BIT 161B : Lab Experiment #3
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Profiling RNA Transcript Expression with Microarray Databases

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Abstract

This experiment serves to investigate the effects of environmental stresses on the expression of NHX genes with the use of databases and online visualization resources. The Bio-Analytic Resource from University of Toronto provided a series of stress trials with the reported northern blot results of mRNA abundance in Arabidopsis. These resources yielded results that indicated that salt stress induces the transcription of NHX antiporters. We believe these results further confirm our prior understanding of NHX antiporters and NHX gene expression.

Introduction and Objectives

The expansion of collected DNA sequence data in recent years has allowed for the widespread implementation of genomic strategies associated with information technology. Expressed sequence tags and whole genome sequencing has led to the accumulation of millions of data points in the NCBI and TAIR (the Arabidopsis Information Resource) which are used to develop maps, visualizations, and models. Microarrays use this plethora of data to analyze nucleic acid abundance in RNA populations of multiple samples. With this microarray data, the localized abundance of RNA expression for any given gene in a completely sequenced genome can be quickly recorded and shared online. Access to this data allows us to investigate the expression of our genes of interest, the NHX genes, in a variety of physiological categories on arabidopsis without doing any PCR or blot tests ourselves. We will be using TAIR to find accession numbers that identify our genes, BAR(Bio-Analytic Resource) to get visualizations of RNA expression in a developmental model of Arabidopsis, microarray, and Affymetrix oligonucleotide array data to get a virtual northern blot test also located on the BAR site. These online resources are to be utilized for an analysis of expression of the NHX genes in Arabidopsis models.

Methods

The entirety of the procedure was carried out as expected from the methods outlined in the lab manual.

Results

The database information we gathered indicated spatially and temporally varying expression between the six NHX genes.

NHX 1	AT5G27150
NHX 2	AT3G05030
NHX 3	AT5G55470
NHX 4	AT3G06370
NHX 5	AT1G54370
NHX 6	AT1G79610

Above are the accession numbers found on TAIR for the NHX genes we set out to investigate. These numbers allow us to search databases with a universal code representing our genes.

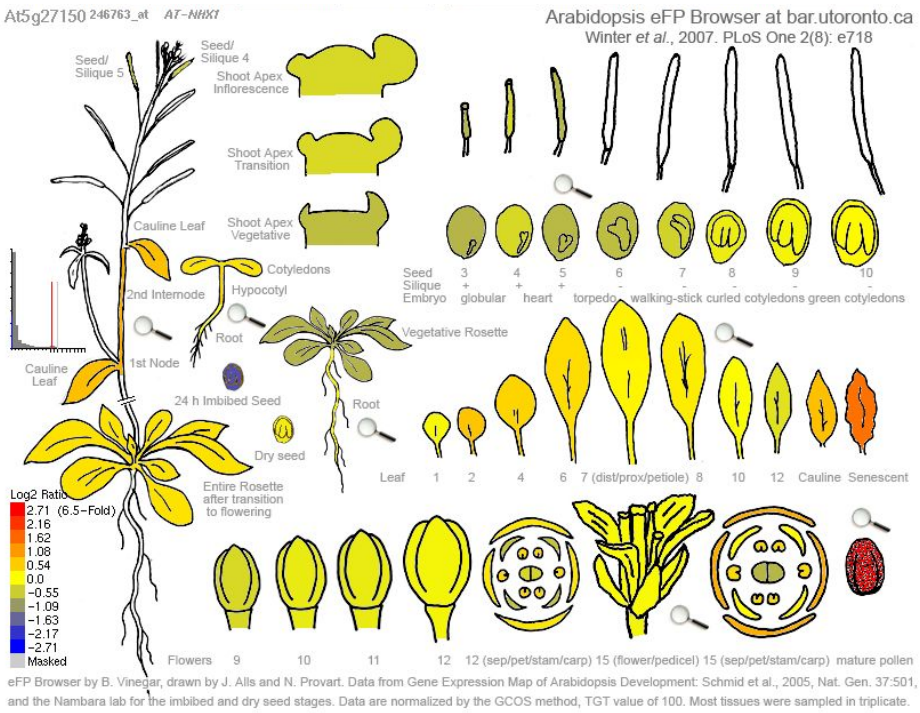

Figure 1. The Arabidopsis eFP Browser application produced the above diagram. It is a developmental map highlighting the presence of mRNA corresponding to NHX1.

According to the map, NHX1 is expressed mostly in the leaves and buds of the plant, increasing slightly as the plant matures or as leaves senesce.

