

Alfalfa Research in Molecular Plant Pathology Laboratory,  
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The project was formally initiated in 2013 in order to acquire fundamental knowledge on the genetic basis of adaptive mechanisms leading to stress tolerance in alfalfa. The following advances were made toward achieving the research objectives:

- I. We completed the first deep sequencing-based profiling of the alfalfa transcriptome in response to salinity stress. Salt-responsive genes were identified and assigned to functional classes, and gene candidates with roles in adaptation to salinity were identified. The data will be useful in understanding the molecular mechanisms of salinity tolerance in alfalfa.
- II. Our *in silico* analysis of available transcriptomic data revealed and systematized alfalfa transcription factors (TFs), proteins that govern responses to the environment by regulating gene expression. Only scattered information on separate TFs was available for alfalfa prior to this work. All data were assembled into a simple open-access database named AlfalfaTFDB (<http://plantpathology.ba.ars.usda.gov/alfalfatfdb.html>). Integrated TFs repertoires of *Medicago sativa* will also provide an important tool for studying regulation of gene expression in other complex non-model species of agricultural significance.
- III. Natural antisense transcripts (NATs) are long non-coding RNAs complementary to the messenger (sense) RNA. Only recently it has become recognized that many NATs play important biological roles. Prior to our work, no information was available on NATs and other lncRNAs in alfalfa. In this study, we discovered that some genes, differentially expressed under salinity stress, generated NATs. Their expression changed under salinity conditions, suggesting a fine-tuning mechanism of gene regulation by NATs in response to salt stress.
- IV. Root-knot nematodes (RKN) can inflict significant damage to alfalfa. Studies on global gene expression profiling in alfalfa infected with RKN or any other plant parasitic nematode were absent in the literature. We performed root transcriptome analysis of resistant and susceptible alfalfa cultivars infected with RKN *Meloidogyne incognita*, a major pest worldwide. Candidate genes that contribute to protection against *M. incognita* in alfalfa were identified.
- V. Novel methodology is needed for functional genomics studies in alfalfa to gain critical insights for breeding programs. To overcome this deficiency, we are developing VIGS (virus-induced gene silencing) technology that so far has not been implemented in alfalfa research as a tool for alfalfa functional genomics. We have chosen an economically unimportant alfalfa pathogen, *Alfalfa latent virus* (ALV), for which no complete genomic sequence was available. We achieved the first complete genome sequence of ALV and the entire viral cDNA was cloned into a plasmid vector that was engineered to express foreign sequences. The vector is undergoing green house testing. We expect that the VIGS approach will greatly accelerate gene function studies in alfalfa.
- VI. Bacterial stem blight of alfalfa caused by *P. syringae* pv. *syringae* occurs in the central and western U.S. and yield losses reportedly can be as high as 50% of the first harvest. To obtain more information on the pathogen, we performed a large scale transcriptome profiling of *P. syringae* pv. *syringae* in the viable but nonculturable state (VBNC) and identified bacterial genes and pathways associated with this condition. These findings offer insights into molecular mechanisms of bacterial pathogenicity and survival strategy.

We believe that knowledge gained through these studies will contribute toward our understanding of stress tolerance in alfalfa and serve as a resource for alfalfa breeding programs.