

Using Complex Networks for Identifying Potentially New Saline Stress Responsive Genes in Rice

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Abstract. Abiotic stresses are a main cause of extensive agricultural production losses worldwide. This paper proposes a workflow to identify stress responsive genes in organisms: on input RNA sequencing read counts measured for genotypes under control and treatment conditions, and biological replicates, it outputs a collection of characterized genes, potentially relevant to treatment. Technically, the proposed approach is both a generalization and an extension of WGCNA. It is showcased with a systematic study on rice (*Oryza sativa*), a major food source that is known to be highly sensitive to salt stress. A total of 6 modules are detected as relevant in the response to salt stress in rice: 3 modules of 3 genes each, all associated with shoot K content, 2 modules of 3 genes associated with shoot biomass, and 1 module of 4 genes associated with root biomass. These genes may act as potential targets for the improvement of salinity tolerance in rice cultivars.

1 Introduction

Abiotic stresses are key factors that can negatively influence plant development and productivity. They are a main cause of extensive agricultural production losses worldwide [15]. Soil salinity is one of the most devastating abiotic stresses, causing reduction in the cultivable land, crop quality, and productivity. It has been estimated that 20% of total cultivated and 33% of irrigated agricultural lands worldwide are already affected by high salinity. Moreover, due to the human activities and natural causes, salinized areas are gradually increasing every year and are expected to reach 50% by the end of year 2050 [22]. Salinity tolerance and susceptibility in plants is known to be the result of elaborated interactions between morphological, physiological, and biochemical processes that are regulated, in the end, by multiple genes in different parts of a genome [19]. Therefore, identifying groups of stress responsive genes may lead to crop improvement in terms of salinity tolerance and, ultimately, contribute solutions to the general problem of food sustainability in the years to come.

This paper proposes a workflow to identify stress responsive genes in organisms, which is known to be a complex quantitative trait. It takes as input RNA sequencing read counts measured for genotypes under control and treatment conditions (and representing gene expression profiles of the target organism), and

biological replicates. In order to discover key genes and their interaction with phenotypes related to treatment tolerance, the approach requires a collection of phenotypic traits (under control and under treatment), measured for the given genotypes. The output of the workflow is a collection of characterized genes, potentially relevant to treatment, yielding insight on the possible behavior of specific genes and the role they may play in functional pathways in response to the studied treatment of the organism of interest. The proposed workflow can thus take advantage of transcriptomic data for different organisms and conditions, based on the current availability of high-throughput technologies that include microarrays and RNA sequencing, to study the reaction of organisms under different environmental stimuli, such as salt stress.

Technically, the proposed approach is both a generalization and an extension of Weighted Gene Co-expression Network Analysis (WGCNA) [13], a widely applied workflow that has been successfully used for identifying target genes related to diseases and cancer in several organisms [24]. The general idea behind each approach is to identify specific modules in a network of genes after a sequence of normalization and filtering steps. The proposed approach is considered a *generalization* of WGCNA because module detection can now recognize overlapping communities, which may have more biological meaning given the overlapping regulatory domains of systems that generate co-expression [10]. This is achieved by using Hierarchical Link Clustering (HLC) [2]. It is also an *extension* of WGCNA because additional steps and information are added to the workflow: namely, some networks in the intermediate steps are forced to be scale-free [?] and LASSO regression [25] is employed to select the most significant modules of phenotypical responses to stress. The advantage of using HLC as clustering method is its ability to detect overlapping modules, since biological components are involved in multiple functions and therefore biological communities tend to be highly overlapping. On the other hand, LASSO is a regularized regression technique widely used in variable selection, thanks to its ability to obtain zero regression coefficients for the less relevant variables [8]. Moreover, LASSO is especially useful in problems where the number of variables is much larger than the number of samples, which may be the case more often than desired. The proposed workflow is also modular, since other module detection and selection techniques could be used, instead HLC and LASSO, respectively.

