Aluminum tolerance in the model legume *Medicago truncatula*

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Introduction Aluminum (Al) is the most abundant metal found in the earth's crust, comprising up to 7% of its mass. At low pH, Al becomes soluble and available to plants, resulting in inhibition of root elongation and reduced plant growth. Aluminum toxicity associated with acid soils has been a major obstacle in alfalfa (*Medicago sativa*) production. The objective of this study is to identify genes that are differentially expressed under normal and Al stress conditions in the model legume *M. truncatula*, with the long term goal of using these genes to improve cultivated alfalfa.

Materials and methods A hydroponics procedure was used to screen 272 accessions of *M. truncatula* obtained from the USDA National Plant Germplasm System for aluminum tolerance. Sterilized and sprouted *M. truncatula* seeds (Cohn *et al*., 2001), placed in plastic mesh-bottomed cups with the roots threaded through the mesh, were floated in a hydroponics culture tank containing 25 L of a modified Blaydes media (Blyades, 1966), with 25% macronutrients (0.5mM CaCl2), pH 4.3, both with and without 50M Al. After five days, seedling root lengths were measured, and relative root length was calculated. Root tips from tolerant and sensitive genotypes 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 gene expression in response to Al toxicity in an Al sensitive genotype and an Al tolerant genotype. Seedlings were grown without aluminum for 5 days in modified Blaydes medium with 25% macronutrients, 0.5mM $CaCl_2$, and pH 4.3. Seedlings were then transferred to fresh medium with 50 µM Al, and control and Al stressed root tips are harvested at 0 h, 1 h, 6 h, and 24 h. Root tip mRNA was isolated at each time point, and cDNA was prepared from the isolated RNA. Labelled cDNA was hybridized to 16K *M. truncatula* 70mer oligonucleotide microarrays.

Results Tolerance of Al was normally distributed, and sensitive and tolerant genotypes were identified. Images of root tips stained with lumogallion indicate that root tip cells of Al tolerant genotypes accumulate less Al than those of Al sensitive genotypes. Microarray results indicate differences in gene expression between Al sensitive and Al tolerant genotypes.

Conclusions Variability for Al tolerance exists within the USDA collection of *M. truncatula*. Therefore, it is feasible to construct a population segregating for Al tolerance, with the objective of mapping quantitative trait locus for Al tolerance in *M. truncatula*, and this work is currently underway. Microarray results could aid in identifying candidate genes for Al tolerance.

References

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