

1 **MultiGWAS: An integrative tool for Genome**
2 **Wide Association Studies (GWAS) in tetraploid**
3 **organisms**

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12 **Abstract**

13 **Summary:** The Genome-Wide Association Studies (GWAS) are essential to
14 determine the genetic bases of either ecological or economic phenotypic variation
15 across individuals within populations of wild and domesticated species. For
16 this research question, current practice is the replication of the GWAS testing
17 different parameters and models to validate the reproducibility of results. How-
18 ever, straightforward methodologies that manage both replication and tetra-
19 ploid data are still missing. To solve this problem, we designed the MultiG-
20 WAS, a tool that does GWAS for diploid and tetraploid organisms by execut-
21 ing in parallel four software, two for polyploid data (GWASpoly and SHEsis)
22 and two for diploids data (PLINK and TASSEL). MultiGWAS has several ad-
23 vantages. It runs either in the command line or in an interface. It manages
24 different genotype formats, including VCF. It executes both the full and naïve
25 models using several quality filters. Besides, it calculates a score to choose the
26 best gene action model across GWASPoly and TASSEL. Finally, it generates sev-
27 eral reports that facilitate the identification of false associations from both the
28 significant and the best-ranked association SNP among the four software. We
29 tested MultiGWAS with tetraploid potato data. The execution demonstrated
30 that the Venn diagram and the other companion reports (i.e., Manhattan and
31 QQ plots, heatmaps for associated SNP profiles, and chord diagrams to trace as-
32 sociated SNP by chromosomes) were useful to identify associated SNP shared
33 among different models and parameters. Therefore, we confirmed that Multi-
34 GWAS is a suitable wrapping tool that successfully handles GWAS replication
35 in both diploid and tetraploid organisms.

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37 **Keywords:** GWAS, tetraploids, SNPs, polyploids, software

38

1 Introduction

39 The Genome-wide association studies (GWAS) are used to identify which variants
40 through the whole genome of a large number of individuals are associated with a
41 specific trait (Begum et al., 2012; Cantor et al., 2010). This methodology started
42 with humans and several model plants, such as rice, maize, and *Arabidopsis* (Cao
43 et al., 2011; Han and Huang, 2013; Korte and Farlow, 2013; Lauc et al., 2010;
44 Tian et al., 2011). Because of the advances in the next-gen sequencing technology
45 and the decline of the sequencing cost in recent years, there is an increase in the
46 availability of genome sequences of different organisms at a faster rate (Ekblom
47 and Galindo, 2011; Ellegren, 2014). Thus, the GWAS is becoming the standard tool
48 to understand for both model and non-model organisms. This increment includes
49 complex species such as polyploids (Fig. 1) (Ekblom and Galindo, 2011; Santure
50 and Garant, 2018).

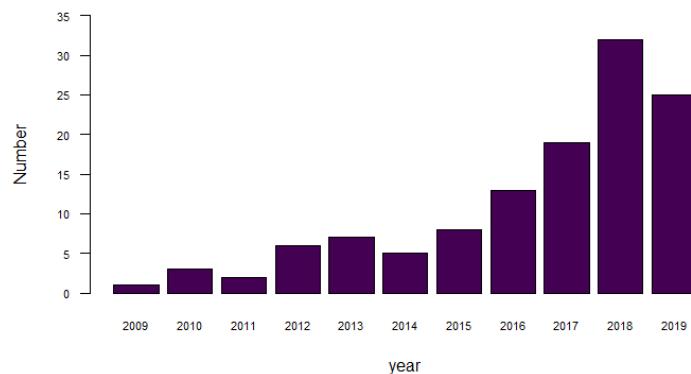


Figure 1: The number of peer-reviewed papers that contains the keywords "GWAS" and "polyploid" in the PubMed database between 2009 and 2019.

51 The GWAS for polyploid species has fourth related challenges. First, as all
52 GWAS, we should replicate the study as a reliable method to validate the results
53 and recognize real associations. This replication involves finding the same associa-
54 tions either in several replicates from the study population using the same software
55 or testing different GWAS tools among the same study population. This approach
56 involved the use of different parameters, models, or conditions, to test how con-
57 sistent the results are (De et al., 2014; Pearson and Manolio, 2008). However, the
58 performance of different GWAS software could affect the results. For example, the
59 threshold *pvalue* for SNP significance change through four GWAS software (i.e.,
60 PLINK, TASSEL, GAPIT, and FaST-LMM) when the sample size varies (Yan et al.,
61 2019). It means that well-ranked SNPs from one package can be ranked differently
62 in another.

63 Second, there are very few tools focused on the integration of several GWAS
64 software, to make comparisons under different parameters and conditions across
65 them. As far as we know, there is only two software with this service in mind, such
66 as iPAT and easyGWAS.

67 The iPAT allows running in a graphic interface three well-known command-line
68 GWAS software such as GAPIT, PLINK, and FarmCPU (Zhang et al., 2018). However,
69 the output from each package is separated. On the other hand, the easyGWAS
70 allows both running a GWAS analysis on the web using different algorithms and
71 combining several GWAS results. This analysis runs independently of the capacity
72 of the computer and the operating system. However, the output of the different
73 algorithms is presented separately, and it needs either several datasets to obtain
74 the different GWAS results to make replicates or GWAS results already computed
75 (Grimm et al., 2017). Thus, for both software iPAT and easyGWAS, output results
76 from different software but miss integration to make comparisons.

77 Third, although there is different GWAS software available to repeat the analy-
78 sis under different conditions (Gumpinger et al., 2018), most of them are designed
79 exclusively for the diploid data matrix (Bourke et al., 2018). Therefore, it is often
80 necessary to "diploidizing" the polyploid genomic data in order to replicate the
81 analysis. The consequences of this process are that force to simplify the complexity
82 of polyploid data(Ferrão et al., 2018).

83 Finally, and related to the previous point, the GWAS on polyploids generates a
84 new level of complexity to understand how allele dosage affect the phenotype ex-
85 pression on quantitative traits. Therefore, any tool that compares among software
86 but also models different gene action will contribute to gain a better understand-
87 ing in how redundancy or complex interaction among alleles affect the phenotype
88 expression and the evolution of new phenotypes among polyploid species (Bourke
89 et al., 2018; Ferrão et al., 2018; Rosyara et al., 2016).

90 To settle all the above fourth challenges, we developed the MultiGWAS tool that
91 performs GWAS analyses for tetraploid species using four software in parallel. Our
92 tool include GWASPoly (Rosyara et al., 2016) and the SHEsis tool (Shen et al.,
93 2016) that accept polyploid genomic data, and PLINK (Purcell et al., 2007) and
94 TASSEL (Bradbury et al., 2007) with the use of a "diploidized" genomic matrix. The
95 tool deals with input file formats, data preprocessing, search for associations by
96 running four GWAS tools in parallel, and creation of comparative reports from the
97 output of each software to help the user to decide more intuitively the true or false
98 associations.

