

Agronomic, physiological, and molecular characterization of salt tolerant alfalfa (*Medicago sativa*)

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Alfalfa is an important forage crop in the semiarid regions of the western United States. Saline soils in this region can 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 alfalfa tolerance to saline conditions, three cycles of recurrent selection for survival under salt treatment in the greenhouse has been completed according to Peel et al (2004) wherein plants were subjected to saline irrigation up to EC of 18.0 dS m⁻¹. Two sativa and two falcata type alfalfa populations were developed. Agronomic performance in saline field conditions, physiological mechanisms of salt tolerance, and molecular genetic differences of the selected alfalfas are unknown. One line (CkSltn 15-2) from a sativa-type population and one line (BC79 11-1) from a falcata-type population were selected for physiological and molecular analysis.

Evaluation of forage yield over two years in non-saline and saline field locations indicate that selected alfalfa CkSltn 15-2 and BC79 11-1 have a slight loss of yield compared to parental lines in a non-saline environment, but under salt stress yielded better (107%) or as well as (100%) the parents, respectively. Six individual genotypes for each experimental line and their parents were recovered from the field and clonally propagated in the greenhouse for physiological and molecular analysis. After replicated treatments at EC of 9.0 dS m⁻¹ and control conditions, measures of chlorophyll content index were obtained, and plant tissues were harvested for analysis of electrolyte content and gene expression analysis. CkSltn 15-2 had higher chlorophyll content at high EC days and lower total shoot electrolyte content than its parents. BC79 11-1 had higher chlorophyll content under both control and salt conditions and had no difference in total electrolyte content compared to its parents. This suggests that different mechanisms of salt tolerance may have been selected in the two lines.

To identify genetic changes in the selected alfalfas, preliminary studies utilized suppression subtraction hybridization (SSH) to identify genes that were differentially expressed in salt-selected and non-selected genotypes under salt stress. Forty-seven gene sequences were identified that had differential expression, ten which had 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 had no known gene functions, but had homology to genomic sequences of *Medicago*, *Oryza*, and *Lotus*, whereas ten genes were novel and had no homology. Affymetrix *Medicago* genome arrays were utilized to conduct more extensive assessment of differential gene expression in selected versus non-selected CkSltn 15-2 and BC79 11-1 genotypes under saline and control irrigation. Results from these microarray experiments will be discussed.

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.