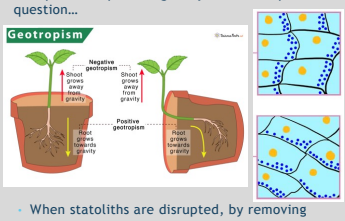
Ohio Wesleyan University

Identifying gravitropic phenotypes of co-expressed gene mutants of the gravity-regulated protein ROSY-1

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Introduction:

- How plants respond to gravity is still an open question…



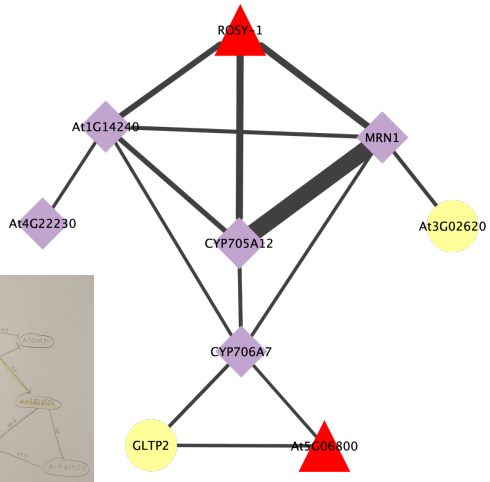
  - When statoliths are disrupted, by removing knocking down the gene that makes starch, the gravity response is disrupted…
    - But not gone.
- Our lab explores 124 genes found to be expressed differently in wildtype and starchless mutants; this difference occurs in contrast pattern to the gravity response.
  - ROSY-1 is less expressed in response to gravity in wildtype plants, but more expressed in response to gravity in starchless mutants.
  - But what does this really tell us about this gene's role in the gravity response?
- ROSY-1 – interact**O**r **O**f **SY**naptotagmin-**1** – is a gene of unknown function.
  - Appears to play an inhibitory role in the gravity response.
    - Knock-out mutants have a *faster* gravity phenotype.
  - Contains a binding site for synaptotagmin-1 and stigmasterol.
- Genes do not exist in a bubble. It is the interaction of genes and their proteins that cause the body to work the way it does.
  - **Research Question:**
    - **Can understanding the genes *interacting* with ROSY-1 tell us more about ROSY-1's role in the gravity response?**

Methods:

- *Co-expression - Manual*
  - Used ten databases provided by The Bio-Analytic Resource for Plant Biology to find the top 100 genes co- and anti-expressed with ROSY-1.
  - We then compared this resultant list to our 124 genes of interest.
- *Co-expression - Automatic*
  - 124 genes submitted into the ATTED-II database to calculate Z-scores for expected co-expression.
    - Interactions with Z-scores larger than 3.0 counted as significant.
- *Differential Co-expression*
  - Normalized RNA-seq data from previous research submitted through an *adapted* WGCNA analysis (package: multiWGCNA).
    - Co-expression modules constructed at multiple levels of expression treatments (see Figure 2C for example).
    - Power level of 12 chosen.
    - Module Connectivity value of 0.40
      - Does that gene belong in that module?
    - Currently playing with low tree cut heights to find statistically relevant but differentially expressed modules.



Legend
- Blue Arrow – mutant responds to gravity slower than Wildtype
- Red Triangle – mutant responds to gravity quicker than Wildtype
- Purple Diamond – mutant gravity response is not significantly different from Wildtype
- Yellow Circle – mutant gravity phenotype unknown



