies have been more variable than accuracies achieved using bi-parental populations  
383 (Spindel and McCouch 2016).

384 Cross-validation (CV) schemes are used in genomic prediction to estimate the accuracy  
385 with which predictions for different traits and environments can be made (Burgueño *et al.* 2012;  
386 Zhang *et al.* 2015; Saint Pierre *et al.* 2016; Velu *et al.* 2016; Sukumaran *et al.* 2017a, 2017b;  
387 Monteverde *et al.* 2018; Roorkiwal *et al.* 2018). In this study, two CV schemes (CV1- predicting  
388 the performance of newly developed lines, and CV2- predicting the performance of lines that  
389 have been evaluated in some environments, but not in others) were used. The utility of these  
390 schemes indicated that prediction values for newly developed lines (CV1) were generally lower  
391 (0.39 for the ZAM panel, 0.53 for DH1 and 0.44 for DH2) than the values for lines which have  
392 been evaluated in different but correlated environments (CV2; 0.71, 0.70 and 0.51 for the ZAM  
393 panel, DH1 and DH2, respectively). Such observations indicate the importance of using  
394 information from correlated environments when predicting the performance of inbred lines.  
395 However, selection of new lines without field testing, as simulated in CV1 allows shortening of  
396 the generation interval (cycle time) by replacing the time-intensive phenotypic evaluation for Zn  
397 with genomic-estimated breeding values. But, the quality of prediction accuracy may be lower  
398 such that the annual rate of genetic progress in a GS program is compromised (Burgueño *et al.*  
399 2012). So, the ultimate decision of how a breeding scheme should be structured could depend on  
400 the compromise between the desired prediction accuracy and the generation interval (Burgueño  
401 *et al.* 2012).

402 Genotype by environment interaction is an important factor affecting kernel Zn  
403 concentration in maize and genomic prediction models that incorporate G x E may enhance the  
404 potential of GS for biofortification breeding. For different crop species and traits, genomic  
405 prediction models which incorporated G x E achieved higher prediction accuracies in both CV1  
406 and CV2 schemes relative to models which did not include G x E (Burgueño *et al.* 2012; Guo *et*

407 *al.* 2013; Jarquín *et al.* 2014; Lopez-Cruz *et al.* 2015; Zhang *et al.* 2015; Monteverde *et al.*  
408 2018). In this study, the impact of modeling G x E variance structures for multi-environment  
409 trials was investigated and results indicated that the average predicted values from M3 (G x E  
410 model) were higher (0.39 and 0.44 for CV1 and 0.71 and 0.51 for CV2) than the values from M2  
411 (non-G x E; 0.37 and 0.43 for CV1-M2, 0.69 and 0.50 for CV2-M2) for the ZAM panel and  
412 DH2. These findings agree with those reported on Zn concentration in wheat (Velu *et al.* 2016),  
413 providing evidence that incorporating G x E in GS models can enhance their power and  
414 suitability for improving maize for kernel Zn concentration. Conversely, the average predicted  
415 values for CV1 and CV2 of DH1 were higher in M2 (0.53 and 0.70) than in M3 (0.53 and 0.69).  
416 Except for differences in population size (112 lines vs 143 lines), this was unexpected since DH1  
417 and DH2 were grown in the same environments.

418         The gains in prediction accuracies for the GS model that accounted for G x E were  
419 dependent on the correlation between environments and CV method used. In this study, the  
420 phenotypic correlations between environments were all positive (ranging from 0.58 to 0.62 for  
421 DH1, 0.29 to 0.46 for DH2 and 0.61 to 0.66 for the ZAM panel). Such correlations can be  
422 exploited using multi-environment models to derive predictions that use information from across  
423 both the lines and environments (Burgueño *et al.* 2012). For instance, although the phenotypic  
424 correlations between environments for DH2 were positive (0.29 to 0.46), the lowest average  
425 prediction value (0.51) for CV2 was observed for this population. This was expected because  
426 CV2 uses phenotypic information from genotypes which have already been tested; hence,  
427 effectively exploiting the correlations between environments (Burgueño *et al.* 2012; Jarquín *et*  
428 *al.* 2014; Crossa *et al.* 2015; Pérez-Rodríguez *et al.* 2015; Saint Pierre *et al.* 2016; Monteverde *et*  
429 *al.* 2018). However, for CV1, the information between environments could only be accounted for

