

9 Running title: GWAS in random cross populations.

10 KEYWORDS association mapping; GWAS; linkage disequilibrium; inbred lines panel; RIL.

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14 **ABSTRACT** Genome-wide association studies (GWAS) with plant species have employed inbred
15 lines panels. Our objectives were to present additional quantitative genetics theory for GWAS,
16 evaluate the relative efficiency of GWAS in non-inbred and inbred populations and in an inbred
17 lines panel, and assess factors affecting GWAS. Fifty samples of 400 individuals from populations
18 with linkage disequilibrium were simulated. Individuals were genotyped for 10,000 single
19 nucleotide polymorphisms (SNPs) and phenotyped for traits controlled by 10 quantitative trait loci
20 (QTLs) and 90 minor genes, assuming different degrees of dominance and heritabilities of 40 and
21 80%. The average SNP density was 0.1 centiMorgan and the QTL heritabilities ranged from 3.2 to
22 11.8%. To increase the QTL detection power, the additive-dominance model must be fitted for traits
23 controlled by dominance effects but must not be fitted for traits showing no dominance. The power
24 of detection was maximized increasing the sample size to 400 and the false discovery rate (FDR) to
25 5%. The average power of detection for the low, intermediate, and high heritability QTLs were 9.7,
26 32.7, and 87.7%, respectively. Under sample size of 400 the observed FDR was equal to or lower
27 than the specified level of significance. The association mapping was highly precise. The analysis
28 of the inbred random cross population provided essentially the same results from the non-inbred
29 population. The inbred lines panel provided the best results concerning the low and intermediate
30 heritability QTL detection power, FDR, and mapping precision. The FDR is mainly affected by
31 population structure, compared to relationship information.

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INTRODUCTION

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Association mapping is a high-resolution method for mapping quantitative trait locus (QTL) based on linkage disequilibrium (LD) (Yu and Buckler 2006). Linkage disequilibrium is commonly defined as the non-random association of alleles at two loci carried on the same gamete, caused by their shared history of mutation and recombination (Weir 2008). Association mapping has been successful in detecting genes controlling human diseases and quantitative traits in humans and plant and animal species (Pearson and Manolio 2008; Zhu *et al.* 2008; Barendse *et al.* 2007). There are two main association mapping strategies: the candidate gene approach, which focuses on polymorphisms in specific genes that control the traits of interest, and the genome-wide association study (GWAS), which surveys the entire genome for polymorphisms associated with complex traits (Rafalski 2010).

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With the advent of high-throughput genotyping and sequencing technologies, breeders have used GWAS to identify genes underlying quantitative trait variation. Compared to QTL mapping, which has precision in the range of 10^5 to 10^7 base pairs (Yu and Buckley 2006), the main advantage of GWAS is a more precise identification of candidate genes (Zhu *et al.* 2008). Another advantage is the use of a breeding population instead of one derived by crossing two inbred or pure lines (Flint-Garcia *et al.* 2005). However, as highlighted by Weir (2010), the efficiency of GWAS is considerably affected by relatedness and population structure, which can generate spurious association between unlinked marker and QTL. Rafalski (2010) emphasized that the choices of population (due to the degree of LD and genotypic variation), marker density, and sample size are crucial decisions for achieving greater power of QTL detection. Ingvarsson and Street (2011) discussed the influence of population size, extent of LD, trait heritability (precision of phenotyping), and population structure on GWAS efficiency, highlighting that studies with plant species should greatly increase population size to detect QTLs with lower effect (heritability of 1–2%).

57 Yu *et al.* (2006) proposed a mixed model approach for GWAS analysis called the Q + K (or
58 QK) method, where Q and K are the population structure and kinship matrices, respectively. This
59 method has provided the best results and greatly has improved the control of both type I and type II
60 error rates compared with other methods. Stich and Melchinger (2009) and Yang *et al.* (2010)
61 compared GWAS methods based on simulated and field data. Based on type I error control and
62 power of QTL detection, they concluded that the mixed model approach using only the kinship
63 matrix (K model) to correct for relatedness was more efficient than the approaches controlling only
64 population structure (Q model) and both population structure and relatedness because the spurious
65 associations could not be completely controlled by population structure. Based on simulated inbred
66 lines panel, Bernardo (2013) demonstrated that his models G and QG were superior to the K and
67 QK, respectively, where G indicates a model that uses genome-wide markers to account for QTLs
68 on background chromosomes. The new approach showed a better balance between power of QTL
69 detection and false discovery rate (FDR). Frąszczak and Szyda (2016) also compared GWAS
70 methods. The genomic selection model was the best method compared to the single SNP, the single
71 SNP with a random polygenic effect (K model), and the CAR score regression.

72 Recently, many instances of GWAS have been published with plant species, including barley,
73 sorghum, wheat, rice, sugarcane, soybean and particularly maize (Ingvarsson and Street 2011).
74 From the analysis of 271 inbreds genotyped for 28,626 single nucleotide polymorphisms (SNPs),
75 Bernardo and Thompson (2016) calculated chromosomal effects from the effects of SNP alleles
76 carried on the chromosomes. Many chromosome-inbred combinations showed large chromosome x
77 inbred effects. Pace *et al.* (2015) carried out a GWAS with 384 maize inbred lines evaluated for 22
78 seedling root architecture traits and genotyped with 681,257 SNPs. They identified 268 marker-trait
79 associations. Some of these SNPs were located within or near (less than one kilo base pairs) to
80 candidate genes involved in root development at the seedling stage. Thirunavukkarasu *et al.* (2014)
81 evaluated 240 elite inbred lines of subtropical maize under water stress and used a set of 29,619

82 high-quality SNPs. The GWAS identified 50 SNPs consistently associated with agronomic traits
83 related to functional traits that could lead to drought tolerance. Thirty-one of the significant SNPs
84 were situated near drought-tolerance genes. Schaefer and Bernardo (2013) used GWAS on a
85 collection of 284 historical maize inbred lines and 39,166 SNPs and identified 19 QTLs for
86 flowering time, 13 for kernel composition, and 22 for disease resistance. However, only two
87 candidate genes were suggested: one regulating days to anthesis and one regulating oil
88 concentration.

89 Genome-wide association studies with plant species have employed inbred lines panels. Thus,
90 to our knowledge, no information is available on efficiency of GWAS in open-pollinated
91 populations. Because association mapping has been primarily developed for mapping human genes,
92 the available quantitative genetics theory, as that presented by Weir (2008), based on LD and
93 analysis of case-control, is adequate for non-inbred populations. However, there is a lack of
94 quantitative genetics theory for GWAS in inbred populations and inbred lines panels. In this paper
95 we also evidenced the importance of fitting the additive-dominance model for traits showing
96 unidirectional or bidirectional dominance and the additive model when there is no dominance, to
97 achieve high power of QTL detection. We also highlighted the power of QTL detection, the false
98 discovery rate (FDR), and the precision of GWAS. In summary, our objectives were to present
99 additional quantitative genetics theory for GWAS, evaluate the relative efficiency of GWAS in non-
100 inbred and inbred populations and in an inbred lines panel, and assess factors affecting GWAS,
101 such as sample size and QTL heritability, effect of substitution, and dominance deviation.
102 Importantly, the results for open-pollinated populations are directly applied to human populations.