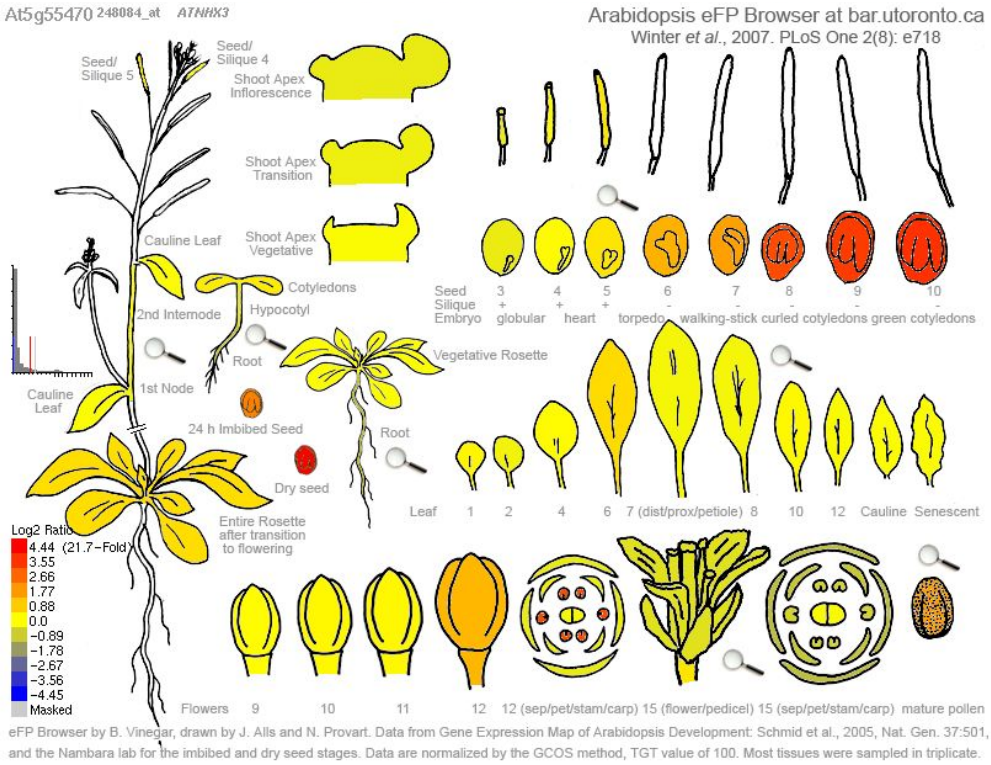

Figure 2. The above image is a developmental expression map of NHX3 mRNA abundance.

The expression of NHX3 is indicated by the map to be fairly even throughout the plant, with particularly high levels in the late stages of the embryo.

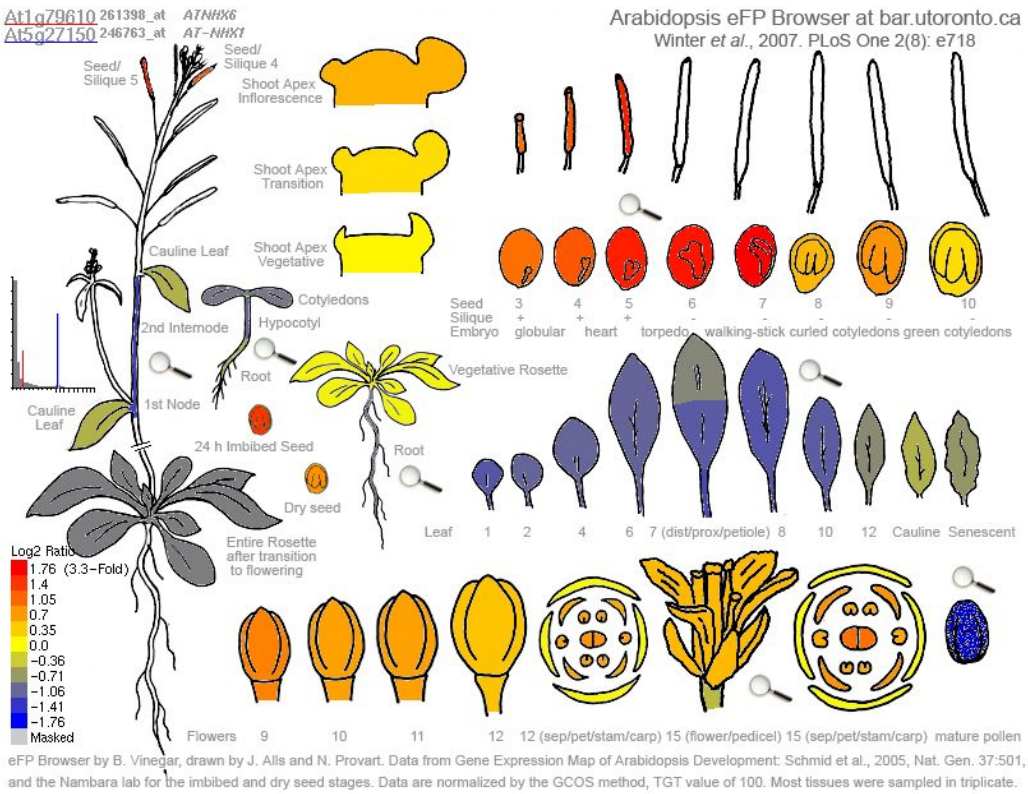

Figure 3. This developmental map displays a comparative analysis of NHX1 and NHX6

NHX1, as we saw in Figure 1, is expressed in the leaves but from this diagram we can see that the expression is much higher than that of NHX6 in the same location. In the embryo however, the expression of NHX6 is much higher than that of NHX1. As the plant matures, the levels of NHX1 expression increase in relation to NHX6 expression levels.