430 through the genomic relationship matrix (Monteverde *et al.* 2018). Hence, the gains in CV1 may  
431 likely attribute to more accurate estimate of environment-specific marker effects (Guo *et al.*  
432 2013). In contrast, when multiple environments are weakly correlated, prediction accuracies  
433 from across environment analyses can be negatively affected relative to prediction accuracies  
434 within environments (Bentley *et al.* 2014; Wang *et al.* 2014; Spindel and McCouch 2016). Thus,  
435 before designing a GS experiment, identifying correlated environments where environments can  
436 differ in terms of site, year or season in which data were collected is of great interest (Spindel  
437 and McCouch 2016).

438           The ability to predict kernel Zn concentration using high-throughput SNP markers  
439 including G x E interactions creates an opportunity for efficiently enhancing Zn concentration in  
440 maize breeding programs. For instance, during early generations of a breeding program, GS can  
441 be utilized to identify genotypes with favorable alleles when numbers of progenies and families  
442 are large. This could potentially reduce the resource-intensive evaluation process and  
443 advancement of false-positive progenies (Velu *et al.* 2016). Coupled with advances in  
444 technologies for assessing Zn, plant scientists can more rapidly measure Zn concentration in  
445 maize kernels using the energy dispersive x-ray fluorescence (XRF) assays (Guild *et al.* 2017).  
446 Thus, with more validations and model refinements, GS can potentially accelerate the breeding  
447 process to enhance Zn concentration in maize for a wider range of environments.

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## CONCLUSION

450           The moderate to high prediction accuracies reported in this study shows that GS can be  
451 used in maize breeding to improve kernel Zn concentration. Assuming two possible seasons of  
452 Zn evaluation per year, the predicted genetic gains can be estimated from prediction accuracies

453 and genetic variances of the training populations. The genetic variances for the ZAM panel, DH1  
454 and DH2 were 12.38, 12.20 and 14.88, and prediction accuracies were 0.71, 0.70 and 0.51,  
455 respectively. If the inbreds in each predicted population are ranked based on their predicted Zn  
456 values and the top 10% selected, then their expected average Zn values can be estimated from  
457 the proportion of inbreds selected, their respective training population genetic variances,  
458 prediction accuracies and the time interval for evaluating the lines. With reference to this, the  
459 expected average values of Zn are approximately 31  $\mu\text{g/g}$  for the ZAM panel, 30  $\mu\text{g/g}$  for DH1  
460 and 27  $\mu\text{g/g}$  for DH2. These averages are higher than the averages of the respective training  
461 populations ( $\sim 27 \mu\text{g/g}$  for the ZAM panel,  $\sim 25 \mu\text{g/g}$  for DH1 and  $\sim 26 \mu\text{g/g}$  for DH2) suggesting  
462 that the prediction accuracies achieved are sufficient to select at least 10% of the predicted  
463 inbreds with higher Zn concentration.

464         The prediction accuracies were of lower quality when genomic predictions were  
465 conducted across populations. When the ZAM panel was used as the training population,  
466 prediction accuracies for DH1, DH2 and DH1+DH2 were 0.15, -0.10 and 0.09, respectively.  
467 When DH1 and DH2 were used as a training and prediction set for each other, prediction  
468 accuracies were 0.08 and 0.16 (Unpublished data). These prediction accuracies are considerably  
469 lower than those reported in this study and the differences may be attributed to: (i) weak genetic  
470 relationships between the training and prediction population sets and (ii) different methods of  
471 analysis because the prediction accuracies reported in this study were partly achieved by  
472 modelling the random-effects environment structure to account for G x E while for the  
473 unpublished data, the random-effects environment structure of G x E was not included.