The proposed workflow is showcased with a systematic study on rice (*Oryza sativa*), a major food source that is known to be highly sensitive to salt stress [6]. RNA-seq data was accessed from the GEO database [1] (accession number GSE98455). It corresponds to 57845 gene expression profiles of shoot tissues measured for both control and salt condition in 92 accessions of the Rice Diversity Panel 1. As output, 6 modules are detected as relevant in the response to salt stress in rice: 3 modules of 3 genes each, all associated with shoot K content, 2 modules of 3 genes associated with shoot biomass, and 1 module of 4 genes associated with root biomass. These genes may act as potential targets for the improvement of salinity tolerance in rice cultivars. From the 19 genes, all but 3 genes (associated with K content), were also identified as differentially expressed

for at least one of the 92 accessions, suggesting that those genes are strong candidates as stress responsive genes. Only 2 of the 16 differentially expressed genes, both from the module related with shoot biomass, are named and have an associated protein product: Spermidine hydroxycinnamoyltransferase 2 (SHT2) and Lipoxigenase. In other words, further studies are needed to elucidate the detailed biological function of the remaining 14 genes that have not been named so far, which may have a potential relevance in stress responsive mechanisms to salt conditions in rice. The goal is that the results reported in this paper may allow biologist to develop new rice cultivars with higher resistance to salinity.

2 Preliminaries

This section presents preliminaries on networks, hierarchical link clustering and the LASSO linear regression technique.

2.1 Co-expression network

A network is an undirected graph $G = (V, E)$ where $V = \{v_1, v_2, \dots, v_n\}$ is a set of *vertices* or *nodes* and $E = \{e_1, e_2, \dots, e_q\}$ is a set of *edges* or *links* that connect vertices. In a gene co-expression network, each node corresponds to a gene. A pair of genes is connected if they show similar expression patterns. A simple and unweighted network can be represented by an adjacency matrix $A \in \{0, 1\}^{n \times n}$ that is symmetric with a positive one in the positions (v_i, v_j) and (v_j, v_i) whenever there is an edge connecting vertices v_i and v_j , and zeros elsewhere. Co-expression networks are of biological interest because the co-expressed genes are usually controlled by the same transcriptional regulatory pathway, functionally related or members of the same pathway or metabolic complex.

2.2 Hierarchical Link Clustering

The Hierarchical Link Clustering (HLC) algorithm was proposed by Ahn et al. [2]. The HLC approach represents communities as groups of links (rather than nodes), and each node inherits all memberships of its links and can thus belong to multiple, overlapping communities. It maps links to nodes and connects them if a pair of links shares a node. The similarity between two links e_{ik} and e_{jk} is computed using the Jaccard index

$$S(e_{ik}, e_{jk}) = \frac{|n(i) \cap n(j)|}{|n(i) \cup n(j)|}, \quad (1)$$

where $n(i)$ denotes the set containing exactly node i and its neighbors. The algorithm uses single-linkage hierarchical clustering to build a dendrogram in which each leaf is a link from the original network and branches represent link communities. Hierarchical clustering algorithms repeatedly merge groups until all elements are members of a single cluster. For the purpose of finding meaningful

communities, it is crucial to know where to partition the dendrogram. In this case, the most relevant communities are established at the maximal partition density D , a function based on link density inside communities measuring the quality of a link partition. The partition density D has a single global maximum along the dendrogram in almost all cases, because its value is the average density at the top of the dendrogram (a single giant community with every link and node) and it is very small at the bottom of the dendrogram (most communities consists of a single link). In particular, it is the case that $D = 1$ when every community is a fully connected clique and $D = 0$ when each community is a tree. If a community is less dense than a tree (i.e. when the community subgraph has disconnected components), then such a community contribute negatively to D , which can take negative values. The minimum density inside a community is $-2/3$, given by one community of two disconnected edges. Since D is the average of the intra-community density, there is a lower bound of $-2/3$ for D . Computing D at each level of the link dendrogram can help the purpose of picking the best level to cut, although meaningful structure could exist above or below the threshold. The output of cutting is a set of node clusters, where each node can participate in multiple communities.

2.3 Least Absolute Shrinkage Selector Operator

The Least Absolute Shrinkage Selector Operator (LASSO) is a regularized linear regression technique. It combines a regression model with a procedure of contraction of some parameters towards zero and selection of variables, imposing a restriction or a penalty on the regression coefficients. In other words, LASSO solves the least squares problem with restriction on the L_1 -norm of the coefficient vector. It can be especially useful to solve problems where the number of variables (e.g., genes) n is much greater than the number of samples m (i.e., $n \gg m$).

