



University of Kentucky
UKnowledge

International Grassland Congress Proceedings

XXI International Grassland Congress / VIII
International Rangeland Congress

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

J. J. Li
Zhejiang University, China

X. W. Li
Zhejiang University, China

Z. S. Xia
Zhejiang University, China

Y. M. Wu
Zhejiang University, China

J. X. Liu
Zhejiang University, China

Follow this and additional works at: <https://uknowledge.uky.edu/igc>

 Part of the [Plant Sciences Commons](#), and the [Soil Science Commons](#)

This document is available at <https://uknowledge.uky.edu/igc/21/12-1/46>

The XXI International Grassland Congress / VIII International Rangeland Congress took place in Hohhot, China from June 29 through July 5, 2008.

Proceedings edited by Organizing Committee of 2008 IGC/IRC Conference

Published by Guangdong People's Publishing House

This Event is brought to you for free and open access by the Plant and Soil Sciences at UKnowledge. It has been accepted for inclusion in International Grassland Congress Proceedings by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

Application of SSH and a macroarray to identify genes involved in aluminum tolerance of alfalfa (*Medicago sativa* L.)

J.J. Li, X.W. Li, Z.S. Xia, Y.M. Wu, J.X. Liu

College of Animal Sciences, Zhejiang University, Hangzhou 310029, P. R. China, E-mail: well-being365@163.com

Key words: alfalfa, suppression subtractive hybridisation, macroarray, aluminum tolerance, cDNA library

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 CaCl₂, pH 4.5, solution either with or without 40 μM AlCl₃. 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 BLAST^x 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.

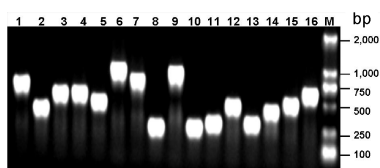


Figure 1 Analysis of selected clones (lanes 1-16) in the SSH library of alfalfa, M is molecular mass marker DL2000.

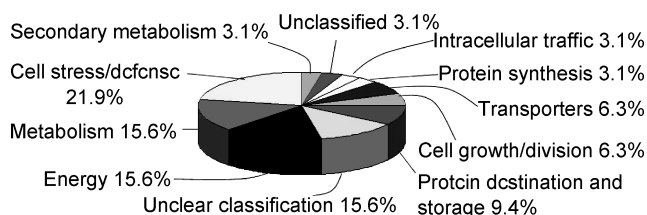


Figure 2 Categorization of putative functions of the alfalfa ESTs based on results from BLAST^x analysis of the NCBI/GenBank database.

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.