Discovery and Characterization of miRNAs in *Medicago truncatula* and Alfalfa

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MicroRNAs (miRNAs) are small, non-coding RNAs that regulate the expression of target genes associated with plant growth, development, defense against pathogens and adaptation to environmental stress responses via transcriptional or post-transcriptional regulation of gene expression. Identification of miRNAs associated with abiotic stress responses and their target genes in alfalfa (*Medicago sativa L.*), a perennial forage legume, can contribute to the development of breeding strategies aimed at improving forage production. Several studies have identified miRNA levels dynamically regulated in various plant species when exposed to stress including drought, heat, aluminum and phosphorus. Specific miRNAs whose expression was substantially altered in response to abiotic stresses were used to query the *M. truncatula* and alfalfa genome assemblies to obtain the sequences of pre-miRNAs. Primers were designed to amplify the pre-miRNA sequences and enable the formation of the characteristic fold-back stem-loop or hairpin structure. The PCR amplicons were excised and used for sequencing. Confirmed miRNA fragments were independently cloned into a transformation vector to produce stable transgenic events in *M. truncatula* and alfalfa. Computational methods were used to predict the putative target genes (including transcription factors) of abiotic stress-induced miRNAs. Quantitative real-time PCR enables us to quantify the levels of miRNAs and their target genes in roots and above-ground biomass under various growing conditions (limited water availability, nutrient deficiencies, pH and aluminum). Understanding mechanisms of post-transcriptional regulation can facilitate the development of novel strategies to advance alfalfa improvement efforts aimed at increasing productivity under drought and other abiotic stress conditions.