

NetREx Tutorial

About App

NetREx is a Network-based Rice Expression Analysis Server hosted in the form of a web application.

One can explore the genes that are differentially co-expressed across four different stress conditions (drought, cold, flood and osmosis) and two hormone treatments (ABA, JA) in different tissues, root and shoot. The correlation between the genes is depicted by Pearson correlation coefficient and top 30 ranked correlations for a gene are represented as its neighbours in HRR30.

The weighted gene co-expression network has been constructed each for root and shoot tissues under four different stress conditions and two hormone treatments using differentially expressed genes (identified for every time-point at 2-fold change and p-value < 0.5). This resulted in a number of co-expressed modules. For a given set of genes, the user can query interactions between them across different stress/hormone treatments, in different tissues and whether belonging to the same co-expressed module or not.

Tools and Features

In NetREx various viewers are provided for different analysis:

- Heatmaps – depicts expression of user chosen genes
- Network Viewer – provides interactions between the chosen genes under different stress/hormone treatment/tissue
- Network Neighbourhood Viewer – displays interactions between the chosen genes and its first neighbours (extracted from HRR network)
- Module Viewer – clusters of co-expressed genes

The user can search through the database using the following three search options:

- Based on WGCNA Modules
- Based on Tissue
- Based on Stress/Hormone treatment
- Based on Pathways

Query Form

The user has to first fill the query form which requires the following information:

- **Species** : (Oryza Sativa) The plant species for which the genes are to be viewed.
- **Tissue Type** : (Root / Shoot) The tissue type for the corresponding genes.

- **Condition** : Currently hosting 4 abiotic conditions (Drought, Cold, Flood, Osmosis) and 2 Phytohormone change (ABA and JA)
- **Gene List** : A comma separated set of genes (max 300) needs to be specified whose networks and expression is to be viewed for the above species, condition and tissue.

One could click on the **Load Example** button to load an example set of query and press Send to access the viewers.

Validation Page

Upon submission of the query, one gets redirected to the validation page which displays the Species, Tissue and Condition that was specified and the list of genes that have been found to be Differentially Expressed for the specified condition.

A gene is classified as valid if it is differentially expressed in at least 2 time-points for a given stress condition.



There are tabs for Network and Expression Viewer underneath that could be clicked to load the corresponding visualisation.

Network Viewer



Network Viewer displays the HRR subnetwork of the filtered set of DEGs queried genes for the specified tissue and condition. The nodes correspond to the genes and the edge represents a correlation between the two genes if they have a similar expression profile across time points.

View

For the visualization of the dense co-expression networks, we have implemented Force Atlas 2 in the Sigma JS module which helps in representing nodes belonging to a cluster close together while allowing independent clusters to be spatially separated. The size and colour of the nodes provides additional information about the genes, such as how well it is connected in the network, is it up- or down regulated as given below.

With the combination of colors, shapes and user interactions, maximum information is tried to convey for the queried genes.

- Visuals
 - Size: The nodes with greater size represents an overall higher degree in the complete HRR network for a given tissue.
 - Color: Different coloring options are present in the side panel that could be switched on to view different information.
 - Up/Down DEGs : Red for up regulated and Blue for Down regulated
 - Module Membership : Colors based on WGCNA module membership of the gene

- Default View : Color gradient based proportional to size
 - Shape: Any general gene has a circular shaped node while a transcription factor is shown as a triangular shaped node. The edges are curved shaped for better visibility.
 - Node Label: To avoid overcrowding in the network, the labels of all the nodes are not shown all at once. The labels of only those nodes are displayed that cross a certain node size threshold across different zooms.
- Interaction
 - Pan: Click and drag with the mouse at the stage area of the graph (i.e. part where there are no nodes and edges present) to pan the network.
 - Zoom: Double click or scroll to change the zoom level. Zooming also resets the hover highlighted nodes.
 - Hover: On hovering over a particular node, its label and its first neighbours gets highlighted.
 - Expand () : For a bigger view, the expand button could be clicked to hide the options and increase the view size.
 - Reset () : If any particular node or pathway is highlighted, reset button could be clicked to remove the highlights and reset the coloring.

Options

The option panel rests beside the view and its visibility could be toggled by clicking on the expand button in the top right corner of the view.

