

MultiGWAS: A tool for GWAS analysis on tetraploid organisms by integrating -four GWAS software

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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 perform GWAS analysis in tetraploid organisms by executing in parallel and integrating the results from four existing GWAS packages: 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 packages, including (1) Genomica data management from different input formats (2) Data cleaning using different control quality filters, (3) Data conversion for package file formats (4) GWAS Execution in the for packages using two GWAS models, the full model with control for population structure and individual relatedness and the Naive model without any control (5) Summary statisticswith tables and plots describing intuitively the significant association found by both each one and across four packages, which helps users to check for false-positive or false-negative results.

1 Introduction

The Genome-Wide Association Studies (GWAS) is used to identify which variants through the whole genome of a large number of individuals are associated with a specific trait (CITES). This methodology started with humans and several model plants, such as rice, maize, and *Arabidopsis* [26, 14]. Because of the advances in the next-gen sequencing technology and the decreasing of the sequencing cost in recent years, there is an increase in genome sequences in non-model organisms at a faster rate [7, 8]. Therefore, several research projects pursue, for the first time, the genetic bases of an ecological or economic phenotypic variation through GWAS analysis for non-model wild plants and crops that often are polyploids [7, 21].

The GWAS for polyploid species has three related challenges. First, as all GWAS analyses, 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 an independent population sample under the same software or using an independent technology using the same population sample [6, 16]. Both approaches involve the use of different parameters, models, or conditions, to test how consistent the results are.

Second, although there are many GWAS packages available to repeat the analysis under different conditions [11], most of them are designed exclusively for the diploid **data matrix** [3]. Therefore, it is often necessary to "diploidizing" the polyploid genomic data in order to replicate the analysis.

Third, the performance of different GWAS software could affect the results. However, there are few tools focused in the integration of the GWAS software outputs to make comparisons under different parameters and conditions. For example, comparing four packages for diploid species (i.e., PLINK, TASSEL, GAPIT, and FaST-LMM) using different sample sizes for plant data found that the threshold *Pvalue* for SNP significance change for each package [27]. It means that well-ranked SNPs from one package can be ranked differently in another, causing difficulty in selecting the most credible associations when results from each package are analyzed separately. Besides, the software iPAT facilitates the use of three popular command-line GWAS packages GAPIT, PLINK, and FarmCPU [30]. However, results from the execution of each package are separated. Therefore, the problem of interpreting and selecting the best associations persists. In comparison, the easyGWAS cloud platform, perform, share, and compare the results of GWAS [10]. This platform offers two types of analysis: the first is an intersection analysis that searches associations that were found significant in more than one dataset. The second is a meta-analysis that searches associations mutually supported by several datasets. Both types are based on different datasets with the same GWAS parameters to confirm or search for new associations. The limitation of this approach is when the data set is unique or has a small sample of individuals.

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 [20] and the SHEsis tool [23] that accept polyploid genomic data, and PLINK [18] and TASSEL [4] with the use of a "diploidized" genomic matrix. The tool deals with preprocessing data, running four GWAS tools in parallel, and create reports that compare the results and help the user decide more intuitively the true or false associations.

2 Methods

The MultiGWAS tool has three main consecutive steps: the adjustment, the multi analysis, and finally, the integration (Fig. 1). In the adjustment step, MultiGWAS processes the configuration file, cleans and filters the genotype and phenotype, and "diploidize" the genomic data. Then, during the multi analysis, each GWAS tool runs in parallel. Finally, in the integration step, the MultiGWAS tool scans the

