MultiGWAS: A tool for GWAS analysis on tetraploid organisms by integrating the results of four GWAS software

L. Garreta[1], I. Cerón-Souza[1], M.R. Palacio[2], and P.H. Reyes-Herrera[1]

[1]Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), CI Tibaitatá, Kilómetro 14, Vía a Mosquera, 250047, Colombia
[2]Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), CI El Mira, Kilómetro 38, Vía Tumaco Pasto, Colombia

June 12, 2020

Abstract

Summary: The Genome-Wide Association Studies (GWAS) are essential to determine the association between genetic variants across individuals. One way to support the results is by using different tools to validate the reproducibility of the associations. Currently, software for GWAS in diploids is well-established but for polyploids species is scarce. Each GWAS software has its characteristics, which can cost time and effort to use them successfully. Here, we present MultiGWAS, a tool to do GWAS analysis in tetraploid organisms by executing in parallel and integrating the results from four existing GWAS software: two available for polyploids (GWASpoly and SHEsis) and two frequently used for diploids (PLINK and TASSEL). The tool deals with all the elements of the GWAS process in the four software, including (1) the use of different control quality filters for the genomic data, (2) the execution of two GWAS models, the full model with control for population structure and individual relatedness and the Naive model without any control. The summary report generated by MultiGWAS provides the user with tables and plots describing intuitively the significant association found by both each one and across four software, which helps users to check for false-positive or false-negative results.

MultiGWAS generates five summary results integrating the four tools. (1) Score tables with detailed information on the associations for each tool. (2) Venn diagrams of shared SNPs among the four tools. (3) Heatmaps

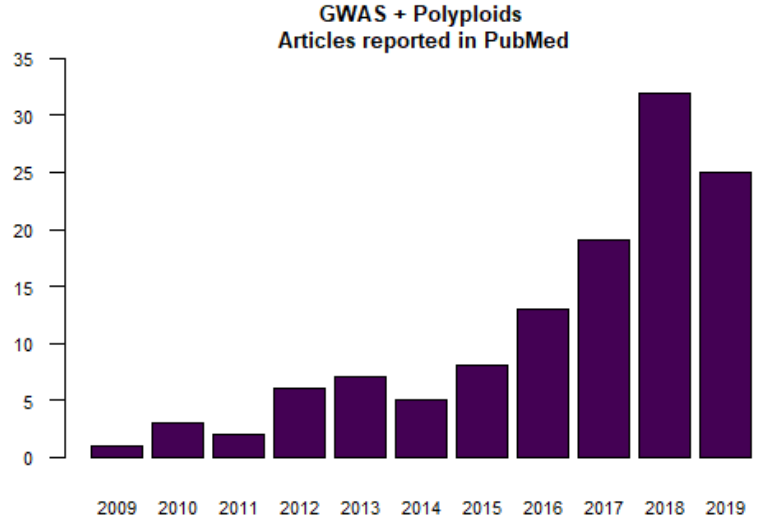


Figure 1: Timeline for articles reported for GWAS studies on polyploid species in PubMed. We present data for completed years.

of significative SNP profiles among the four tools. (4) Manhattan and QQ plots for the association found by each tool. And (5) Chord diagrams for the chromosomes vs. SNP by each tool. **Contact:** phreyes@agrosavia.co

Keywords: GWAS, tetraploids, SNPs,XXX

1 Introduction

The Genome-Wide Association Study (GWAS) is used to identify which variants through the whole genome of a large number of individuals are associated with a specific trait [6, 2]. This methodology started with humans and several model plants, such as rice, maize, and *Arabidopsis* [20, 30, 7, 19, 15]. Because of the advances in the next-gen sequencing technology and the decline of the sequencing cost in recent years, there is an increase in the availability of genome sequences of different organisms at a faster rate [10, 11]. Thus, the GWAS is becoming the standard tool to understand the genetic bases of either ecological or economic phenotypic variation for both model and non-model organisms. This increment in GWAS includes complex species such as polyploids (Fig 1) [10, 26].

The GWAS for polyploid species has three related challenges. First, as all GWAS, we should replicate the study as a reliable method to validate the results

and recognize real associations. This replication involves finding the same associations either in several replicates from the study population using the same software or testing different GWAS tools among the same study population. This approach involved the use of different parameters, models, or conditions, to test how consistent the results are [9, 17]. However, the performance of different GWAS software could affect the results. For example, the threshold $pvalue$ for SNP significance change through four GWAS software (i.e., PLINK, TASSEL, GAPIT, and FaST-LMM) when sample size varies [31]. It means that well-ranked SNPs from one package can be ranked differently in another.

Second, although there are many GWAS software available to repeat the analysis under different conditions [14], most of them are designed exclusively for the diploid data matrix [4]. Therefore, it is often necessary to "diploidizing" the polyploid genomic data in order to replicate the analysis.

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

The iPAT allows running in a graphic interface three well-known command-line GWAS software such as GAPIT, PLINK, and FarmCPU (Chen and Zhang, 2018). However, the output from each package is separated. 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 [13]. Thus, for both software iPAT and easyGWAS, the integrative and comparative outputs among software or algorithms are missing.

To solve all the three challenges above, we developed the MultiGWAS tool that performs GWAS analyses for tetraploid species using four software in parallel. Our tool include GWASpoly [25] and the SHEsis tool [28] that accept polyploid genomic data, and PLINK [23] and TASSEL [5] with the use of a "diploidized" genomic matrix. The tool deals with preprocessing data, running four GWAS tools in parallel, and create comparative reports from the output of each software to help the user to decide more intuitively the true or false associations.

