

Using Gene Arrays as Tools to Develop Alfalfa as a Biomass Crop

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Biomass crops have been in the news quite a bit lately. That is because there is a need to decrease U.S. dependence on foreign oil imports and reduce greenhouse-gas emissions. Many crop and non-crop plants are currently being considered as cellulosic feedstocks for producing ethanol. Among those most commonly mentioned are switchgrass, Miscanthus, corn (stover), and fast-growing hybrid poplar. Alfalfa is generally overlooked as a biomass feedstock, but should not be. This widely grown crop has a number of advantages compared to other potential feedstocks. Alfalfa is a high-yielding, perennial, non-food crop that fixes atmospheric nitrogen, improves soil quality and provides environmental benefits. And biomass alfalfa cultivars could easily be incorporated into a rotation with corn.

Scientists at the USDA-Agricultural Research Service, Plant Science Research Unit located at the University of Minnesota, St. Paul campus have been working for over a decade to develop alfalfa as a biomass crop. The model that is being proposed involves using the leaves as a protein supplement for livestock and the stems as a feedstock for cellulosic ethanol production. Significant progress toward this goal has been made. JoAnn Lamb, alfalfa breeder, has developed a large-stemmed, non-lodging, biomass-type alfalfa experimental germplasm. Lamb and other scientists at the St. Paul location have also developed a management strategy to increase stem yield while maintaining leaf yield. Researchers found the best strategy for biomass alfalfa involves seeding at a lower density and harvesting at the green pod maturity stage. When this biomass management system is combined with biomass-type alfalfa (ORCA-WTS), biomass production increased by 40% and theoretical ethanol yield doubled compared to hay-type alfalfa (MP-2000) grown under a hay management system (Figure 1).

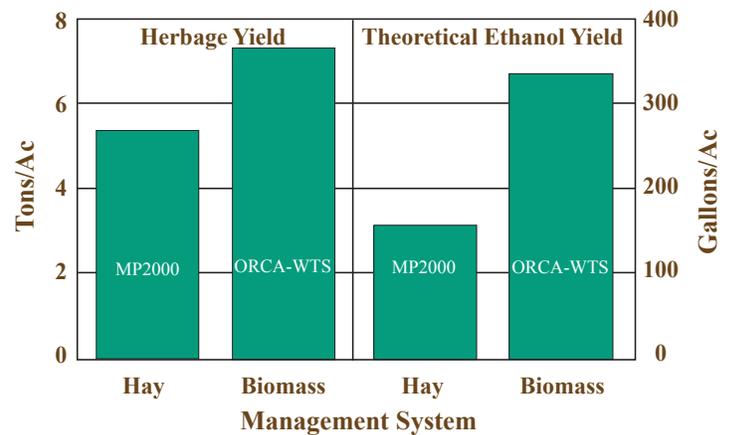
An important next step in developing alfalfa as a cellulosic feedstock is modifying the composition of cell walls in stems. Plant cells are surrounded by a cell wall that provides mechanical support along with many other important functions. Cell walls consist largely of cellulose, hemicellulose, pectin and lignin. Alfalfa stems that have more cellulose and less lignin in their cell walls will yield more ethanol. Alfalfa breeders have spent many years selecting for more cellulose and less lignin in cell walls to improve forage quality for ruminant animals. However, because alfalfa is a perennial with a large, complex genome, selecting for these traits in a conventional breeding program is a slow process, requiring many plant generations and years of selection by breeders. Time required to improve alfalfa as a cellulosic feedstock could be shortened if researchers were able to identify key genes regulating cellulose and lignin production. It is estimated that alfalfa has over 40,000 genes. Finding key genes regulating cellulose and lignin levels in stems is difficult – in many ways it is like trying to find the proverbial needle in a haystack.

With the development of a new technology called gene arrays, researchers now have a way to identify these key genes. Researchers are using gene arrays to compare gene expression in alfalfa plants that differ in the amount of cellulose and lignin in stems. One of these lines, line 252, has high amounts of cellulose and lignin in stem cell walls. In contrast, line 1283 has low amounts of cellulose and lignin. By comparing gene expression (activity) in these two lines, the list of genes can be narrowed to those that are most important in regulating cellulose and lignin. For example, in the high-cellulose alfalfa line 252, researchers should find genes involved in making cellulose that are up-regulated (more active) compared to the same genes in the low-cellulose line 1283.