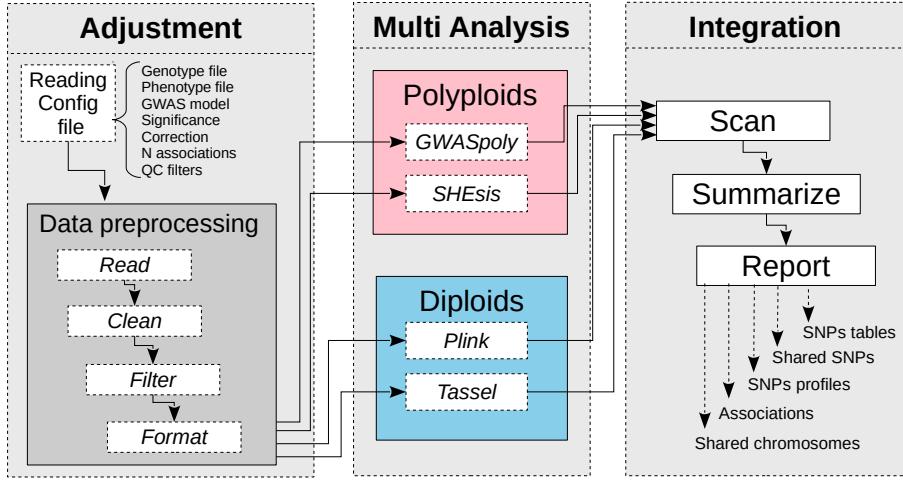


Figure 1: MultiGWAS flowchart has three steps: adjustment, multi analysis, and integration. The first step deals with input data management, reading the configuration file and preprocessing the input genomic data (genotype and phenotype). The second step deals with GWAS analysis, configuring and running the four packages in parallel. And the third step deals with summarizing and reporting results using different tabular and graphical visualizations.

output files from the four packages (i.e., GWASPoly, SHEsis, PLink, and TASSEL).

MultiGWAS generates a summary of all results that contains the following tabular and graphical visualizations: score tables with detailed information of associations, shared SNPs visualization using Venn diagrams, SNP profiles using heatmaps, associations visualizations using Manhattan and QQ plots, and chromosomes vs SNP visualizations using chord diagrams.

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 read and processed, the genomic data files (genotype and phenotype) are preprocessed by 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 2). The genotype file uses SNP markers in rows and samples in columns (Figure 2a). The phenotype file uses samples in rows and traits in columns (Figure 2b) with the first column corresponding to the sample name and the second column to trait value.

| | |
|--|--|
| Marker,Chrom,Pos,Indiv01,Indiv02,Indiv03,... | Individual,Traitname Indiv01, 3.59 Indiv02, 4.07 Indiv03, 1.05 ... |
| a | b |

Figure 2: 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 correspond to the columns for the samples content. **b.** Phenotype file format, where “Individual” and “Traitname” are the column names for the individual and trait names, 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 [28] and includes control for structure (Q) and relatedness between samples (K), whereas the *naive model* does not include any type of correction. Both models are based on linear regression approaches and variations of them are implemented by the four GWAS packages used by MultiGWAS. 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) [28], which is expressed with the following equation:

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

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

Genome-wide significance: GWAS searches SNPs associated with the phenotype 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 [11, 20], and MultiGWAS uses an α of 0.05 for the four GWAS packages. But the threshold is adjusted according to each package, as some packages as GWASpoly and TASSEL calculates the SNP effect for each genotypic class using different gene action models (see “Multi analysis stage”). So, 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 common methods for multiple hypothesis testing are the false discovery rate (FDR) and the

Bonferroni correction. The latter is the default method used by MultiGWAS, which is one of the most stringent methods. However, instead of adjusting the *p-values*, 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 [25, 13]. Many of low *p-value* associations, closer to being significant, are discarded due to the stringent significance levels, and consequently increasing 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, we present each association with the 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 Hardy-Weinberg 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 [11] 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, individuals and SNPs with poor quality are removed by considering the previous selected quality-control filters and their thresholds,

At this point, 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,

$TTTT \rightarrow TT$. Moreover, for tetraploid heterozygous genotypes, the conversion depends on the reference and alternate alleles calculated for each position (e.g., $AAAT \rightarrow AT, \dots, CCCG \rightarrow CG$).

After this process, the genomic data, genotype and phenotype, are converted 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) [22]. 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 of kinship coefficients calculated with the PLINK 2.0 built-in algorithm [5]**.

be integrated in our multiGWAS tool, two designed specifically for polyploid species as many important crops are polyploids: GWASpoly [20] and SHEsis [24], and another two designed for diploids species and extensively used in humans and plants: PLINK [18, 5] and TASSEL [4], respectively.

As MultiGWAS implements two types of GWAS analysis, naive and full, each tool is called in two different ways.