2 Method

The MultiGWAS tool has three main consecutive steps: the adjustment, the multi analysis, and the integration (Fig. 2). In the adjustment step, MultiGWAS processes the configuration file. Then it cleans and filters the genotype and phenotype, and MultiGWAS "diploidize" the genomic data. Next, during the multi analysis, each GWAS tool runs in parallel. Subsequently, in the integration step, the MultiGWAS tool scans the output files from the four packages (i.e., GWASPoly, SHEsis, PLink, and TASSEL). Finally, it generates a summary of all

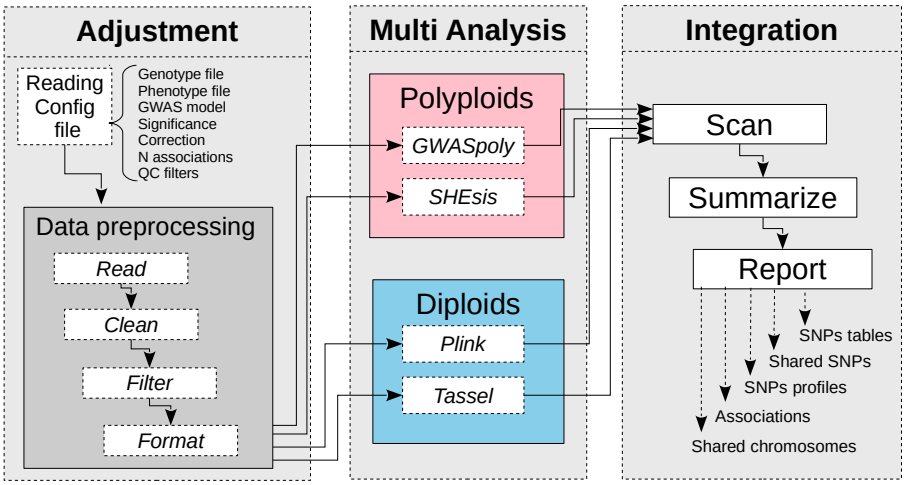


Figure 2: MultiGWAS flowchart has three consecutive steps: adjustment, multi analysis, and integration. The adjustment step manages the input data, reads the configuration file, and preprocessing the input genomic data (genotype and phenotype). The multi analysis step configures and runs the four GWAS packages in parallel. The integration step summarizes and reports results using different tabular and graphical visualizations.

results that contains score tables, Venn diagrams, SNP profiles, and Manhattan plots.

2.1 Adjustment stage

MultiGWAS takes as input a configuration file where the user specifies the genomics data along with the parameters that will be used by the four tools. Once the configuration file is processed, MultiGWAS preprocess the data that is cleaning, filtering, and checking data quality. The output of this stage corresponds to the inputs for the four programs at the Multi Analysis stage.

2.1.1 Reading configuration file

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

Input genotype and phenotype files: Currently, MultiGWAS uses two input files, one for genotype and the other for the phenotype. Both data correspond to data matrices with column and row names (Figure 3). The genotype file uses SNP markers in rows and samples in columns (Figure 3a). The phenotype file uses samples in rows and traits in columns (Figure 3b) with the first column corresponding to the sample name and the second column to trait value.

```
Marker,Chrom,Pos,Indiv01,Indiv02,Indiv03,...
c2_41437,0,805179,AAAG,AAGG,AAGG,...
c2_24258,0,1252430,AAGG,AGGG,GGGG,...
c2_21332,0,3499519,TTCC,TTCC,TTCC,...
...
```

a

```
Individual,Traitname
Indiv01, 3.59
Indiv02, 4.07
Indiv03, 1.05
...
```

b

Figure 3: **MultiGWAS genotype and phenotype formats**. Both files are in CSV format (Comma Separated Values) and contain as first row the header labels of the columns. Although the header labels are arbitrary, the column order is obligatory. **a.** Genotype file format, where "Marker", "Chrom", and "Pos", correspond to the names for marker name, chromosome, and position in the first three columns respectively. The next columns names correspond to the individual names and the column content correspond to the genotype of each individual. **b.** Phenotype file format, where "Individual" and "Traitname" are the column for the individual ID and the column for the numerical value of the trait, respectively.

GWAS model: MultiGWAS is designed to work with quantitative phenotypes and can run GWAS analysis using two types of statistical models that we have called *full* and *naive* models. The *full model* is known in the literature as the Q+K model [32] and includes a control for structure (Q) and relatedness between samples (K). In contrast, the *naive model* does not include any correction. Both models are linear regression approaches, and the four GWAS packages used by MultiGWAS implemented variations of them. The *naive* is modeled with Generalized Linear Models (GLMs, Phenotype + Genotype), and the *full* is modeled with Mixed Linear Models (MLMs, Phenotype + Genotype + Structure + Kinship). The default model used by MultiGWAS is the *full model* (Q+K) [32], following this equation:

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

The vector y represents the observed phenotypes depends on the following factors: the fixed effect vector β, the SNP effects vector α, the population effect vector ν, the polygene background effect vector μ, and, the residual effect vector e. The Q, modeled as a fixed effect, refers to the incidence matrix for subpopulation covariates relating y to ν. Moreover, X, S, and Z are incidence matrices of ones and zeros relating y to β, α, and μ, respectively.

Genome-wide significance: GWAS searches SNPs associated with a phenotype trait in a statistically significant manner. A threshold or significance level α is specified and compared with the *p-value* derived for each association score. Standard significance levels are 0.01 or 0.05 [14, 25], and MultiGWAS uses an α of 0.05 for the four GWAS packages. However, the adjustment of the threshold is according to each package. For example, GWASpoly and TASSEL calculate the SNP effect for each genotypic class using different gene action models (see "Multi analysis stage"). Therefore, the number of tested markers may be different in each model (see below) that results in different *p-value* thresholds.

Multiple testing correction: Due to the massive number of statistical tests performed by GWAS, it is necessary to perform a correction method for multiple hypothesis testing and adjusting the *p-value* threshold accordingly. Two

standard methods for multiple hypothesis testing are the false discovery rate (FDR) and the Bonferroni correction. The latter is the default method used by MultiGWAS because it is one of the most rigorous. MultiGWAS adjust the threshold below which a *p-value* is considered significant, that is α/m, where α is the significance level and *m* is the number of tested markers from the genotype matrix.

Number of reported associations: Criticism has arisen in considering only statistically significant associations as the only possible correct associations [29, 18]. Many low *p-value* associations are closer to being significant, are discarded due to the stringent significance levels, and, consequently, increase the number of false negatives. To help to analyze both significant and non-significant associations, MultiGWAS provides the option to specify the number of best-ranked associations (lower *p-values*), adding the corresponding *p-value* to each association found. In this way, it is possible to enlarge the number of results, and we can observe replicability in the results for different programs. Nevertheless, MultiGWAS always presents each associated SNP with its corresponding *p-value*.