103 **MATERIALS AND METHODS**

104 The following theory aims to prove that a significant association between a SNP and a
105 quantitative trait in an open-pollinated population, in a sample of recombinant inbred lines (RILs),
106 and in an inbred lines panel, after correcting for population structure, depends on the LD between

107 the SNP and at least one of the QTLs that affect the trait. Further, we present the parametric values
 108 of the average effect of a SNP substitution and LD measure in these association mapping
 109 populations, increasing the quantitative genetics knowledge on GWAS previously provided by
 110 human geneticists in the context of random population samples, case-control, and family-based
 111 studies.

112 **Quantitative genetics theory for GWAS in random cross populations**

113 Consider a biallelic QTL (alleles **B/b**) and a SNP (alleles **C/c**) located in the same
 114 chromosome, and a population (generation 0) of a random cross species. Assuming LD, the joint
 115 gamete and joint genotype probabilities in the population are presented by Weir (2008). The QTL
 116 genotypic values are $G_{\mathbf{BB}} = m_b + a_b$, $G_{\mathbf{Bb}} = m_b + d_b$, and $G_{\mathbf{bb}} = m_b - a_b$, where m_b is the
 117 mean of the genotypic values of the homozygotes, a_b is the deviation between the genotypic value
 118 of the homozygote of higher expression and m_b , and d_b is the dominance deviation (the deviation
 119 between the genotypic value of the heterozygote and m_b). The average genotypic values of
 120 individuals with the SNP genotypes **CC**, **Cc**, and **cc** are

$$121 \quad G_{\mathbf{CC}} = \frac{1}{p_c} \left(f_{22} G_{\mathbf{BBCC}} + f_{12} G_{\mathbf{BbCC}} + f_{02} G_{\mathbf{bbCC}} \right)$$

$$= M + 2q_c \kappa_{bc} \alpha_b + \left(-2q_c^2 \kappa_{bc}^2 d_b \right) = M + 2\alpha_{\mathbf{C}} + D_{\mathbf{CC}} = M + A_{\mathbf{CC}} + D_{\mathbf{CC}} = m_c + a_c$$

$$122 \quad G_{\mathbf{Cc}} = \frac{1}{2p_c q_c} \left(f_{21} G_{\mathbf{BBCc}} + f_{11} G_{\mathbf{BbCc}} + f_{01} G_{\mathbf{bbCc}} \right)$$

$$= M + (q_c - p_c) \kappa_{bc} \alpha_b + 2p_c q_c \kappa_{bc}^2 d_b = M + (\alpha_{\mathbf{C}} + \alpha_{\mathbf{c}}) + D_{\mathbf{Cc}} = M + A_{\mathbf{Cc}} + D_{\mathbf{Cc}} = m_c + d_c$$

$$123 \quad G_{\mathbf{cc}} = \frac{1}{q_c} \left(f_{20} G_{\mathbf{BBcc}} + f_{10} G_{\mathbf{Bbcc}} + f_{00} G_{\mathbf{bbcc}} \right)$$

$$= M + \left(-2p_c \kappa_{bc} \alpha_b \right) + \left(-2p_c^2 \kappa_{bc}^2 d_b \right) = M + 2\alpha_{\mathbf{c}} + D_{\mathbf{cc}} = M + A_{\mathbf{cc}} + D_{\mathbf{cc}} = m_c - a_c$$

124 where p is the frequency of the major allele (**B** or **C**), $q = 1 - p$ is the frequency of the minor allele
 125 (**b** or **c**), f_{ij} is the probability of the individual with i and j copies of the allele **B** of the QTL and the
 126 allele **C** of the SNP ($i, j = 2, 1$, or 0) (for simplicity, we omitted the superscript (0) - for generation 0
 127 - in all parameters that depend on the LD measure of generation -1),
 128 $M = m_b + (p_b - q_b)a_b + 2p_bq_b d_b$ is the population mean, $\kappa_{bc} = \left[\frac{\Delta_{bc}^{(-1)}}{p_c q_c} \right]$,
 129 $\alpha_b = a_b + (q_b - p_b)d_b$ is the average effect of a gene substitution, $\alpha_C = q_c \kappa_{bc} \alpha_b$ and
 130 $\alpha_c = -p_c \kappa_{bc} \alpha_b$ are the average effects of the SNP alleles, and A and D are the SNP additive and
 131 dominance values. $\Delta_{bc}^{(-1)} = P_{\mathbf{BC}}^{(-1)} P_{\mathbf{bc}}^{(-1)} - P_{\mathbf{Bc}}^{(-1)} P_{\mathbf{bC}}^{(-1)}$ is the measure of LD in the gametic pool of
 132 generation -1 (Kempthorne 1957), where $P^{(-1)}$ indicates a joint gamete probability. Another
 133 common measure of LD is the square of the correlation between the values of the alleles at the two
 134 loci ($r_{bc}^{(-1)}$) in the gametic pool of generation -1 (Hill and Robertson 1968). Note that
 135 $\Delta_{bc}^{(-1)} = r_{bc}^{(-1)} \sqrt{p_b q_b p_c q_c}$. The average effect of substituting the allele **C** for **c** is
 136 $\alpha_{\text{SNP}} = \alpha_C - \alpha_c = \kappa_{bc} \alpha_b$. The dominance deviation for the SNP is $d_{\text{SNP}} = \kappa_{bc}^2 d_b$. The other
 137 SNP parameters are $m_c = M + (q_c - p_c) \alpha_{\text{SNP}} - (1 - 2p_c q_c) d_{\text{SNP}}$, $a_c = \alpha_{\text{SNP}} - (q_c - p_c) d_{\text{SNP}}$,
 138 and $d_c = d_{\text{SNP}}$. The GWAS and genomic selection models commonly fit the SNP parameters a
 139 (values 1, 0, and -1 for SNP genotypes **CC**, **Cc**, and **cc**, respectively) and d (values 0, 1, and 0 for
 140 SNP genotypes **CC**, **Cc**, and **cc**, respectively).

141 Assuming no QTL in LD with the SNP ($\Delta_{bc}^{(-1)} = 0$), $G_{\mathbf{CC}} = G_{\mathbf{Cc}} = G_{\mathbf{cc}} = M$. Thus, the
 142 identification of the QTL can be based on testing the hypothesis that there is no difference between
 143 these genotypic means. Assuming thousands of SNPs, it is necessary to employ a Bonferroni-type

144 procedure to control the type I error when there are multiple-comparisons, as that proposed by
 145 Benjamini and Hochberg (1995). Note that $\alpha_{\text{SNP}} = a_c + (q_c - p_c)d_{\text{SNP}}$, where $a_c = G_{\text{CC}} - m_c$,
 146 $d_{\text{SNP}} = G_{\text{CC}} - m_c$, and $m_c = (G_{\text{CC}} + G_{\text{cc}})/2$.

