

Understanding Segregation Distortion in Diploid Alfalfa

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Alfalfa (*Medicago sativa*) is a widely cultivated forage crop that is susceptible to inbreeding depression due to its outcrossing nature, resulting in the accumulation of deleterious alleles within the genome. The effect of deleterious alleles can be observed in segregating (e.g., F₂) populations, both phenotypically as dwarf, albino, or other mutant plants, or genetically in the form of segregation distortion. We have identified segregation distortion regions throughout the genome; genetic ratios are often skewed towards excess heterozygosity (Li et al., 2011). In this experiment, we have developed multiple F₂ and advanced intercross segregating populations derived from multiple diploid alfalfa genotypes that are characterized by geographic and genetic diversity. The objective of this experiment is to understand the pervasiveness of segregation distortion toward excess heterozygosity and ultimately, to identify the genetic loci and/or mechanisms responsible for the phenomenon.

We have characterized four F₂ populations to date, including inter- and intra-subspecies crosses between the two main subspecies of diploid alfalfa: *M. sativa* subsp. *caerulea* (C) and subsp. *falcata* (F). These populations are termed "F×F" (PI494662 × PI502449), "F×C" (PI502449 × PI464717), "C×F" (PI464717 × PI502449), and "C×C" (PI577547 × PI641380). A single plant from each accession was used to create these populations. Plants were genotyped with the DarTag system of 3,000 SNP markers (Zhao et al., 2023). A large percentage of polymorphic markers within each of these populations shows segregation distortion (Table 1), mostly toward excess heterozygosity. We are currently generating haplotype-resolved whole genome sequences and RNA-seq to help find the genes associated with these segregation distortion loci and will conduct field trials to relate distorted loci with forage biomass yield QTL.

Table 1. Characteristics of the DArTag markers for the aforementioned main F₂ populations used in analysis. "Distorted" markers were counted as those with X^2 $p < 0.005$. As seen in the table, segregation distortion is prevalent in all of these crosses.

	F x F	F x C	C x F	C x C
Population Size	66	92	80	80
# Polymorphic markers	529	927	911	598
# Non-dist. markers	125	336	321	304
# Distorted markers	404	591	590	294
% Distortion	76.37%	63.75%	64.76%	49.16%

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