Consider a dataset consisting of m samples, each of which consists of n covariates and a single outcome. Let y_i be the outcome and $x_i := (x_1, \dots, x_n)$ be the covariate vector for the i -th sample. The objective of LASSO is to solve

$$\min \left\{ \sum_{i=1}^m \left(y_i - \sum_{j=1}^n \beta_j x_{ij} \right)^2 \right\}, \quad \text{subject to} \quad \sum_{j=1}^n |\beta_j| \leq s. \quad (2)$$

Equivalently, in the Lagrangian form, it minimizes

$$\sum_{i=1}^m \left(y_i - \sum_{j=1}^n \beta_j x_{ij} \right)^2 + \lambda \sum_{j=1}^n |\beta_j| \quad (3)$$

where s is the regularization penalty and $\lambda \geq 0$ is the corresponding Lagrange multiplier. Since the λ value determines the degree of penalty, the accuracy of the model depends on its choice. Cross-validation is often used to select the regularization parameter, choosing the one that minimizes the mean-squared error.

3 The Workflow

The proposed workflow uses RNA-seq read counts, representing gene expression levels, as input data. More precisely, it uses n_0 gene expression profiles of an organism, measured for m different genotypes under control and treatment conditions, and r biological replicates. This raw data is represented as a matrix $D_0 \in \mathbb{N}_0^{n_0 \times 2mr}$. In order to discover key genes and their interaction with phenotypes related to treatment tolerance, the approach also requires a set of p phenotypic traits, measured for the m genotypes. The phenotypic data is seen as a matrix $P \in \mathbb{R}^{2m \times p}$ containing two phenotypic values per genotype, one under control condition and a second one under treatment condition. The proposed workflow is depicted in Figure 1. This section explains the five macro-processes (A)-(E) of the proposed workflow. In comparison with WGCNA, it adds the macro-step (D) and generalizes macro-steps (A)-(C).

3.1 Data pre-processing

The goal of the data pre-processing stage is to build matrices P_{ell} and L_1 representing, respectively, the changes in phenotypic values and expression levels between control and treatment condition, from RNA-seq and phenotypic data found in matrices D_0 and P , respectively.

The RNA-seq data cannot be directly interpreted. Therefore, a normalization process is applied to deal with the problem of possible biases affecting the quantification of results. The suggested normalization technique for correcting library size and RNA composition bias is DESeq2 [14]. The normalized data is represented as a matrix $D_1 \in \mathbb{R}^{n_0 \times 2mr}$, and the biological replicates of each genotype are averaged and represented as a matrix $D_2 \in \mathbb{R}^{n_0 \times 2m}$. The genes exhibiting low variance or low expression are removed from D_2 , thus identifying a subset of size $n_1 \leq n_0$ of the original genes. The control and treatment data is separated into the matrices $C \in \mathbb{R}^{n_1 \times m}$ and $T \in \mathbb{R}^{n_1 \times m}$, respectively. The matrix entries c_{ij} in C and t_{ij} in T represent, respectively, the normalized expression level of gene i in accession j . Control and treatment data is also separated from phenotypic data P , obtaining the P_c and P_t matrices of dimensions $m \times p$.

In the above configuration, the changes in expression levels and phenotypic values, between control and treatment conditions, are measured in terms of logarithmic ratios. In the case of expression levels, the log ratios are represented in the Log Fold Change matrix $L_0 \in \mathbb{R}^{n_1 \times m}$, where $\ell_{ij} = \log_2(t_{ij}/c_{ij})$. Similarly, the log ratios of the phenotypic data are computed and represented in the $P_\ell \in \mathbb{R}^{m \times p}$ matrix.

The final step of the data pre-processing is to filter L_0 by removing rows (e.g., genes) with low variance in the differential expression patterns, obtaining a new matrix L_1 of dimensions $n_2 \times m$, with $n_2 \leq n_1$.

