

UNDERSTANDING ADAPTIVE RESPONSES TO DROUGHT STRESS IN ALFALFA (*Medicago sativa* L.)

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Drought stress is a significant factor limiting agricultural productivity. Alfalfa (*Medicago sativa* L.) is an important forage legume species worldwide due to its biomass production, relative feed value, persistence and symbiotic nitrogen fixation capabilities. Productivity and persistence in alfalfa are reduced under drought stress. Integrating phenomics, genomics, metabolomics and transcriptomics approaches can provide valuable insights on the complex drought tolerance mechanisms and inform breeding strategies aimed at increasing productivity under limited water availability. The objectives of this research were to evaluate genetic variation for biomass production under drought stress in alfalfa and assess physiological, transcriptomics and metabolomics adaptations deployed in response to drought stress. A total of 233 diverse alfalfa accessions were evaluated in a rain-fed field environment for biomass yield, relative water content, agronomic performance and field persistence. The most promising accessions were selected from the field and further evaluated in the greenhouse under well-watered and water-limited conditions to simulate drought stress conditions. Soil moisture sensors and data loggers were used to monitor the progression of drought impact in real-time including severe drought stress determined based on volumetric water content. Alfalfa genotypes with contrasting biomass production under drought stress also differed in leaf and root phenotypes, physiological responses, transcriptome dynamics and their metabolic profiles. The high yielding plants grown under drought stress maintained a higher osmotic potential and accumulated higher amounts of osmoprotectants (sugars, polyols, amino acids, carbohydrates, organic acids, lipids and secondary metabolites) in shoots and roots compared to the drought sensitive genotypes. Further, RNA-sequencing of contrasting genotypes grown with and without drought stress revealed differential expression of transcription factors (bZIP, Myb, bHLH, NAC, AP2/ERF and WRKY), genes involved in the biosynthesis of osmoprotectants and those associated with the photosynthetic machinery. Overall, integration of complementary -omics based approaches can be used to identify adaptive responses to drought stress and inform breeding decisions aimed at increasing productivity of alfalfa cultivars under limited water availability.