

WHAT IS ON THE HORIZON FOR ALFALFA & BIOTECH?

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ABSTRACT

Biotechnology is concentrated in two evolving research areas, genomics and transgenics. For the genomics approach, molecular markers combined with sequencing data, high throughput machinery, and sophisticated data analysis allow determinations of complex gene expression. The use of an annual relative of cultivated alfalfa, *Medicago truncatula*, as the reference species for all legumes is likewise viewed as a big plus for using genomic technologies for alfalfa research. Transgenics involve the movement of specific and useful genes into the crop of choice and is sometimes referred to as genetic engineering. A summary of biotech research by the North American Alfalfa Improvement Conference in 2004 showed these biotech approaches are now a large part of alfalfa cultivar development and basic research. However, biotech approaches are both expensive, and in the case of transgenics, controversial, requiring much planning and even partnerships with others. The Noble Foundation and the Consortium to Improve Alfalfa is presented as a model of what may need to be done when using biotechnology for alfalfa improvement. However, the irony is not whether basic biotechnology research is increasing in alfalfa, because it is, but whether we will be able to deliver useful biotech traits directly to the farmer due to costs and concerns with regulatory agencies and the public.

Key Words: alfalfa, biotechnology, breeding, cultivar, genomics, transgenics.

INTRODUCTION

The future of alfalfa cultivar development will surely include the new and evolving areas of genomic and transgenic technologies. Genomics research received great publicity with the successful completion of the project to completely sequence the DNA of the human genome. Plant species soon followed suit with an annual relative of cultivated alfalfa, *Medicago truncatula*, chosen as the reference species for all legumes. Approximately, 50% of the sequencing goals for *M. truncatula*, have now been achieved (Young et al. 2004). These sequencing data, combined with high throughput machinery and data analysis (e.g. bioinformatics), allows determinations of gene expression here to fore not possible. From this understanding, new and innovative methods for improving cultivated alfalfa will evolve.

Transgenics involve the movement of specific and useful genes into the crop of choice and is sometimes referred to as genetic engineering. Scientists using this approach have already shown success in introducing genes which make many important row crops resistant to insects, viruses, and herbicides. It is also been very useful in creating unique plants that allow basic research to be conducted on physiological and biochemical pathways. As will be discussed elsewhere in this

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symposium, the introduction of genes for resistance to the herbicide Roundup may likely be the first of these transgenes to be introduced into alfalfa.

Although the use of genomics for basic research purposes is not controversial, there has been great controversy surrounding the use of transgenes for crop improvement, especially when transferring genes between two unrelated organisms. This has created a very costly regulatory climate, to go along with inherent costs of obtaining freedom to operate for using the gene and the enabling technologies in order to bring a transgene into the alfalfa seed market.

WHAT IS HAPPENING TODAY

The North American Alfalfa Improvement Conference (NAAIC) meets every two years and publishes a “Use of Biotechnology Research in Alfalfa Improvement” report (Brummer et al. 2004). The report is summarized for this presentation in Table 1 and demonstrates the high level of activity currently underway in biotech research and its application in alfalfa and related species. Biotechnology research in alfalfa, especially to study and/or incorporate complex traits, is therefore in a time of increased emphasis and success throughout the world. For example, at the Fourth International Symposium on Molecular Breeding of Forage and Turf held in May 2003 in Dallas, Texas, there were approximately 200 scientists in attendance from 19 countries. Research talks were many and varied on every aspect of basic biotechnology in forage and turf grasses and legumes, but the use of alfalfa in this research was very prevalent (Hopkins et al. 2003). This symposium, and many others like it, is direct proof that research in this area is intense and growing. Ironically, it is not basic research that has not moved forward, but the application of that research to solve on-farm problems.