2.2.1 GWASpoly

GWASpoly [20] is an R package designed for GWAS in polyploid species used in several studies in plants [2, 9, 22, 29]. 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, and duplex dominant. 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, employing all gene action models to find associations and reporting the top N best-ranked (the SNPs with lowest p-values), where N is defined by the user in the 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 another program designed for polyploid species that includes single locus association analysis, among others. It is based on a linear regression model, and it has been used in some studies of animals and humans [19, 15].

MultiGWAS is using the version 1.0 which does not take account for population structure or relatedness, however 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 diploids species. It was developed for humans but it is applicable to any species [17]. PLINK includes a range of analysis, 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 is used to achieve both types of analysis, naive and full. For the full analysis, population structure is estimated using the first five principal components calculated with the PLINK 1.9 built in algorithm. But relatedness is estimated from the kinship coefficients calculated with the PLINK 2.0 built in algorithm, removing the close relatives or individuals with first-degree relatedness.

2.2.4 TASSEL

TASSEL is another common GWAS program based on the Java software. It was developed for maize and it has been used in several studies in plants [1, 30], but like PLINK, it is applicable to any species. For association analysis, TASSEL includes the general lineal model (GLM) and mixed linear model (MLM) that accounts for population structure and relatedness. *And, in the same manner that GWASPoly, TASSEL provides three gene action models to calculate the SNP effect of each genotypc class: general, additive, and dominant, and so the significance threshold depends of 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 is achieved by the GLM, and full GWAS is achieved by 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 individual SNP and the trait of interest. The SNPs are considered statistically significant,

and so possible true associations, when their *p-value* drops below a predefined significance threshold. But, most GWAS packages compute differently *p-values* with the possibility to compute them too high or too low. If *p-values* are too high, then it would lead to false negatives or SNPs with true 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 closer 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 in which the significance threshold is adjusted as α/m , where α is the significance level defined by the user in the configuration file, and m is the number of tested markers 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, but for both GWASpoly and TASSEL packages, which use eight and three gene action models, respectively, m is equal to the number of test performed in each model, which is different between models.

2.3.2 Selection of significant and best-ranked associations

After corrections, significant associations are selected as the ones with *p-values* falling below a significant threshold, which is calculated for each GWAS package. But, as described above, it is equally important to know the best-ranked associations, closer to being statistically significant, as they may represent important associations to consider for posterior analysis.

In the case of GWAS packages with only one gene action model (PLINK and SHESIS), the best-ranked associations are selected from the top N identified by the package. But, in the case of GWAS packages with several gene action models (GWASpoly and TASSEL), the best-ranked associations are selected as the top N from the “best action model”, the one with more shared SNP associations, in other words, from the action model that identifies more associations that are also identified in the other models.

2.3.3 Integration of results

At this stage, **MultiGWAS integrates** the results to evaluate reproducible results among tools (Fig 3). But, it still reports a summary for 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 asses the degree of the test statistic inflation.

