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

Abdelali Hannoufa, Banyar Aung, Margaret Gruber, Lisa Amyot, Min Yu

Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, N5V4T3, Canada
Biology Department, Western University, 1151 Richmond Street, London, ON, N6A5B7, Canada
Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, S7N0X2, Canada

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.