

THE FUTURE OF ALFALFA AND FORAGE CROPS

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ABSTRACT

A diversity of forage species and forage ecosystems suited to different management practices exists in the United States. The future of cultivar development in forages includes the new and evolving areas of genomic and transgenic technologies. Genomic research gained momentum with the publication of the complete DNA sequence of the human genome and has since expanded to plant species including the model legume *Medicago truncatula*, which is a close relative of alfalfa. The integration of sequence information obtained using high throughput approaches with phenotypic data for traits of agronomic importance and biotechnology enable the development of genetic solutions to abiotic and biotic factors limiting forage productivity. The strategy used to integrate biotechnology for alfalfa improvement includes partnerships through the Consortium for Alfalfa Improvement. The utilization of molecular breeding approaches integrating the latest technologies will allow the implementation of sustainable and profitable integrated systems to increase the efficiency of cultivar development to meet existing and future needs of alfalfa and forage crop production.

Key Words: abiotic stress, alfalfa, biofuel, biotechnology, molecular breeding

INTRODUCTION

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 Noble 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. Therefore, other target traits include improving their rate of digestion by ruminant animals through decreasing lignification, and removing naturally occurring toxic alkaloids.

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The Consortium for Alfalfa Improvement is made up of researchers from the 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 consortium's major goal is to re-design alfalfa as a major forage source. Therefore, the first two initiatives by the consortium focus on improving protein utilization and cell wall digestibility via lignin reduction. The potential impact of these traits justifies the use of conventional and biotechnology approaches and includes leveraging of resources at the Noble Foundation with FGI for commercialization with enabling technologies and facilities for controlled assessment of the benefits of the enhanced forage in animal and dairy nutrition at USDFRC.

ALFALFA PRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important forage legume species and the third most valuable crop in the country (\$8 billion per annum). Alfalfa is a perennial species that requires little or no nitrogen fertilizer because of its ability to perform symbiotic nitrogen fixation. Due to the production of more protein per hectare than grain or oilseed crops, alfalfa is highly desirable for hay production and pasture for livestock. Dormant-specific alfalfa cultivars with additional traits such as grazing tolerance (Bouton et al., 1993), low bloat potential (Goplen et al., 1993) and insect resistance (McCaslin, 1994), are now being marketed in the main alfalfa growing regions. Alfalfa has been an important component of sustainable agricultural systems for many years and is set to become an integral player in a sustainable bio-fuel industry (Ebert, 2007), especially if factors limiting its productivity are overcome. Productivity in tetraploid alfalfa is limited by multiple abiotic and biotic stress factors. Therefore, continued research on alfalfa breeding and management is necessary to expand adaptation of alfalfa to hostile environments with less-than-optimal soil conditions, limited water availability, and resistant to insect pests and diseases to maximize yield and economic return. The goals of the forage legume breeding and genomics program at the Noble Foundation are to identify genetic solutions for abiotic and biotic limitations in forage legume productivity and to develop molecular technologies to improve the efficiency of cultivar development.

ABIOTIC STRESS TOLERANCE

Drought. Drought is considered the most important cause of yield reduction in crop plants (Bohnert and Jensen, 1996). Soils with low water availability constitute about 45% of the U.S. land surface and drought alone accounted for 41% of the insurance indemnities for crop losses between 1940 and 1978 (Boyer, 1982). In most of the Southern Great Plains and western U.S., inadequate surface water supplies, declining water tables, and high water pumping costs limit alfalfa production (Ray et al., 1999). Drought stress on perennial forages reduces forage yield and limits persistence. A collaborative project with New Mexico State University aims to identify genetic mechanisms associated with water-use-efficiency (WUE), drought tolerance and forage biomass production in two tetraploid alfalfa populations derived from a cross between genotypes of *M. sativa* subsp. *sativa* var. Chilean (low WUE) and *Medicago sativa* subsp.

falcata, var. Wisfal (high WUE). Forage biomass yield was evaluated under both irrigated and drought conditions at two locations (NM and OK) and used to identify molecular markers associated with increased yields under both water regimens. Current efforts include evaluating a core collection of alfalfa germplasm including various subspecies and genotypes collected from arid regions to identify additional sources of useful genetic variation in the drought-stress response. The feasibility of using biotechnology to enhance drought tolerance in alfalfa is also being evaluated.