100 2 Method

101 The MultiGWAS tool has three main consecutive steps: the adjustment, the multi
102 analysis, and the integration (Fig. 2). In the adjustment step, MultiGWAS processes
103 the configuration file. Then it cleans and filters the genotype and phenotype, and
104 MultiGWAS "diploidize" the genomic data. Next, during the multi analysis, each
105 GWAS tool runs in parallel. Subsequently, in the integration step, the MultiGWAS
106 tool scans the output files from the four packages (i.e., GWASPoly, SHEsis, PLINK,

Revisar. Antes: "On the other hand, the easyGWAS allows running a GWAS analysis on the web using different algorithms. This analysis could run independently of both the computer capacity and operating system. However, it needs either several datasets available or a dataset with a large number of individuals to make replicates in order to compare among algorithms. Moreover, the output from different algorithms is separated"

PAULA: de nuevo acá no es allele dosage sino gene action lo modifique la segunda vez que lo mencionas

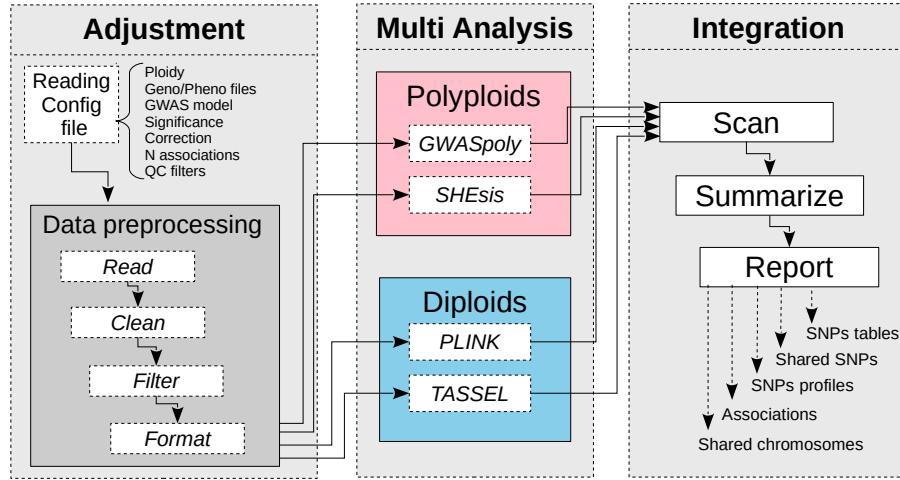


Figure 2: MultiGWAS flowchart has three steps: adjustment, multi analysis, and integration. In the first step, the user uploads the input data management, reading the configuration file, and reading and preprocessing the input data (genotype and phenotype). In the second step, the users perform the GWAS analysis, configuring and running the four packages in parallel. And the third step, the user can browse the summarizing and reporting results using different tabular and graphical visualizations.

107 and TASSEL). Finally, it generates a summary of all results that contains score tables,
 108 Venn diagrams, SNP profiles, and Manhattan plots.

109 2.1 Adjustment stage

110 MultiGWAS takes as input a configuration file where the user specifies the genomics
 111 data along with the parameters that will be used by the four tools. Once the config-
 112 uration file is read and processed, the genomic data files (genotype and phenotype)
 113 are preprocessed by cleaning, filtering, and checking data quality. The output of this
 114 stage corresponds to the inputs for the four programs at the Multi Analysis stage.

115 2.1.1 Reading configuration file

116 The configuration file includes the following settings that we briefly describe:

117 **Ploidy:** Numerical value for the ploidy level of the genotype, currently MultiGWAS
 118 supports diploids and tetraploids genotypes (2: for diploids, 4: for tetraploids).

119 **Genotype and phenotype input files:** MultiGWAS uses two input files, one for
 120 genotype and one for the phenotype. Genotypes files can be either in GWASpoly
 121 format (Rosyara et al., 2016) using SNP markers in rows and samples in columns

122 (Fig. 3.a) or Variant Call Format (VCF) (Fig.3.b) which is transformed into GWAS-
 123 poly format using NGSEP 4.0.2 (Tello et al., 2019). The phenotype file contains
 124 only one trait and uses a matrix format with the first column for the sample names
 125 and the second column for the trait values (Fig. 3.c).

a.	Marker, Chrom, Pos, sample01, sample02, sample03, ... c2_41437, 0, 805179, AAGG, AAGG, AAGG, ... c2_24258, 0, 1252430, AAGG, AGGG, GGGG, ... c2_21332, 0, 3499519, TTCC, TTCC, TTCC, ...	Individual, Trait sample01, 3.59 sample02, 4.07 sample03, 1.05
b.	##fileformat=VCFv4.2 ##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype"> #CHROM POS ID REF ALT QUAL FILTER INFO FORMAT sample01 sample02 sample03 0 805179 c2_41437 A G . . PR GT 0/1/1/0 0/1/1/0 0/1/0/0 0 1252430 c2_24258 G A . . PR GT 0/1/0/0 0/1/1/0 0/0/1/0 0 3499519 c2_21332 T C . . PR GT 0/1/1/0 0/1/1/1 0/1/1/0	

Figure 3: Examples of MultiGWAS input file formats. Figures a and b show genotype files in GWASpoly and VCF formats, respectively, while figure c shows a phenotype file in a matrix format. a. Genotype file in GWASpoly format containing column headers and with the first three columns for markers names, chromosomes and positions. The following columns correspond to the marker data of the samples in "ACGT" format (e.g. AAGG, CCTT for tetraploids, AG, CT for diploids). b. Genotype file in VCF format with metadata (first two lines) and header line. The following lines contain genotype information of the samples for each position. VCF marker data can be encoded as simple genotype calls (GT format field, e.g. 0/0/1/1 for tetraploids or 0/1 for diploids) or using the NGSEP custom format fields (Tello et al., 2019): ACN, ADP or BSDP. c. Phenotype file in a matrix format with column headers and sample names followed by their trait values. Both GWASpoly genotype and phenotype files are in CSV (Comma Separated Values format).

126 **GWAS model:** MultiGWAS is designed to work with quantitative phenotypes and
 127 can run GWAS analysis using two types of statistical models that we have called *full*
 128 and *naive* models. The *full model* is known in the literature as the Q+K model (Yu et
 129 al., 2006) and includes control for structure (Q) and relatedness between samples
 130 (K), whereas the *naive model* does not include any type of correction. Both models
 131 are based on linear regression approaches and variations of them are implemented
 132 by the four GWAS packages used by MultiGWAS. The *naive* is modeled with Gener-
 133 alized Linear Models (GLMs, Phenotype + Genotype), and the *full* is modeled with
 134 Mixed Linear Models (MLMs, Phenotype + Genotype + Structure + Kinship). The
 135 default model used by MultiGWAS is the *full model* (Q+K) (Yu et al., 2006), which
 136 is expressed with the following equation:

$$y = X\beta + S\alpha + Q\nu + Z\mu + e$$

137 where y is the vector of observed phenotypes; β is a vector of fixed effects other
 138 than SNP or population group effects; α is a vector of SNP effects (Quantitative
 139 Trait Nucleotides); ν is a vector of population effects; μ is a vector of polygene
 140 background effects; e is a vector of residual effects; Q , modeled as a fixed effect,
 141 refers to the incidence matrix for subpopulation covariates relating y to ν ; and X ,
 142 S and Z are incidence matrices of 1s and 0s relating y to β , α and μ , respectively.

