Genomic analysis of alfalfa for the development of salt and drought tolerant germplasm for breeding programs

Iqbal I1\*, Perumal S1, Biligetu B2, Sharpe A1\*

*E-mail of corresponding author:* *Irfan.iqbal@gifs.ca* *;* *Andrew.sharpe@gifs.ca*

1 Global Institute for Food Security, University of Saskatchewan, Saskatoon, CANADA

2 Department of Plant Sciences, University of Saskatchewan, Saskatoon, CANADA

Alfalfa (*Medicago sativa* L.), also known as the queen of forages, holds significant importance as a legume forage crop due to its high yield and nutritional quality. Cultivated alfalfa (*Medicago sativa* ssp. sativa L.) is a perennial, self-incompatible, autotetraploid species. In Canada, alfalfa is cultivated across various climatic regions, facing challenges such as salt and drought stress which adversely affect its production. This project aims to enhance alfalfa breeding programs by developing genomic resources for higher-yielding cultivars with increased tolerance to salt and drought stress. Utilizing PacBio long-read sequencing, contig-level assemblies were created for PI212798 (diploid) and Beaver (tetraploid) lines, yielding genome sizes of 889 Mb (N50: 26.75 Mb) and 2064 Mb (N50: 10.23 Mb) respectively, with BUSCO analysis indicating >99% completeness for both assemblies. Currently, a HiC-based scaffolding approach is underway to develop pseudo-chromosome scale assemblies, followed by gene annotation and repeat analysis. Additionally, Illumina short-read sequencing was employed for genotyping by sequencing (GBS) to assess genetic diversity across a broad panel of alfalfa germplasm. Pre-screening for salt stress identified 8 potential salt-tolerant lines. The outcomes of this project will facilitate the development of highly contiguous and high-quality genome assemblies and a pangenome, serving as foundational genomic resources. These resources will expedite the future breeding efforts and identify key genes/QTLs associated with salt and drought tolerance, thereby expediting the development of salt and drought-tolerant alfalfa cultivars.