Quality control filters: A control step is necessary to check the input data for genotype or phenotype errors or poor quality that can lead to spurious GWAS results. MultiGWAS provides the option to select and define thresholds for the following filters that control the data quality: Minor Allele Frequency (MAF), individual missing rate (MIND), SNP missing rate (GENO), and HardyWeinberg threshold (HWE):

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

MultiGWAS does the MAF filtering and uses the PLINK package [14] for the other three filters: MIND, GENO, and HWE.

2.1.2 Data preprocessing

Once the configuration file is processed, the genomic data is read and cleaned by selecting individuals present in both genotype and phenotype. Then, based on previous selected quality-control filters and their thresholds, MultiGWAS remove individuals and SNPs with poor quality.

During this step, the format "ACGT" suitable for the polyploid software GWASpoly and SHEsis, is "diploidized" for PLINK and TASSEL. The homozygous tetraploid genotypes are converted to diploid thus: AAAA→AA, CCCC→CC, GGGG→GG, TTTT→TT. Moreover, for tetraploid heterozygous genotypes, the conversion depends on the reference and alternate alleles calculated for each position (e.g., AAAT→AT, ... , CCCG→CG).

After this process, MultiGWAS convert the genotype and phenotype data to the specific formats required for each of the four GWAS packages.

2.2 Multi analysis stage

MultiGWAS runs in parallel using two types of statistical models specified in the parameters file, the Full model (Q+K) and Naive (i.e., without any control) where Q refers to population structure and K refers to relatedness, calculated by kinship coeficients across individuals [27]. The Full model (Q+K) controls for both population structure and individual relatedness. For population structure, MultiGWAS uses the Principal Component Analysis (PCA) and takes the top five PC as covariates. For relatedness, MultiGWAS uses kinship matrices that TASSEL and GWASpoly calculated separately, and for PLINK and SHEsis, relatedness depends on kinship coefficients calculated with the PLINK 2.0 built-in algorithm [8].

2.2.1 GWASpoly

GWASpoly [25] is an R package designed for GWAS in polyploid species used in several studies in plants [3, 12, 27, 33]. GWASpoly uses a Q+K linear mixed model with biallelic SNPs that account for population structure and relatedness. Also, to calculate the SNP effect for each genotypic class, GWASpoly provides eight gene action models: general, additive, simplex dominant alternative, simplex dominant reference, duplex dominant alternative,duplex dominant, diplo-general, and diplo-additive. As a consequence, the number of statistical test performed can be different in each action model and so thresholds below which the *p-values* are considered significant.

MultiGWAS is using GWASpoly version 1.3 with all gene action models available 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 poly-

ploid species. However, their use is mainly in diploids animals and humans [24, 21].

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 [22]. 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 [1, 34]. 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.

2.3 Integration stage.

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

2.3.1 Calculation of *p-values* and significance thresholds

GWAS packages compute *p-value* as a measure of association between each SNP and the trait of interest. The real associations are those their *p-value* drops below a predefined significance threshold. However, the four GWAS packages

compute differently *p-values* with the consequence to compute them too high or too low. If *p-values* is too high, it would lead to false negatives or SNPs with real associations with the phenotype, but that does not reach the significance threshold. Conversely, if *p-values* are too low, then it would lead to false positives or SNPs with false associations with the phenotype, but that reaches the significance threshold.

To overcome these difficulties, in the case of too high *p-values*, MultiGWAS identifies and reports both significant and best-ranked associations (the ones closest to being statistically significant). Whereas, in the case of too low *p-values*, MultiGWAS provides two methods for adjusting *p-values* and significance threshold: the false discovery rate (FDR) that adjust *p-values,* and the Bonferroni correction, that adjusts the threshold.

By default, MultiGWAS uses the Bonferroni correction that uses the significance level α/m (defined by the user in the configuration file), and m (the number of tested markers) to adjust the significance threshold in the GWAS study. However, the significance threshold can be different for each GWAS package as some of them use several action models to calculate the SNP effect of each genotypic class. For both PLINK and SHEsis packages, which use only one model, m is equal to the total number of SNPs. However, for both GWASpoly and TASSEL packages, which use eight and three gene action models, respectively, m is equal to the number of tests performed in each model, which is different between models.

2.3.2 Selection of significant and best-ranked associations

MultiGWAS selects two groups of associations from the results of each GWAS package: statistically significant and best-ranked. The latter equally important to the former as they are associations with lowest *p-values* not reaching the significance threshold but representing interesting associations for further analysis (possible false negatives).

The significant associations are selected from the ones with *p-values* falling below a significant threshold, calculated for each GWAS package; and the best-ranked associations as the *N* ones closer to being statistically significant, with N defined by the user in the configuration file.

The selection of these groups takes into account whether the GWAS package uses only one gene action model, as PLINK and SHEsis do, or uses several ones, as GWASpoly and TASSEL do. In the first case, there is only one resulting set of associations and the selection is straighforward, as described above. However, in the second case, there are several resulting sets of associations (one for each model), and MultiGWAS takes the significant and best-ranked associations from the best gene action model. Action models are scored taking into account the number of shared SNPs between models and the inflation factor, with the following equation:

$$score(M_i) = \frac{\sum_{all\ models} SharedSNPs(M_i)}{\sum_{all\ models} MaxSharedSNPs} + 1 - \mid 1 - \lambda(M_i) \mid$$

where M_i is the gene action model i, with i from $1..n$, for a GWAS package with n gene action models; $SharedSNPs(M_i)$ is the number of SNPs identified at once in both the model M_i and the other models (shared SNPs); $MaxSharedSNPs$ is the max number of shared SNPs by each model; and $\lambda(M_i)$ is the inflation factor for the model M_i.

The score is high when a model M_i both identifies a high number of shared SNPs and has an inflation factor λ close to 1. Conversely, the score is low when the model M_i both identifies a small number of shared SNPs and has an inflation factor λ either low (close to 0) or high ($\lambda > 2$). In any other case, the score is balanced between the number of shared SNPs and the inflation factor.