143 **Genome-wide significance:** GWAS searches SNPs associated with the phenotype
 144 in a statistically significant manner. A threshold or significance level α is specified
 145 and compared with the p -value derived for each association score. Standard signif-
 146 icance levels are 0.01 or 0.05 (Gumpinger et al., 2018; Rosyara et al., 2016), and

147 MultiGWAS uses an α of 0.05 for the four GWAS packages. But the threshold is
148 adjusted according to each package, as some packages as GWASpoly and TASSEL
149 calculates the SNP effect for each genotypic class using different gene action models
150 (see “Multi analysis stage”). So, the number of tested markers may be different in
151 each model (see below) that results in different *p-value* thresholds.

152 **Multiple testing correction:** Due to the massive number of statistical tests per-
153 formed by GWAS, it is necessary to perform a correction method for multiple hy-
154 pothesis testing and adjusting the *p-value* threshold accordingly. Two common
155 methods for multiple hypothesis testing are the false discovery rate (FDR) and the
156 Bonferroni correction. The latter is the default method used by MultiGWAS, which
157 is one of the most stringent methods. However, instead of adjusting the *p-values*,
158 MultiGWAS adjust the threshold below which a *p-value* is considered significant,
159 that is α/m , where α is the significance level and m is the number of tested markers
160 from the genotype matrix.

161 **Number of reported associations:** Criticism has arisen in considering only sta-
162 tistically significant associations as the only possible correct associations (Kaler and
163 Purcell, 2019; Thompson et al., 2011). Many of low *p-value* associations, closer to
164 being significant, are discarded due to the stringent significance levels, and conse-
165 quently increasing the number of false negatives. To help to analyze both signif-
166 icant and non-significant associations, MultiGWAS provides the option to specify
167 the number of best-ranked associations (lower *p-values*), adding the corresponding
168 *p-value* to each association found. In this way, it is possible to enlarge the number
169 of results, and we can observe replicability in the results for different programs.
170 Nevertheless, we present each association with the corresponding *p-value*.

171 **Quality control filters:** A control step is necessary to check the input data for
172 genotype or phenotype errors or poor quality that can lead to spurious GWAS re-
173 sults. MultiGWAS provides the option to select and define thresholds for the follow-
174 ing filters that control the data quality: Minor Allele Frequency (MAF), individual
175 missing rate (MIND), SNP missing rate (GENO), and Hardy-Weinberg threshold
176 (HWE):

- 177 • **MAF of x :** filters out SNPs with minor allele frequency below x (default 0.01);
- 178 • **MIND of x :** filters out all individuals with missing genotypes exceeding $x*100\%$
179 (default 0.1);
- 180 • **GENO of x :** filters out SNPs with missing values exceeding $x*100\%$ (default
181 0.1);
- 182 • **HWE of x :** filters out SNPs which have Hardy-Weinberg equilibrium exact test
183 *p-value* below the x threshold.

184 MultiGWAS does the MAF filtering, and uses the PLINK package (Gumpinger et al.,
185 2018) for the other three filters: MIND, GENO, and HWE.

186 **GWAS tools:** List of names of the four GWAS software to run and integrate into
187 MultiGWAS analysis. They are GWASpoly and SHEsis (designed for polyploid data),
188 and PLINK and TASSEL (designed for diploid data).

189 **2.1.2 Data preprocessing**

190 Once the configuration file is processed, the genomic data is read and cleaned by se-
191 lecting individuals present in both genotype and phenotype. Then, individuals and
192 SNPs with poor quality are removed by considering the previous selected quality-
193 control filters and their thresholds,

194 At this point, the format "ACGT" suitable for the polyploid software GWAS-
195 poly and SHEsis, is "diploidized" for PLINK and TASSEL. The homozygous tetra-
196 ploid genotypes are converted to diploid thus: AAAA→AA, CCCC→CC, GGGG→GG,
197 TTTT→TT. Moreover, for tetraploid heterozygous genotypes, the conversion de-
198 pends on the reference and alternate alleles calculated for each position (e.g., AAAT
199 →AT, ... ,CCCG→CG).

200 After this process, the genomic data, genotype and phenotype, are converted to
201 the specific formats required for each of the four GWAS packages.

202 **2.2 Multi analysis stage**

203 MultiGWAS runs in parallel using two types of statistical models specified in the
204 parameters file, the Full model (Q+K) and Naive (i.e., without any control) where
205 Q refers to population structure and K refers to relatedness, calculated by kinship
206 coefficients across individuals (Sharma et al., 2018). The Full model (Q+K) controls
207 for both population structure and individual relatedness. For population structure,
208 MultiGWAS uses the Principal Component Analysis (PCA) and takes the top five PC
209 as covariates. For relatedness, MultiGWAS uses kinship matrices that TASSEL and
210 GWASpoly calculated separately, and for PLINK and SHEsis, relatedness depends on
211 kinship coefficients calculated with the PLINK 2.0 built-in algorithm (Chang et al.,
212 2015).

213 **2.2.1 GWASpoly**

214 GWASpoly (Rosyara et al., 2016) is an R package designed for GWAS in polyploid
215 species used in several studies in plants (Berdugo-Cely et al., 2017; Ferrão et al.,
216 2018; Sharma et al., 2018; Yuan et al., 2019). GWASpoly uses a Q+K linear mixed
217 model with biallelic SNPs that account for population structure and relatedness.
218 Also, to calculate the SNP effect for each genotypic class, GWASpoly provides eight
219 gene action models: general, additive, simplex dominant alternative, simplex dom-
220 inant reference, duplex dominant alternative, duplex dominant, diplo-general, and
221 diplo-additive. As a consequence, the number of statistical test performed can be
222 different in each action model and so thresholds below which the *p*-values are con-
223 sidered significant.

224 MultiGWAS is using GWASpoly version 1.3 with all gene action models available
225 to find associations. The MultiGWAS reports the top *N* best-ranked (the SNPs with

lowest *p*-values) that the user specified in the *N* input configuration file. The *full* model used by GWASpoly includes the population structure and relatedness, which are estimated using the first five principal components and the kinship matrix, respectively, both calculated with the GWASpoly built-in algorithms.

2.2.2 SHEsis

SHEsis is a program based on a linear regression model that includes single-locus association analysis, among others. The software design includes polyploid species. However, their use is mainly in diploids animals and humans (Meng et al., 2019; Qiao et al., 2015).

MultiGWAS is using version 1.0, which does not take account for population structure or relatedness. Despite, MultiGWAS externally estimates relatedness for SHEsis by excluding individuals with cryptic first-degree relatedness using the algorithm implemented in PLINK 2.0 (see below).

2.2.3 PLINK

PLINK is one of the most extensively used programs for GWAS in humans and any diploid species (Power et al., 2016). PLINK includes a range of analyses, including univariate GWAS using two-sample tests and linear regression models.