474         This study also showed that higher prediction accuracies can be achieved when some of  
475 the lines are predicted using previous information about them collected from correlated

476 environments. The multi-environment model (M3) which included the interaction between  
477 markers, and the environment gave higher prediction accuracy both in CV1 and CV2 for the  
478 association panel and DH2 compared with the models which only included main effects (M1 and  
479 M2) indicating the importance of accounting for G x E in genomic prediction.

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481

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## TABLES

**Table 1 Pedigree and average concentration of kernel Zn ( $\mu\text{g/g}$ ) concentration for the parents of the DH populations**

DH population	Pedigree	Parent1	Parent2	Zn ( $\mu\text{g/g}$ )	
				Parent1	Parent2
DH1	CML503/CLWN201	CML503	CLWN201	31.21	22.62
DH2	CML 465/CML451	CML465	CML451	31.55	27.88

729 **Table 2 Descriptive statistics for kernel Zn concentration in (A) the ZAM panel and (B) DH**  
 730 **populations grown in each environment, variance components and broad-and narrow sense**  
 731 **heritabilities.**

732 **A**

Population	Population size	Location	Mean $\pm$ se ( $\mu\text{g/g}$ )	$\sigma_G^2$ <sup>a</sup>	$\sigma_{GE}^2$ <sup>a</sup>	$H^2$
ZAM panel	923	Agua Fria 2012	26.15 $\pm$ 0.15	12.04	2.42	0.85
		Celaya 2012	25.06 $\pm$ 0.14			
		Agua Fria 2013	29.53 $\pm$ 0.16			
		Across	<b>26.94 <math>\pm</math> 0.10</b>			

733 **B**

Population	Population size	Location	Mean $\pm$ se ( $\mu\text{g/g}$ )	$\hat{h}^2$
DH1	112	Celaya 2014	25.38 $\pm$ 0.48	0.83
		Tlaltizapan 2015	24.01 $\pm$ 0.38	
		Tlaltizapan 2017	24.53 $\pm$ 0.37	
		Across	<b>24.65 <math>\pm</math> 0.26</b>	
DH2	143	Celaya 2014	27.96 $\pm$ 0.39	0.76
		Tlaltizapan 2015	24.08 $\pm$ 0.33	
		Tlaltizapan 2017	24.64 $\pm$ 0.37	
		Across	<b>25.59 <math>\pm</math> 0.22</b>	

734 Broad-sense heritability  $H^2$  of Zn in each environment and across environments

735 Narrow-sense heritability  $\hat{h}^2$  of Zn across environments

736 <sup>a</sup>variance due to genotypes  $\sigma_G^2$  and the interaction between genotypes and the environment  $\sigma_{GE}^2$  significant

737 at  $P < 0.001$

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741 **Table 3 Phenotypic correlation between environments for kernel Zn**

	DH1	DH 2	ZAM Panel
<sup>a</sup> Env1 vs Env2	0.62	0.46	0.63
<sup>a</sup> Env1 vs Env3	0.58	0.29	0.66
<sup>a</sup> Env2 vs Env3	0.62	0.45	0.61

742 Phenotypic correlation coefficients were significant at  $\alpha = 0.001$

743 <sup>a</sup>DH populations; Env 1, Env2 and Env 3=Celaya,2014, Tlaltizapan, 2017 and Tlaltizapan 2017,

744 respectively.

745 <sup>a</sup>ZAM panel; Env 1, Env2 and Env 3= Agua Fria, 2012, Celaya, 2012 and Agua Fria 2013, respectively.

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747 **Table 4 Correlations (mean  $\pm$  SD) between observed and genomic estimated breeding values for**  
 748 **kernel Zn in the three environments for three GBLUP models for cross-validations CV1 and CV2 of**  
 749 **the ZAM panel**