Before providing a simplified overview of how gene arrays are used for measuring gene expression in alfalfa, how genes control plant traits will be briefly reviewed. All plant cells contain DNA within the cell nucleus. A gene, the basic unit of heredity, is a segment of DNA. Each of the thousands of genes in alfalfa contains coded information that directs cells to perform specific functions. Not all genes in alfalfa stems are expressed or “turned on.” Those genes that are expressed make a molecule called messenger RNA (mRNA) that transfers this coded genetic information out of the nucleus and into the cytoplasm. The coded information in the mRNA is then translated to make proteins. Many people may think of proteins as food, but most proteins are enzymes. Enzymes are tiny “nanomachines” that carry on the chemical reactions in the cell. Many enzymes are responsible for the assembly of cell walls in alfalfa stems.

Now back to identifying the key genes that regulate cellulose and lignin levels in stems of lines 252 and 1283. Gene arrays are used to identify genes that are expressed or “turned on” in cells. Figure 2A shows a gene array called a GeneChip[®] made by Affymetrix. The actual gene array is on a chip located in the square window. If one could peer into the window with a powerful microscope, rows and columns of DNA segments called probes would be seen arrayed over the silicon chip. Each alfalfa gene has been divided up in eleven segments (probes) and each probe has been permanently fixed on the chip in a specific location. In the case of the Medicago GeneChip[®] used, thousands of gene probes are arrayed on the chip.

Figure 1.



The first step is to purify the mRNA from the stems of each alfalfa line. The mRNA is labeled with a fluorescent tag that emits light when exposed to light – like safety vests worn by highway workers. The fluorescently-labeled mRNA molecules from the stems of each line are then applied to separate GeneChips[®]. Since an mRNA molecule is complementary to its parent gene – the gene that originally made the mRNA – it will bind to the probes (spots) on the GeneChip[®] where the DNA of the parent gene is located. The GeneChip[®] is placed in a laser scanner and laser light is applied.

Genes (probes) that are expressed will emit light because the fluorescently-labeled mRNA binds to the gene from which it was derived. If one looked at the chip under high magnification, thousands of light and dark spots would be seen (Figure 2B). Each spot is the location of one of the gene probes on the chip. This pattern shows genes that are not expressed (dark spots), genes that are expressed at a low level (dim spots) and genes that are highly-expressed (bright spots). The scanner collects the light intensity data for the thousands of spots on the chip. Computer analysis from all the probes identifies which genes in alfalfa lines 252 and 1283 are expressed and the level of expression based on the brightness of the spot.

Researchers recently completed the analysis of the GeneChip[®] data for alfalfa lines 252 and 1283. As one might expect for a species with over 40,000 genes, many genes show differences in expression between the two lines. However, researchers focused on genes previously suggested to be involved in making cellulose and lignin. Several key genes that appear to regulate these cell wall components in alfalfa stems were identified.

The key genes that were identified will be used to speed up selection of high cellulose/low lignin cultivars in the conventional breeding program. For example, these genes can be developed as markers that will be useful in selecting for high cellulose/low lignin lines. In addition, researchers can use the tools of molecular biology to genetically engineer alfalfa cultivars to increase expression of key cellulose genes and decrease expression of key lignin genes. Both approaches should shorten the time required to develop a biomass alfalfa cultivar that yields higher levels of ethanol. However, work remains to be done prior to achieving this goal. Hopefully, in the future, alfalfa will no longer be the “overlooked” feedstock, but a widely used renewable, biomass crop that is profitable for farmers to grow and reduces the nation’s dependence on foreign oil imports.

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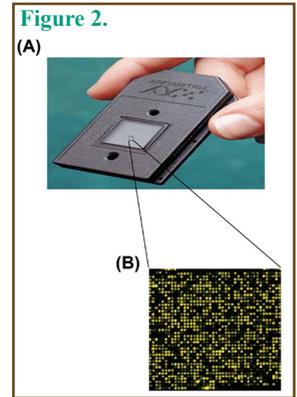


Figure 2. (A) Affymetrix GeneChip[®] (courtesy of Affymetrix). (B) Magnified view of a small region on the chip (Photo source: Juanita Martinez and Angelina Rodriguez, University of New Mexico, Albuquerque).