MultiGWAS is using two versions of PLINK: 1.9 and 2.0. Linear regression from PLINK 1.9 performs both naive and full model. For the full model, the software calculates the population structure using the first five principal components calculated with a built-in algorithm integrated into version 1.9. Moreover, version 2.0 calculates the kinship coefficients across individuals using a built-in algorithm that removes the close individuals with first-degree relatedness.

2.2.4 TASSEL

TASSEL is another standard GWAS program based on the Java software developed initially for maize but currently used in several species (Álvarez et al., 2017; Zhang et al., 2018). For the association analysis, TASSEL includes the general linear model (GLM) and mixed linear model (MLM) that accounts for population structure and relatedness. Moreover, as GWASPoly, TASSEL provides three-gene action models to calculate the SNP effect of each genotypic class: general, additive, and dominant, and so the significance threshold depends on each action model.

MultiGWAS is using TASSEL 5.0, with all gene action models used to find the *N* best-ranked associations and reporting the top *N* best-ranked associations (SNPs with lowest *p*-values). Naive GWAS uses the GLM, and full GWAS uses the MLM with two parameters: population structure that uses the first five principal components, and relatedness that uses the kinship matrix with centered IBS method, both calculated with the TASSEL built-in algorithms.

263 2.3 Integration stage.

264 The outputs resulting from the four GWAS packages are scanned and processed to
265 identify both significant and best-ranked associations with *p-values* lower than and
266 close to a significance threshold, respectively.

267 2.3.1 Calculation of *p-values* and significance thresholds

268 GWAS packages compute *p-value* as a measure of association between each SNP and
269 the trait of interest. The statistically significant associations are those their *p-value*
270 drops below a predefined significance threshold. Since a GWAS analysis performs
271 a large number of tests to look for possible associations, one for each SNP, then
272 some correction in the *p-values* is needed to reduce the possibility of identifying
273 false positives, or SNPs with false associations with the phenotype, but that reach
274 the significance threshold.

275 MultiGWAS provides two methods for adjusting *p-values* and significance thresh-
276 old: the false discovery rate (FDR) that adjust *p-values*, and the Bonferroni cor-
277 rection, that adjusts the threshold. By default, MultiGWAS uses the Bonferroni
278 correction that uses the significance level α/m , with α defined by the user in the
279 configuration file, and m as the number of tested markers to adjust the significance
280 threshold in the GWAS study.

281 However, the significance threshold can be different for each GWAS package as
282 some of them use several action models to calculate the SNP effect of each genotypic
283 class. For both PLINK and SHEsis packages, which use only one model, m is equal
284 to the total number of SNPs. However, for both GWASpoly and TASSEL packages,
285 which use eight and three gene action models, respectively, m is equal to the number
286 of tests performed in each model, which is different between models.

287 Furthermore, most GWAS packages compute both *p-values* and thresholds differ-
288 ently, with the consequence that significant associations identified by one package
289 do not reach the threshold of significance in the others. This results in the loss of
290 real associations, the so-called false negatives. To overcome these difficulties, Multi-
291 GWAS reports two sets of associations: significant and best-ranked (those closest to
292 being statistically significant), as described below.

293 2.3.2 Selection of significant and best-ranked associations

294 MultiGWAS reports two groups of associations from the results of the four GWAS
295 packages: the statistically significant associations with *p-values* below a threshold
296 of significance, and the best-ranked associations with the lowest *p-values*, but not
297 reaching the limit to be statistically significant. However, they are representing
298 interesting associations for further analysis (possible false negatives).

299 In the case of PLINK and SHEsis, which have a unique gene action model, the
300 associations are as described above. But, in the case of GWASpoly and TASSEL,
301 which have eight and three models respectively, MultiGWAS automatically selects
302 the "best gene action model" from each package and takes the associations from it.

303 This selection within GWASPoly and TASSEL has three criteria: the inflation factor
304 (I), the shared SNPs (R) and the significant SNPs (S).

305 Each gene action model is scored using the following equation:

306

$$score(M_i) = I_i + R_i + S_i$$

307 where $score(M_i)$ is the score for the gene action model M_i , with i from 1.. k ,
308 for a GWAS package with k gene action models. I_i is the score for the inflation
309 factor defined as $I_i = 1 - |1 - \lambda(M_i)|$, where $\lambda(M_i)$ is the inflation factor for the
310 M_i model. R_i is the score of the shared SNPs defined as $R_i = \sum_{j=1}^k |M_i \sim M_j|$, where
311 $|M_i \sim M_j|$ is the number of SNPs shared between M_i and M_j models, normalized by
312 the maximum number of SNPs shared between all models. And, S_i is the number of
313 significant SNPs of model M_i normalized by the total number SNPs shared among
314 all models.

315 The score is high when an M_i model has an inflation factor λ close to 1, iden-
316 tifies a high number of shared SNPs, and contains one or more significant SNPs.
317 Conversely, the score is low when the M_i model has an inflation factor λ either
318 low (close to 0) or high ($\lambda > 2$), identifies a small number of shared SNPs, and
319 contains 0 or few significant SNPs. In any other case, the score results from the
320 balance among the inflation factor, the number of shared SNPs, and the number of
321 significant SNPs.

322 **2.3.3 Integration of results**

323 At this stage, MultiGWAS integrates the results to evaluate reproducible results
324 among tools (Fig 4). However, it still reports a summary of the results of each
325 tool:

- 326
- A Quantile-Quantile (QQ) plots for the resultant *p-values* of each tool and
327 the corresponding inflation factor λ to assess the degree of the test statistic
328 inflation.
 - A Manhattan plot of each tool with two lower thresholds, one for the best-
329 ranked SNPs, and another for the significant SNPs.

330 To present the replicability, we use two sets: (1) the set of all the significative SNPs
331 provided by each tool and (2) the set of all the best-ranked SNPs. For each set,
332 we present a Venn diagram that shows SNPs predicted exclusively by one tool and
333 intersections that help to identify the SNPs predicted by one, two, three, or all the
334 tools. Also, we provide detailed tables for the two sets.