2.3.3 Integration of results

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

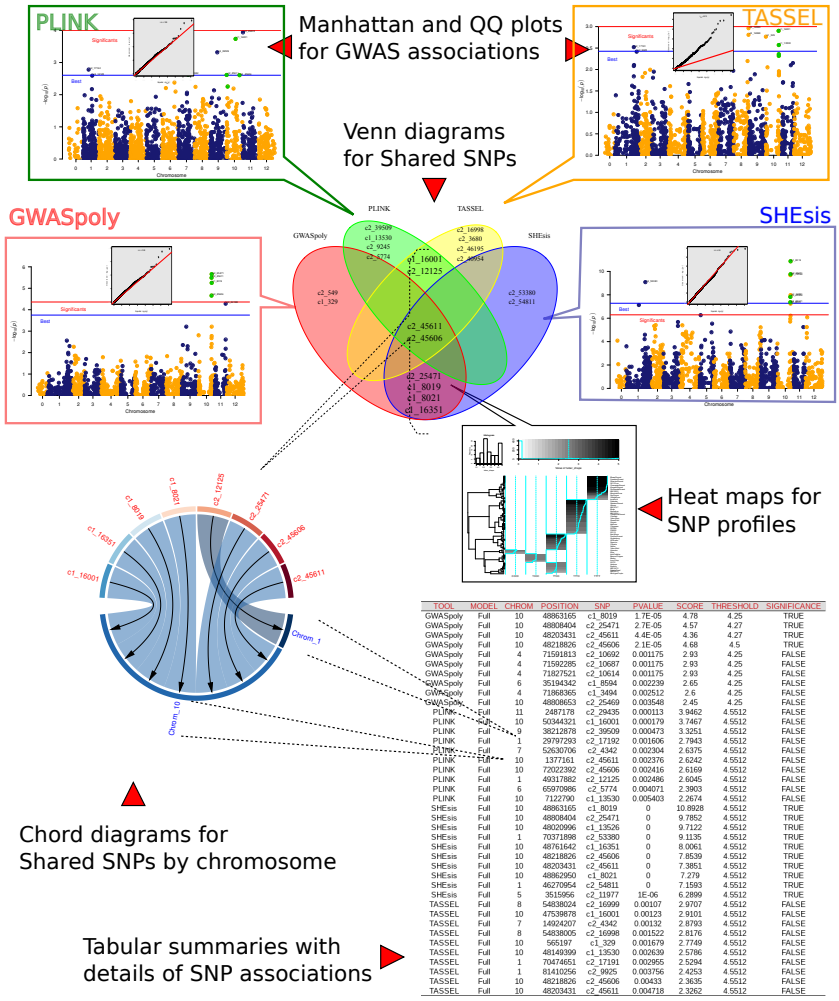
- A Quantile-Quantile (QQ) plots for the resultant *p-values* of each tool and the corresponding inflation factor λ to assess the degree of the test statistic inflation.
- A Manhattan plot of each tool with two lower thresholds, one for the best-ranked SNPs, and another for the significant SNPs.

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

For each SNP identified more than once, we provide what we call the SNP profile. That is a heat diagram for a specific SNP, where each column is a genotype state AAAA, AAAB, AABB, ABBB, and BBBB. Moreover, each row corresponds to a sample. Samples with close genotypes form together clusters. Thus to generate the clusters, we do not use the phenotype information. However, we present the phenotype information in the figure as the color. This figure visually provides information regarding genotype and phenotype information simultaneously for the whole population. We present colors as tones between white and black for color blind people.

MultiGWAS generates a report, one document with the content previously described. Besides, there is a folder with the individual figures just in case the user needs one. In the supplementary information, we include a report and a description of the report content (supplementary information XXX)

In the following section, we present the results applied to a public dataset.



TOOL	MODEL	CHROM	POSITION	SNP	PVALUE	SCORE	THRESHOLD	SIGNIFICANCE
GWASpoly	Full	10	48863165	c1_8019	1.7E-05	4.78	4.25	TRUE
GWASpoly	Full	10	48808404	c2_25471	2.7E-05	4.57	4.27	TRUE
GWASpoly	Full	10	48203431	c2_45611	4.4E-05	4.36	4.27	TRUE
GWASpoly	Full	10	48218826	c2_45606	2.1E-05	4.68	4.5	TRUE
GWASpoly	Full	4	71591813	c2_10692	0.001175	2.93	4.25	FALSE
GWASpoly	Full	4	71592285	c2_10687	0.001175	2.93	4.25	FALSE
GWASpoly	Full	4	71827521	c2_10614	0.001175	2.93	4.25	FALSE
GWASpoly	Full	6	35194342	c1_8594	0.002239	2.65	4.25	FALSE
GWASpoly	Full	4	71868365	c1_3494	0.002512	2.6	4.25	FALSE
GWASpoly	Full	10	48808653	c2_25469	0.003548	2.45	4.25	FALSE
PLINK	Full	11	2487178	c2_29435	0.000113	3.9462	4.5512	FALSE
PLINK	Full	10	50344321	c1_16001	0.000179	3.7467	4.5512	FALSE
PLINK	Full	9	38212878	c2_39509	0.000473	3.3251	4.5512	FALSE
PLINK	Full	1	29797293	c2_17192	0.001606	2.7943	4.5512	FALSE
PLINK	Full	7	52630706	c2_4342	0.002304	2.6375	4.5512	FALSE
PLINK	Full	10	1377161	c2_45611	0.002376	2.6242	4.5512	FALSE
PLINK	Full	10	72022392	c2_45606	0.002416	2.6169	4.5512	FALSE
PLINK	Full	1	49317882	c2_12125	0.002486	2.6045	4.5512	FALSE
PLINK	Full	6	65970986	c2_5774	0.004071	2.3903	4.5512	FALSE
PLINK	Full	10	7122790	c1_13530	0.005403	2.2674	4.5512	FALSE
SHEsis	Full	10	48863165	c1_8019	0	10.8928	4.5512	TRUE
SHEsis	Full	10	48808404	c2_25471	0	9.7852	4.5512	TRUE
SHEsis	Full	10	48020996	c1_13526	0	9.7122	4.5512	TRUE
SHEsis	Full	1	70371898	c2_53380	0	9.1135	4.5512	TRUE
SHEsis	Full	10	48761642	c1_16351	0	8.0061	4.5512	TRUE
SHEsis	Full	10	48218826	c2_45606	0	7.8539	4.5512	TRUE
SHEsis	Full	10	48203431	c2_45611	0	7.3851	4.5512	TRUE
SHEsis	Full	10	48862950	c1_8021	0	7.279	4.5512	TRUE
SHEsis	Full	1	46270954	c2_54811	0	7.1593	4.5512	TRUE
SHEsis	Full	5	3515956	c2_11977	1E-06	6.2899	4.5512	TRUE
TASSEL	Full	8	54838024	c2_16999	0.00107	2.9707	4.5512	FALSE
TASSEL	Full	10	47539878	c1_16001	0.00123	2.9101	4.5512	FALSE
TASSEL	Full	7	14924207	c2_4342	0.00132	2.8793	4.5512	FALSE
TASSEL	Full	8	54838005	c2_16998	0.001522	2.8176	4.5512	FALSE
TASSEL	Full	10	565197	c1_329	0.001679	2.7749	4.5512	FALSE
TASSEL	Full	10	48148399	c1_13530	0.002639	2.5786	4.5512	FALSE
TASSEL	Full	1	70474651	c2_17191	0.002955	2.5294	4.5512	FALSE
TASSEL	Full	1	81410256	c2_9925	0.003756	2.4253	4.5512	FALSE
TASSEL	Full	10	48218826	c2_45606	0.00433	2.3635	4.5512	FALSE
TASSEL	Full	10	48203431	c2_45611	0.004718	2.3262	4.5512	FALSE

