Medicago GeneChips: A New Tool for Alfalfa Improvement

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Alfalfa plants have somewhere between 35,000-45,000 genes within their 32 chromosomes. At different times during plant growth and development, different sets of genes are turned on or "expressed" and are used to produce many RNA copies of the expressed genes which are then made into proteins. These proteins control every aspect of the plant's life cycle, from seed germination through herbage production to seed formation. Until recently, only one to a few expressed genes in alfalfa could be studied at one time. This technological limitation has severely hampered the ability to identify the genes needed for alfalfa improvement. A major step forward for measuring activity of expressed genes occurred with GeneChips. First created to study human gene expression, GeneChips have recently been developed for many crop plants.

Measuring 2 cm^2 in size, a GeneChip is a silicon wafer on which short strands of DNA have been synthesized. Each chip contains over a million DNA strands (probe sets), each in a unique known location on the wafer. Each gene is represented by 11 probe sets on the chip. To use the chip for gene expression study, RNA is extracted from cells and applied to the chip. Spots on the chip where RNA finds matching DNA segments allow researchers to simultaneously follow the expression of all genes in an organism.

In September 2005, a *Medicago* GeneChip was released by Affymetrix, a company specializing in the development and manufacturing of GeneChips.The *Medicago* GeneChip includes probe sets for genes of three different organisms. The majority of the genes (about 52,700 probe sets) are from barrel medic (*Medicago truncatula*), an annual forage species grown widely in Australia, that is closely related to alfalfa (*M. sativa*). Barrel medic has become a widely researched plant for understanding biology of legumes. In addition, there are probe sets for approximately 1,800 alfalfa genes and all of the genes from the bacterium *Sinorhizobium meliloti*, which is the microsymbiont that nodulates alfalfa for fixing nitrogen. Having both plant and bacterial genes on the same GeneChip allows researchers to study how plant growth or environmental conditions affect both the host plant and its nitrogen fixing bacterial partner.

While alfalfa is an economically important forage crop world-wide, its genetic complexity has hindered progress in understanding the genes controlling important agronomic traits. At the DNA sequence identity level, genes in barrel medic and alfalfa are about 95% identical. The question is whether or not the *Medicago* GeneChip can be used to identify expressed genes in alfalfa. The assumption is that the barrel medic probe sets on the GeneChip should detect the majority of expressed alfalfa genes. ARS laboratories set out to test this assumption by analyzing the expression of genes in leaves and roots of 14-day-old seedlings of alfalfa and barrel medic. On the GeneChip, 24,371-28,668 probes were active in barrel medic root and leaf tissues, and 21,526-23,202 probes were active in alfalfa root/leaf samples. This was good news for alfalfa research as the GeneChip was found to be applicable for the expression of thousands of genes that can lead to the discovery of agronomically important genes in alfalfa.

Currently, the GeneChip is being used to study biological nitrogen fixation, as well as nitrogen and phosphorus stress in alfalfa. Insights into the mechanisms involved in bacterial symbiosis as well as nutrient acquisition in alfalfa have the potential to improve biological nitrogen fixation and reduce costly fertilizer applications. Work is also underway to measure expression of all alfalfa genes throughout plant development as a step to understand important biological processes, particularly those involved in root structure, carbohydrate storage, and pathogen defense, and to identify key genes regulating these processes. This new *Medicago* genomics research tool will be of great utility for the discovery and characterization of genes for improving stand persistence, forage quality, pest and disease resistance, and other traits needed for increased economic vitality of alfalfa and environmentally friendly agricultural production.