335 For each SNP identified more than once, we provide what we call the SNP pro-
336 file. That is a heat diagram for a specific SNP, where each column is a genotype
337 state AAAA, AAAB, AABB, ABBC, and BBBB. Moreover, each row corresponds to a
338 sample. Samples with close genotypes form together clusters. Thus to generate
339 the clusters, we do not use the phenotype information. However, we present the
340 phenotype information in the figure as the color. This figure visually provides in-
341 formation regarding genotype and phenotype information simultaneously for the
342

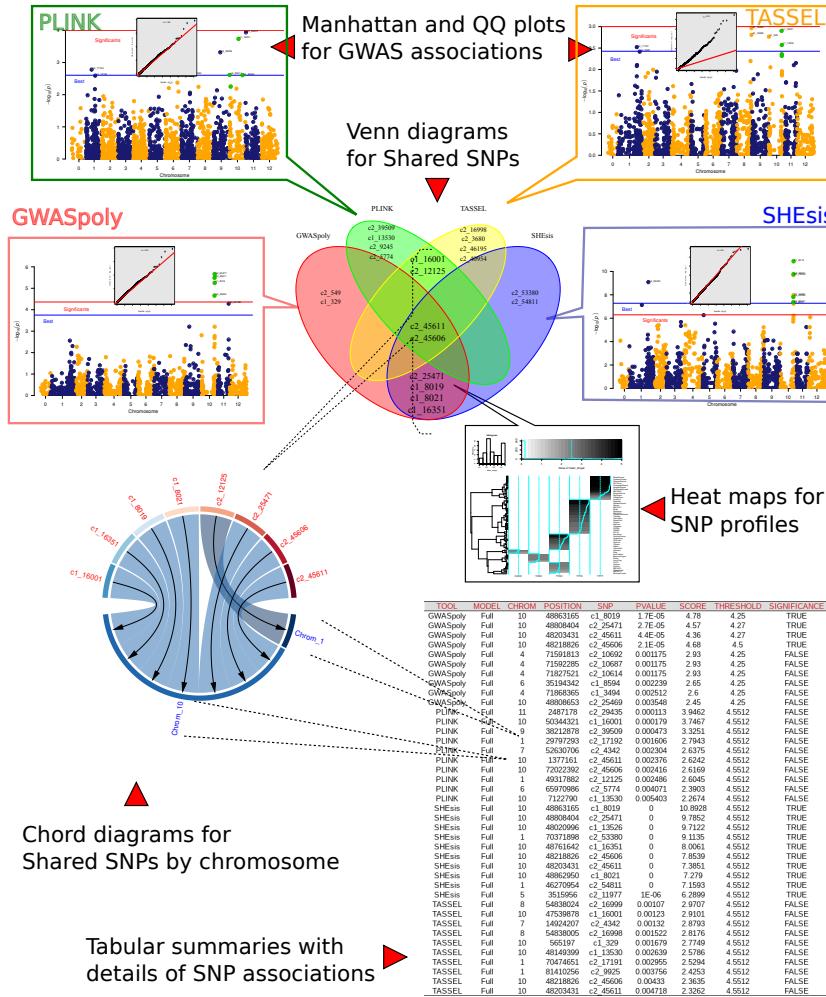


Figure 4: Reports presented by MultiGWAS. For each tool, first a QQ plot that assesses the resultant p -values. Second, a Manhattan plot for each tool with two lines, blue and red, respectively, is the lower limit for the best ranked and significative SNPs. We present two Venn diagrams, one for the significative SNPs and one for N best-ranked SNPs of each tool. We show the results for GWAsPoly, PLINK, TASSEL, and SHEsis in red, green, yellow, and blue. For each SNP that is in the intersection, thus, that is predicted by more than one tool, we provide an SNP profile. SNPs by chromosome chord diagrams show that the strongest associations are limited to few chromosomes. Furthermore, we present tabular summaries with details of significant and best-ranked associations.

343 whole population. We present colors as tones between white and black for color
344 blind people.

345 MultiGWAS generates a report, one document with the content previously de-
346 scribed. Besides, there is a folder with the individual figures just in case the user
347 needs one (Supplementary Material 1).

348 In the following section, we present the results of the functionality of the tool
349 applied on the diversity panel for the tetraploid potato, which has open access for
350 genotyped and phenotyped dataset as part of the USDA-NIFA Solanaceae Coordi-
351 nated Agricultural Project (SolCAP) (Hirsch et al., 2013).

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supplementary material

352 3 Results

353 All four GWAS packages adopted by MultiGWAS use linear regression approaches.
354 However, they often produce different association results for the same input. Com-
355 puted *p-values* for the same set of SNPs are different between packages. Therefore,
356 SNPs with significant *p-values* for one package maybe not significant for the oth-
357 ers. Alternatively, well-ranked SNPs in one package may be ranked differently in
358 another.

359 To highlight these differences in the results across the four packages, MultiGWAS
360 produces five types of results combining graphics and tables to compare, select, and
361 interpret the set of possible SNPs associated with a trait of interest. The outputs
362 include:

- 363 • Manhattan and Q-Q plots to show GWAS associations.
- 364 • Venn diagrams to show associations identified by single or several tools.
- 365 • Heat diagrams to show the genotypic structure of shared SNPs.
- 366 • Chord diagrams to show shared SNPs by chromosomes.
- 367 • Score tables to show detailed information of associations for both summary
368 results from MultiGWAS and particular results from each GWAS package

369 The complete reports generated by MultiGWAS for both types of analysis, full
370 and naive, applied to the diversity panel of tetraploid potato are in the supplemen-
371 tary information at <https://github.com/agrosavia-bioinformatics/multiGWAS-Supplementary>.

revisa hipervinculo

373 3.1 Manhattan and QQ plots for GWAS associations

374 MultiGWAS uses classical Manhattan and Quantile–Quantile plots (QQ plots) to
375 visualize the results of each package. In both plots, the points are the SNPs and
376 their *p-values* are transformed into scores like $-\log_{10}(p\text{-values})$ (see Fig. 5). The
377 Manhattan plot shows the strength of association of the SNPs (y-axis) distributed at
378 their genomic location (x-axis), so the higher the score, the stronger the association.

379 At the same time, the QQ plot compares the expected distribution of p -values (y-
380 axis) with the observed distribution (x-axis).

381 MultiGWAS adds distinctive marks to both plots to identify different types of
382 SNPs: (a) In the Manhattan plots, the significant SNPs are above a red line and the
383 best-ranked SNPs are above a blue line. Also, SNPs shared between packages are
384 coloured green (See Fig. 6.b). (b) In the QQ plots, a red diagonal line indicates
385 the expected distribution under the null hypothesis of no association of SNPs with
386 the phenotype, both distributions should coincide, and most SNPs should lie on the
387 diagonal line. Deviations for a large number of SNPs may reflect inflated p -values
388 due to population structure or cryptic relatedness. But, few SNPs deviate from the
389 diagonal for a truly polygenic trait (Power et al., 2016).

