

Variation in Soluble & Crude Protein Across Diverse Alfalfa Germplasm & Implications for Crop Improvement

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Alfalfa (*Medicago sativa* L.) is a high-biomass, nitrogen-fixing perennial legume with substantial protein yield potential, yet its utilization remains largely confined to forage systems. Expansion of alfalfa as a protein crop is limited by insufficient characterization of functional protein fractions, post-harvest protein instability, and a lack of integration between compositional measurements and genetic analysis. Soluble protein (SP), in particular, remains under-characterized relative to crude protein (CP), despite its greater relevance to extractability and downstream applications.

The objective of this work is to quantify variation in protein and associated compositional traits across diverse alfalfa germplasm and to identify genomic regions associated with these traits. A panel of approximately 250 genetically distinct accessions, including high-protein lines, was evaluated under multi-environment field conditions in Minnesota using a randomized complete block design across two locations and multiple years. Whole-biomass samples were collected, and oven-dried for analysis. Soluble protein was quantified using a bicinchoninic acid (BCA) assay, while crude protein was determined via Dumas combustion (LECO CN828). Fiber fractions, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), were measured to contextualize compositional relationships.

Substantial variation in SP, CP, and fiber traits was observed across genotypes and environments, with strong environmental influence on trait expression, consistent with multi-location trends. Genome-wide association analysis using DArTag SNP markers identified marker–trait associations for protein and fiber traits, providing an initial set of candidate genomic regions. Marker analysis identified differences in SP and CP.

Wet chemistry measurements generated in this study are being used to develop near-infrared reflectance spectroscopy (NIR) calibration models, enabling high-throughput prediction of protein and compositional traits across broader germplasm panels. This integration of compositional analysis and genomic data provides a framework for improving selection efficiency.

Overall, this work demonstrates that meaningful variation in protein-related traits exists within diverse alfalfa germplasm and can be leveraged to inform breeding strategies. These findings support the identification of superior high-protein accessions and contribute to ongoing efforts to advance alfalfa as a higher-value protein crop for both feed and emerging applications.