## Genome-Wide Association & Genomic Prediction of Root System Architecture in Alfalfa

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Alfalfa root system architecture (RSA) is a complex trait with low heritability, making it challenging to improve through conventional breeding programs. However, the identification of molecular markers associated with RSA and the ability to predict genotypes with desirable characteristics for RSA can significantly enhance drought tolerance and nutrient intake in alfalfa. The objective of this study was to identify molecular markers associated with RSA in alfalfa and implement GBLUP as a genomic prediction model to identify genotypes with desirable traits for RSA. A population of 1,188 plots was phenotyped for four RSA traits: taproot diameter (TD), lateral roots distance (LD), lateral root number (LN), and fibrous score (FS). Genotyping was performed on more than two thousand individual plants using a panel of 3,000 Diversity Array Technologies (DArTag) markers, resulting in 2,515 high-quality SNPs after filtering. Fifteen markers were found to be associated with different RSA traits: one for TD, two for LD, one for LN, and 15 for FS, with -log<sub>10</sub>(p-values) up to 15.5. Of particular interest was marker chr5.1\_20774900, with a -log<sub>10</sub>(p-value) of 15.5, located in a locus region with a gene annotated as Starch Synthase, crucial for starch biosynthesis in storage roots. A GBLUP model was trained using the 1,188 plots with phenotypic and genotypic information, employing a ten-fold cross-validation approach. Prediction accuracy and genomic estimated breeding values (GEBVs) for trained genotypes were obtained. LN exhibited a medium accuracy (0.45), while TD, FS, and LD demonstrated high accuracy (0.64, 0.83, and 0.85, respectively). GWAS identified potential markers and candidate genes associated with root development, while genomic prediction facilitated the selection of individual plants with high merit based on RSAassociated traits. The integration of DNA markers and GEBVs will enable the alfalfa breeding community to accelerate genetic gains, leading to the development of biotic drought-tolerant and more productive alfalfa cultivars.