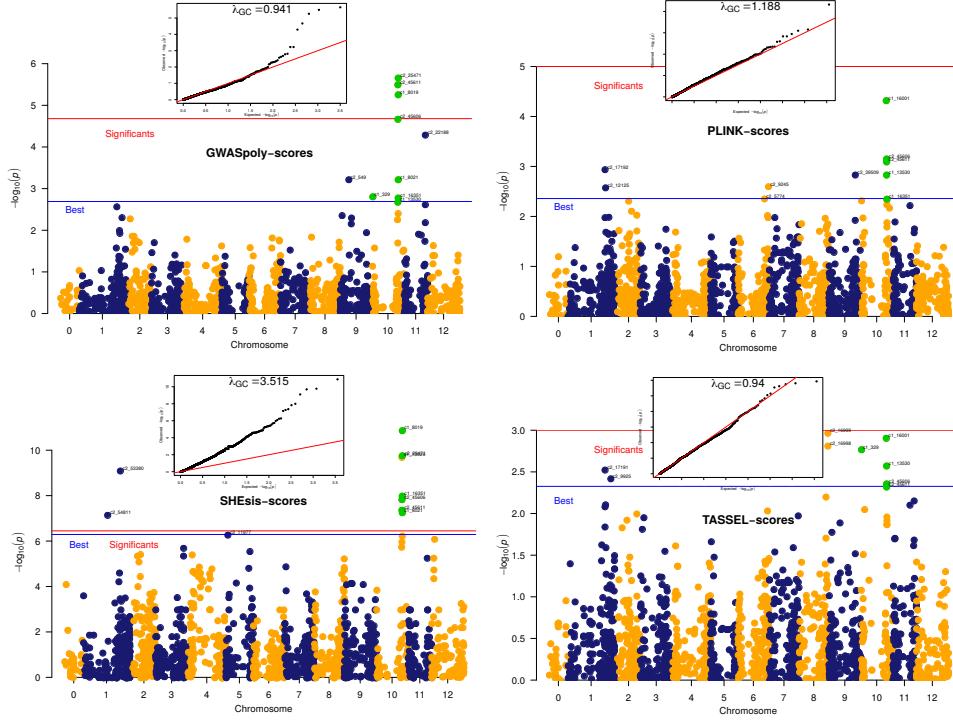


Figure 5: Associations in the tetraploid potato dataset. MultiGWAS shows the associations identified by the four GWAS packages using Manhattan and QQ plots. The tetraploid potato data showed several SNPs shared between the four software (green dots). The best-ranked SNPs are above the blue line, but only GWASpoly and SHEsis identified significant associations (SNPs above the red line) for this dataset. However, the inflation factor given by SHEsis is too high ($\lambda = 3.5$, at the top of the QQ plot), which is observed by the high number of SNPs deviating from the red diagonal of the QQ plot.

390 3.2 Tables and Venn diagrams for single and shared SNPs

391 MultiGWAS provides tabular and graphic views to report the best-ranked and signif-
392 icant SNPs identified by the four GWAS packages in an integrative way (see Figure
393 6). Both *p-values* and significance levels have been scaled as $-\log_{10}(p\text{-value})$ to give
394 high scores to the best statistically evaluated SNPs.

395 First, best-ranked SNPs correspond to the top-scored N SNPs, whether they were
396 assessed significant or not by its package, and with N defined by the user in the
397 configuration file. These SNPs appears in both a SNPs table (Figure 6.a), and in a
398 Venn diagram (Figure 6.b). The table lists them by package and sorts by decreasing
399 score, whereas the Venn diagram emphasizes if they were best-ranked either in a
400 single package or in several at once (shared).

401 Second, the significant SNPs correspond to the ones valued statistically signifi-
402 cant by each package. They appear in a Venn diagram (Figure 6.c), and in the SNPs
403 table, marked with significance TRUE (T) in the table of the Figure 6.a.

a.

TOOL	MODEL	GC	SNP	CHR	POS	PVALUE	SCR	THR	SGN
GWASPoly	additive	0.96	c2_25471	10	48808	0.000002	5.67	4.50	T
GWASPoly	additive	0.96	c2_45611	10	48203	0.000003	5.51	4.50	F
GWASPoly	additive	0.96	c1_8019	10	48863	0.000005	5.27	4.50	T
GWASPoly	additive	0.96	c2_45606	10	48218	0.000021	4.68	4.50	F
GWASPoly	additive	0.96	c2_22188	11	40777	0.000050	4.30	4.50	F
GWASPoly	additive	0.96	c2_549	9	16527	0.000580	3.23	4.50	F
GWASPoly	additive	0.96	c1_8021	10	48862	0.000589	3.23	4.50	F
GWASPoly	additive	0.96	c1_329	10	56519	0.001514	2.82	4.50	F
GWASPoly	additive	0.96	c1_16351	10	48761	0.001622	2.79	4.50	F
PLINK	additive	1.19	c1_16001	10	47539	0.000047	4.33	4.55	F
PLINK	additive	1.19	c2_45606	10	48218	0.000688	3.16	4.55	F
PLINK	additive	1.19	c2_45611	10	48203	0.000786	3.10	4.55	F
PLINK	additive	1.19	c2_17192	1	70472	0.001123	2.95	4.55	F
PLINK	additive	1.19	c2_39509	9	50174	0.001440	2.84	4.55	F
PLINK	additive	1.19	c1_13530	10	48149	0.001443	2.84	4.55	F
PLINK	additive	1.19	c2_9245	6	57953	0.002455	2.61	4.55	F
PLINK	additive	1.19	c2_12125	1	71450	0.002593	2.59	4.55	F
PLINK	additive	1.19	c2_5774	6	50345	0.004336	2.36	4.55	F
SHEsis	general	1.47	c1_8019	10	48863	0.000000	7.64	4.55	T
SHEsis	general	1.47	c1_13526	10	48020	0.000000	6.94	4.55	F
SHEsis	general	1.47	c2_25471	10	48808	0.000000	6.94	4.55	T
SHEsis	general	1.47	c2_53380	1	70371	0.000000	6.46	4.55	T
SHEsis	general	1.47	c1_16351	10	48761	0.000004	5.45	4.55	T
SHEsis	general	1.47	c2_45606	10	48218	0.000004	5.38	4.55	F
SHEsis	general	1.47	c2_45611	10	48203	0.000010	4.98	4.55	T
SHEsis	general	1.47	c1_8021	10	48862	0.000012	4.93	4.55	F
SHEsis	general	1.47	c2_54811	1	46270	0.000014	4.86	4.55	T
TASSEL	additive	0.86	c2_16999	8	54838	0.000247	3.61	3.89	F
TASSEL	additive	0.86	c2_16998	8	54838	0.000329	3.48	3.89	F
TASSEL	additive	0.86	c2_12125	1	71450	0.003287	2.48	3.89	F
TASSEL	additive	0.86	c1_16001	10	47539	0.006105	2.21	3.89	F
TASSEL	additive	0.86	c2_3680	11	39908	0.006701	2.17	3.89	F
TASSEL	additive	0.86	c2_46195	1	64259	0.007116	2.15	3.89	F
TASSEL	additive	0.86	c2_40954	1	63756	0.011097	1.95	3.89	F
TASSEL	additive	0.86	c2_45606	10	48218	0.011369	1.94	3.89	F
TASSEL	additive	0.86	c2_45611	10	48203	0.012091	1.92	3.89	F

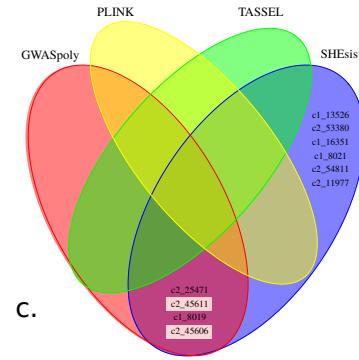
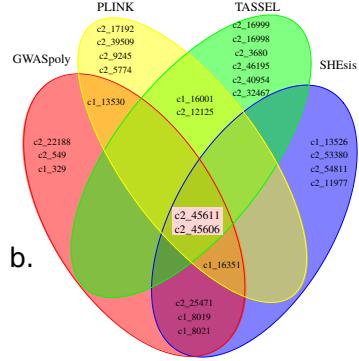


Figure 6: Shared SNPs Views. Tabular and graphical views of SNP associations identified by one or more GWAS packages (shared SNPs). SNPs identified by all packages are marker with red background in all figures. **(a)** Table with details of the N=9 best-ranked SNPs from each GWAS package. Each row corresponds to a single SNP, and the nine columns are tool name, the model used by the tool, genomic control factor (inflation factor), SNP name, chromosome, position in the genome, *p*-value, score as $-\log_{10}(p\text{-value})$, significance threshold as $-\log_{10}(\alpha/m)$ where α is the significance level, and m is the number of tested markers, and significance as true (T) or false (F) whether score $>$ threshold or not. **(b)** Venn diagram of the N=9 best-ranked SNPs. SNPs identified by all packages are in the central intersection. Other SNPs identified by more than one packages are in both upper central and lower central intersections. **(c)** Venn diagram of the significant SNPs (score $>$ threshold).