147 **Quantitative genetics theory for GWAS with inbred lines panel**

148 In general, the inbred lines in a panel represent the genetic variability for the traits being
 149 assessed. Therefore, an inbred lines panel includes inbreds from distinct populations or heterotic
 150 groups. Consider again a QTL (alleles **B/b**) and a SNP (alleles **C/c**) located in the same
 151 chromosome, and that they are in LD in a population (generation 0). Assuming n ($n \rightarrow \infty$)
 152 generations of selfing, the (limits of the) probabilities of the inbreds are (for simplicity, we omitted
 153 again the superscript (0) - for generation 0 - in all parameters that depend on the LD measure of
 154 generation -1)

$$155 \quad f_{22}^{(n)} = f_{22} + \frac{1}{2}(f_{21} + f_{12}) + \frac{1}{4}f_{11} + \frac{1}{2}\left(\frac{1 - 2\theta_{bc}}{1 + 2\theta_{bc}}\right)\Delta_{bc}^{(-1)}$$

$$156 \quad f_{20}^{(n)} = f_{20} + \frac{1}{2}(f_{21} + f_{10}) + \frac{1}{4}f_{11} - \frac{1}{2}\left(\frac{1 - 2\theta_{bc}}{1 + 2\theta_{bc}}\right)\Delta_{bc}^{(-1)}$$

$$157 \quad f_{02}^{(n)} = f_{02} + \frac{1}{2}(f_{01} + f_{12}) + \frac{1}{4}f_{11} - \frac{1}{2}\left(\frac{1 - 2\theta_{bc}}{1 + 2\theta_{bc}}\right)\Delta_{bc}^{(-1)}$$

$$158 \quad f_{00}^{(n)} = f_{00} + \frac{1}{2}(f_{01} + f_{10}) + \frac{1}{4}f_{11} + \frac{1}{2}\left(\frac{1 - 2\theta_{bc}}{1 + 2\theta_{bc}}\right)\Delta_{bc}^{(-1)}$$

159 where θ_{bc} is the frequency of recombinant gametes. The haplotypes are $P_{\mathbf{BC}}^{(n)} = p_b p_c + \Delta_{bc}^{(n)}$,

$$160 \quad P_{\mathbf{Bc}}^{(n)} = p_b q_c - \Delta_{bc}^{(n)}, \quad P_{\mathbf{bC}}^{(n)} = q_b p_c - \Delta_{bc}^{(n)}, \quad \text{and} \quad P_{\mathbf{bc}}^{(n)} = q_b q_c + \Delta_{bc}^{(n)}, \quad \text{where}$$

161 $\Delta_{bc}^{(n)} = \left(\frac{1}{1 + 2\theta_{bc}}\right)\Delta_{bc}^{(-1)}$. Thus, if there is crossing over ($0 < \theta_{bc} \leq 0.5$), the LD in this inbred

162 population is lower than the LD in generation -1. If the SNP and QTL are completely linked (θ_{bc}

163 = 0), the LD in the inbred population is the same LD in generation -1 . The maximum decrease is
 164 50%, achieved with $\theta_{bc} = 0.5$. Compared with the LD in generation 0, the LD in generation n is

165 $\Delta_{bc}^{(n)} = \left[\frac{1}{(1+2\theta_{bc})(1-\theta_{bc})} \right] \Delta_{bc}^{(0)}$. Thus, the maximum decrease is approximately 10%, achieved

166 with $\theta_{bc} = 0.25$. In contrast, after n generations of random crosses

167 $\Delta_{bc}^{(n)} = (1-\theta_{bc})^{n+1} \Delta_{bc}^{(-1)} = (1-\theta_{bc})^n \Delta_{bc}^{(0)}$. Thus, if $0 < \theta_{bc} \leq 0.5$, the maximum decrease is 100%

168 since $\lim_{n \rightarrow \infty} \Delta_{bc}^{(n)} = 0$.

169 If the panel includes double haploid (DH) lines, the LD for the DH lines sampled from a
 170 population is $\Delta_{bc} = \Delta_{bc}^{(0)} = (1-\theta_{bc}) \Delta_{bc}^{(-1)}$. Thus, the LD in a sample of DH lines is greater than the
 171 LD in a sample of inbred lines (up to 12.5% greater when $\theta_{bc} = 0.25$).

172 For the inbreds sampled from a population, we have

173
$$G_{CC}^{(n)} = \frac{1}{f_{.2}^{(n)}} \left[f_{22}^{(n)}(m_b + a_b) + f_{02}^{(n)}(m_b - a_b) \right] = M_{IL} + 2q_c \alpha_{SNP}^{(n)} = M_{IL} + A_{CC}^{(n)}$$

174
$$G_{cc}^{(n)} = \frac{1}{f_{.0}^{(n)}} \left[f_{20}^{(n)}(m_b + a_b) + f_{00}^{(n)}(m_b - a_b) \right] = M_{IL} - 2p_c \alpha_{SNP}^{(n)} = M_{IL} + A_{cc}^{(n)}$$

175 where $M_{IL} = m_b + (p_b - q_b)a_b$ is the inbred population mean, $\alpha_{SNP}^{(n)} = \left(\frac{1}{1+2\theta_{bc}} \right) \alpha_{SNP}$ is the

176 SNP average effect of allele substitution in the inbred population, and A is the SNP additive value

177 for an inbred line. Assuming no QTL in LD with the SNP, $G_{CC}^{(n)} = G_{cc}^{(n)} = M_{IL}$. Notice that

178 $\alpha_{SNP}^{(n)} = \left(G_{CC}^{(n)} - G_{cc}^{(n)} \right) / 2$. In the case of DH lines, $\alpha_{SNP}^* = (1-\theta_{bc}) \alpha_{SNP}$. Thus, assuming

179 $\theta_{bc} = 0$, the SNP average effect of substitution is approximately the same in a non-inbred and in an

180 inbred population (RILs or DH lines).

181 The haplotypes of an inbred lines panel including inbreds from N populations are

$$182 \quad P_{\mathbf{BC}}^{(n)'} = \bar{p}_b \bar{p}_c + \Delta_{bc}^{(n)'}, \quad P_{\mathbf{Bc}}^{(n)'} = \bar{p}_b \bar{q}_c - \Delta_{bc}^{(n)'}, \quad P_{\mathbf{bC}}^{(n)'} = \bar{q}_b \bar{p}_c - \Delta_{bc}^{(n)'}, \quad \text{and} \quad P_{\mathbf{bc}}^{(n)'} = \bar{q}_b \bar{q}_c + \Delta_{bc}^{(n)'},$$

$$183 \quad \text{where } \Delta_{bc}^{(n)'} = \sum_{i=1}^N u_i \left[\Delta_{bc_i}^{(n)} + p_{b_i} p_{c_i} \right] - \left(\sum_{i=1}^N u_i p_{b_i} \right) \left(\sum_{i=1}^N u_i p_{c_i} \right) = \bar{\Delta}_{bc}^{(n)} + \overline{p_b p_c} - \bar{p}_b \bar{p}_c \text{ and } u_i \text{ is the}$$

184 probability of an inbred line belonging to population i. Our simulated data showed that the LD

185 value in an inbred lines panel tends to be lower than the LD in each group of inbreds (that are lower

186 than the LD in the base populations) because it is an admixture of positive and negative LD values.