This panel hosts a set of options for network interaction which are described as follows:

- Coloring Switches : The nodes in the graph can be colored with three different schemes upon toggling the corresponding switches.
 - Default View: By default the nodes in the graph are shaded based on its degree in the complete network. A darker shade corresponds to a higher degree and vice versa.
 - Module Membership: Switching this will make the nodes colored on the basis of the WGCNA module which they belong to.
 - Up/Down DEGs: This scheme enables to view the up/down regulated genes at different time points. From the drop down one could select the time point and switch on the button to enable the coloring. Red indicated up regulated while blue indicated down regulated
- Pathway Visualiser : The KEGG pathways corresponding to the genes are assembled and only those genes pertaining to a specific pathway could be highlighted. To use this one could select the path from the drop down and that would get highlighted.
- Other Condition : To see the corresponding viewers for some other stress condition, one could select that condition from the dropdown and click on the load button to load the viewers for the same set of genes in a new tab.
- Download : The visualisation in the view panel could be downloaded in the form of a jpg image by clicking the Download Image button.

Table

The table corresponding to the above network could be expanded and collapsed using the “Show/Hide Button” underneath the view panel. This table consists of information about the network classified into two sections, Nodes and Edges.

- Edges : Each row corresponds to an edge specifying the source and target gene as well as its PCC and HRR value.
- Nodes : The node section consists of gene information displayed in the network. This information has been further divided into three sections for better visibility, Description, Function and Fold Change.
 - Description : Consists of Transcription Factor (TF) Labelling, Module Name, General Description, MSU_ID and IC4R Expression.
 - On clicking over a module name, the complete network of the corresponding module gets loaded in a new tab.
 - On clicking the “Click!” button under IC4R Expression, the IC4R expression profile of that gene gets loaded in a new tab.
 - Function : Functional annotations like GO, Mapman and KEGG are listed under this section.
 - Fold Change : Fold change and p-values across different timepoints are listed under this section

Upon hovering over a row in the Node Section, the corresponding node and its first neighbours gets highlighted in the network.

Apart from the above features in the table there also exists a search bar corresponding to both nodes and edges where the entered text is searched across all the sections.

Network Neighbourhood Viewer


The neighbourhood of the queried genes could be viewed by this viewer. After specifying the number of neighbours (by default 50 and max 100) that is intended to be displayed, upon clicking the “Network Neighbourhood Viewer” button, the viewer gets loaded. The top 50 or the user specified number of neighbours are chosen on the basis of their overall Rank in the complete network.

The original genes are bordered with fluorescent green and have their size increased to make them distinct from the neighbours.

This viewer has similar functionalities as that of Network Viewer.

Expression Viewer

The Expression Viewer is a heatmap based visual of the expression profile of the queried genes across different timepoints. Two heatmaps are shown, first corresponding to the fold change expression values while the second corresponding to the p-values. Using the plugin clustergrammer js for the heatmap, it hosts a range of functionalities that could be of use.

- Sorting : On double clicking the row or the column headers, the corresponding row or column gets sorted based on the expression values.
- Zoom : Scrolling up/down over the heatmap, zooms in and out correspondingly
- Pan : When in a zoomed state, click and drag to pan the heatmap.
- Hover : On hovering over a tile in the heatmap for over a second, the corresponding value of the tile pops up.
- Snapshot (): To save a snapshot of the heatmap, click on this button.

Browse

The complete dataset could be browsed with the aid of three different browsers that have been provided in the application.

WGCNA Module Browse

For viewing the network and data of the WGCNA modules, this browser could be used. A form specifying the tissue type and the module color is provided, whose corresponding table and network containing the top 100 nodes in this browser can be viewed. Apart from the nodes and edges, the table also contains GO information about the corresponding module.

Stress Condition Browse

Having a form similar to Query Form, this browser is used to view the complete table of all the genes corresponding to the specific tissue type and stress condition. Since this data is huge, it takes a minute time to load the table after submitting the form. No visualization in this case.

Pathway Browse

To select genes based on KEGG pathways, instead of manually entering gene lists to load their viewers, this browsing option could be used. In this, first the species, the tissue type and the stress condition is specified to load all the KEGG pathways. Now using the multi-select tree format of the KEGG loaded paths, genes could be ticked to select them. After the selection of upto max 100 genes, the send button can be clicked to load the corresponding viewers from the selected genes under the specified tissue and stress condition.

The multi-select tree under KEGG pathways, also has the option for a search bar where the entered text is searched across pathway names and genes.

Datasets

The complete datasets using which the graph was constructed could be downloaded under this section. It contains data of the Node Lists for the Root and Shoot Tissue for the different stress condition, and it also has the overall fold change values for the DEGs corresponding to root and shoot tissue.