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.

3 Results

Most of the GWAS packages used by MultiGWAS are based on a linear regression approaches, but they often produce dissimilar association results for the same input. For example, computed *p-values* for the same set of SNPs are different between packages; SNPs with significant *p-values* for one package may be not significant for the others; or well-ranked SNPs in one package may be ranked differently in another.

To alleviate these difficulties, MultiGWAS produces five types of outputs using different graphics and tabular views, these outputs are intended to help users to compare, select, and interpret the set of possible SNPs associated with a trait of interest. The outputs include:

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

As an example of the functionality of the tool, here we show the outputs reported by MultiGWAS in the tetraploid potato diversity panel, genotyped and phenotyped as part of the USDA-NIFA Solanaceae Coordinated Agricultural Project (SolCAP) [16]. The complete report from MultiGWAS for the naive and full model is in the Supplementary information (`https://github.com/agrosavia-bioinformatics/multiGWAS`)

3.1 Manhattan and QQ plots for GWAS associations

MultiGWAS uses classical Manhattan and Quantile–Quantile plots (QQ plots) to visualize the results of GWAS analysis from each package. In both plots, SNPs are represented by dots and their *p-values* are transformed to scores as $-log_{10}(p\text{-}values)$ (see Figure 5). The Manhattan plot displays the SNP association strength (y-axis) distributed in their genomic location (x-axis), so the higher the score the stronger the association. Whereas the QQ plot is used to visually compare the expected distribution of *p-values* (y-axis) vs. the observed distribution (x-axis), so under the null hypothesis of no association of SNPs with the phenotype, both distributions should coincide, and most SNPs should lie on a diagonal line.

MultiGWAS adds special marks to the Manhattan and QQ plots to help identify different types of SNPs: (a) In Manhattan plots, significant SNPs are above a red line, best-ranked SNPs are above a blue line, and shared SNPs (See Figure

6.b) are colored in green (b) In QQ plots, a red diagonal line indicates the expectation, so potential associations can be observed when the number of SNPs deviating from the diagonal is small, as in the case of monogenic traits, or when this number is somewhat higher, as in the case of truly polygenic traits. However, deviations for a high number of SNPs could reflect inflated *p-values* owing to population structure or cryptic relatedness.

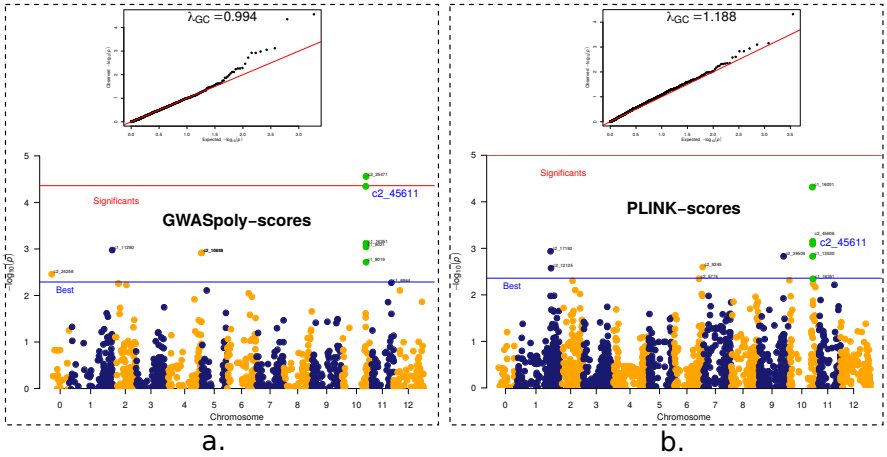


Figure 5: MultiGWAS visualization of associations. MultiGWAS creates Manhattan and QQ plots for GWAS results of each GWAS packages. Here we show the plots for one tetraploid package, GWASpoly (a), and other diploid package, PLINK (b).