		Prediction accuracy in CV1		
Population	Environment	M1 <sup>a</sup>	M2	M3
	Agua Fria, 2012	-0.01 $\pm$ 0.04	0.33 $\pm$ 0.01	0.34 $\pm$ 0.02
ZAM panel (923)	Celaya, 2012	0.004 $\pm$ 0.04	0.43 $\pm$ 0.01	0.47 $\pm$ 0.01
	Agua Fria, 2013	-0.001 $\pm$ 0.03	0.34 $\pm$ 0.01	0.35 $\pm$ 0.01
	<b>Average</b>	<b>-0.001 <math>\pm</math> 0.03</b>	<b>0.37 <math>\pm</math> 0.01</b>	<b>0.39 <math>\pm</math> 0.01</b>
		Prediction accuracy in CV2		
Population	Environment	<sup>a</sup> M1	M2	M3
	Agua Fria, 2012	0.71 $\pm$ 0.00	0.71 $\pm$ 0.00	0.72 $\pm$ 0.00
ZAM panel (923)	Celaya, 2012	0.64 $\pm$ 0.00	0.68 $\pm$ 0.00	0.72 $\pm$ 0.00
	Agua Fria, 2013	0.67 $\pm$ 0.00	0.67 $\pm$ 0.00	0.69 $\pm$ 0.01
	<b>Average</b>	<b>0.67 <math>\pm</math> 0.00</b>	<b>0.69 <math>\pm</math> 0.00</b>	<b>0.71 <math>\pm</math> 0.00</b>

750 <sup>a</sup>Models: M1= Environment +Line; M2 = Environment + Line + Genomic; M3 = Environment + Line +

751 Genomic + Genomic  $\times$  Environment

752 **Table 5 Correlations (mean  $\pm$  SD) between observed and genomic estimated breeding values for**  
 753 **Zn in the three environments for three GBLUP models for cross-validation CV1 of DH populations**

Population	Environment	Prediction accuracy in CV1		
		M1 <sup>a</sup>	M2	M3
DH1	Celaya, 2014	-0.05 $\pm$ 0.10	0.52 $\pm$ 0.04	0.51 $\pm$ 0.04
	Tlaltizapan, 2015	-0.02 $\pm$ 0.12	0.52 $\pm$ 0.05	0.51 $\pm$ 0.05
	Tlaltizapan, 2017	-0.01 $\pm$ 0.10	0.56 $\pm$ 0.05	0.55 $\pm$ 0.05
	<b>Average</b>	<b>-0.03 <math>\pm</math> 0.10</b>	<b>0.53 <math>\pm</math> 0.04</b>	<b>0.52 <math>\pm</math> 0.04</b>
DH2	Celaya, 2014	0.05 $\pm$ 0.08	0.47 $\pm$ 0.03	0.50 $\pm$ 0.04
	Tlaltizapan, 2015	0.03 $\pm$ 0.08	0.45 $\pm$ 0.03	0.45 $\pm$ 0.03
	Tlaltizapan, 2017	0.04 $\pm$ 0.08	0.35 $\pm$ 0.03	0.35 $\pm$ 0.04
	<b>Average</b>	<b>0.04 <math>\pm</math> 0.06</b>	<b>0.43 <math>\pm</math> 0.03</b>	<b>0.44 <math>\pm</math> 0.02</b>

754 <sup>a</sup>Models: M1= Environment +Line; M2 = Environment + Line + Genomic; M3 = Environment + Line +  
 755 Genomic + Genomic  $\times$  Environment

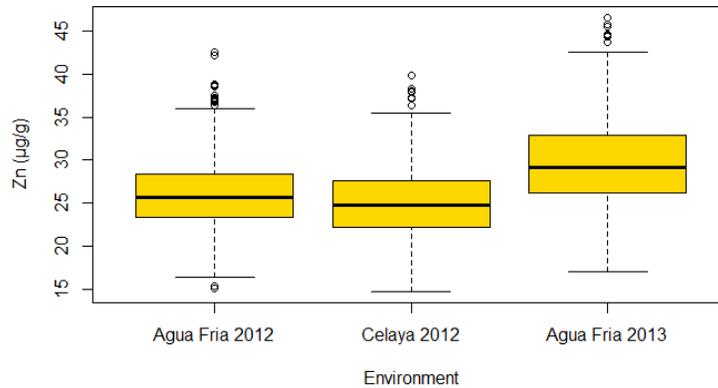
756 **Table 6 Correlations (mean  $\pm$  SD) between observed and genomic estimated breeding values for**  
 757 **Zn in the three environments for three GBLUP models for cross-validation CV2 of DH populations**

