

Aluminum Tolerance in the Model Legume *Medicago truncatula*

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Aluminum (Al) toxicity associated with acid soils has been a major obstacle in alfalfa (*Medicago sativa*) production in the U.S. The mechanism of Al tolerance in *Medicago* is unknown. The objective of this study is to investigate the mechanism of Al tolerance in the model legume *M. truncatula*, and to identify genes that are differentially expressed under normal and Al stress conditions, with the long term goal of using these genes to improve cultivated alfalfa. A hydroponics procedure was used to screen 321 accessions of *M. truncatula* obtained from the National Plant Germplasm System (NPGS) for aluminum tolerance. Root tips from genotypes identified as sensitive and tolerant from the hydroponics assay were stained with the Al-sensitive stain lumogallion (3-[2,4 dihydroxyphenylazo]-2-hydroxy-5-chlorobenzene sulfonic acid), and visualized using confocal microscopy. Microarray analysis was used to investigate relative gene expression level in response to Al stress in an Al sensitive genotype. Al sensitive seedlings were grown without aluminum for 5 days in modified Blaydes medium with 25% macronutrients, 0.5mM CaCl₂, pH 4.3. The seedlings were then transferred to medium with 25 μM Al. Control and Al stressed root tips are harvested at 0 h, 30 min, 1 h, 3 h, 6 h, 12 h and 24 h, and total RNA was isolated for microarray hybridization. The microarray chips used contained 16,000 70-mer oligos that were designed from *M. truncatula* EST sequences and spotted in triplicate. Microarray results from different time-points will be presented. Relative root growth in Al and Al free hydroponics medium was used to assign each genotype an Al tolerance index (TI). The TI ranged from 0.4 to 1.13, with 2.2% of accessions between 0.4 and 0.49 (highly sensitive), 94.6 % between 0.5 and 0.9 (sensitive), and 3.1% between 0.91 and 1.13 (tolerant). Images of root tips stained with lumogallion indicate that root tip cells of the Al tolerant genotypes accumulate less Al than those of Al sensitive genotypes. This evidence supports an exclusion mechanism of Al tolerance in *M. truncatula*.

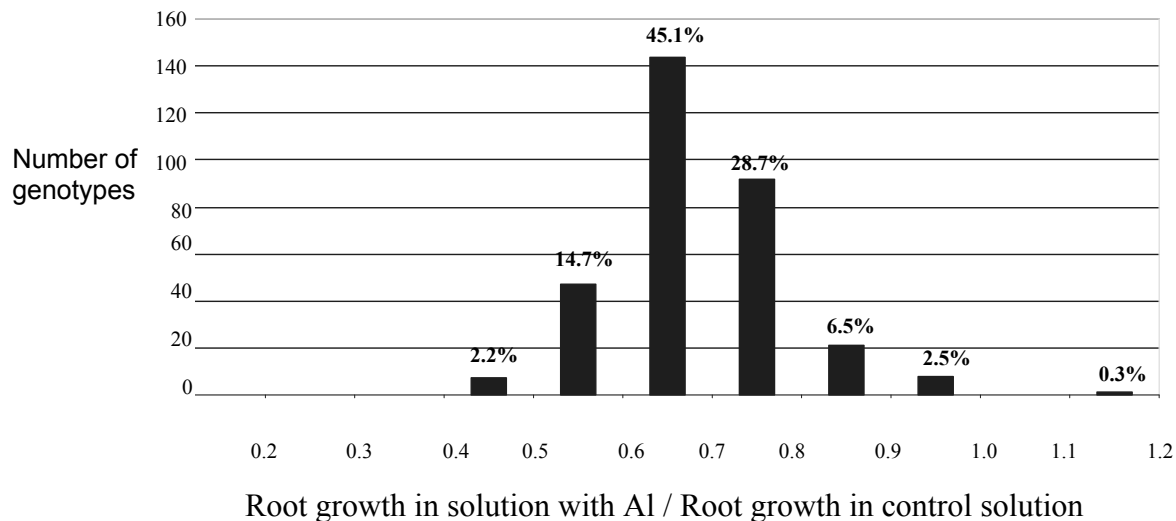


Fig. 1. Distribution of 321 *M. truncatula* accessions by Al tolerance index.