3.2 Tables and Venn diagrams for single and shared SNPs

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

First, best-ranked SNPs correspond to the top-scored *N* SNPs, wheter they were assesed significant or not by its package, and with *N* defined by the user in the configuration file. These SNPs are shown both in a SNPs table (Figure 6.a) and in a Venn diagram (Figure 6.b). The table lists them by package and sorts by decreasing score, whereas the Venn diagram shows them emphasizing if they were best-ranked either in a single package or in several at once (shared). And second, the significant SNPs correspond to the ones assesed statistically significant by each package, they are shown in a Venn diagram (Figure 6.c), and they are also shown in the SNPs table, marked with significance TRUE (T) in the table of the Figure6.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	T
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	T
GWASpoly	additive	0.96	c2_22188	11	40777	0.000050	4.30	4.50	F
GWASpoly	additive	0.96	c2_549	9	16527	0.000589	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	T
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	T
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	T
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

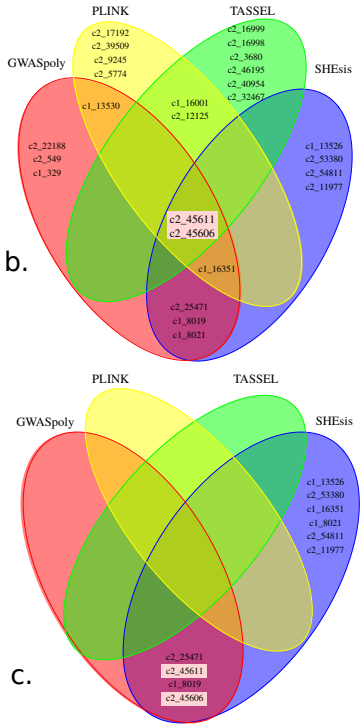


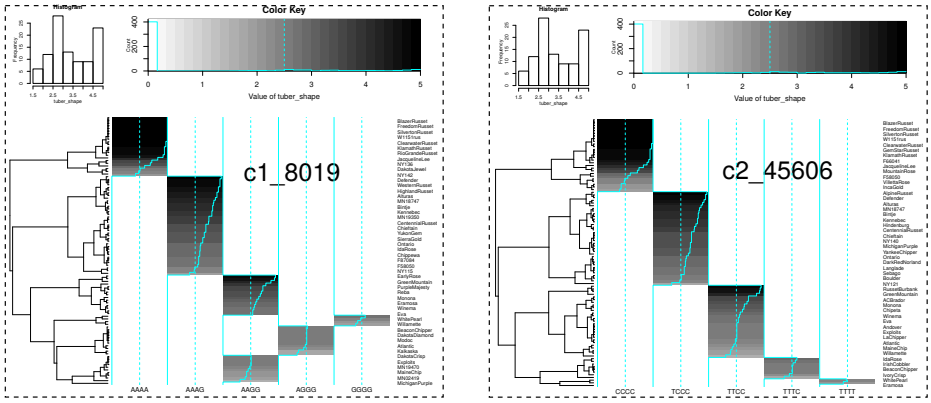
Figure 6: Shared SNPs Views. Tabular and graphical views of SNP associations identified by one or more GWAS packages (shared SNPs). SNPs identified by all packages are marker with red background in all figures **(a)** Table with details of the N=9 best-ranked SNPs from each GWAS package. Each row corresponds to a single SNP and the 9 columns are: tool name, 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 located in the central intersection. Other SNPs identified by more than one packages are located in both upper central and lower central intersections. **(c)** Venn diagram of the significant SNPs (score > threshold).

3.3 Heat diagrams for structure of shared SNPs

MultiGWAS creates a two-dimensional representation, called 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, ABBB, 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 column, there is a solid cyan line with the mean of each column and a broken cyan line that indicates how far the cell deviates from the mean.

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



a. b.

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).

3.4 Chord diagrams for SNPs by chromosome

Generally, in a typical GWAS analysis the strongest associations are signaled by several nearby-correlated SNPs located in the same chromosome, as in manhattan plots, where these associations form neat peaks with several SNPs showing the same signal. Conversely, no peaks are shown when few SNPs correlate with a trait.

However, when the analysis is performed by several GWAS packages, as MultiGWAS does, it can identify correlated SNPs between packages that show the same signal, what is presented by MultiGWAS through chord diagrams. For exampele, the Figure 8.a shows the chord diagram for the shared SNPs from the best-ranked associations previously described in the Figure 6.b. It can be observed that most SNPs relate to chromosome 10 and only one to chromosome 1, which is also observed in the manhattan plots from each GWAS package (Figure 8.b).

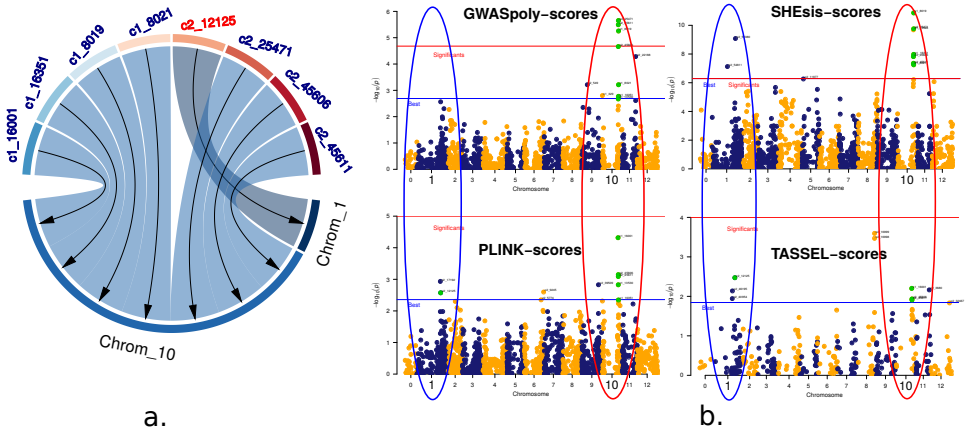


Figure 8: SNPs by chromosome. The figure shows how the best-ranked SNPs relate to chromosomes. **(a)** Chord diagram showing that most SNPs related to chromosome 10. SNPs are at the top of the diagram, chromosomes at the botton, and associations are represented by arrows drawn from SNPs to their chromosomes. The more associations identified in one chromosome, the wider the space of its sector. **(b)** Manhattan plots from each GWAS packages showing two important locations of associations: chromosome 1 and chromosome 10, marked with a blue and red ellipsis, respectively.