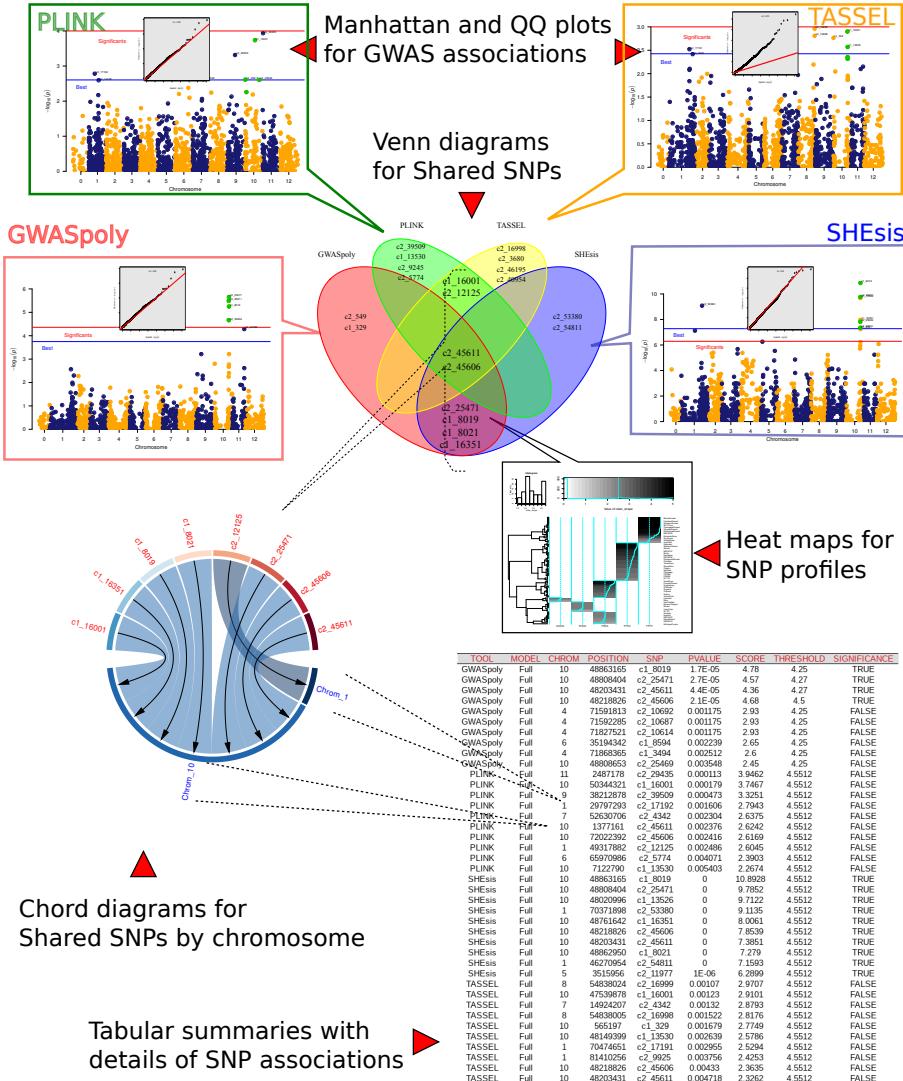


Figure 3: Reports presented by MultigWAS. For each tool, first a QQ plot that assesses the resultant p-values. Second, a Manhattan plot for each tool with two lines, blue and red, respectively, is the lower limit for the best ranked and significative SNPs. We present two Venn diagrams, one for the significative SNPs and one for N best-ranked SNPs of each tool. We show the results for GWAStoly, PLINK, Tassel, and SHEsis in red, green, yellow, and blue, respectively. For each SNP that is in the intersection; thus, that is predicted by more than one tool we provide SNP profile. Chord diagrams for SNPs by chromosome showing how the strongest associations are mostly found on few chromosomes. And we also present tabular summaries with details of significant and best-ranked associations.

- 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. In addition, 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. And 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.

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) [12]. 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 4). 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 5.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.

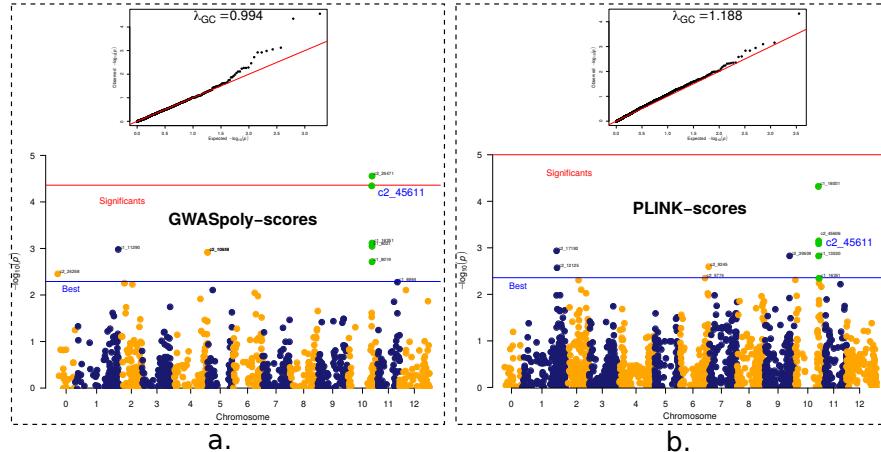


Figure 4: MultiGWAS visualization of associations. MultiGWAS creates Manhattan and QQ plots for GWAS results of each GWAS packages. Here we show the plots for one tetraploid package, GWASPoly (a), and other diploid package, PLINK (b).

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 5). 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, whether they were assessed 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 5.a) and in a Venn diagram (Figure 5.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 assessed statistically significant by each package, they are shown in a Venn diagram (Figure 5.c), and they are also shown in the SNPs table, marked with significance TRUE (T) in the table of the Figure 5.a.

