Characterization of the Functional Microbiome of Tobacco
Progress Report
December 2015

Summary

For our first trial we decided to use only the aerial portion of the plant (Stem+leaves). Plants were treated with the inoculants corresponding to stocks S413, S41 and S343 for 12 hours and placed in pots with potting mix. They were grown under greenhouse conditions and monitored for morphological evaluation. Harvesting occurred after 3 weeks post-completing germination.

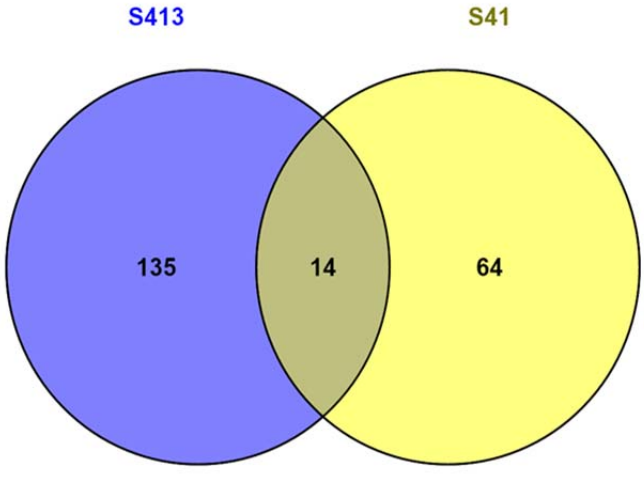
After plant material was processed and RNA was extracted, we proceeded to create libraries for sequencing using a Poly (A)-tag sequencing (also known as Poly(A)-seq which is considered as a better alternative for RNA-seq) approach. A total of 3 biological replicates were used for each treatment, including control.

To date, we have been able to obtain and analyze the data from treatments S413, S41 and Control. A new library set is in the process of being sequence in the following weeks in which treatment S343 is included.

Preliminary results and interpretation

Gene expression patterns showed that when control plants were compared with treated plants (S413 and S41) different sets of genes were downregulated in relationship to stress induced scenarios (S413) and in others they were upregulated (S41).

A total of 150 genes were obtained for S413 which function was determined and in its majority was dominated by intracellular processes, protein folding and binding and regulation. Only one gene from the 150 was specifically indicated to be related to a response to a biotic stress or stimulus. On the contrary, S41 plants showed that from a total of 78 genes, a vast majority were strictly related to DNA, RNA and mRNA binding and defense response patterns, in which hormones like Auxin, Ethylene and Jasmonic Acid came across as highly expressed (Supplementary data 1).



Only 14 genes overlapped among treatments (Figure 1) in which most of them are linked to general metabolic processes and only one is related to biotic stress.

Figure 1.- Number of downregulated genes between S413 compared to S41 treated plants.

For downregulated genes we observed that most of the stress related genes like: heat shock proteins, misfolded proteins and biotic and abiotic genes were dominating the list, which is to what S41 shows. For this last one, photosynthesis related genes were almost ~80% represented as downregulated (Supplementary data1).

A greater amount of genes seen to be downregulated for S41 treated plants, being almost double to the number of genes in S413 (Figure 2).

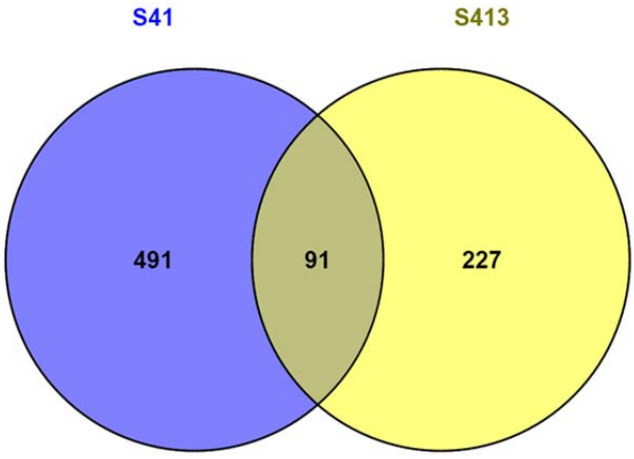


Figure 2.- Number of upregulated genes between S413 compared to S41 treated plants.

Morphological data showed that the growth in S41 is slower than it is S413 plants, which gives as a result a much smaller phenotype when compared to the control. Based on preliminary data obtained, we could possibly link the fast development and greater amount of traits that are evaluated in this project to a “relax” state in which plants treated with S413 seem to be. On the contrary, S41 plants slower development could be related to feeling stressed and taking a longer time to adapt to the environment in which they are growing.

This preliminary data shows that understanding what could be happening to the plant after being exposed to the bacterial strain prior completing germination, may be a better method to select for bacterial strains to inoculate seed and help reduce stress to the plant and therefore increasing the growth of it.

Future work

We are currently working on new libraries for roots of treated plants. Our ultimate goal is to be able to put together the patterns of gene expression of the plant as a whole, which could be used to have a better comparison to the rest of our data in which we have evaluated aerial and underground portions of the plant.

The new libraries will include all the treatments of our original plant growth promoters (S413 and S343), plus our slow developing treatment (S41). Samples will be run in a High-seq platform to obtain a better amount of reads and better coverage of the genome.