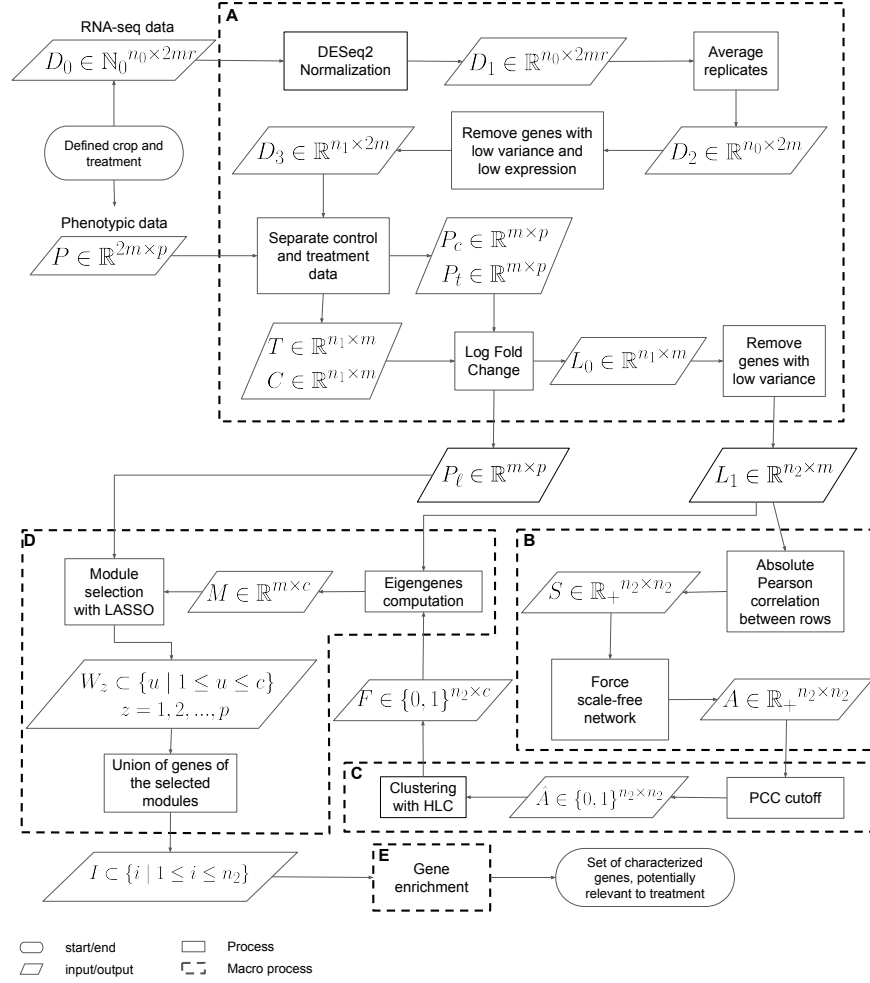


Fig. 1. The proposed workflow comprising five macro-steps: A. Data pre-processing, B. Co-expression network construction, C. Co-expression module identification, D. Modules association to phenotypic traits, and E. Genes enrichment.

3.2 Co-expression network construction

A gene co-expression network connect genes with similar expression patterns across biological conditions. The purpose of this stage is to describe how to build the co-expression network A from the Log Fold Change matrix L_1 , capturing the relationship between genes according to the change in expression levels between the two studied conditions. These co-expression patterns are meaningful for the identification of genes not yet associated with the response to the treatment condition.

The Log Fold Change matrix L_1 is used to build the co-expression network following the first two steps of the WGCNA methodology [13]. First, the level of concordance between gene differential expression profiles across samples is measured. To this end, the absolute value of the Pearson correlation coefficient is used as the similarity measure between genes and the resulting values are stored in the similarity matrix $S \in \mathbb{R}_+^{n_2 \times n_2}$. Second, the matrix S is transformed into an adjacency matrix $A \in \mathbb{R}_+^{n_2 \times n_2}$ where each entry $a_{ij} = (s_{ij})^\beta$ encodes the connection strength between each pair of genes. In other words, the elements of the adjacency matrix are the similarity values up to the power $\beta > 1$ so the degree distribution will fit a scale-free network. These networks contain many nodes with very few connections and a small number of hubs with high connections. In a strict scale-free network the logarithm of $P(k)$ (i.e., the probability of a node having degree k) is approximately inversely proportional to the logarithm of k (i.e., the degree of a node). So the parameter β is chosen as the smallest value of β such that the R^2 of the linear regression between $\log_{10}(p(k))$ and $\log_{10}(k)$ is close to 1 (e.g., $R^2 > 0.85$).