Genomics has a costly investment upfront, but has less controversy for actual use in the cultivar development process. However, the cost of using transgenics in alfalfa cultivar development is two-fold; the patenting issues enabling the use of a specific technology and regulatory trials with the resulting germplasm. The patenting issues are simply a business decision of whether obtaining freedom to operate from the patent holder is a possible and justified cost. The regulatory costs are more problematic. First, there has not been a new crop de-regulated in several years when one defines a “new crop” as one that has never been in commercial production while containing a biotech trait. Since alfalfa would represent a “new crop” by that definition, then there is concern that even doing all requisite safety trials may not be enough to insure de-regulation. Second, two crops, creeping bentgrass and alfalfa, are currently being assessed by USDA-APHIS for de-regulation for the Roundup Ready gene, a 1980s technology that is currently found in millions of acres of corn, soybean, and cotton. Many companies are therefore watching very intently the final disposition of these two applications. The fact that the creeping bentgrass application has now been in the process longer than any crop to date is not encouraging.

A FUTURE MODEL

The Samuel Roberts Noble Foundation, a non-profit research and outreach organization located in southern Oklahoma, is a good example of the approach needed in this new technology driven climate. The Foundation’s mission is to assist farmers and ranchers in the Southern Great Plains

region of the USA with their agricultural needs. There are currently three operating divisions at Noble: Plant Biology, a research group emphasizing basic research in plant molecular biology; Agriculture, a consulting and outreach group working directly with farmers in the region and conducting on-farm research to support the outreach mission; Forage Improvement, the forage cultivar development and technology application arm of the Foundation. Although each division works within its own area, the real strengths result when they work across divisions and leverage the abilities of each. For example, genes and technologies discovered by Plant Biology are passed to Forage Improvement for conventional breeding and application of biotechnology to produce a cultivar which is then passed to Agriculture for direct on-farm demonstration and farmer use.

The challenges faced by southern Great Plains agriculture can be broadly classified into concerns with dependability, cost, and ease of use, nutritive quality, and environmental desirability of production. Of these, dependability is the critical characteristic needed in this harsh environment. Therefore, the main target species for the Foundation are perennial forages such as tall fescue, bermudagrass, hardinggrass, western wheatgrass, alfalfa, and red and white clover. The target traits are ones that directly impact dependability and survival and include drought and heat tolerance and pest resistance. However, poor nutritive quality, and less often, low levels of animal toxins, are often found in these species. So, other target traits include improving their rate of digestion by ruminant animals through decreasing lignification, and removing naturally occurring toxic alkaloids.

The Foundation's Forage Improvement Division approaches the incorporation of useful genes that govern these traits almost exclusively with conventional selection and breeding techniques. In this approach, as much of the known plant germplasm, often from the USDA plant germplasm system, is collected and screened for the target traits. Sometimes, the traits are very complex and not contained in a species' primary germplasm. For this scenario, biotechnology approaches are an option for trait incorporation.

The Forage Improvement Division, therefore, has the option of turning to the new biotechnologies to help with incorporation of difficult traits into our cultivars. Genomics is a costly investment upfront, but has less controversy for actual use in the cultivar development process. In Plant Biology Division, there has been a concentrated effort to produce genetic sequences of genes expressed by *Medicago truncatula*. These are called ESTs and have been useful for producing molecular markers to locate and follow important genes for aluminum, drought, and disease resistance.

However, due to the cost and controversy of using transgenics, it is our option of last resort. Transgenic biotechnologies do provide very powerful and useful alternatives to not having the trait altogether, but the main question for us is this: Is the trait of such value and impact that it will justify a biotechnological approach? The answer to this question for alfalfa involved an additional component to the Foundation's existing model. This component was achieved through the Consortium to Improve Alfalfa.