3.3 Heat diagrams for the structure of shared SNPs

MultiGWAS creates a two-dimensional representation, called the SNP profile, to visualize each trait by individuals and genotypes as rows and columns, respectively (Figure 7). At the left, the individuals are grouped in a dendrogram by their genotype. At the right, there is the name or ID of each individual. At the bottom, the genotypes are ordered from left to right, starting from the major to the minor allele (i.e., AAAA, AAAB, AABB, ABAA, BBBB). At the top, there is a description of the trait based on a histogram of frequency (top left) and by an assigned color for each numerical phenotype value using a grayscale (top right). Thus, each individual appears as a colored line by its phenotype value on its genotype column. For each

414 column, there is a solid cyan line with the mean of each column and a broken cyan
 415 line that indicates how far the cell deviates from the mean.

416 Because each multiGWAS report shows one specific trait at a time, the histogram
 417 and color key will remain the same for all the best-ranked SNPs.

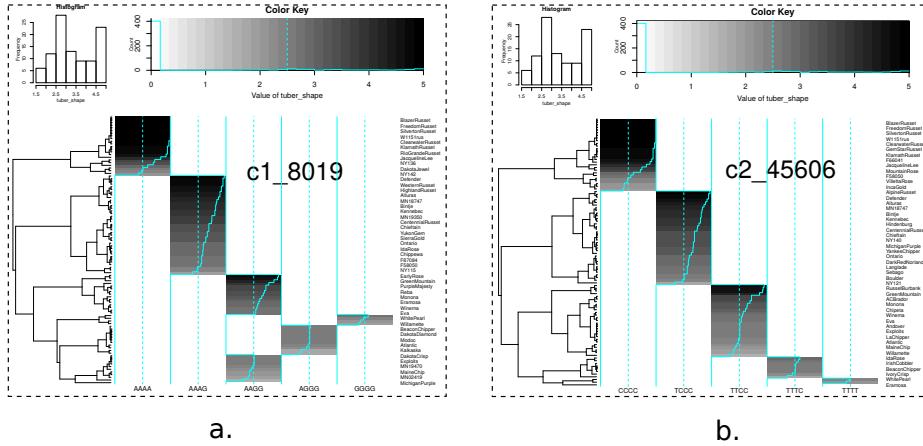


Figure 7: SNP profiles. SNP profiles for two of the best-ranked significant SNPs shown in the figure 6.b. (a) SNP c2_45606 best-ranked by the four packages (central intersection of the Venn diagram Figure 6.b) (b) SNP c1_8019 best-ranked by the two tetraploid packages (Figure 6.b), and also identified as significant by the same packages (at the bottom of the Figure 6.a).

418 3.4 Chord diagrams for SNPs by chromosome

419 The chord diagrams visualize the location across the genome of the best-ranked
 420 associated SNPs shared among the four packages and described in the table 6.a.
 421 Thus, in the case of the tetraploid potato, we found that they are located mostly in
 422 chromosome 10 (Figure 8.a). This visualization complements the manhattan plots
 423 from each GWAS package (Figure 8.b).

424 4 Availability and Implementation

425 The core of the MultiGWAS tool runs under R and users can interact with the tool by
 426 either a command-line interface (CLI) developed in R or a graphical user interface
 427 (GUI) developed in Java (Figure 10). Source code, examples, documentation, and
 428 installation instructions are available at <https://github.com/agrosavia-bioinformatics/multiGWAS>.

430 4.1 Input parameters

431 MutiGWAS uses as the only input a simple configuration text file with the values
 432 for the main parameters that drive the analysis. To create the configuration text
 433 file, users can choose either a text editor or the MultiGWAS GUI application. If

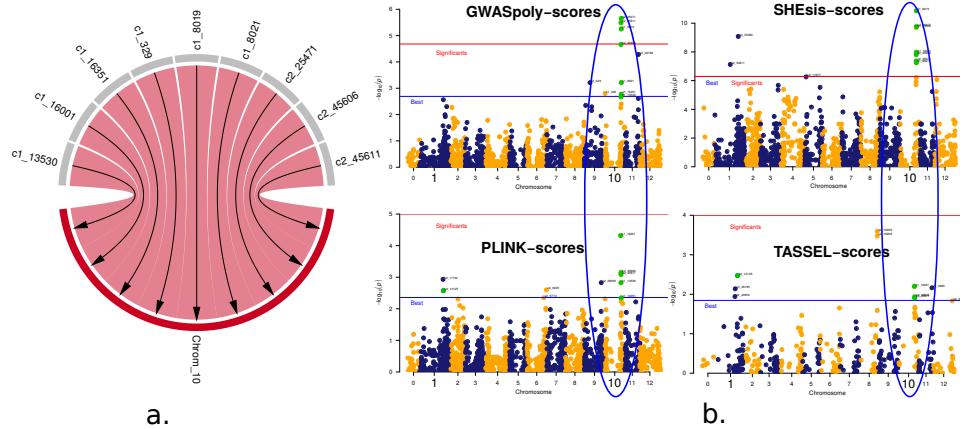


Figure 8: SNPs by chromosome. The position of best-ranked SNPs across chromosomes using two different visualizations. (a) Chord diagram showing that best-ranked SNPs located in chromosome 10. The SNPs are at the top and the chromosomes at the bottom. The arrows connect the best-ranked SNPs with their position in the chromosomes. (b) Manhattan plots from each GWAS packages showing two important locations of associations, chromosome 1 and chromosome 10, marked with blue and red ellipsis, respectively.