187 **Simulation**

188 We simulated 50 samples of populations with LD using the software *REALbreeding* (Viana *et*

189 *al.* 2017, 2016, 2013; Azevedo *et al.* 2015). This software has been developed by the first author

190 using the program *REALbasic 2009*. Population 1, generation 0, is a composite of two populations

191 in linkage equilibrium. Population 1, generations 10s and 10r10s, were obtained from Population 1,

192 generation 0, assuming 10 generations of selfing and 10 generations of random crosses followed by

193 10 generations of selfing, respectively, assuming sample sizes of 100 and 400, respectively.

194 Populations 2, 3, and 4, generation 10s, are also inbred populations (10 generations of selfing)

195 derived from composites of two populations, also assuming a sample size of 100. The parents of

196 populations 2 and 3 were assumed to be non-improved and improved populations, respectively. An

197 improved population was defined as having frequencies of favorable genes greater than 0.5, while a

198 non-improved population was defined as having frequencies less than 0.5. A composite is a Hardy-

199 Weinberg equilibrium population with LD for only linked markers and genes. In the case of a

$$200 \quad \text{composite of two populations in linkage equilibrium, } \Delta_{bc}^{(-1)} = \left(\frac{1 - 2\theta_{bc}}{4} \right) \left(p_b^1 - p_b^2 \right) \left(p_c^1 - p_c^2 \right),$$

201 where the indices 1 and 2 refer to the parental populations. Under random crosses, we instructed

202 *REALbreeding* to generate two descendents by plant (one as male and one as female) and to allow

203 selfing. Under selfing, *REALbreeding* used the single seed descent process. Thus, the individuals in

204 generations 0 and the derived inbred lines are non-related and the individuals in generation 10r10s
205 can be related.

206 Based on our input, *REALbreeding* randomly distributed 10,000 SNPs, 10 QTLs and 90 minor
207 genes (QTLs of lower effect) in 10 chromosomes (1,000 SNPs and 10 genes by chromosome). The
208 average SNP density was 0.1 cM. The genes were distributed in the regions covered by the SNPs.
209 Four, three, two, and one QTLs were inserted in chromosomes 1, 5, 9, and 10, respectively. We also
210 specified one SNP within each QTL (with same frequency) and a minimum distance between linked
211 QTLs of 10 cM. To allow *REALbreeding* to compute the phenotypic value for each genotyped
212 individual, we informed the minimum and maximum genotypic values for homozygotes, proportion
213 between the parameter a for a QTL and the parameter a for a minor gene ($a_{\text{QTL}}/a_{\text{mg}}$), degree of
214 dominance ($(d/a)_i$, $i = 1, \dots, 100$), direction of dominance, and broad sense heritability.
215 *REALbreeding* saves two main files, one with the marker genotypes and another with the additive,
216 dominance, and phenotypic values (non-inbred populations) or the genotypic and phenotypic values
217 (inbred populations). The true additive and dominance genetic values or genotypic values are
218 computed from the population gene frequencies (random values), LD values, average effects of
219 gene substitution or a deviations, and dominance deviations. The phenotypic values are computed
220 from the true population mean, additive and dominance values or genotypic values, and from error
221 effects sampled from a normal distribution. The error variance is computed from the broad sense
222 heritability.

223 We simulated three popcorn traits. The minimum and maximum genotypic values of
224 homozygotes for grain yield, expansion volume, and days to maturity were 30 and 180 g per plant,
225 15 and 65 mL/g, and 100 and 170 days, respectively. We defined positive dominance for grain yield
226 ($0 < (d/a)_i \leq 1.2$), bidirectional dominance for expansion volume ($-1.2 \leq (d/a)_i \leq 1.2$), and no
227 dominance for days to maturity ($(d/a)_i = 0$). The broad sense heritabilities were 40 and 80%. These
228 values can be associated with individual and progeny assessment, respectively. Assuming $a_{\text{QTL}}/a_{\text{mg}}$

229 = 10, the QTL heritabilities ranged from 3.2 to 11.8%. The GWAS was performed in population 1,
230 generations 0 and 10r10s, and in the inbred lines panel obtained from inbreds of the populations 1
231 through 4, generation 0 (generations 10s). To assess the influence of the sample size on the GWAS
232 efficiency, we considered sample sizes of 400 and 200. Thus, we used 100 or 50 inbreds from
233 populations 1 through 4 to generate the inbred lines panel.

234 Statistical analyses

235 The analysis of the Q + K linear mixed model was performed with the software *GWASpoly*
236 (Rosyara *et al.* 2016) fitting the additive and additive-dominance models for the open-pollinated
237 population and the additive model for the RILs and inbred lines panel. For the population structure
238 analysis, we used *Structure* software (Falush *et al.* 2003) and fitted the admixture model with
239 correlated allelic frequencies and the no admixture model with independent allelic frequencies. The
240 number of SNPs, sample size, burn-in period, and number of MCMC (Markov chain Monte Carlo)
241 replications were 100 (10 random SNPs by chromosome), 400 (simulation 1), 10,000, and 40,000,
242 respectively. The number of populations assumed (K) ranged from 1 to 7, and the most probable K
243 value was determined based on the inferred plateau method (Viana *et al.* 2013). The population
244 structure analysis evidenced four subpopulations (data not shown).

245 To classify each significant association as true or false, we used a program developed in
246 *REALbasic 2009* by the first author. The classification criterion was based on the difference
247 between the position of the SNP and the position of a true QTL (candidate gene). If the difference
248 was less than or equal to 2.5 cM (Yu *et al.* 2008), the association was classified as true. The GWAS
249 efficiency was assessed based on the power of QTL detection (probability of rejecting H_0 when H_0
250 is false; control of the type II error), FDR (control of the type I error), and bias in the estimated
251 QTL position (precision of mapping) (Li *et al.* 2010). We used Benjamini-Hochberg FDR of 5 and
252 1% to control the type I error (Benjamini and Hochberg 1995).

253 **Data availability**

254 *REALbreeding* is available upon request. The data set is available at
255 <https://dx.doi.org/10.6084/m9.figshare.3201838.version3>. Supplemental file S1 contains detailed
256 description of all data files (SNP and QTL positions, SNP genotypes, and phenotypic values). Data
257 citation:

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259 Carla Bastos; Fonseca e Silva, Fabyano (2017): Efficiency of genome-wide association study in
260 random cross populations. figshare. <https://dx.doi.org/10.6084/m9.figshare.3201838.version3>

261 **RESULTS**

262 Our first result from the open-pollinated population, assuming sample size of 400 and FDR of
263 1%, was disappointing since most grain yield QTLs of high heritability showed low power of
264 detection. For example, the power of detection for the QTLs with heritabilities of 8.4, 9.4, and
265 11.6% were 4.2, 8.3, and 10.4%, respectively (Figure 1a). We realized that the problem was the
266 high dominance deviation for these QTLs (the greatest values among the 10 QTLs). This explained
267 the relatively low coefficients of determination for the linear regression models relating QTL
268 detection power and heritability, especially with sample size of 200 and FDR of 5% (45, 55, and
269 19%) (Figure 1a, c, and Figure 2a). The solution to this problem was to fit the additive-dominance
270 model for grain yield and expansion volume. Regardless of the sample size and FDR, for 76% of
271 the grain yield and expansion volume QTLs the detection power was increased (Figures 1b, d, and
272 Figure 2b). Previously undetected QTLs showed detection power ranging from 2.3 to 52.3%. The
273 increase in the detection power for previously detected QTLs ranged from 0.8 to 2,300% (244.6%
274 on average), mainly with sample size of 400 and FDR of 1%. A consequence of fitting the additive-
275 dominance model for grain yield and expansion volume was an increase in the coefficients of
276 determination for the linear regression models relating QTL detection power and heritability,
277 especially with lower sample size (R^2 of 79, 81, and 46%) (Figure 1b, d, and Figure 2b). Unlike, the
278 additive-dominance model should not be fitted for traits showing no dominance. Fitting the

279 additive-dominance model for days to maturity, for 82% of the QTLs the detection power decreased
280 from 6.6 to 100.0% (48.4% on average), regardless of sample size and FDR.

