

## Genetic Analysis of Complex Traits in Alfalfa

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Despite the importance of alfalfa (*Medicago sativa* L.) to animal nutrition and sustainable agricultural practices, breeding efforts over the last twenty years resulted in little to no progress for forage yield in the upper Midwestern US. Multiple issues underlie this problem, but our lack of understanding of the genetics of complex agronomic traits is certainly one of the more important factors. The autotetraploid nature of alfalfa hinders our understanding of its genetics and slows breeding progress. However, the application of molecular marker technology for genetic linkage mapping and, in combination with phenotypic data, for QTL (quantitative trait loci) mapping provides a way of improving our knowledge of alfalfa genetics by identifying regions of the alfalfa genome associated with important traits.

We crossed the subspecies *M. sativa* subsp. *falcata* and *M. sativa* subsp. *sativa* and created a fullsib, F<sub>1</sub> population consisting of 200 genotypes. Using this population, we created genetic linkage maps of both parental genomes (there is no recombination between parental genomes in an F<sub>1</sub> population) using 65 RFLP probes and 111 EST-SSR primers. Both parental maps consist of eight consensus linkage groups that correspond to the eight basic chromosomes of the alfalfa genome. Each of the eight consensus linkage groups is further divided into four co-segregation groups that correspond to the four homologues of each alfalfa chromosome. The maps are about 470 cM in length and have an average of 18 loci per consensus linkage group or about 1 loci every 3 cM. This represents good marker coverage of the alfalfa genome and a high level of saturation resulting in a good framework for our QTL studies. About a third of the marker alleles exhibit distorted segregation patterns. However, this is consistent with most of the previous alfalfa mapping studies and caused no difficulties in the mapping process. The use of EST-SSRs allowed us to more highly saturate the maps and also linked this map to that of the model legume *M. truncatula*.

We grew the population at three field locations (Ames and Nashua, IA and Ithaca, NY) and collected phenotypic data for a variety of traits (including forage yield, harvest height, and regrowth) over three years (1999-2001). There was a large amount of transgressive segregation within the population for each of the measured traits with some genotypes significantly outperforming the high parent and some genotypes significantly underperforming the low parent. While there was significant genotype-by-environment interaction for each of the traits, broad-sense heritabilities were fairly high (typically above 0.7), indicating the importance of the underlying genetics. By combining the molecular marker data with the phenotypic data, we identified potential QTL (regions of the alfalfa genome) associated with these traits. In particular, we identified regions, on linkage groups 2 and 7, significantly associated with forage yield ( $R^2$  values ranging from ~ 6 % to 10 %).

The results of this study indicate that both major subspecies of cultivated alfalfa contain alleles associated with important agronomic traits. They also provide a framework for further work focused on developing a marker-assisted selection program for alfalfa improvement. The use of markers in conjunction with improved selection methodology, such as hybrid alfalfa development, may provide the key to overcoming the stagnation in forage yield gains and may improve the efficiency of the selection process.