Differential gene expression in salt-tolerant alfalfa (Medicago sativa)

Ivan W. Mott and Michael D. Peel

USDA, Agriculture Research Service, Forage and Range Research Laboratory, Logan, Utah

Alfalfa is an important forage crop in the western United States. In semiarid regions, limited water and hot, dry climates frequently cause salinity concentrations that limit or prevent crop production. Alfalfa has been characterized as a moderately salt sensitive species with a threshold of 4.0 dS m^{-1} electrical conductivity (EC). To improve the tolerance to saline conditions, we have completed three cycles of recurrent selection for relative salt tolerance in alfalfa using a greenhouse selection protocol following Peel et al (2004) wherein plants were subjected to saline irrigation up to EC of 18.0 dS m^{-1} .

Alfalfa populations from the second cycle of selection were field tested under non-stressed, irrigated and salt-stressed, non-irrigated conditions. In comparisons of selected alfalfa for relative forage production, preliminary results showed that the top lines from one of the selected populations averaged 36% higher forage production than the check cultivars. The highest producing selected alfalfas produced 2.58 Mg/ha under salt-stressed conditions compared to 2.37 and 1.29 Mg/ha for the salt tolerant checks Mesa Sirsa and Malone, respectively. The populations from the third cycle of selection are currently undergoing field evaluation in non-stressed irrigated site and a saline-soil with saline irrigation water (EC 6.0) site.

To identify genetic changes in the selected alfalfas, we used Suppression Subtraction Hybridization (SSH) to identify genes that are differentially expressed in salt-selected and non-selected alfalfa genotypes under salt stress. Total RNA was extracted from individual seedlings grown under salt stress. RNA from three plants from each cycle of selection were pooled for subtraction. SSH was utilized to make cDNA libraries enriched for transcripts with differing expression levels between the selected alfalfas. cDNA clones were screened for differential expression using ³²P-labeled cDNA probes. Forty-seven gene sequences were identified that had differential expression. Ten genes have known cellular functions including photosynthesis (5), metabolism (2), cell wall extension (1), nucleic acid binding (1), and translation (1). The majority (37) of differentially expressed genes have no known gene functions. Of those, 27 have homology to genomic sequences of Medicago, Oryza, and Lotus whereas ten genes are novel and have no homology to any nucleotide sequences in the NCBI database.

Reference

Peel MD, Waldron BL, Jensen KB, Chatterton NJ, Horton H, Dudley LM (2004) Screening for salinity tolerance in alfalfa: A repeatable method. Crop Science 44: 2049-2053.