Population	Environment	Prediction accuracy in CV2		
		M1 <sup>a</sup>	M2	M3
DH1	Celaya, 2014	0.67 $\pm$ 0.02	0.68 $\pm$ 0.02	0.68 $\pm$ 0.03
	Tlaltizapan, 2015	0.70 $\pm$ 0.02	0.71 $\pm$ 0.02	0.70 $\pm$ 0.02
	Tlaltizapan, 2017	0.67 $\pm$ 0.02	0.70 $\pm$ 0.02	0.69 $\pm$ 0.02
	<b>Average</b>	<b>0.68 <math>\pm</math> 0.01</b>	<b>0.70 <math>\pm</math> 0.01</b>	<b>0.69 <math>\pm</math> 0.01</b>
DH2	Celaya, 2014	0.46 $\pm$ 0.016	0.53 $\pm$ 0.02	0.56 $\pm$ 0.02
	Tlaltizapan, 2015	0.50 $\pm$ 0.020	0.55 $\pm$ 0.02	0.55 $\pm$ 0.02
	Tlaltizapan, 2017	0.40 $\pm$ 0.023	0.43 $\pm$ 0.02	0.43 $\pm$ 0.02
	<b>Average</b>	<b>0.45 <math>\pm</math> 0.02</b>	<b>0.50 <math>\pm</math> 0.01</b>	<b>0.51 <math>\pm</math> 0.01</b>

758 <sup>a</sup>Models: M1= Environment +Line; M2 = Environment + Line + Genomic; M3 = Environment + Line +  
759 Genomic + Genomic × Environment

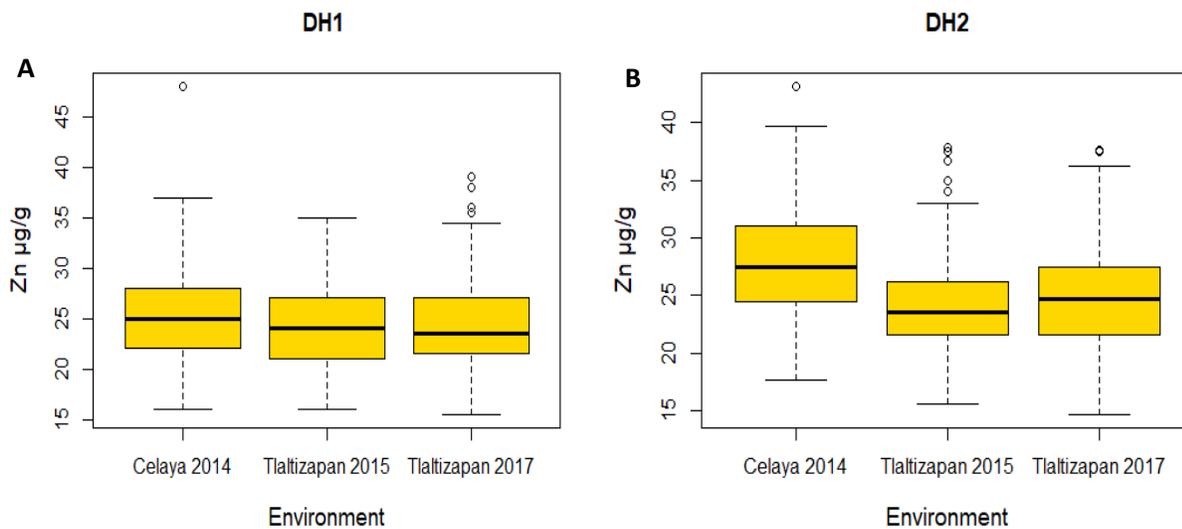
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## FIGURES