3.3 Co-expression module identification

Next step in the workflow is to identifying communities (also called modules) from the co-expression network structure and dynamics represented in A . The idea is to cluster genes with similar patterns of differential expression change. Membership in these modules may overlap in biological contexts, because modules may be related to specific molecular, cellular or tissue functions and the biological components (i.e. genes) may be involved in multiple functions. Thus, unlike WGCNA, the adjacency matrix A is used to detect overlapping (rather than non-overlapping) communities, using the Hierarchical Link Clustering (HLC) algorithm (see section 2).

As a preliminary step, matrix A is transformed, into an unweighted network $\hat{A} \in \{0, 1\}^{n_2 \times n_2}$ before applying the clustering algorithm. To this end, the Pearson Correlation Coefficient (PCC) cutoff is determined using the approach described in [3]. The number of nodes, edges, and the network density is determined for different PCC cutoffs. Around the most biological relevant PCC cutoff, the number of nodes presents a linear decrease, and the density of the network reaches its minimum, while below this value the number of edges rapidly increases. Considering this, a cutoff is selected such that gene pairs which have a correlation score higher than the threshold are considered to have significant

co-expression relationship. Above the cutoff, the entries of matrix A become 1 and below the cutoff A values becomes 0. The application of the HLC algorithm organizes the n_2 genes of matrix \hat{A} into c modules, where each gene can belong to one, multiple, or no module at all. This information is represented as an affiliation matrix $F \in \{0, 1\}^{n_2 \times c}$, where $f_{iu} = 1$ if node i is member of module u .

3.4 Module association to phenotypic traits

To identify the most relevant groups (modules) of genes, associated with the phenotypic response to a specific treatment in an organism, the proposed workflow uses a LASSO based approach. Each module is represented by an eigengene, which is defined as the first principal component of such module. An eigengene can be thought of as an average differential expression profile for each community: it is computed from the Log Fold Change Matrix L_1 and the affiliation matrix F . Given a module u , the affiliation matrix is used to identify the genes belonging to u and then the corresponding rows of the matrix L_1 are selected to compute the first principal component of u . Each principal component becomes a column of the matrix $M \in \mathbb{R}^{m \times c}$. These profiles are associated with each phenotypic trait using the least absolute shrinkage and selection operator (LASSO). In this context, the eigengenes (i.e., the columns of M) act as regressor variables and each phenotypic trait (i.e., each column of P_ℓ) is used as an outcome variable.

The output after applying LASSO is a set W_z of modules for each phenotypic trait z , where $W_z \subset \{u \mid 1 \leq u \leq c\}$ for $z = 1, 2, \dots, p$. The target genes I for downstream analysis, which may be important in the treatment response, are the union of genes belonging to the selected modules, that is $I = \cup_{z=1}^p W_z$, where $I \subset \{i \mid 1 \leq i \leq n_2\}$.

3.5 Gene Enrichment

The goal of this final stage of the process is to characterize the genes identified in previous stage with additional information, helping to elucidate their possible behavior and role in the response to the studied treatment.

A simple analysis to made with the selected genes is to identify the differentially expressed genes in set I . That is, to select those genes in I having an absolute value of the log fold change of at least 2 ($|\ell_{ij}| \geq 2$) for at least one sample. This represents genes level of expression is quadrupled (up or down) from control to treatment condition; these genes are strong candidates for treatment responsive genes.

Also functional category enrichment can be done by, e.g., searching for gene ontology annotations in databases such as QuickGO [4]. Such annotations can provide evidence of biological implications of the target genes in the treatment-tolerance mechanisms. Furthermore, QuickGO can be used to identify genes with reported protein products, which can be used to perform additional relevant analysis reviewing their reported protein-protein interactions in other databases,

such as STRING [23]. The interactions include direct (physical) and indirect (functional) associations; they stem from computational prediction, from knowledge transfer between organisms, and from interactions aggregated from other (primary) databases. This information can give new insight on how the selected genes are involved in functional pathways that can be related with the treatment of interest.