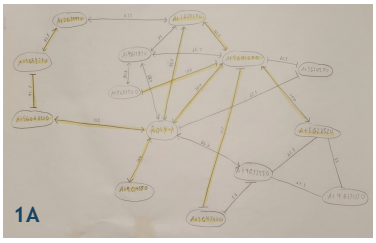
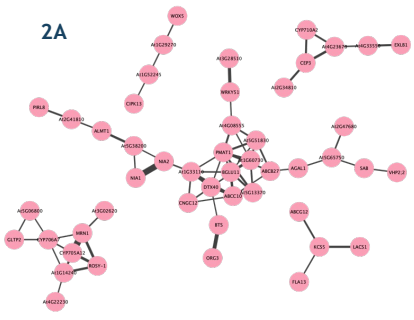
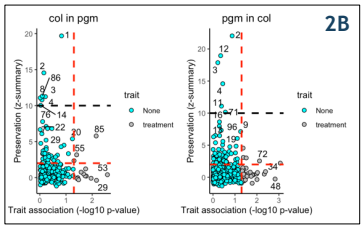
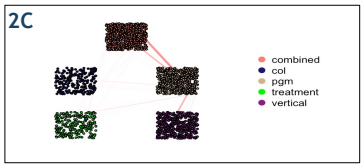
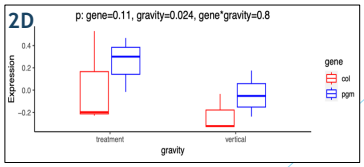


Figure 1 - Of the 124 genes submitted to the ATTED-II database, 8 possessed significant co-expression interactions with/near ROSY-1. Thicker connections correspond to higher significance in z-score. One gene indirectly connected to ROSY-1, AT5G06800 – a transcription factor, has a knockout mutant with a faster gravity phenotype. Five of the eight genes have knockout mutants with nonsignificant gravity phenotypes. Two of the eight are still unknown. **1A** shows an earlier iteration of the co-expression network discovered through a manual technique (see Methods).









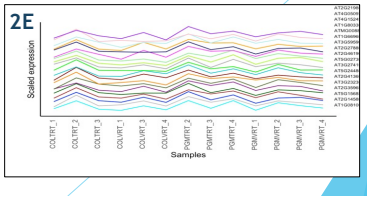


Figure 2 - **A)** Co-expressed networks within our 124 contrast genes. ATTED-II further found four other networks in our contrast genes with 49 of the 124 genes showing above-threshold co-expression. **B)** Genotype-specific preservation analysis of the modules found using multiWGCNA. We found 720 potential modules within our original RNA-seq data; we are now examining these modules through a genotype/treatment-specific preservation analysis. At least a dozen modules found are specific to each genotype (COL-1 vs PGM-1). **C)** Movement of genes within modules in the combined network construction and the genotype/treatment network constructions. Module 72 was found to have minimal preservation in COL-1 samples relative to PGM-1 samples. Genes within this module can be seen to move almost exclusively to modules discovered in PGM-1 samples. Potential movement between vertical, but not treatment, samples too. **D)** Expression levels of Module 72. Genes within module 72 have higher expression during gravity stimulation than during vertical. This effect appears more pronounced in the PGM-1 samples than in the COL-1 samples. **E)** Relative expression among samples of the top 20 genes in module 72. Expression levels of the top 20 show high synchrony within PGM-1 samples than COL-1 samples, supporting **2B** and **2C**.

Discussion:

- Work in the lab continues to explore the individual gravitropic phenotypes of these genes. My work has found evidence of potential, previously unknown, interactions between our genes of interest. With these networks, **we can construct hypotheses** to test *why* knocking out a certain gene causes a specific phenotype.
  - Do certain genes have a regulatory relationship during the gravity response?
  - Does one appear to inhibit or facilitate the response of the other?
  - Many of the genes ATTED-II identified without our contrast genes as being co-expressed with ROSY-1 show no gravitropic phenotype.
    - Do these genes play no role in the gravitropic phenotype of ROSY-1? Or is their role tangential and so redundancy can pick up the slack of their mutant deficit?
- Next steps are to search for genetic relationships that appear unique to the gravity response.
  - multiWGCNA allows exploration of these potential co-expression networks, that we have some insight on due to ATTED-II, with specificity of genotype and treatment type.
  - We are currently playing with lower tree cut-heights to allow network construction of modules with more biologically relevant affiliations to specific genotypes/treatment types within modules.
  - We plan to use the Preservation functions of multiWGCNA to find these biologically relevant modules and and the genes that make them up.
  - Comparisons between these biologically relevant modules and the ones broadly produced by ATTED-II should allow a more thoughtful idea of these genes and their role in the gravity response of plants specifically.

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