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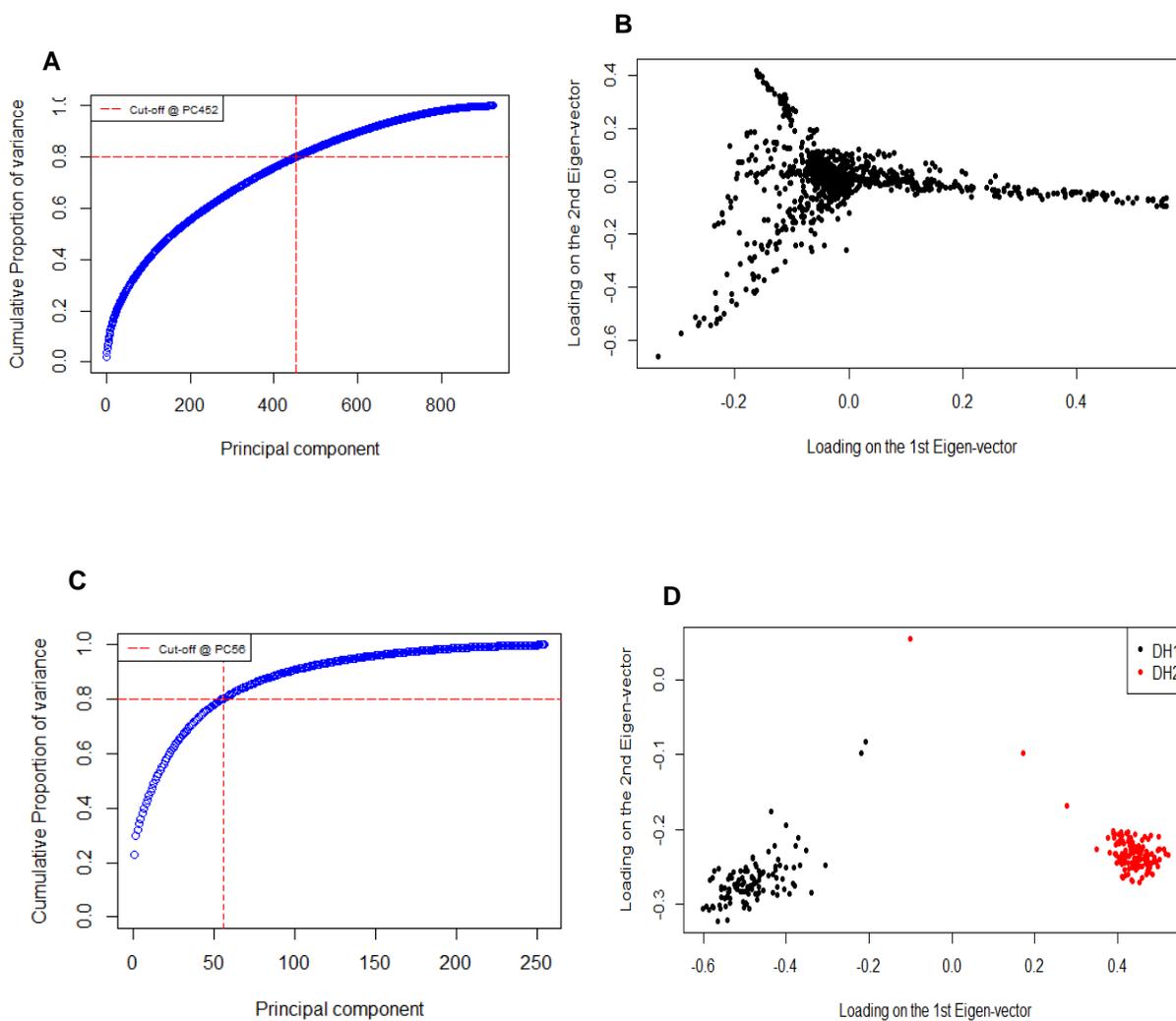
762 **Figure 1** Box plots for kernel Zn (µg/g) in the ZAM panel in three environments (Agua Fria, 2012, Celaya,  
763 2012 and Agua Fria, 2013)



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765 **Figure 2** Box plots for kernel Zn (µg/g) for (A) DH1 and (B) DH2 in three environments (Celaya 2014,  
766 Tlaltizapan, 2015 and Tlaltizapan, 2017)

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770 **Figure 3** Scree plots (*A and C*) and loadings of the first two eigenvectors (*B and D*) of the covariance  
771 matrices derived from markers for the ZAM panel (*A and B*) and for the DH populations (*C and D*)

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