281 Defining low, intermediate, and high heritability QTLs as those with heritability values less
282 than or equal to 3.5%, between 3.6 and 7.5%, and greater than 7.5%, respectively, the power of
283 detection was maximized increasing the sample size from 200 to 400 and the FDR from 1% to 5%
284 (Table 1). It is important to highlight that under sample size of 400 the observed FDR was equal to
285 or lower than the specified level of significance. Assuming sample size of 400 and FDR of 5%, the
286 average power of detection for the low, intermediate, and high heritability QTLs were 9.7, 32.7, and
287 87.7%, respectively. The minimum and maximum values were 2 and 29.5%, 5.5 and 94%, and 62.0
288 and 100.0%. The observed FDR was 3.8%. Decreasing the sample size to 200 decreased the QTL
289 detection power (28, 67 and 62% for the low, intermediate and high heritability QTLs, respectively)
290 and increased the observed FDR to 9.4%. Concerning the bias in the QTL (candidate gene) position,
291 it should be also highlighted that at least 97% (assuming sample size of 200 and FDR of 5%) of the
292 QTLs were declared by the SNP within it, and that the number of significant SNPs within the range
293 of 2.5 cM was very low, regardless of sample size and FDR. The average number of significant
294 SNPs within the range of 2.5 cM varied from 0.1 to 1.0. The average bias in the QTL position from
295 a significant SNP within the range of 2.5 cM varied from approximately 0.2 to 0.4 cM.
296 Furthermore, we also observed that the QTL detection power has low correlation with the QTL
297 average effect of substitution (0.1) and QTL dominance deviation (0.2). The correlation between
298 QTL detection power and QTL heritability ranged from 0.68 to 0.90, proportional to the sample
299 size.

300 The analysis of the RILs from the open-pollinated population provided essentially the same
301 results (similar magnitude of the statistics) concerning QTL detection power, control of the type I
302 error, and mapping precision (Figure 2c, d, and Table 1). The only significant difference was the
303 absence of low heritability QTLs. We can highlight a slightly better control of the type I error also.

304 Thus, the QTL detection power was also maximized assuming sample size of 400 and FDR of 5%
305 and the observed FDR was 1.8% in this scenario. The decrease in the sample size significantly
306 decreased the power of QTL detection (54 and 65% for the intermediate and high heritability QTLs,
307 respectively) and increased the FDR (to 7.3%) too. Regarding of the inbred lines panel, it is
308 impressive to realize that this population provided an improvement in the outstanding results
309 offered by the non-inbred and inbred open-pollinated populations, concerning low and intermediate
310 heritability QTL detection power, control of the type I error, and mapping precision (Figure 3a, b,
311 and Table 1). The increase in the low heritability QTL detection power ranged from 74 to 177%.
312 For the intermediate heritability QTLs the increase ranged from 39 to 64%. The observed FDR was
313 reduced in up to 72% and the bias in the QTL position decreased between 80 and 87%. The QTL
314 detection power was also maximized assuming sample size of 400 and FDR of 5%, combined with
315 an observed FDR of 2.7%. Similarly to non-inbred and inbred open-pollinated populations, the
316 decrease in the sample size significantly decreased the power of QTL detection (54, 61 and 61% for
317 the low, intermediate, and high heritability QTLs, respectively) but the FDR was unaffected.

318 Finally, it is important to highlight that the FDR is mainly affected by population structure,
319 compared to relationship information. Assuming non-inbred population, sample size of 400 and
320 FDR of 1%, ignoring the relationship information (by ignoring the polygenic effect), the observed
321 FDR was unaffected (1.1 vs. 1.4%) but the number of significant associations outside the 2.5 cM
322 interval was drastically increased (from practically zero to 28; Table 1). As will be discussed
323 further, these are not all false-positive associations but due to LD between the SNPs and one or
324 more QTLs in the chromosome. With RILs, because the level of LD is lower than in the non-inbred
325 population, the observed FDR was 1.2% but the number of significant associations outside the 2.5
326 cM interval achieved approximately 7. For inbred lines panel, ignoring only the relationship
327 information the FDR was 0.8% but ignoring only population structure increased the FDR to 29.5%
328 Ignoring the relationship information and population structure in the analysis of the inbred lines

329 panel determined thousands of significant associations in all chromosomes, increasing drastically
330 the FDR (to approximately 60%; data not shown). In these scenarios it was not possible to detect
331 QTLs because many significant associations were observed along the length of a chromosome or in
332 one or more large chromosome regions, especially for chromosomes 1 (four QTLs) and 5 (three
333 QTLs) (data not shown).

334 **DISCUSSION**

335 The presented theory proves that a significant association from a GWAS in a non-inbred or
336 inbred random cross population and in an inbred lines panel, correcting for population structure and
337 relatedness, is due to LD between the SNP and one or more linked QTLs. The theory also shows
338 that GWAS provides estimation of the average effect of a SNP substitution (and consequently the
339 estimation of SNP effects). Using SNP effects to measure chromosome and chromosome x inbred
340 effects, Bernardo and Thompson (2016) showed that GWAS also provide dissection of the
341 germplasm architecture for quantitative traits. Schaefer and Bernardo (2013) estimated SNP effects
342 and identified candidate genes and QTL hot spots (chromosome regions with previously mapped
343 QTLs) for days to flowering, kernel composition, and disease resistance. Based on the theory
344 presented, only if there is a single QTL in LD with a significant SNP, if the SNP is within the QTL,
345 and if QTL and SNP alleles have the same frequency it is adequate to consider the SNP average
346 effect of substitution as the QTL average effect of substitution. We additionally provided the
347 parametric values of SNP effects commonly fitted in the GWAS and genomic selection models, and
348 the genotype and gametic probabilities and the parametric LD values in a completely inbred
349 population and in an inbred lines panel. The LD in a group of RILs are lower than the LD in the
350 non-inbred population and the LD value in an inbred lines panel tends to be lower than the LD in
351 each group of inbreds (that are lower than the LD in the base populations) because it is an
352 admixture of positive and negative LD values.