The Consortium to Improve Alfalfa is made up of researchers from Noble Foundation, the U.S. Dairy Forage Research Center (USDFRC) in Madison, WI, and Forage Genetics International

(FGI), a commercial alfalfa research and seed company. The purpose of the consortium is to improve important characteristics of alfalfa such as nutritional content and digestibility. The first two initiatives by the consortium will focus on improving protein utilization and cell wall digestibility via lignin reduction. Therefore, the consortium major goal is to re-design alfalfa as a major forage source. This would be of such impact as to justify use of any biotechnologies. It also brings to bear additional resources to leverage with those of the existing Noble model: commercialization with enabling technologies from FGI and abilities in animal and dairy nutrition including facilities for controlled assessment of the forage with USDFRC. The Noble Foundation, and especially its partnership in the Consortium to Improve Alfalfa, is therefore a future model of what may need to be done to justify the costs when using biotechnology for alfalfa improvement.

CONCLUSIONS

The irony is not whether basic biotechnology research is increasing in alfalfa, because as shown above, it is, but whether we will be able to deliver useful biotech traits directly to the farmer. Consortia of various partners like those described for the Consortium to Improve Alfalfa will also be important to bring the fruits of these new technologies to researchers and farmers alike. However, it is hoped that more funding will be available to help the regulatory agencies in assessing the question of real versus perceived risks. At the end of the day, these agencies will need to make decisions on what are the real risks, establish a rigorous regulatory process to assess these risks, oversee the regulatory process in a fair manner, and make a decision! We can all then move forward based strictly on the value of the traits to the well-being of the environment, the farmer, American agriculture, and all citizens of this country.

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Table 1. Summary of alfalfa biotechnology research as reported by the North American Alfalfa Improvement Conference (Brummer et al. 2004).

USA	
Arizona State Univ.	Over-expression of salt tolerance genes
Forage Genetics Int'l, West Salem, WI	Commercialization of Roundup Ready gene and down regulation of lignin genes to increase digestibility
Iowa State Univ.	Identifying alfalfa genes controlling yield and winter-hardiness
New Mexico State Univ.	Identifying genes controlling salt and drought stress
Purdue Univ.	Cloning genes for vegetative storage proteins
Samuel Roberts Noble Foundation, Ardmore, OK	Developing molecular markers, studying down regulation of lignin genes, insertion of genes for condensed tannins, identifying and introgression of drought and aluminum tolerance genes
USDA-ARS, Beltsville, MD	Developing molecular markers and using markers for identifying genes for yield and winter survival.
USDA-ARS, Madison, WI	Characterize genes controlling post-harvest proteolysis.
USDA-ARS, St. Paul, MN	Insertion of genes to allow remediation of atrazine and genes to control pectin in cell walls.
USDA-ARS, Prosser, WA	Using molecular techniques for quick identification of disease pathogens.
Univ. of California, LA	Expression of plant genes controlling nodulation and nitrogen fixation.
Washington State Univ. and USDA-ARS	Molecular markers to characterize diversity among alfalfa accessions.
Canada	
Agriculture and Agri-Food Canada. Saskatoon	Alter expression of condensed tannin genes to reduce bloat, greenhouse gases, and protein bypass
Agriculture and Agri-Food Canada. Ste-Foy	Identification of genes for improved persistence, yield, and cold tolerance.
Medicago Inc., Ste-Foy	Using alfalfa as factory to produce pharmaceutical products
Univ. of Guelph	Genetic engineering to modify winter-hardiness.
Mexico	
National Univ. of Mexico	Using anti-sense and over-expression approaches to study nodulation and nitrogen fixation
Europe	
AgroBioInstitute, Bulgaria	In vitro selection to increase tolerance to abiotic stresses
INRA, Toulouse, France	Genomic approaches for nitrogen fixation, disease resistance, and abiotic stress tolerance.
BAP, Toulouse, France	Induction of plant defenses during disease infection via genetic and cellular approaches.
INRA, Lusignan, France	Developing molecular markers for candidate genes for aerial morphogenesis and genetic mapping.
Univ. of Perugia, Italy	Study of aluminum tolerance genes and examining methodology to remove antibiotic resistance during selection of transgenes.