

## The miR156 gene regulatory network controls forage yield and other traits in alfalfa

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MicroRNA156 (miR156) regulates many aspects of plant development, including juvenile to adult transition, shoot branching, and flowering time by post-transcriptional silencing of members of the *SQUAMOUS PROMOTER-LIKE* (*SPL*) gene family. Here, we investigated miR156 function in *Lotus japonicus* (Lotus) and *Medicago sativa* (alfalfa). In Lotus, miR156 cleaves transcripts of two *SPL* genes, *Lj-SPL1* and *Lj-SPL2*. Lotus plants overexpressing miR156 showed enhanced branching, delayed flowering time, and reduced nodulation. We also cloned and overexpressed an alfalfa homologue of a miR156 precursor and identified its target genes, comprising three *SPL* genes (*SPL6*, *SPL12* and *SPL13*) and two members of the *WD40* family (*WD40-1* and *WD40-5*). Transgenic alfalfa plants overexpressing miR156 showed up to a 2.5-fold increase in vegetative biomass, enhanced shoot branching and root length, reduced internode length resulting in more nodes, increased secondary branches and leaves per stem, higher leaf trichome density, and a moderate delay in flowering time. The multitude of traits affected by miR156 are likely due to the large network of genes that are regulated by miR156 and its target genes. Thus, these genes can be exploited in improving yield and quality of alfalfa forage.