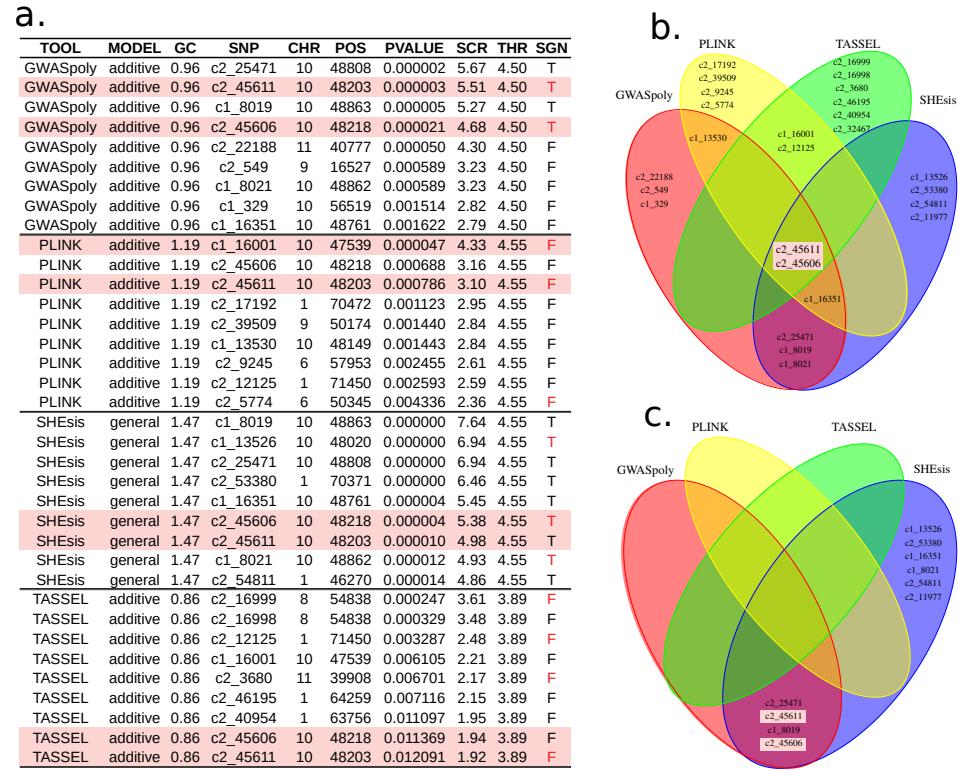


Figure 5: Shared SNPs Views. Tabular and graphical views of SNP associations identified by one or more GWAS packages (shared SNPs). SNPs identified by all packages are marker with red background in all figures (a) Table with details of the $N=9$ best-ranked SNPs from each GWAS package. Each row corresponds 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 6). 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, ABBC, 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.

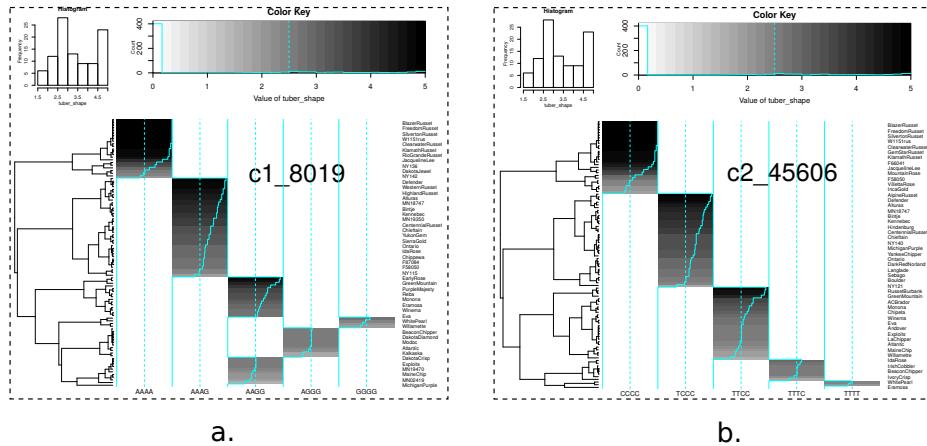


Figure 6: SNP profiles. SNP profiles for two of the best-ranked significant SNPs shown in the figure 5.b. (a) SNP c2_45606 best-ranked by the four packages (central intersection of the Venn diagram Figure 5.b) (b) SNP c1_8019 best-ranked by the two tetraploid packages (Figure 5.b), and also identified as significant by the same packages (at the bottom of the Figure 5.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 example, the Figure 7.a shows the chord diagram for the shared SNPs from the best-ranked associations previously described in the Figure 5.b. It can be observed that most