353 To our knowledge, this is the first study on GWAS efficiency in open-pollinated population.
354 The results are impressive and show that the identification of candidate genes can be highly
355 efficient, depending on sample size and QTL heritability. LD is for sure another important factor
356 affecting GWAS (Weir 2008). Thus, based on a sample of 400 individuals and defining a level of
357 significance of 5%, the power of detection of low ($\leq 3.5\%$), intermediate (3.6-7.5%), and high (\geq
358 7.6) heritability QTLs can achieve approximately 30, 90, and 100%, respectively (10, 30, and 90%
359 on average). This result is achieved keeping the FDR below 5% and is associated with a very low
360 number of significant associations close to the QTL (highly precise mapping), besides the
361 significant SNP within the QTL. This means that GWAS efficiency is maximized when there is at
362 least one SNP within each QTL, with the same allelic frequency. This seems very restrictive and,
363 unfortunately, is. To achieve high efficiency when there is not a SNP within each QTL, high LD
364 between a SNP close to the QTL and greater sample size are required, especially for low heritability
365 QTLs. In a random cross population the LD measure depends also on the SNP and QTL allele
366 frequencies. Thus, significant associations involving few SNPs with the same QTL can be observed,
367 including SNPs that are tens of mega base pairs (or centiMorgans) from the QTL. In reality, a
368 closely linked QTL and SNP can have a lower LD value compared to a more distant QTL and SNP
369 pair. In populations with low level of LD, significant associations are expected to occur for only
370 SNPs within the QTL or located very close to the QTL (within a few hundred base pairs), which
371 favors the identification of a candidate gene for the QTL. In this scenario, a QTL would be declared
372 based on one to a small number of significant associations spanning a chromosome region of a few
373 kilo base pairs (not mega base pairs or centiMorgans).

374 Field results have demonstrated that GWAS are best carried out with a large sample size (Yu
375 and Buckler 2006). According to Flint-Garcia *et al.* (2005), increasing the population size increases
376 the number of individuals with rare alleles, thus improving the power to test the association between
377 these rare alleles and the trait of interest. Yu *et al.* (2008) showed that the gain in the GWAS

378 efficiency by increasing sample size was evidenced by increased power of QTL detection and
379 smaller FDR, mainly with heritability of 0.7 in comparison with a heritability of 0.4. Based on a
380 simulation study, Long and Langley (1999) demonstrated that approximately 500 individuals
381 should be genotyped for 20 SNP loci within the candidate gene region to detect marker-trait
382 associations for QTLs that account for as little as 5% of the phenotypic variation. They observed
383 that more power was achieved by increasing the population size than by increasing the SNP density
384 within the candidate gene region.

385 Our most significant contribution to the knowledge on GWAS is the empirical proof that the
386 additive-dominance model must be fitted for traits controlled by dominance (uni- or bidirectional),
387 to increase the QTL power of detection. We additionally evidenced that the additive-dominance
388 model must not be fitted for traits determined only by additive gene effects, to avoid a decrease in
389 the QTL detection power. This is probably due to over fitting. Further, we provided results for
390 comparing GWAS in non-inbred and inbred random cross populations, and in an inbred lines panel.
391 The inbreeding did not affect the GWAS efficiency, but RILs, if available, can be interesting to
392 maximize the QTL heritabilities, since they allow standard experimental procedures (local control
393 and replication) and the assessment of SNP x environment interaction. Compared to GWAS in an
394 inbred lines panel, GWAS in random cross population was less efficient, i.e., showed lower power
395 of QTL detection for the low and intermediate heritability QTLs, slightly inferior control of false-
396 positive associations, and higher bias in QTL position. This is due to higher genetic variability in
397 the inbred lines panel since the average LD in the population 1, generation 0, is higher than the
398 average LD in the inbred lines panel (average absolute Δ equal to 0.0403 and 0.0249, respectively).
399 The genetic variability in the inbred lines panel is 9 to 13 times greater, depending on the trait (data
400 not shown).

401 According to Flint-Garcia *et al.* (2005), the inbred lines panel exploits the rapid breakdown of
402 LD in diverse maize lines, enabling very high resolution for QTL mapping. Population structure

403 results from constructing a panel with inbreds from various breeding programs and distinct heterotic
404 groups, which can cause false-positive marker-trait associations if the data is not corrected (Yan *et*
405 *al.* 2009). The lowest parametric LD values for the inbred lines panel occurred in published studies
406 (Yan *et al.* 2009, Remington *et al.* 2001). Moreover, with the inbred lines panel, generally, only
407 SNP loci within the QTL showed significant association, which is a highlighted result from GWAS
408 that can serve as a basis for a fine mapping strategy for marker-assisted selection and map-based
409 cloning genes (Gupta *et al.* 2005).

410 Our results are comparable to previous GWAS with field and simulated data. Concerning the
411 QK model, Bernardo (2013) observed that the power of QTL detection and number of false-positive
412 associations were proportional to the sample size. Assuming FDR of approximately 1% and an
413 average QTL heritability of approximately 5%, the power of detection increased from 13 to 45%
414 when the sample size increased from 384 to 1,536. In the study of Yang *et al.* (2010) the QTL
415 detection power was relatively low for QTLs with heritability lower than 10% but increased
416 significantly with the increase in the population size. Assuming sample size of 155, the power of
417 detection was 16.5, 59.2, and 87.6% for the low (1%), intermediate (5%) and high ($\geq 10\%$)
418 heritability QTLs, respectively. Yu *et al.* (2008) investigated the genetic and statistical properties
419 (power of QTL detection and FDR) of the nested association mapping (NAM) design. With 5,000
420 genotypes, they achieved an average power of QTL detection of 57% (with a range of 30 to 85%)
421 when considering two trait heritabilities (0.4 and 0.7) and two different numbers of QTL controlling
422 the trait (20 and 50). They also observed that a higher heritability always gave higher QTL
423 detection power, particularly for QTL with moderate to small effect. However, the FDR values
424 were high, ranging from 9 to 23%.

425 Concerning the relevance of relatedness, even if due to identity by state, and population
426 structure correction, our findings agreed with previous knowledge that the best GWAS model must
427 include a polygenic effect - to eliminate significant associations outside of the QTL interval

428 (including false-positive associations) - and a population structure effect - to control the type I error,
429 as highlighted by Yu et al (2006) and Bernardo (2013), among others. Stich and Melchinger (2009)
430 and Yang *et al.* (2010) observed best control of spurious associations by the K model. In the study
431 of Flint-Garcia (2005) the population structure effect was significant, explaining 9.3% of the
432 phenotypic variation, on average.

433 The GWAS in plant breeding has been effective for identifying candidate genes for
434 quantitative traits such as plant architecture, kernel composition, root development, flowering time,
435 drought tolerance, pathogen resistance, and metabolic processes (Zhu *et al.* 2008). Our study
436 provided the following additional knowledge: 1) the additive-dominance model must be fitted for
437 traits controlled by dominance effects but must not be fitted for traits controlled only by additive
438 effects, to achieve high power of QTL detection; 2) with sample size of 400 and level of
439 significance of 5%, the power of detection for the low, intermediate, and high heritability QTLs can
440 achieve approximately 30, 90, and 100%, respectively; 3) under sample size of 400, the observed
441 FDR was equal to or lower than the specified level of significance; 4) GWAS in random cross
442 populations is highly precise, since at least 97% of the QTLs were detected by the SNP inside it and
443 the number of significant associations outside of the QTL interval (2.5 cM) is very low; 5)
444 inbreeding does not affect the GWAS efficiency; 6) identity by state is important to control
445 significant associations outside of the QTL interval; and 7) in random cross populations, FDR is
446 mainly affected by population structure, compared to relationship information. Based on our
447 evidence, breeders can employ non-inbred and inbred populations for GWAS while taking into
448 account that the level of LD should be high, the sample size should be higher than that necessary for
449 QTL mapping, and the QTL heritability should be intermediate to high to achieve greater power of
450 QTL detection and precise mapping of candidate genes.

