# Genetic Relationship \& Diversity of 14 Alfalfa Populations Collected From Long-Term Grazing Sites 

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Information on local adaptation of alfalfa populations under long-term grazing is largely lacking. The objectives of this study were to evaluate phenotypic and genomic variations of alfalfa populations from long-term grazing sites, and to identify adaptive loci associated with environmental factors under long-term grazing across four soil zones of Saskatchewan, Canada. In this study, 14 alfalfa populations were collected from long-term grazing sites (>25 yrs) across four soil zones of Saskatchewan. Seven agro-morphological and three nutritive values traits were evaluated from 2018 to 2020. The genotyping-by-sequencing (GBS) data of the 14 alfalfa populations and 11 commercial alfalfa cultivars released between 1926-1980 were used to conduct genetic diversity and genotype-environment association (GEA) analyses. The STRUCTURE analysis showed that the 14 alfalfa populations had varying percentages of the alfalfa sub-species Medicago sativa and M. falcata. In addition, there was a genetic shift by soil zone with the populations from the Black and Brown soil zones being the most unique, inferred by discriminant analysis of principal components (DAPC). Significant differences ( $p<0.05$ ) were observed for seven agro-morphological and three nutritive value traits among alfalfa populations and soil zones. The highest yielding four populations (MacDowall, Duck Lake, Dalmeny and Arcola) was clustered closely with the $M$. sativa sub-species according to the best linear unbiased prediction (BLUP) values of 10 agro-morphological traits. The genotype-environment association (GEA) found 70 SNPs that were significantly associated with eight environmental factors of the long-term grazing sites. Candidate genes underlying these environmental factors were associated with a variety of proteins, which were involved in plant growth and development, and plant responses to abiotic stresses, i.e., high salinity, drought, and cold, and biotic stress, i.e., defense against pathogens.