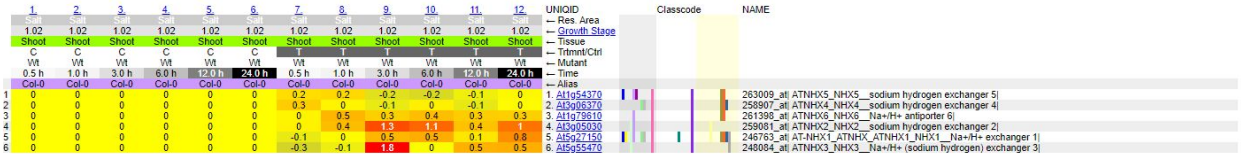

Figure 4. Virtual Northern Blot of Shoot Salt Stress

In these northern diagrams, intensity of color indicates the logarithmically adjusted level of mRNA abundance for a certain gene at a certain time during the experiment. The chosen settings produced results from ten days of seedling growth on media plates. The above image displays the levels of each gene in the shoots of a plant under salt stress conditions. We can see a slight increase in expression in NHX2 and NHX3 at hour 3. Expression of NHX5 saw a slight decrease.

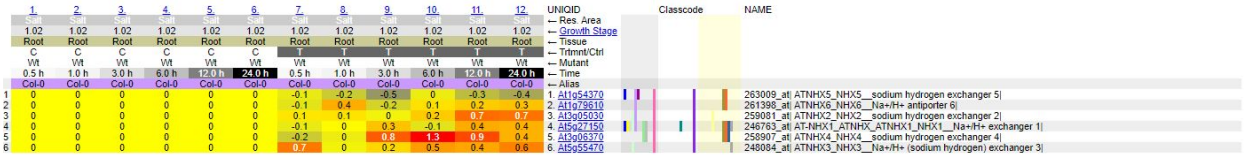

Figure 5. Virtual Northern Blot of Root Salt Stress

The above image depicts mRNA abundance in the roots of plants subject to salt stress. Here again, there is an increase in NHX4 expression and a decrease in NHX5 but the disparity from control measurements is greater.

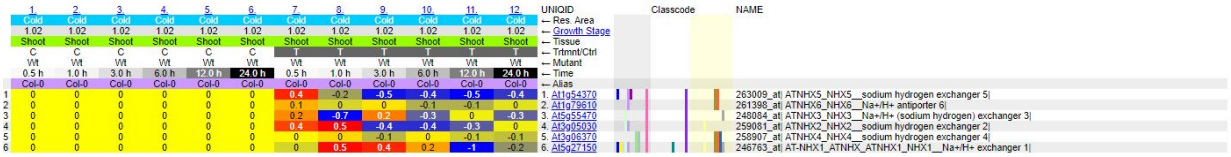

Figure 6. Virtual Northern Blot of Shoot Cold Stress

Here we have data on the response of plant shoots to cold stress. There is considerable suppression of each NHX gene following after a sharp increase in expression for each gene except NHX4.

Discussion

This experiment was a successful foray into in silico analysis of mRNA expression. The differences in expression we observed in this experiment between NHX1 and NHX6 were supported by the findings of our earlier investigation where we used the NCBI database to compare the DNA sequences of both genes. The NHX1 sequence had shown greater similarities to NHX2, NHX3, and NHX4 while NHX5 and NHX6 were more similar to each other than the rest. From this evidence we hypothesized that the functions and expression of NHX5 and NHX6 would be largely dissimilar to the function and expression of NHX genes 1 through 4. That evidence has been corroborated by the observed disparities in expression as mRNA abundance in the development maps. Another point of investigation for our experiment was to determine the effects of stresses on NHX expression based on the northern blot data. We observed that salt stress caused an increase in NHX expression in the roots and shoots of the trial plants. This is in line with our understanding of the role NHX genes play in producing ion antiporters. In a situation of high sodium soil for example, the plant benefits from increased NHX response since maintaining pH and water potential equilibrium will require a greater abundance of antiporters suited to transport Na+ ion across semi-permeable membranes.

Conclusion

This lab experiment was an exploration into the online resources that allow of in silico analysis of gene expression. The accessibility of this precise scientific data to anyone with the initiative to seek it is a momentous step toward the application of information technology in the fields of

genetics. The BAR resources visually organize the results of thousands of trials in a way that allow for quick analysis and comparison. With these tools, geneticists can quickly retrieve and interpret data necessary for many research applications.

References

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