451

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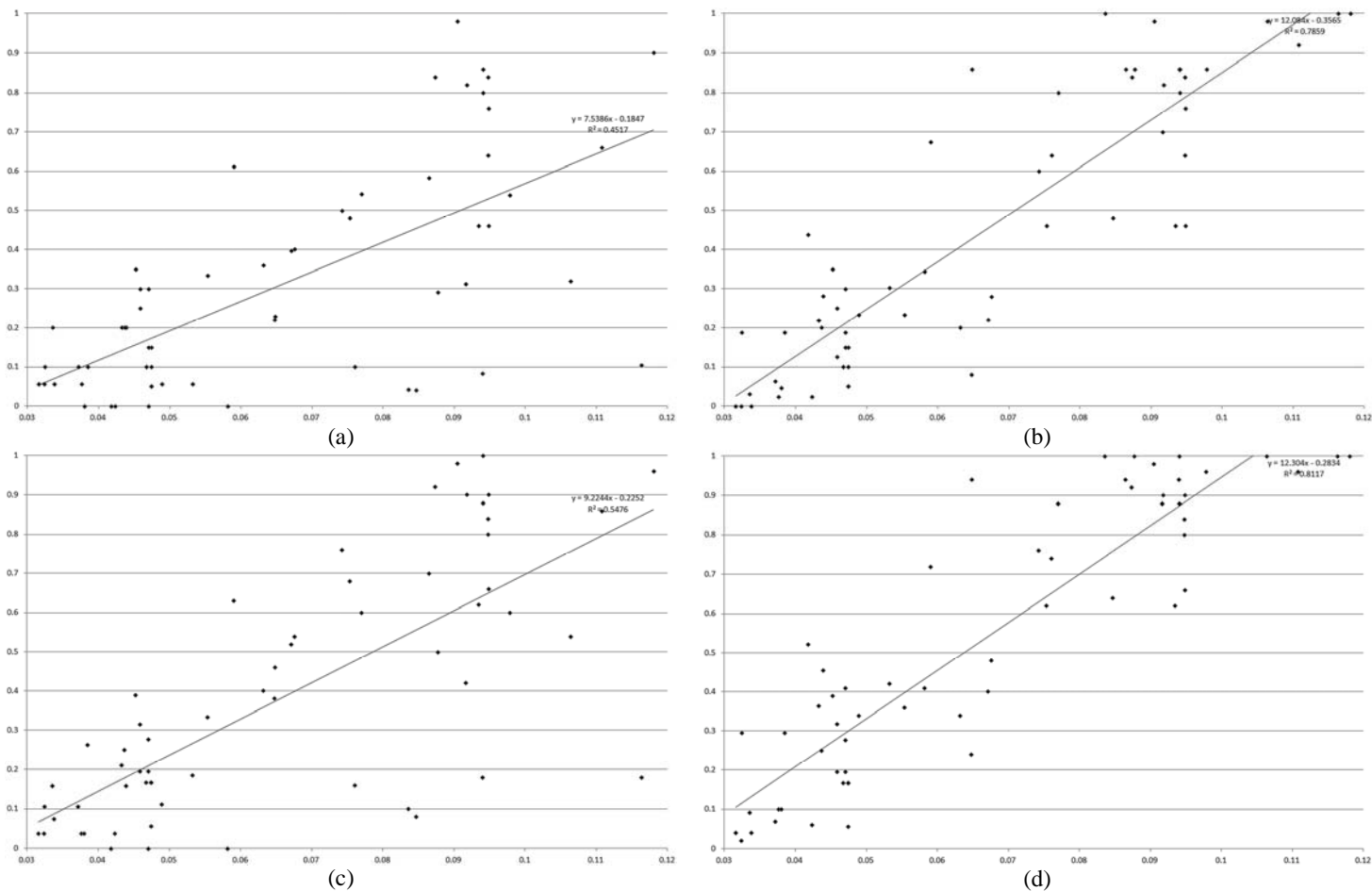
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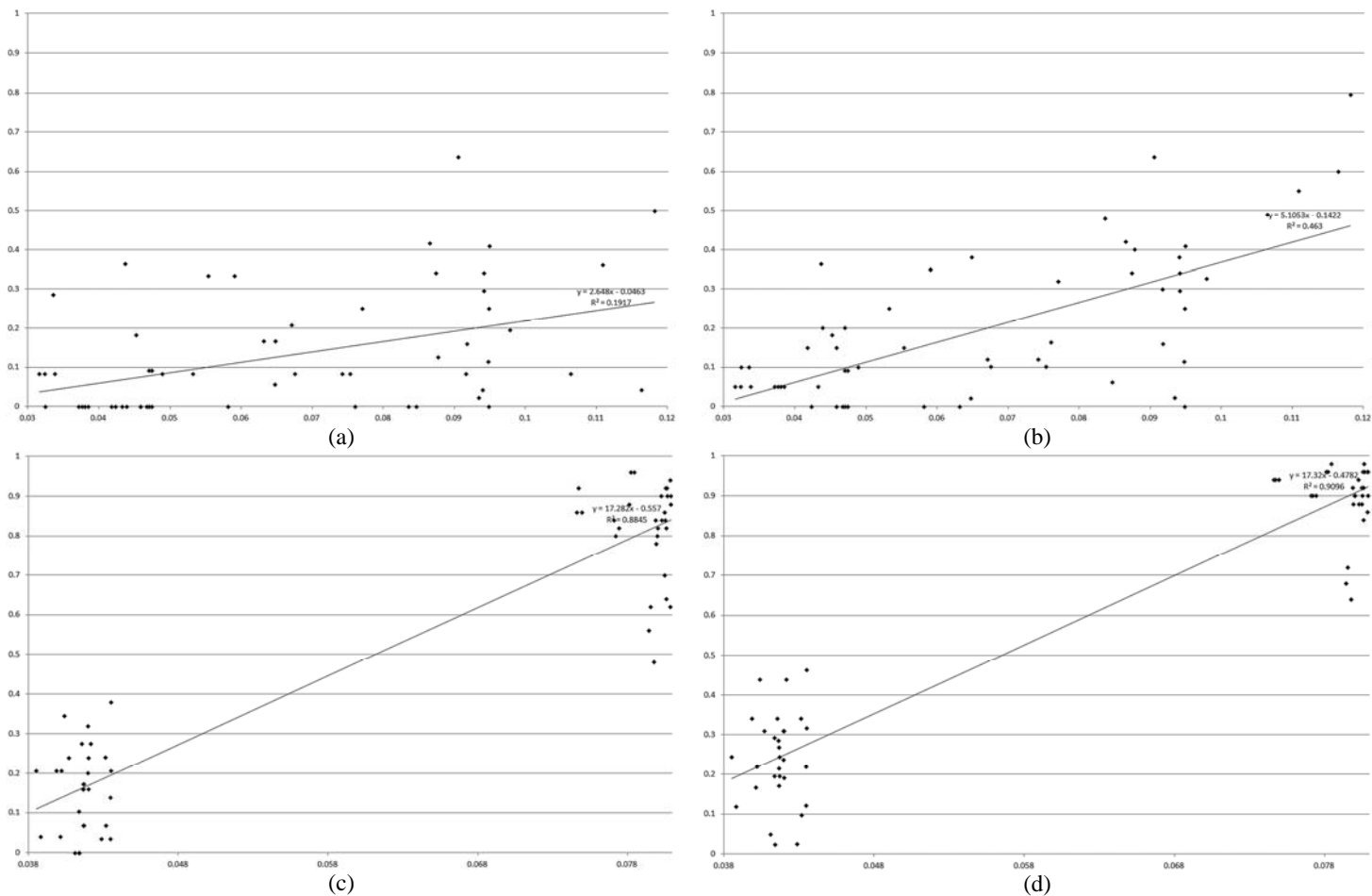
530 **Table 1** Average power of detection (%) for the low ($\leq 3.5\%$), intermediate (3.6-7.5%), and high ($\geq 7.6\%$) heritability QTLs, false discovery rate (%),
 531 correlations between power and QTL effect of substitution, dominance deviation, and heritability, bias in the QTL position for significant associations
 532 outside the QTL (cM), and number of significant SNPs outside the 2.5 cM interval, regarding population 1, generations 0 (open-pollinated) and 10r10s
 533 (RILs), and an inbred lines panel, five models, two sample sizes, and two levels of significance

