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Civitas Alters Grass Leaf Genetic Expression

Bill Kreuser and Frank S. Rossi, Ph.D., Cornell University

Civitas is a novel horticultural oil used to increase plant defense and overall health in turfgrass. Specifically it enhances a form of inate plant immunity called induced systemic resistance (ISR). This type of resistance enhances disease resistances through broad changes in gene expression and gene priming (Djonovic et al., 2007; Cho et al., 2007; Kwon et al., 2010; Shoresh et al., 2010). Application of Civitas to Agrostis stolonifera was found to enhance expression of genes in the jasmonic acid pathway which initiates ISR and primes genes related to pathogen defense (Cortes-Barco et al., 2010a and b). In addition to ISR, Civitas application to turfgrass enhances clipping yield and causes a brief period of non-lethal phytotoxicity. Since the phytotoxicity is short-lived, the plant likely has a mechanism to repair the damaged cuticle. Clearly application of Civitas affects plant physiology and gene expression on a broad scale.

Analysis of the activity, or expression, of all genes in the plant is called transcriptome analysis. Studying the transcriptome is a relatively new and powerful tool to understand plant development, or in this case, how Civitas alters the expression of every gene within the plant's genome. Advancement of next generation DNA sequencing technology as fostered the development of RNA-sequencing for transcriptome analysis. This technique involves removal of and quantification of messenger RNA molecules, the blueprints used to assemble new proteins, to understand which processes are being turned on or off within the plant. Although a relatively new technology, RNA-seq has successfully analyzed the transcriptome of mice, humans, yeast, Arabidopsis thaliana, and maize.

RNA-seq is an ideal platform to understand how Civitas affects the various aspects of turfgrass physiology. The model grass Brachypodium distachyon transcriptome was analyzed following Civitas application because it is a model plant with more genetic resources than with common grasses (Li et al., 2010). The transcriptome was quantified at the leaf base and leaf apex, zones of cell division and elongation and zone of cell maturation and photosynthetic activity, respectively during a five day time course. This analysis will be used to further understand how processes such as growth rate and defense pathways respond to Civitas at the molecular level. Specifically, pathways involved in recovery from

phytotoxicity, carbon partitioning, regulation of photosynthetic and respiratory, biosynthesis of phytohormones, production of secondary metabolites, and expression of proteins involved in growth such as expansins and XET are of particular interest.

MATERIALS AND METHODS Nine mature Brachybodium distachyon plants were grown in a growth chamber at Cornell University. Plant were watered daily and fertilized with a complete fertilizer mix to aid in establishment. The treatments included Civitas alone (2.3 ml m-2), Civitas with the Harmonizer pigment (16:1 v/v) or water (control) replicated three times. A subset of leaves were taken from the plants 3, 24, and 72 h after Civitas application, cut in half, pooled together for each treatment, and flash frozen with liquid N2. This created a time-course from two distinct regions of leaf in response to each main treatment. The mRNA was extracted from the leaves using the Trizol reagent (Invitrogen) and was further purified with the RNAeasy Mini RNA kit (Qiagen). RNA purification, cDNA synthesis and amplification followed the methods developed by Wang et al. (2012; Fig. 1). The cDNA libraries were ligated to HiSeq 2000 primers and sequence with the Illumina Genome Analyzer 2 at the Weill Cornell Medical School.

RESULTS & DISCUSSION Plants were successfully established and treated with Civitas three weeks after germination. Messenger RNA was successfully extracted from the 18 samples and cDNA molecules were successfully constructed (Table 1). The mRNA is turned into cDNA because cDNA is much more stable than RNA for sequencing. Primers are then attached, through ligation, to the small cDNA strands to identify which cDNA came from which treatment during sequencing, much like a bar code. However, there was a problem with the ligation of the HiSeq Primers to the cDNA molecules. The primers did not connect to the cDNA properally. As a result, the primers bound with other primers and not the desired cDNA. This became apparent when the sample was sequenced. The expected cDNA read length was approximately 300 base pairs; however the actual "read" length of a majority of the reads were 66 and 121 base pairs in lenghh which is similar to the length of the primers (Figure 2).

Currently this experiment is being run again. Treatments will be similar to those in the first run with an additional treatment factor of light intensity to stimulate phytotoxicity. However, the rapid advancement of genetic data analysis now allows for the use of non-model plants for RNA-seq analysis. The current experiment will use a perennial ecotype of Poa annua instead of B. distachyon. This makes the results of this experiment even more representative and useful to improving our understanding of the mechanism and role Civitas plays on turfgrass growth and physiology. Despite our setbacks during the first experiment, the potential advancement offered by transcriptome analysis easily justifies the time and expense to redo the experiment. We hope to have insightful results by early fall 2013.

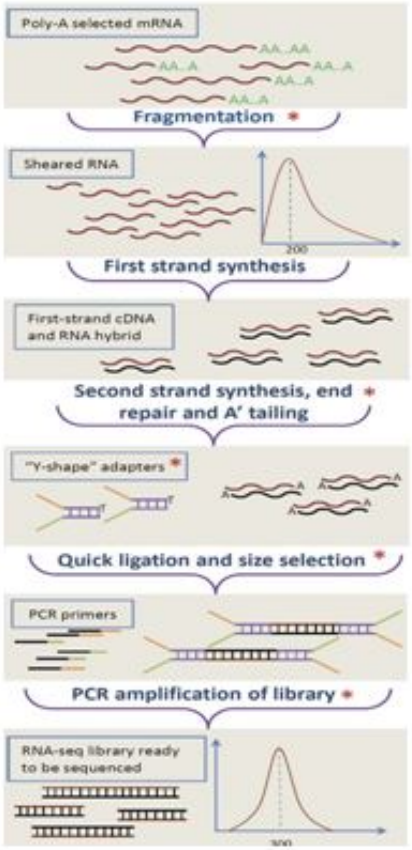

Figure 1. The process of cDNA library construction from mRNA (from Wang et al., 2012). "Y-shape" adaptors were not correctly bonded to the cDNA during the quick ligation and size selection step. This caused the failure of this experiment.

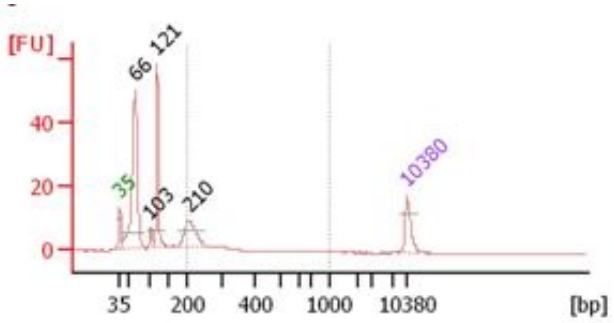

Figure 2. The size of the DNA sequence reads. The peaks were expected to occur at 300 base pair (bp).

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