
Shifts in Allele Frequencies in Alfalfa Breeding Populations during Cycles of Phenotypic Selection for Whitefly Resistance

Alyssa R. Nedley¹, Maria J. Monteros*¹, Yuanhong Han¹, Patrick Thomas², Ananta R. Acharya⁴, Yanling Wei^{1,3}, Min Jiu,^{2,5} Lunji Wang^{2,5}, Frances Holzer², E. Charles Brummer³, Larry R. Teuber^{3†}, Linda Walling²

¹The Samuel Roberts Noble Foundation, Ardmore, OK. USA; ²Department of Botany and Plant Sciences, University of California, Riverside, CA. USA; ³Department of Plant Sciences, University of California, Davis, CA. USA. ⁴Dow AgroSciences, Indianapolis, IN. USA, ⁵College of Food and Bioengineering, Henan University of Science and Technology, Luoyang, China. [†]Deceased.

*Corresponding author: mjmonteros@noble.org

Alfalfa (*Medicago sativa* L.) is an autotetraploid, outcrossing species with high levels of genetic diversity. Whiteflies belonging to the *Bemisia tabaci* species complex are one of the major biotic stresses that threaten the sustainability of crops. The genetic variability for whitefly resistance in alfalfa breeding programs was captured during multiple cycles of selection to generate two resistant generations (C+4 and C+7) with significant gains compared to the C-1 (susceptible) population. DNA was isolated from four bulked pools of samples from each population and used for genotyping-by-sequencing (GBS). Sequence reads from the bulks were used for SNP discovery that enabled the identification of a region on chromosome 8 with significant changes in allele frequencies between the C-1 and C+7 populations. The allele frequency changes on chromosome 8 associated with whitefly resistance (21 to 34%) differed from the allele frequencies identified at non-target loci (1 to 9%). Further, individuals from the three breeding cycles were evaluated for whitefly resistance/susceptibility in the greenhouse and rated as highly resistant when 95% of the nymphs were in their 1st or 2nd instar, or susceptible based on a higher percentage of nymphs in their 3rd or 4th instar. Four resistant and four susceptible alfalfa genotypes were further genotyped with SNPs in the target region and used to confirm the relationship between a given allele and whitefly resistance. The identification of SNPs in key genes represent potential candidates for genome editing as well as tangible targets to develop molecular breeding resources aimed at integrating whitefly resistance in alfalfa.

Keywords: genotyping-by-sequencing, BSA, alfalfa, high-resolution melting, whitefly resistance, allele frequencies, breeding.