434 users prefer a text file, it must have the parameter names and values separated by a
 435 colon, filenames enclosed in quotation marks, and TRUE or FALSE values to indicate
 436 if filters are applied. If the users prefer the GUI applications, they can create the
 437 configuration file using the input parameter view. In any case, this file must have
 438 the structure shown in the Figure 9.

```

default:
  ploidy      : 4
  genotypeFile : "example-genotype-tetra.csv"
  phenotypeFile : "example-phenotype.csv"
  significanceLevel : 0.05
  correctionMethod : "Bonferroni"
  gwasModel    : "Full"
  nBest        : 10
  filtering    : TRUE
  MAF          : 0.01
  MIND         : 0.1
  GENO         : 0.1
  HWE          : 1e-10
  tools         : "GWASpoly SHEsis PLINK TASSEL"
  
```

Figure 9: Configuration file for MultiGWAS. The input parameters include the ploidy level of the organism (2: for diploids, 4: for tetraploids). The input genotype/phenotype filenames. The genome-wide significance threshold. The method for multiple testing correction. The GWAS model. The number of associations to report. The quality control filters choosing TRUE or FALSE. The filters are minor allele frequency, individual missing rate, SNP missing rate, and Hardy-Weinberg threshold. Finally, the GWAS packages selected for the analysis.

439 4.2 Using the command line interface

440 The execution of the CLI tool is simple. It only needs to open a Linux console,
441 change to the folder where the configuration file was created, and type the name of
442 the executable tool followed by the filename of the configuration file, like this:

443 `multiGWAS Test01.config`

444 Then, the tool starts the execution, showing information on the process in the
445 console window. When it finishes, the results are in a new subfolder called “*out-*
446 *Test01*. The results include a complete HTML report containing the different views
447 described in the results section, the source graphics and tables supporting the re-
448 port, and the preprocessed tables from the results generated by the four GWAS
449 packages used by MultiGWAS.

450 4.3 Using the graphical user interface

451 The interface allows users to save, load or specify the different input parameters
452 for MultiGWAS in a friendly way (Fig. 10). The input parameters correspond to the
453 settings included in the configuration file described in subsection 2.1.1. It executes
454 by calling the following command from a Linux console:

455 `jmultiGWAS`

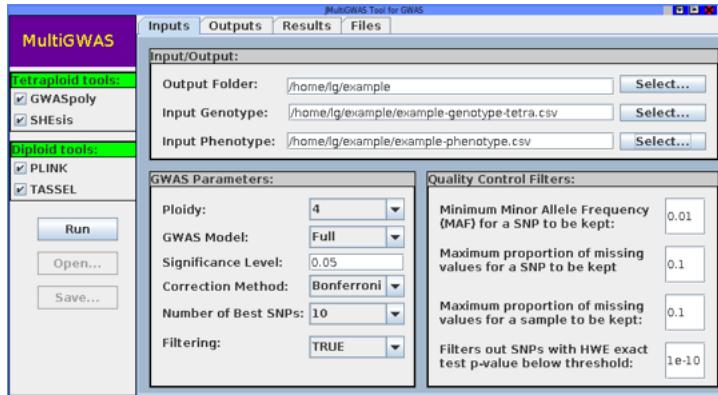


Figure 10: Main view of the MultiGWAS graphical user interface. The interface has a toolbar at the left side and four tabs at the top. In the toolbar, users can select the GWAS packages (Two for tetraploids and two for diploids). The analysis starts with the current parameters or loading a previously saved configuration. In the Input tab, users can set the parameters and quality control filters. The Output tab shows the execution of each process. In the Results tab, users can browse the HTML report of the current analysis generated by the tool. Finally, in the Files tab, users can browse the source files of each software and can access the produced data across the analysis.

456

Corregido, Revisar

Luis, revisa la leyenda de la Fig. 10, si estás conforme con esta descripción de la figura. Traté de hacerla más descriptiva

458 **5 Discussion**

459 The reanalysis of potato data with MultiGWAS showed that this tool is handy to im-
460 prove the GWAS in tetraploid species. Through MultiGWAS performance, we could
461 test its effectiveness to answer some of the challenges associating phenotypic in a
462 polyploid organism. They include the integration and replication among param-
463 eters and software, the diploidization of polyploid data, and the incorporation of
464 allele dosage models (Dufresne et al., 2014).

465 The main advantage of MultiGWAS is that replicate the GWAS analysis among
466 four software and integrate the results obtained across software, models and param-
467 eters. Depending on the software, users usually have to choose between sensitivity
468 or specificity. But using MultiGWAS, users do not have to choose between both
469 approaches because they can observe their effect in the analysis within the same
470 environment.

471 Another difficulty for replication among software is the variability of structures
472 for the genomic input data. Currently, the most common format for next-generation
473 sequencing variant data is the VCF (Variant Call Format) (Danecek et al., 2011;
474 Ebbert et al., 2014) . One of the advantages of VCF is the versatility to summarize
475 important genome information for hundreds or thousands of individuals and SNP,
476 including information about levels of ploidy. MultiGWAS different from most of
477 GWAS software available allows the VCF as an input (but see VarStats tool in VTC).

478 Moreover, the MultiGWAS is the unique tool as far as we know that allows us to
479 compare the effect of diploidized the tetraploid data in the performance of the anal-
480 ysis directly. The graphic outputs are a handy approach to find similar results. The
481 SNP profile allows identifying what the significant associations detected by more
482 than one software are. Furthermore, although MultiGWAS check for significative
483 SNPs based on the p-value, it is essential to go back to the data and check if the
484 SNP is a real association between the genotype and phenotype. For this purpose,
485 the SNP profile gives visual feedback for the accuracy of the association.

486 Finally, the MultyGWAS allows comparing among the gene action models that
487 offer GWASPoly and TASSEL. GWASpoly (Rosyara et al., 2016) provides models of
488 different types of polyploid gene action including additive, diploidized additive, du-
489 plex dominant, simplex dominant, and general. On the other hand, TASSEL (Brad-
490 bury et al., 2007) also models different types of gene action for diploids general,
491 additive and dominant. To choose among models, We propose an automatic se-
492 lection of the gene action model for both tools based on a balance between three
493 criteria: the inflation factor, the replicability of identified SNPs and the significance
494 of identified SNPs. This inflation index is a new tool for comparison that do not
495 offer either GWASPoly or TASSEL. This automatic strategy will help to understand
496 the gene action model for the trait of interest. Even though the main focus is on
497 the resultant SNPs, the model has assumptions that reflect the gene actions for a
498 specific phenotype.

499 On the other hand, although MultyGWAS does not solve the uncertainty in
500 the allele dosage and null alleles, However the active comparison among models
501 that MultiGWAS addresses the search of the inheritance mechanisms by comparing
502 among two designed for polysomic inheritance software (Rosyara et al., 2016; Shen

503 et al., 2016) with two software for disomic inheritance (Bradbury et al., 2007; Pur-
504 cell et al., 2007). Understanding the inheritance mechanisms for polyploids organ-
505 ism is an open question. For autopolyploids, most loci have a polysomic heritage.
506 However, sections of the genome that did not duplicate lead to disomic inheritance
507 for some loci (Dufresne et al., 2014; Lynch and Conery, 2000; Ohno, 1970). Thus
508 it is a useful tool for researchers because it looks for significative associations that
509 involve both types of inheritance.

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519 **7 Author Contributions**

520 LG, ICS, and PHRH conceived the idea. LG developed MultiGWAS. MP tested Multi-
521 GWAS. LG, ICS, and PHRH drafted the first version of this manuscript, edited by
522 the other co-authors.

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