Aluminum. Approximately 30% of the soils worldwide are acidic and over 50% of the world's potentially arable lands are acidic (von Uexkull and Mutert, 1995). Acid soil syndrome, which consists of phytotoxicity to excess ions such as aluminum (Al^{+3}) and protons (H^{+}), and a deficiency of essential nutrients including phosphorus (P), is a major factor limiting crop productivity (Delhaize and Ryan, 1995; Ishitani et al., 2004). Because very small concentrations of Al^{+3} can inhibit and damage the root system (within minutes of exposure), it also significantly reduces yields due to an insufficient intake of water and other nutrients (Kochian, 1995; Kochian et al., 2004). Heavy applications of limestone and P fertilizer are commonly used to prevent growth losses associated with acid soil syndrome (Al^{+3} and H^{+} toxicity, and P deficiency), but these amendments are often not economical or practical due to higher energy inputs and because the buffering capacity (the amount of lime required to change the pH a given amount) of various soil types can greatly increase establishment costs (Haby et al., 2002).

The mechanisms underlying resistance to Al toxicity in forage legumes have yet to be determined and to date, no alfalfa germplasm with Al tolerance is commercially available. Our strategy is to utilize a combination of tissue culture, nutrient solution and soil-based assays to evaluate populations developed at the Noble Foundation and at the University of Georgia to combine Al tolerance in alfalfa with desirable agronomic characteristics. Ongoing efforts to confirm molecular markers associated with Al tolerance will allow us to implement molecular breeding strategies to develop improved alfalfa cultivars which can be productive in acid and Al-toxic soils.

BIOTIC STRESS TOLERANCE

Alfalfa productivity is limited by multiple insect and disease pests. For example, potato leafhopper (PLH) feeding in alfalfa causes losses in biomass yield and nutritive quality (Hutchins and Pedigo, 1990; Sulc et al., 2004). We are using bulk segregant analysis to identify molecular markers associated with alfalfa potato leafhopper resistance. The goal is to use these markers in an applied breeding program to develop alfalfa cultivars with durable PLH resistance. The methods and techniques being utilized have the potential to address other biotic stress factors limiting alfalfa productivity such as aphids and nematodes.

DIGESTIBILITY AND BIOFUELS

Alfalfa. Improving the digestibility of forages translates to enhanced animal performance and reduces excessive animal waste. Researchers at the Noble Foundation have been successful in manipulating lignin composition and levels in alfalfa and other forages to improve their digestibility and the conversion of biomass to biofuels (Chen and Dixon, 2007). Alfalfa lines independently down-regulated in all key enzymatic steps of the monolignol biosynthetic pathway have been developed. Ongoing efforts aim to understand the biological mechanisms underlying agronomic traits of alfalfa lines with altered lignin composition to enhance the efficiency of the lignocellulosic biomass conversion process while maintaining good agronomic performance. These lignin manipulation techniques should allow development of plants capable of producing ethanol much more efficiently in a way that is cost effective.

Switchgrass. Switchgrass is a perennial grass native to North America. It is a tall, sod forming species that is currently used mainly for hay, summer pasture, and erosion control in the Great Plains region. Switchgrass has very unique characteristics that make it useful as forage and pasture. First, it is the earliest warm season grass to break winter dormancy in the southern region; with growth reaching 4 to 6 tons by June 15 depending on rainfall. This growth is also very high in nutritional quality (60 to 70% TDN and 10 to 12% crude protein). During this period, we have found animal gains, depending of stocking rate, of 2.2 to 3.5 lbs per day and total gains of 100 to 200 lbs per acre. Interest in this grass peaked when it was identified by the U.S. Department of Energy (DOE) as a primary target for development as a dedicated energy crop because of its potential for high fuel yields, hardiness, and ability to be grown in diverse areas. Switchgrass is relatively drought-tolerant, grows well on marginal cropland and shouldn't require heavy fertilizing or intensive management practices.

The economics of growing alfalfa and other forages such as switchgrass as energy crops will be determined by a combination of variables including market demand, input requirements and costs, government support programs, value for alternative use of the land, and monetary returns for the crop itself or its products (Bouton, 2007). Despite years of research and the development of improved biofuels feedstocks through government research programs, little has been done to definitively establish these economics. Therefore, the Foundation's work in bioenergy crops will continue to focus on using alfalfa and other species such as switchgrass as dual purpose crops for forages and potential bioenergy. Ongoing efforts on molecular breeding, management, and biotechnology to increase forage biomass yields will also be used to address the input vs. output model to obtain a profitable bioenergy enterprise.

