2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

UTILIZATION OF AN INTRASPECIFIC HYBRID POPULATION FOR SALT TOLERANCE STUDIES

Sarah M. Higbie, Fei Wang and Jinfa Zhang
New Mexico State University
Las Cruces, NM
James McD Stewart
University of Arkansas
Fayetteville, AR
Thea Wilkins
Davis, CA

Abstract

High soil salinity is a worldwide problem that results in visible damage at the whole plant level and can ultimately lead to lowered crop productivity and yield quality. Due to the narrow understanding of salt tolerance mechanisms at the molecular level, crude method for controlling high soil salinity like rotating crops for salt remediation and alteration in irrigation practices have little affect on increasing overall crop tolerance to high salinity. A viable alternative to these practices is to develop crop varieties better adapted to salinized conditions.

Materials and Methods

A BC_1F_1 derived BC_2F_1 population was developed from *G. hirsutum* DP33B and *G. hirsutum* PI501500 (Mac Stewart, The University of Arkansas, Fayetteville, AR). PI501500 is a semi-wild species discovered close to the ocean shore in Mexico. The saline environment in which PI501500 was discovered suggests that PI501500 has adapted to grow in a higher salt environment and may possess genes to aid in its survival in high salt conditions. This population, due to the possibility of increased salt tolerance genes was used to investigate the genetic and molecular basis of salt tolerance in cotton.

Up to five seeds from 110 BC_1F_1:BC_2F_1 progenies were planted in 4 inch pots and watered normally for two weeks. Progenies with at least 2 plants (a total of 99 progenies) were then divided into two groups (salt treated and control) and arranged in a paired randomized design. Each plant was watered with either 100 ml 200 mM NaCl (experimental salt treated) or 100 ml tap water (control).

Plant height and fresh weight, of both treated and control plants, were measured at 0,7,14 and 21 days after salt treatment (DAT). The percent reduction in plant height (treated – control) at 21 DAT was chosen as a viable indicator of salt tolerance or susceptibility. At 21 DAT, final plant height was taken along with fresh weight before all plants were repotted for seed increase. ANOVA analysis was conducted on all the data. The 21 DAT height data was found to be highly significant at P=0.05.

Results and Discussion

From the 21 DAT height data, five of the most sensitive progenies (with a total height reduction of greater than 51% of the control) and five of the most tolerant progenies (with a total height reduction of less than 27% of the control) were selected for microarray studies. The progenies were divided into two groups and half were treated daily with 100 ml of 200 mM NaCl for 7 days, while the control plants were watered daily with 100 ml tap water. The third and fourth leaves from the meristem were harvested and the tissue was bulked (tolerant or control) at 7 DAT. Total RNA was isolated from the bulked tissue. Total RNA was labeled with Cy3/Cy5 (Amersham, Piscataway, NJ) using the SuperScript™ Indirect cDNA Labeling System (In Vitrogen, Carlsbad, CA; Cat# L1014-02). Target was hybridized to a 12, 227 spot *G. arboreum* fiber gene chip on which 70-mers were developed from NR fiber ESTs (Thea Wilkens, UC, Davis). Chips were scanned using GenePix 4100A (Axon Instruments, Union City, CA) and GenePix Pro 5.0 software. Significant genes ($p \leq 0.01$, $-1 < \log_2 < +1$) were identified using SAS. Twenty-five genes were identified as significant in the Cy5 channel (13 had known function, 12 had unknown function); 690 genes were identified as significant in the Cy3 channel.

The genes identified in theCy5 channel were either up regulated in the tolerant or down regulated in the susceptible. The genes identified were from pathways known to be associated with salt stress. These pathways include sugar/osmolyte metabolism, cell wall structure and cellular membrane synthesis. The genes identified in the Cy5

channel are listed in Table 1. Clone ID refers to the *G. arboreum* clone from which the 70-mer was developed for the microarray. The gene names were obtained by searching the TIGR CGI (The Institute for Genomic Research Cotton Gene Index; www.tigr.org).

Table 1. List of genes identified as significant in the tolerant bulk analysis

Gene Name	Clone ID	Stress	P value	log_2 ratio
Sugar/Osmolyte Metabolism				
Sorbitol Dehydrogenase	GA_Ea0018A03r	Osmotic, Insect	0.01	1.79
Serine/Threonine Kinase	GA_Ea0016I14f	Osmotic	0.002	1.67
Glycerolipid Metabolism				
1-Acylglycerol-3-P Acyltransferase	GA_Ed0054C03r	Temp., Osmotic	0.009	1.64
Fatty Acid Metabolism				
Fatty Acid Elongase	GA_Ea0032I19f	Osmotic, Temp.	0.0005	1.20
AMP-Binding Protein	GA_Ed0030E03r	Temp., Osmotic	0.01	1.71
Pyrimidine Metabolism				
Dihydropyrimidinase	GA_Eb0004C07f	Osmotic	0.007	1.08
Cell Wall/Structure/Transport				
Extensin Precursor	GA_Eb0035K12f	Osmotic	0.006	1.46
Endomembrane Protein EMP70	GA_Ed0004G02f	Temp., Osmotic	0.005	1.16
Pherophorin-dz1 Protein	GA_Ea0027N08f	Osmotic, Wound	0.006	1.76
Transcription/Translation				
60S Ribosomal Subunit L5	GA_Ea0021J02f	Osmotic	0.004	1.50
60S Ribosomal Subunit L24	GA_Ea0032J03f	Osmotic	0.004	1.84
SCARECROW Transcription Factor	GA_Ea0031G20r	Osmotic	0.002	1.10

Work is now underway to identify these genes and to confirm, by northern blot analysis or quantitative RT-PCR, the salt responsiveness of these genes. Subsequently, genes identified as salt responsive will be used to develop molecular markers for candidate gene mapping in salt tolerance.