Population	Model	Sample size	Sig. level	Power of detection			FDR	Correlation with power			Bias	Sig. assoc.
				Low	Int.	High		Eff. sub.	Dom. dev.	Herit.		
Open-pollinated	Add.-Dom. ^a (K model)	400	1%	4.4	23.6	79.0	1.13	0.093	0.213	0.886	0.16	0.1
			5%	9.7	32.7	87.7	3.76	0.140	0.234	0.901	0.37	1.0
	Add. (K model)	200	5%	7.0	10.8	33.2	9.44	0.097	0.227	0.680	0.20	0.6
			400	1%	9.3	18.8	51.9	0.25	-0.106	-0.285	0.672	0.07
	Add. -Dom. ^a (K = I model)	400	5%	8.2	24.2	62.7	1.57	-0.079	-0.245	0.740	0.13	0.2
			200	5%	10.7	7.8	19.8	8.64	-0.114	-0.252	0.438	0.06
RILs	Add. (K model)	400	1%	7.1	36.9	81.0	1.42	0.074	0.164	0.686	0.91	28.3
	Add. (K = I model)	400	1%	-	22.4	80.9	0.12	0.008	-	0.940	0.17	0.0
			5%	-	30.3	89.3	1.83	-0.022	-	0.954	0.33	0.2
	Add. (K = I model)	200	5%	-	13.9	31.3	7.30	-0.007	-	0.697	0.24	0.3
Inbred lines panel	Add. (Q + K model)	400	1%	-	33.1	82.4	1.16	0.022	-	0.871	0.82	6.7
			5%	12.2	35.4	85.2	0.36	0.030	-	0.977	0.02	0.1
	Add. (Q + K = I model)	200	5%	26.5	45.6	93.6	2.73	0.021	-	0.985	0.05	0.2
			400	5%	12.2	17.7	36.4	2.61	-0.029	-	0.793	0.04
	Add. (K model)	400	1%	15.3	37.4	87.9	0.80	0.029	-	0.974	0.18	0.4
Add. (K model)	400	1%	24.7	41.4	91.0	29.5	0.002	-	0.979	0.01	0.1	

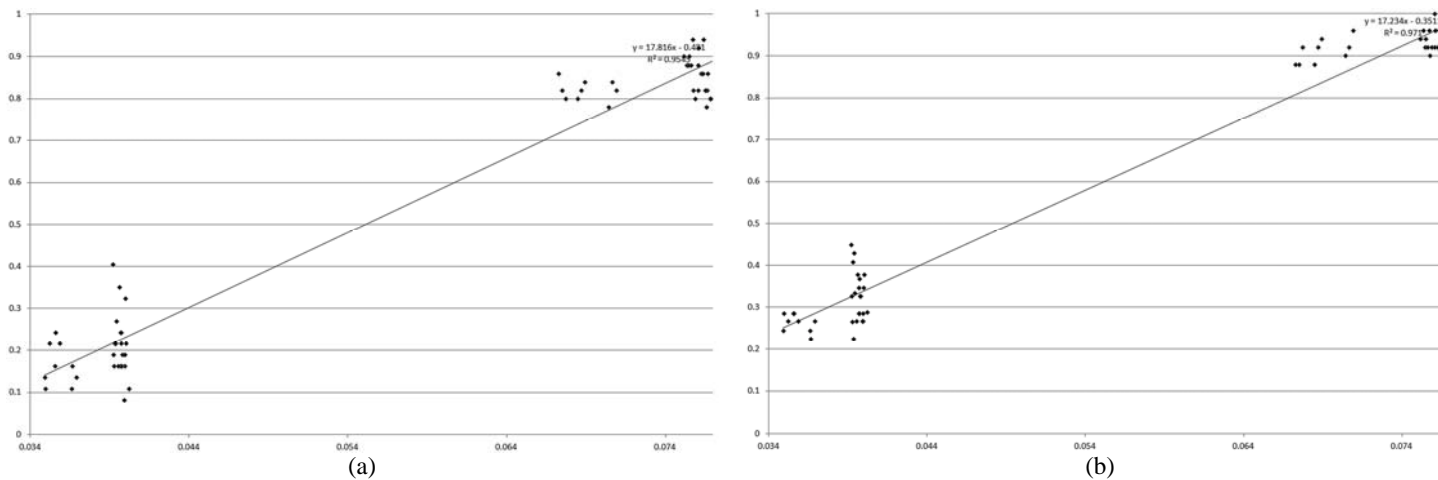
^aFor grain yield and expansion volume.



534 **Figure 1** Relationship between QTL heritability (X axis) and power of detection (Y axis) concerning population 1, generation 0, and QTLs determining
 535 grain yield, expansion volume, and days to maturity, assuming additive (a, c) and additive-dominance models (b, d), sample size of 400, and levels of
 536 significance of 1 (a, b) and 5% (c, d).



537 **Figure 2** Relationship between QTL heritability (X axe) and power of detection (Y axe) concerning population 1, generations 0 (a, b) and 10r10s (c,
 538 d), and QTLs determining grain yield, expansion volume, and days to maturity, assuming additive (a, c, d) and additive-dominance models (b), sample
 539 sizes of 400 (c, d) and 200 (a, b), and levels of significance of 1 (c) and 5% (a, b, d).



540 **Figure 3** Relationship between QTL heritability (X axe) and power of detection (Y axe) concerning the inbred lines panel and QTLs determining grain
541 yield, expansion volume, and days to maturity, assuming sample size of 400 and levels of significance of 1 (a) and 5% (b).