4 Case Study

This section presents a case study on the identification of genes that may respond to saline stress according to the methodology presented in section ?? . The RNA-seq data was obtained from GEO database [1] (accession number GSE98455). This data corresponds to $n_0 = 57845$ gene expression profiles of shoot tissues measured for both control and salt condition in $m = 92$ accessions of the Rice Diversity Panel 1, with $r = 2$ biological replicates. A total of $p = 3$ phenotypic traits are used: shoot K^+ content, root biomass and shoot biomass. These traits were measured for the same 92 genotypes, under control and salt stress conditions, and can be found in the supplementary information of [5].

4.1 Data pre-processing

DESeq2 normalization is applied to the raw data and the biological replicates are averaged. Genes exhibiting low variance are identified as those with ratio of upper quantile to lower quantile smaller than 1.5 and are removed from the normalized data. Genes with low expression, corresponding to those having more than 80% samples with values smaller than 10, are also removed. A total of $n_1 = 8928$ genes are kept after this filtering process.

From the Log Fold Change matrix L_0 , genes whose difference between upper quantile and lower quantile greater than 0.25 are removed. Therefore, the resulting matrix L_1 contains the log ratios of $n_2 = 8928$ genes. The logarithmic ratios of the phenotypic data, for the 92 accessions and the 3 traits, is also computed.

4.2 Co-expression network construction

The Log Fold Change matrix L_1 is used to compute the corresponding similarity matrix. For this network, it is observed that $\beta = 3$ is the smallest integer such that the $R^2 \geq 0.8$. Figure 2 depicts the degree distribution of the similarity matrix (left) and the degree distribution of the adjacency matrix (right), which is the degree distribution of a scale-free network with $R^2 = 0.8$ with $\beta = 3$.

The resulting adjacency matrix A represents a complete graph $G = (V, E)$, with $|V| = 8928$ genes and $|E| = 39850128$ edges.

4.3 Co-expression module identification

The adjacency matrix A is transformed into an unweighted network \hat{A} applying the approach described in [3], based on the density of the network combined

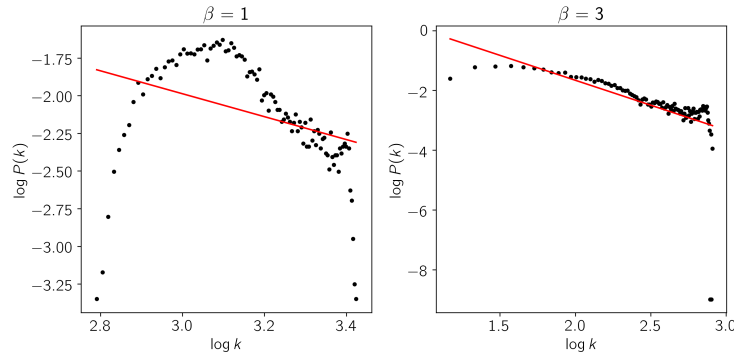


Fig. 2. Degree distribution of A (left) and \hat{A} (right)

with the decreasing number of nodes and edges with higher PCC values. The cutoff value is set to 0.2, thus keeping only the connections above this threshold and removing the isolated nodes. The resulting adjacency matrix \hat{A} has 5810 connected genes and accounts for 16875145 edges.

After applying the HLC algorithm, a total of 4131 genes are distributed in $c = 5143$ overlapping modules of at least 3 genes. Figure 3 presents a histogram of the overlapping percentage of these genes, measured as the proportion of modules to which each gene belongs. The first bar of the histogram represents the genes with zero overlap, corresponding to 28% of the total genes; remaining 72% represents the genes belonging to more than one module.