DNA MARKERS AND BIOTECHNOLOGY

Molecular markers. Molecular markers are specific pieces of DNA sequences. Each molecular marker is located in a specific place in the chromosomes of a plant. Molecular markers are similar to road signs because they provide information on the location of a particular DNA sequence. There are many different types of molecular markers, but they are all used to detect differences in DNA sequences. The value and uses of various types of DNA markers have been shaped in large part by contemporary innovations in marker technologies that increased

throughput and reduced costs per data point. Molecular markers can be used to improve efficiency and/or accuracy in plant breeding programs and have been recognized as a cost-effective alternative to visual selections. Improvements in sequencing and technologies to screen large plant populations are being driven, in part, by the great interest that the medical and human genetics communities have in using molecular markers to find and map genes associated with human diseases. We are currently using these technologies to address issues of importance to agriculture, biotechnology and energy production in alfalfa and other forage crops.

Quantitative traits. Certain traits, such as flower color, are determined by the action of one or a few genes (qualitative) and are easy to see (yellow vs. purple flowers). Other traits, such as biomass yield, are affected by the action of many genes (quantitative), and require some sort of measurement (the kg of biomass produced or the height of a plant). Most traits of agronomic importance, such as the amount of biomass produced, are complex and affected by the action of many genes. Molecular markers are used to identify specific segments of DNA in a plant chromosome which contain the genes responsible for a particular trait, and these are known as quantitative trait loci (QTL). These loci or fixed positions in a chromosome are defined by specific pieces of DNA and molecular markers are used to tag these positions. Researchers can then use the molecular markers tagging these QTL to track the genes in a given position during the breeding process. For example, if you cross a plant with resistance to a pathogen with a susceptible plant, you can use molecular markers associated with resistance genes (or QTL) to identify the plants from the resulting cross that will also be resistant. Overall, once you find the molecular markers associated with a given trait, you can use the DNA of a plant to anticipate its performance (resistant vs. susceptible) without actually challenging the plant with the pathogen.

Biotechnology. The report from the “Use of Biotechnology Research in Alfalfa Improvement” Committee of the North American Alfalfa Improvement Conference highlights the level of ongoing biotechnology research throughout the world and its application in alfalfa and related species (Brunner et al., 2008). However, the cost of using transgenics including patenting issues to use specific technologies and regulatory trials for field evaluations of the resulting material are important considerations for commercial production of a forage species with a biotech trait. Therefore, the main consideration is whether the trait has significant value and impact to justify using a biotechnology approach.

The use of molecular markers and biotechnology can contribute to targeted breeding or engineering of plants that are able to thrive under adverse environmental conditions by increasing our understanding of mechanisms used for growth, development, and adaptation to abiotic and biotic stress factors. The conservation of genetic mechanisms across species and the transfer of knowledge from one species to another can also lead to major innovations in crop improvement via marker-assisted breeding (Dangl et al., 2008). Existing infrastructure for using molecular markers at the Noble Foundation is an attractive option for those interested in implementing marker-assisted breeding strategies to develop enhanced alfalfa cultivars. Access to the latest alfalfa cultivars by producers and ranchers would allow them to reduce inputs, increase efficiency, and be competitive in the world market. Molecular markers and biotechnology offer additional opportunities to address target traits, facilitate the development of enhanced cultivars, integrate multiple desirable traits, such as forage quality, abiotic and biotic stress and select parents to maximize biomass yields.

CONCLUSION

Interaction of breeders and farmers has identified key traits with high impact potential including enhanced water-use-efficiency, tolerance to drought and aluminum toxicity, resistance to disease and insect pests, increased biomass yield, nutritional quality, and persistence. The availability of reliable phenotypic assays, molecular breeding tools and efforts to leverage information from model species will enhance the identification of genes underlying these complex value-added traits and greatly facilitate the development of enhanced forage legume cultivars. Alfalfa and switchgrass are set to become important components in the bio-fuel industry driven by new cultivars with higher biomass yields and enhanced nitrogen use efficiency in agricultural production systems. Integration of molecular breeding approaches and the latest technologies will contribute to the sustainability of profitable forage crop production systems to meet current and future demands for these crops.

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