Application of SSH and a Macroarray to Identify Genes Involved in Aluminum Tolerance of Alfalfa (*Medicago sativa* L.)

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Introduction In acidic soils , the performance of alfalfa is very poor due to its low degree of tolerance to aluminum toxicity . However , high genetic variability in different cultivars of alfalfa in their ability to tolerate Al-toxicity (Li .2006) indicated that some cultivars have evolved mechanisms to survive Al-stress conditions . The objective of the present study was to identify genes involved in aluminum tolerance of alfalfa .

Materials and methods Three-day-old seedlings of an Al-tolerant cultivar of alfalfa (*Medicago sativa* L . cv xiaoguang) , prepared as described by Xia et al (2007) , were exposed for 24 hours to a 0 .5 mM $CaCl_2$, pH 4 .5 , solution either with or without 40 μM $AlCl_3$. Total RNAs were extracted from root tips using TRIzol Reagent (Invitrogen , America) and mRNAs were purified from total RNA using an Oligotex ® mRNA Mini Kit (QIAGEN , Germany) . cDNAs were generated and used for suppressive subtraction hybridization (SSH) with a Clontech PCR-Select™ cDNA Subtraction Kit (Clontech , USA) using cDNA from Al-exposed plants and non-Al-exposed plants as tester and driver , respectively . Products from the secondary suppression PCR were inserted into pGEM ®-T Easy Vectors and transformed into JM109 *Escherichia coli* cells for blue-white selection . cDNA macroarray dot-blots were used to confirm differential expression of the clones and performed according to the manufacturer's protocol in the DIG-labeling and detection kit (Roche , America) . Selected clones were sequenced . Homology searches were performed against the NCBI database using the BLAST× program .

Results A subtracted cDNA library was constructed by SSH and contained approximately 964 ESTs with an insert size of 250 to 1200 bp as estimated by PCR analysis of 16 randomly selected clones (Figure 1) . Subtraction efficiency was checked by macroarray , and 32 clones were selected that had a hybridization signal clearly stronger on membranes probed with the forward subtracted probe population . Sequencing and BLASTx homology analysis of these selected clones indicated that 28 ESTs (84. 4%) showed high homology with plant genes of known and unknown functions registered in database (Figure 2) , whereas the other four ESTs (15 .6%) database searches failed to show significant similarity with listed sequences , suggesting these may be novel genes .

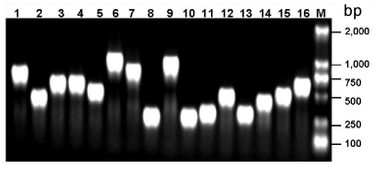

Figure 1 *Analysis of selected clones (lanes 1-16) in the SSH library of alfalfa , M is molecular mass marker DL2000 .*

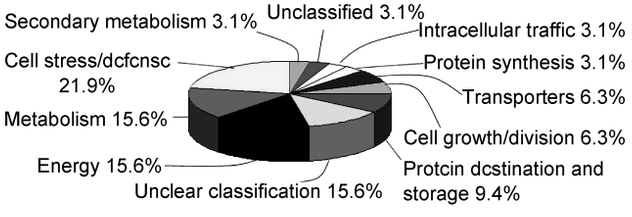

Figure 2 *Categorization of putative functions of the alfalfa ESTs based on results from BLASTx analysis of the NCBI/GenBank database .*

Conclusions We have successfully used SSH and a macroarray to identify 32 genes that are up-regulated in alfalfa during Al-stress . These genes provide a starting point for further functional analysis to determine what role they play in Al-tolerance in alfalfa and may contribute to the improvement of alfalfa through conventional breeding or biotechnology approaches in the future .