Application of Genomic Tools for Enhancing Alfalfa Resistance to Biotic and Abiotic Stresses

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Abstract: Quantitative traits such as biotic and abiotic stress resistance are most likely under the control of multiple genes and interact with environmental factors. Identification of resistance loci that contribute to variation in such complex traits, is a primary challenge in plant breeding and population genetics. In the present study, we used an integrated framework that merges a QTL mapping approach called “genome-wide association (GWA)” with high-throughput genome sequencing methodology called “genotyping by sequencing (GBS)” to map disease and drought resistance loci in alfalfa. This framework provides a statistical basis for analyzing marker-trait association using linkage disequilibrium. We have identified a group of significant GBS markers associated with resistance and there are located on different chromosomes. Validation of the markers is in progress. Once validated, these SNP markers can be used for marker-assisted selection. Quantitative traits such as biotic and abiotic stress resistance are most likely under the control of multiple genes and interact with environmental factors. Identification of resistance loci that contribute to variation in such complex traits, is a primary challenge in plant breeding and population genetics. In the present study, we used an integrated framework that merges a QTL mapping approach called “genome-wide association (GWA)” with high-throughput genome sequencing methodology called “genotyping by sequencing (GBS)” to map disease and drought resistance loci in alfalfa. This framework provides a statistical basis for analyzing marker-trait association using linkage disequilibrium. We have identified a group of significant GBS markers associated with resistance and there are located on different chromosomes. Validation of the markers is in progress. Once validated, these SNP markers can be used for marker-assisted selection.

Alfalfa has the fourth highest production value of all crops in American agriculture. Production of alfalfa is challenged, however, by endemic and emerging diseases and adverse environmental factors. On a national level, diseases and abiotic stresses affecting alfalfa production result in losses of nearly $1 billion annually. Management of soilborne diseases, such as Verticillium wilt, can be difficult because the same pathogens affect several legumes grown in rotation, and persist in the soil for many years. Stem nematode is one of the most serious pests of alfalfa in the United States and causes serious yield, forage quality, and stand losses. Irrigation is necessary for high yields achieved for alfalfa production in the western United States. Climate change and extreme weather adversely impact water availability. Drought resistant and water use efficient alfalfa cultivars are needed to help meet the challenges of finite water resources. Traditional procedures for
selecting resistant varieties are time consuming and imprecise, which limits their utility for breeding resistant varieties. The objectives of this research are: 1) to identify molecular markers in alfalfa associated with resistance to alfalfa Verticillium wilt and stem nematode, and 2) to identify alfalfa molecular markers and germplasm associated with drought tolerance and increased water use efficiency.

1 Identify genes associated with resistance to Verticillium wilt and stem nematode in alfalfa and develop molecular markers for marker-assisted breeding

Verticillium wilt and stem nematode severely affect seedling vigor and yield in alfalfa stands. No rapid means of detection of resistance resources is available using molecular assays and scientists must utilize the alfalfa standard test for evaluating disease resistance which is time consuming and requires greenhouses, bench space and a considerable amount of preparation and labor. Development of molecular markers for high-throughput analysis would significantly reduce the amount of time for identifying source of resistance and accelerate the breeding process. To address this, USDA-ARS Prosser, the Samuel Roberts Noble Foundation, Forage Genetics International (FGI), Pioneer Hi-bred, and Alforex Seeds agreed on a joint effort to identify publically available markers for selection for resistance to Verticillium wilt and stem nematode. Pioneer and Alforex generated and phenotyped biparental populations 188 and 164 individuals, respectively, using parents either resistant or susceptible to Verticillium wilt. We screened the parents and a subset of individuals from both populations with 156 SNP markers, using the high-resolution melting technique. Associations between marker genotypes and Verticillium wilt phenotype were analyzed. Several markers were significantly associated with the trait at p<0.05, but at most 8 percent of the phenotypic variation explained (PVE). No markers were identified for stem nematode at this stage.

To increase the power and resolution of marker-trait association, we are using a genome-wide genotyping platform so-called “genotyping by sequencing (GBS)” which will increase thousands of SNP markers throughout the whole genome. We have genotyped the Pioneer VW population by GBS and obtained nearly 10,000 meaningful GBS markers. Improved methods increased the probability for identifying molecular markers associated with VW resistance. Preliminary result showed strong association of a group of GBS markers with the trait at p<0.001, with the highest PVE of 19%. To confirm this finding, we are going to validate them for VW resistance in different populations. After validation, they can be used in marker-assisted selection for identification of resistance sources. It will facilitate breeding progress and provide alfalfa seed companies with breeding materials for use in the production of improved commercial varieties of alfalfa. It will accelerate the breeding program of resistant varieties, reduce economic losses to growers, increase sustainability, and reduce costs to consumers. Similar procedures will be used for identification of markers associated with stem nematode resistance.

2 Identification of drought-tolerant Medicago accessions and development of alfalfa
populations with drought tolerance and enhanced water use efficiency

Environmental stresses account for approximately 70% of crop yield depression with drought causing the greatest yield losses. Drought tolerance has long been an important trait for improving crop performance under water-limited conditions. Most alfalfa in the Western US is produced under irrigation. Water usage for alfalfa is high, with California’s production consuming more water than any other crop in 2012. Demand for fresh water resources is increasing for growing western urban populations, irrigation for food production, and for providing habitat for fish and other fresh water aquatic species. In addition, reduced snowpack and runoff effects from global climate change indicate future water shortages. As a consequence, increased water use efficiency (WUE) is a key factor for sustainable use of water for alfalfa production and remains an important target trait for alfalfa breeders. Compared with other crop species, little is known about mechanisms by which genetic and physiological factors contribute to drought tolerance and WUE in alfalfa.

Two hundred PIs with potential drought tolerance were selected from arid and semi-arid regions in Northern America, Turkey and former Soviet Union. They have been growing in the field and greenhouse for screening for drought tolerance and WUE in both greenhouse and field conditions. Agronomic and physiological traits including relative leaf water content, osmotic adjustment, canopy temperature, forage quality and biomass under drought were measured. Drought-resistant accessions selected in the above experiments will be crossed with drought susceptible varieties and backcrosses will be made to create F1 populations. Phenotypic screening for drought related traits will be conducted in F1 individuals as described above. Segregating populations for drought tolerance and water use efficiency will be used for mapping QTL associated with drought tolerance and enhanced WUE. Our goal is to understand the genetic basis of drought tolerance and WUE in alfalfa so that we can more efficiently develop stress tolerant alfalfa cultivars. This work will increase our understanding on mechanisms by which genetic and physiological factors affect drought tolerance and enhanced WUE in alfalfa. The information will be used for developing DNA markers associated with drought tolerance and improved WUE for evaluation of their usefulness in developing alfalfa cultivars with sustained productivity of alfalfa under limited-water condition. DNA markers will provide breeders with molecular tools for more rapidly and precisely selecting drought tolerant and improved WUE alfalfa varieties. Identification of alfalfa varieties with resistance to drought and improved WUE will provide resources for breeding abiotic resistant alfalfa. Commercial alfalfa seed companies and public and private scientists will benefit from the availability of improved germplasm and accelerate breeding progress. Growers will have increased opportunity for the profitable cultivation of alfalfa. The general public will benefit from reduced consumer costs and reduced yield loss by biotic and abiotic stresses.