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Identification of Whitefly Resistance Loci in Alfalfa using Genotype-by-Sequencing

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Alfalfa (*Medicago sativa* L.) is an autotetraploid, outcrossing species with high levels of genetic diversity. Whiteflies are one of the major biotic stresses that threaten the sustainability of crops and belong to the *Bemisia tabaci* species complex. Genetic variability for whitefly resistance in alfalfa breeding programs was captured through multiple cycles of selection to generate the resistant generations (C+4 and C+7) with significant gains compared to the C-1 (susceptible) population. For each population, DNA was isolated from four bulked pools of leaves with 25 individuals per bulk and used for genotype-by-sequencing (GBS). DNA samples were digested using *ApeKI* followed by ligation of adaptors and unique barcodes for each bulked sample prior to PCR amplification. Sequencing was performed using an Illumina HiSeq 2000 Platform for SNP identification using UNEAK. The consensus sequence reads were aligned to the *Medicago truncatula* reference genome and only those that aligned were used for further analysis. Loci with the greatest changes in allele frequencies between the C-1 and C+7 populations based on GBS were mostly clustered on chromosome 8. A total of 32 individuals from each population (C-1, C+4, and C+7) were used to further evaluate changes in allele frequencies with SNPs at putative resistance loci on chromosome 8 using high resolution melting analysis (HRM). The shifts in allele frequency ratios (C+7/C-1) at putative R loci on chromosome 8 ranged between 0.21-0.34, while the frequency of SNPs randomly distributed (baseline SNPs) on chromosome 8 and chromosome 2 ranged from 0.01 to 0.09. Thus, the allele frequency enrichment identified on chromosome 8 likely reflects the importance of this region for whitefly resistance. Ongoing efforts include detailed characterization of the genotyped individuals for their whitefly response in the field and in the greenhouse to further evaluate the relationship between allele frequency and insect resistance. The GBS approach using bulked samples is an efficient strategy to quickly identify genomic regions controlling insect resistance that could readily be applied to alfalfa trait-discovery and breeding programs aimed at improving agriculturally important traits.