4 Availability and Implementation

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

4.1 Input parameters

MutiGWAS uses as the only input a simple configuration text file where users set the values for the main parameters that drives the GWAS process. The file can be created either using a general text editor or using the MultiGWAS GUI application (see below). In both cases, the file must have the structure shown in the Figure 9.a, where parameter names and values are separated by colon, filenames are enclosed in quotation marks, and TRUE or FALSE indicates wheter filters are applied or not. In the second case, the user creates the config file in a simple and straightforward way using the input parameter view from the GUI application (see below).

```
default:
  genotypeFile     : "example-genotype.tbl"
  phenotypeFile    : "example- phenotype.tbl"
  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 output folder where results will be written, input genotype/phenotype filenames, genome-wide significance threshold, method for multiple testing correction, GWAS model, number of associations to be reported, filtering with TRUE or FALSE whether to use quality control filters or not. The filters are: minor allele frequency, individual missing rate, SNP missing rate, and Hardy-Weinberg threshold. At the end the tools parameter defines the GWAS packages to be used for the analysis.

4.2 Using the command line interface

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

```
multiGWAS Test01.config
```

Then, the tool starts the execution, showing information of the process in the console window, and when it finishes the results are saved to a new subfolder called *"out-Test01*. Results include a full html report containing the different views described in the results section, along with the original graphics and summary tables created by MultiGWAS and used to create the html report. Additionally, results include the preprocessed tables of the main outputs generated by the four GWAS packages used by MultiGWAS.

4.3 Using the graphical user interface

The MultiGWAS GUI can be executed by calling from a linux console the following command:

```
jmultiGWAS
```

After it opens, it shows a main frame with a tool bar at left and four tabs at the top (Figure 10). From the tool bar, users can select the GWAS packages to use in the analysis–two for tetraploids and two for diploids–, and start the analysis with the current parameters (or with parameters from a previous configuration). And, from the tabs, users can input the MultiGWAS parameters, and view the process and results of the analysis.

.

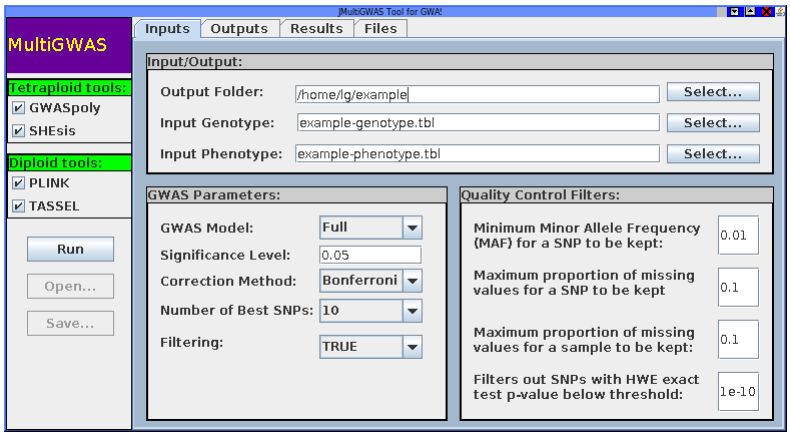


Figure 10: MultiGWAS GUI application. Main view of the MultiGWAS GUI application ("Inputs" view) where users can create the configuration file by setting values for input parameters. The GUI contains other three views: "Outputs" view shows the logs of the running process. "Results" view shows a report in html format with the tabular and graphics described in the results section. And, the "Files" view shows an embedded file manager pointing to the subfolder that contains the files created by MultiGWAS and used to create the report.

5 Discussion

XXXXXXXXXXXXXXXXXXXXXXX

References

[1] María F. Álvarez et al. "Identification of Novel Associations of Candidate Genes with Resistance to Late Blight in Solanum tuberosum Group Phureja". In: *Frontiers in Plant Science* 8 (2017), p. 1040. ISSN: 1664-462X. DOI: 10.3389/fpls.2017.01040. URL: http://journal.frontiersin.org/article/10.3389/fpls.2017.01040/full.

[2] Ferdouse Begum et al. "Comprehensive literature review and statistical considerations for GWAS meta-analysis". In: *Nucleic acids research* 40.9 (2012), pp. 3777–3784.

[3] Jhon Berdugo-Cely et al. "Genetic diversity and association mapping in the colombian central collection of solanum tuberosum L. Andigenum group using SNPs markers". In: *PLoS ONE* 12.3 (2017). ISSN: 19326203. DOI: 10.1371/journal.pone.0173039.

[4] Peter M. Bourke et al. "Tools for Genetic Studies in Experimental Populations of Polyploids". In: *Frontiers in Plant Science* 9 (2018), p. 513. ISSN: 1664-462X. DOI: 10.3389/fpls.2018.00513. URL: http://journal.frontiersin.org/article/10.3389/fpls.2018.00513/full.

[5] Peter J Bradbury et al. “TASSEL: software for association mapping of complex traits in diverse samples”. In: *Bioinformatics* 23.19 (2007), pp. 2633–2635. ISSN: 1367-4803. DOI: 10.1093/bioinformatics/btm308. URL: https://doi.org/10.1093/bioinformatics/btm308.

[6] Rita M Cantor, Kenneth Lange, and Janet S Sinsheimer. “Prioritizing GWAS results: a review of statistical methods and recommendations for their application”. In: *The American Journal of Human Genetics* 86.1 (2010), pp. 6–22.

[7] Jun Cao et al. “Whole-genome sequencing of multiple Arabidopsis thaliana populations”. In: *Nature genetics* 43.10 (2011), p. 956.

[8] Christopher C. Chang et al. “Second-generation PLINK: Rising to the challenge of larger and richer datasets”. In: *GigaScience* 4.1 (2015), pp. 1–16. ISSN: 2047217X. DOI: 10.1186/s13742-015-0047-8. arXiv: 1410.4803.

[9] Rishika De, William S Bush, and Jason H Moore. “Bioinformatics Challenges in Genome-Wide Association Studies (GWAS)”. In: *Clinical Bioinformatics*. Ed. by Ronald Trent. New York, NY: Springer New York, 2014, pp. 63–81. ISBN: 978-1-4939-0847-9. DOI: 10.1007/978-1-4939-0847-9_5. URL: https://doi.org/10.1007/978-1-4939-0847-9%7B%5C_%7D5.

[10] Robert Ekblom and Juan Galindo. “Applications of next generation sequencing in molecular ecology of non-model organisms”. In: *Heredity* 107.1 (2011), pp. 1–15.

[11] Hans Ellegren. “Genome sequencing and population genomics in non-model organisms”. In: *Trends in ecology & evolution* 29.1 (2014), pp. 51–63.

[12] Luís Felipe V. Ferrão et al. “Insights Into the Genetic Basis of Blueberry Fruit-Related Traits Using Diploid and Polyploid Models in a GWAS Context”. In: *Frontiers in Ecology and Evolution* 6 (2018), p. 107. ISSN: 2296-701X. DOI: 10.3389/fevo.2018.00107. URL: https://www.frontiersin.org/articles/10.3389/fevo.2018.00107/full.

[13] Dominik G Grimm et al. “easyGWAS: A Cloud-Based Platform for Comparing the Results of Genome-Wide Association Studies”. In: *The Plant Cell* 29.1 (2017), pp. 5–19. ISSN: 1040-4651. DOI: 10.1105/tpc.16.00551. URL: http://www.plantcell.org/content/29/1/5.

[14] Anja C Gumpinger et al. *Methods and Tools in Genome-wide Association Studies*. Vol. 1819. 2018. ISBN: 9781493986187.

[15] Bin Han and Xuehui Huang. “Sequencing-based genome-wide association study in rice”. In: *Current opinion in plant biology* 16.2 (2013), pp. 133–138.

[16] Candice N. Hirsch et al. “Retrospective view of North American potato (Solanum tuberosum L.) breeding in the 20th and 21st centuries”. In: *G3: Genes, Genomes, Genetics* 3.6 (2013), pp. 1003–1013. ISSN: 21601836. DOI: 10.1534/g3.113.005595.

[17] "How to interpret a genome-wide association study". In: *JAMA - Journal of the American Medical Association* 299.11 (2008), pp. 1335–1344. ISSN: 00987484. DOI: 10.1001/jama.299.11.1335.

[18] Avjinder S Kaler and Larry C Purcell. "Estimation of a significance threshold for genome-wide association studies". In: *BMC Genomics* 20.1 (2019), p. 618. ISSN: 1471-2164. DOI: 10.1186/s12864-019-5992-7. URL: https://doi.org/10.1186/s12864-019-5992-7.

[19] Arthur Korte and Ashley Farlow. "The advantages and limitations of trait analysis with GWAS: a review". In: *Plant methods* 9.1 (2013), p. 29.

[20] Gordan Lauc et al. "Genomics meets glycomics—the first GWAS study of human N-glycome identifies HNF1α as a master regulator of plasma protein fucosylation". In: *PLoS genetics* 6.12 (2010).

[21] Jie Meng et al. "Genome-wide association analysis of nutrient traits in the oyster Crassostrea gigas: Genetic effect and interaction network". In: *BMC Genomics* 20.1 (2019), pp. 1–14. ISSN: 14712164. DOI: 10.1186/s12864-019-5971-z.

[22] Robert A. Power, Julian Parkhill, and Tulio De Oliveira. "Microbial genome-wide association studies: lessons from human GWAS". In: *Nature Reviews Genetics* 18.1 (2016), pp. 41–50. ISSN: 14710064. DOI: 10.1038/nrg.2016.132.

[23] Shaun Purcell et al. "PLINK: A tool set for whole-genome association and population-based linkage analyses". In: *American Journal of Human Genetics* 81.3 (2007), pp. 559–575. ISSN: 00029297. DOI: 10.1086/519795.

[24] Hui Ping Qiao et al. "Genetic variants identified by GWAS was associated with colorectal cancer in the Han Chinese population". In: *Journal of Cancer Research and Therapeutics* 11.2 (2015), pp. 468–470. ISSN: 19984138. DOI: 10.4103/0973-1482.150346.

[25] Umesh R. Rosyara et al. "Software for Genome-Wide Association Studies in Autopolyploids and Its Application to Potato". In: *The Plant Genome* 9.2 (2016), pp. 1–10. ISSN: 1940-3372. DOI: 10.3835/plantgenome2015.08.0073. URL: https://dl.sciencesocieties.org/publications/tpg/abstracts/9/2/plantgenome2015.08.0073.

[26] Anna W Santure and Dany Garant. "Wild GWAS—association mapping in natural populations". In: *Molecular ecology resources* 18.4 (2018), pp. 729–738.

[27] Sanjeev Kumar Sharma et al. "Linkage disequilibrium and evaluation of genome-wide association mapping models in tetraploid potato". In: *G3: Genes, Genomes, Genetics* 8.10 (2018), pp. 3185–3202. ISSN: 21601836. DOI: 10.1534/g3.118.200377.

[28] Jiawei Shen et al. "SHEsisPlus, a toolset for genetic studies on polyploid species". In: *Scientific Reports* 6 (2016), pp. 1–10. ISSN: 20452322. DOI: 10.1038/srep24095. URL: http://dx.doi.org/10.1038/srep24095.

[29] John R Thompson, John Attia, and Cosetta Minelli. “The meta-analysis of genome-wide association studies”. In: *Briefings in Bioinformatics* 12.3 (2011), pp. 259–269. ISSN: 1467-5463. DOI: 10.1093/bib/bbr020. URL: https://doi.org/10.1093/bib/bbr020.

[30] Feng Tian et al. “Genome-wide association study of leaf architecture in the maize nested association mapping population”. In: *Nature genetics* 43.2 (2011), pp. 159–162.

[31] Yan Y. Yan et al. “Effects of input data quantity on genome-wide association studies (GWAS)”. In: *International Journal of Data Mining and Bioinformatics* 22.1 (2019), pp. 19–43. ISSN: 17485681. DOI: 10.1504/IJDMB.2019.099286.

[32] J Yu et al. “A unified mixed-model method for association mapping that accounts for multiple levels of relatedness.” In: *Nature genetics* 38.2 (2006), pp. 203–208.

[33] Jiazheng Yuan et al. “Genome-Wide Association Study of Resistance to Potato Common Scab”. In: *Potato Research* (2019). ISSN: 18714528. DOI: 10.1007/s11540-019-09437-w.

[34] Shengkui Zhang et al. “Genome-wide association studies of 11 agronomic traits in cassava (Manihot esculenta crantz)”. In: *Frontiers in Plant Science* 9.April (2018), pp. 1–15. ISSN: 1664462X. DOI: 10.3389/fpls.2018.00503.