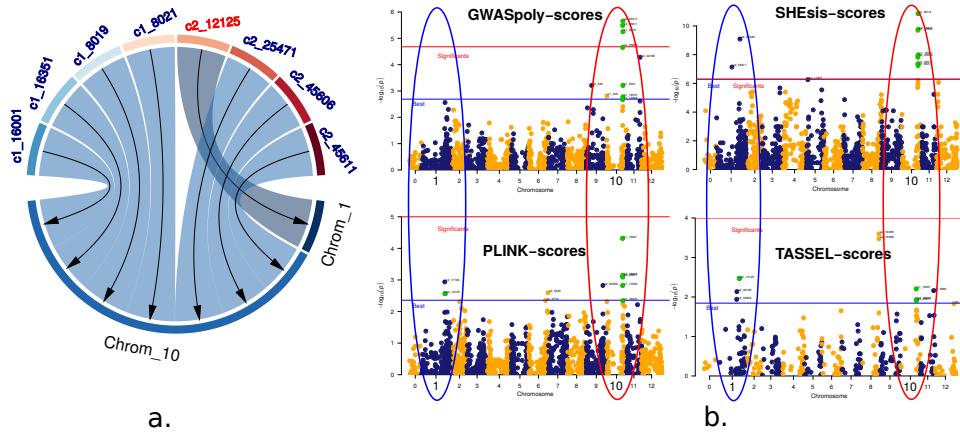


Figure 7: SNPs by chromosome. The figure shows how the best-ranked SNPs relate to chromosomes. (a) Chord diagram showing that most SNPs related to chromosome 10. SNPs are at the top of the diagram, chromosomes at the bottom, and associations are represented by arrows drawn from SNPs to their chromosomes. The more associations identified in one chromosome, the wider the space of its sector. (b) Manhattan plots from each GWAS package showing two important locations of associations: chromosome 1 and chromosome 10, marked with a blue and red ellipsis, respectively.

SNPs relate to chromosome 10 and only one to chromosome 1, which is also observed in the manhattan plots from each GWAS package (Figure 7.b).

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 9). 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 8.a, where parameter names and values are separated by colon, filenames are enclosed in quotation marks, and TRUE or FALSE indicates whether 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).

```

Terminal
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"
11,19           Top

```

Figure 8: 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 9). 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.

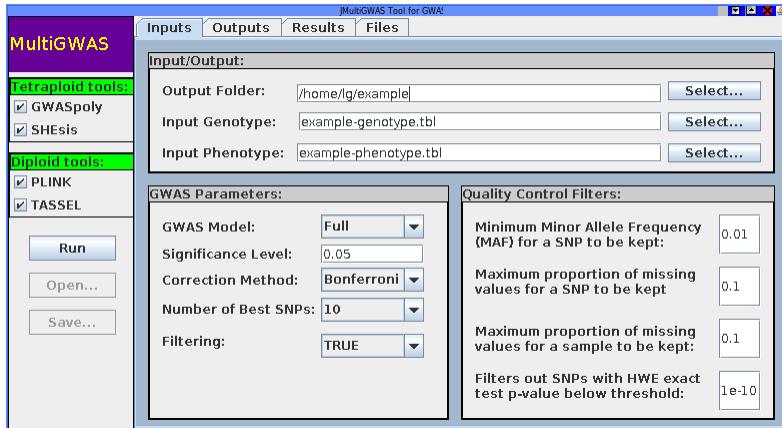


Figure 9: MultiGWAS GUI application. Main view of the MultiGWAS GUI application (“Inputs” view) where users can create the configuration file by setting values for input parameters. The GUI contains other three views: “Outputs” view shows the logs of the running process. “Results” view shows a report 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.

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