Dissecting Alfalfa Dormancy Using Selection Mapping

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Alfalfa adaptation is determined to a large extent by autumn induced (fall) dormancy. Dormant germplasm begins to slow growth due to decreasing photoperiod and temperature during autumn unlike nondormant germplasm which continues to grow if conditions are favorable. Understanding the genes controlling autumn dormancy would be useful in breeding, for example, but ensuring germplasm planted in selection nurseries was of a particular dormancy class. To determine genes involved in dormancy, we used divergently selected populations to evaluate allele frequency changes that might be correlated with dormancy expression. Dr. Teuber selected three cycles for taller and shorter autumn growth in six backgrounds different in dormancy expression. We used Genotyping-by-Sequencing based on Elshire et al. (2011) as modified by our lab (Li et al., 2014, 2015) to evaluate four pools of 24 plants (a total of 96 individuals) for each population. We called SNP using a modified version of GBS-SNP-CROP (BMC Bioinformatics. 2016. 17:29). We also bulked the 96 genotypes into a single in silico pool for each population. We aligned SNP markers to Medicago truncatula and to alfalfa draft scaffolds. We then computed frequencies for each allele in each sample.

For the three CUF101 populations, we found ~85,000 SNP loci that had at least 100 reads/population; ~17,000 were alfalfa specific. The four replicate pools for each population, and their overall pool, cluster together, providing evidence that our GBS markers are robust. In CUF101, of the 85 markers most highly correlated with PC1, 79 were also among the most extreme FST outliers, suggesting these loci are involved with dormancy response.

To identify loci across all genetic backgrounds, we have a problem with analysis because few locai have 100 sequence reads in each of the 18 populations (3 populations x 6 backgrounds). However, we can identify many with at least 30 reads per population. With few reads, however, our estimate of allele frequency is less robust. Thus, we implemented a Baysian model-based approach to account for our uncertainly in frequency estimation and for the effect of genetic background in order identify markers whose allele frequencies have shifted across selection cycles, making them possibly associated with dormancy. Based on our model, we identified allele frequency shifts across our six genetic backgrounds. We identified numerous loci that are associated with dormancy; most chromosomes have some candidate loci. The results are robust, based on repeated runs of the model. Some of the loci identified are expected from the literature (dehydrins, for example), providing support that we are not simply detecting noise.

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