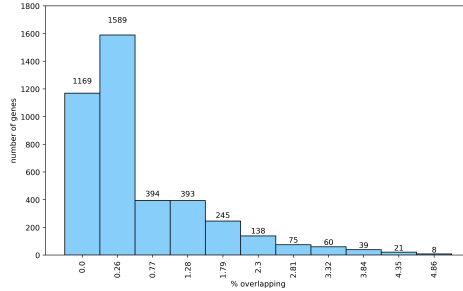


Fig. 3. Overlapping percentage

4.4 Module association to phenotypic traits

The phenotypic traits under study are shoot K^+ content, root biomass and shoot biomass. Figure 4 suggests that there are significant differences in the values of these phenotypic traits between stress and control conditions. This supports

the working hypothesis that these three variables represent tolerance-associated traits in rice under salt stress.

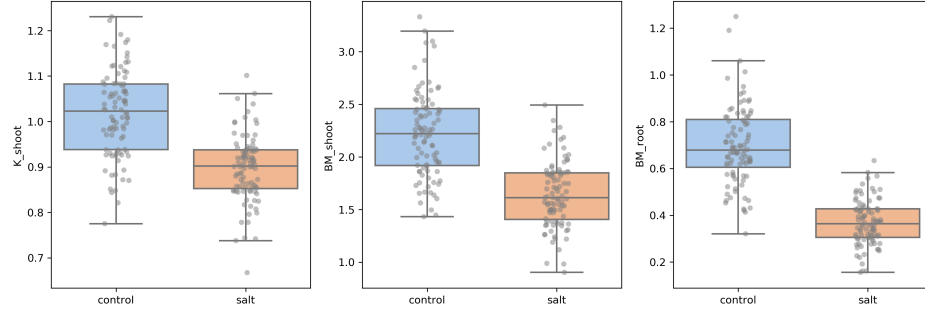


Fig. 4. Phenotypic traits distribution under control and salt stress.

Using the affiliation matrix F derived from the HLC output and the Log Fold Change matrix L_1 , a matrix M is built by computing the eigengene for each of the $c = 5143$ modules. The LASSO technique is applied by using each of the phenotypic traits as the outcome variable, one at a time. As shown in Figure 5, cross-validation is performed for each phenotypical trait in order to select the corresponding regularization parameter λ that minimizes the mean-squared error.

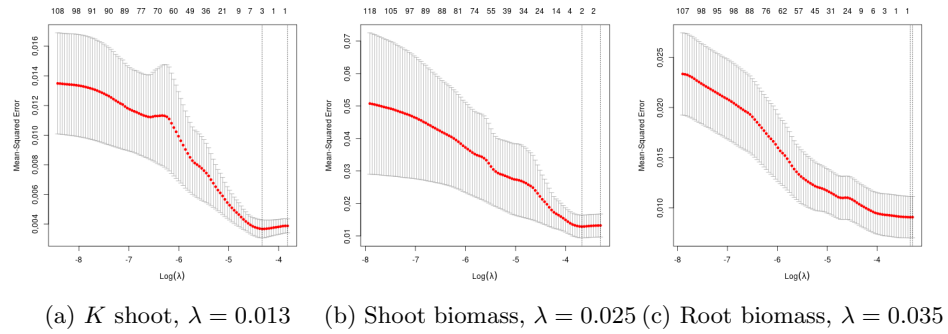


Fig. 5. Cross-validation of the LASSO regularization parameter λ , for each phenotypic trait.

Finally, three LASSO models are adjusted by using the corresponding λ and phenotypical data with the eigengens of matrix M . As result, 6 modules are detected as relevant in the response to salt stress in rice: 3 modules of 3 genes,

each associated with shoot K content; 2 modules of 3 genes associated with shoot biomass; and 1 module of 4 genes associated with root biomass.

4.5 Gene enrichment

From the 19 genes selected by LASSO, all but 3 genes (the ones associated to K content), are also identified as differentially expressed ($|\ell_{ij}| \geq 2$) for at least one of the 92 accessions. This suggests that those genes are strong candidates as stress responsive genes to salt conditions in rice. According to the Quickgo database, only 2 of the 16 differentially expressed genes (both from the module related with shoot biomass) are named and have an associated protein product: Spermidine hydroxycinnamoyltransferase 2 (SHT2) and Lipoxygenase. Figure 6 shows their corresponding 3D protein structures.

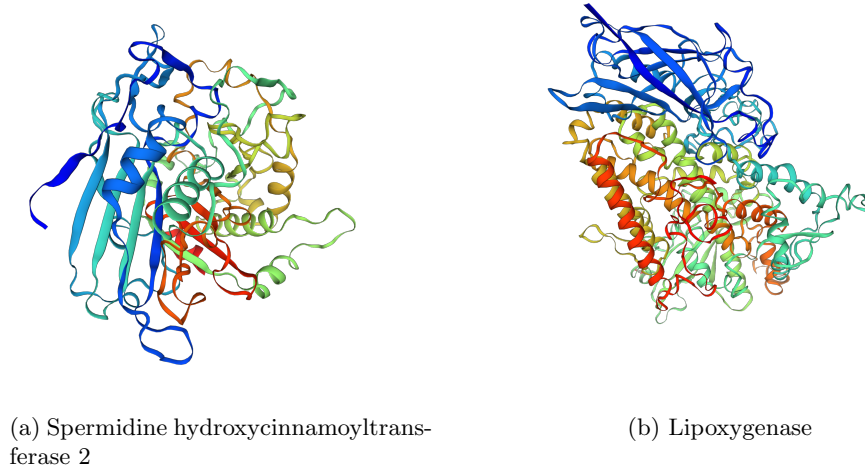


Fig. 6. 3D protein structure of named genes selected by LASSO, available from [23].

Uniprot database [7] reports, on the one hand, that SHT2 contributes to the natural variation of spermidine-based phenolamides in rice cultivars. On the other hand, it is reported that plant lipoxygenase may be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding [7]. This protein is involved in the pathway oxylipin biosynthesis, which is part of Lipid metabolism. Additionally, previous studies in [11,12,16,18,20] provide evidence of biological implications of spermidine and lipoxygenase in tolerance to salt stress in other plants or even in rice cultivars. However, further studies are needed to elucidate the detailed biological function of the remaining 14 genes that have not been named so far, which may have a potential relevance in stress responsive mechanisms to salt conditions in rice.

5 Concluding Remarks

This manuscript provides a detailed description of a network-based analysis methodology for the discovery of key genes responding to a specific treatment in an organism. It links transcriptomic with phenotypic data, and identifies overlapping gene modules.

The proposed methodology is inspired by the workflow suggested in the WGCNA methodology [13]. The main steps are the preprocessing of the gene expression data, the construction of a co-expression network, the detection of modules within the network, the relation of modules with external information (e.g. phenotypic data) and the enrichment of the identified key genes with additional information. The WGCNA methodology and therefore the proposal as well, are structured in a modular way, which allows modifying and exploring different techniques in each step of the process.

The proposed approach is designed to integrate expression data measured under two different conditions (namely, control and treatment), unlike the usually co-expression-based approaches which work with both conditions independently or consider only a single condition. For this purpose, an approach similar to that proposed in [9] is used, where the control and treatment data are compiled in a single matrix using the log fold change measure. Thus, the input to construct the co-expression network is not the expression data, but instead the changes in the expression levels from one condition to the other, making room for capturing the signal of changes caused by the treatment.

Another important feature in the proposed methodology is the module detection technique. The co-expression network is computed as in WGCNA until a scale-free network is obtained. This network is then used to apply the HLC algorithm, a clustering technique capable of detecting overlapping communities. Several approaches of module detection from gene expression have been proposed and were evaluated in [21]. Most of them focuses only on disjoint (non-overlapping) communities, and the described techniques dealing with overlaps are not clustering but biclustering and decomposition methods. It is well known that communities in real networks are overlapping [17]. Thus, the approach presented in this work can be seen as a generalization of the previous approaches such as WGCNA.

The proposed methodology was applied in a case study with rice under salt stress. The results show a group of 14 genes in which 2 of them are related to the response to saline stress according to previous studies, validating the ability of the method to detect this kind of key genes. As future work, other overlapping module detection and selection techniques should be used instead HLC and LASSO, respectively. The combination of these techniques would allow finding target genes for future biological studies that evaluate their potential as genes that respond to salt stress in rice. Also, laboratory experimentation needs to be conducted on via to verify the findings of this paper in relation to salinity stress. Finally, this study can be extended to other stresses and even to other crops.

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