

2005 ANNUAL REPORT TO COTTON INCORPORATED

PROJECT TITLE: Screening Cotton Germplasm for Heat and Osmotic Stress Tolerance
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The initial objective of this work focused on initiating and establishing the validity of a procedure to measure heat stress tolerance to be used to screen the cotton germplasm collection. The initial procedure we developed demonstrated that chlorophyll fluorescence could be used effectively to discriminate heat tolerant germplasm. This involved determining the minimum temperature for the onset of chlorophyll fluorescence. However, the procedure required approximately 20 minutes per sample to conduct. This ultimately is too time-consuming despite the fact that this method gave accurate quantitative information that would have been useful. Additionally, this preliminary approach clearly established that chlorophyll fluorescence was a method that could be utilized to sample the genetic variation for cellular level heat tolerance.

This initial screening method was modified, and a more efficient and rapid chlorophyll fluorescence-based procedure that discriminated the most heat tolerant materials in the germplasm collection from more average heat tolerant materials was developed. Though more rapid this procedure gave less accurate quantitative information on the degree of tolerance. However, this protocol allowed screening of as many as 100 accessions at one time in about 90 minutes, and involved determining the temperature-induced change F_v/F_{max} (chlorophyll fluorescence), a complex measure of chloroplast efficiency after 30 minutes at a series of increasingly stressful temperatures.

Approximately 1700 accessions of the cotton (*Gossypium hirsutum*) germplasm collection were screened using the above primary screen. Twenty-two of these accessions demonstrated F_v/F_{max} fluorescence values after 60 min at 55° C that were at least one standard deviation above the mean of all accessions through two replicated rounds of screening. This data verified the superior chlorophyll fluorescence during episodes of heat stress of these 22 elite accessions. Of the 22 elite accessions, three plants had final fluorescence values greater than two times the standard deviation of the mean in both rounds of screening. These accessions also performed dramatically better than commercial cultivars of cotton grown in Alabama using this technique. Commercial varieties performed essentially the same as the mean for all accessions tested. Because we were supplied limited quantities of seed, it was necessary to grow all 22 elite accessions for seed increase.

A secondary evaluation of the elite accessions was conducted by growing plants of the 22 elite accessions for approximately four weeks in the greenhouse, and then transferring them to a growth chamber where a heat stress of 45° C was continuously applied to the plants. The overall growth and appearance of the accessions was compared with DPL90, a variety considered to be among the most heat and drought tolerant available. While all 22 of the accessions performed better than DPL90, 7 of the elite 22 were clearly superior in performance during heat stress to DPL 90 and the other plants.

This verifies that the elite plants identified via chlorophyll fluorescence will perform significantly better than commercial cotton under extreme heat conditions in growth chambers simulating field conditions. Note that although yield data in high temperature environments

would be superior to the growth chamber studies we have done, these accessions are not adapted to Alabama conditions, and are not commercial quality varieties. Thus, the challenge will be to retain the cellular level heat tolerance that the accessions demonstrate while building into the germplasm developed commercial fiber yield and quality. To that end we are making the initial crosses of the 7 selected accessions with DPL90 and additional germplasm.

In order to efficiently breed for a complex trait like heat tolerance a set of molecular markers that can be utilized to facilitate and expedite the breeding program are being developed. The approach we are using is to determine the profile of genes expressed in both commercial cotton varieties and in our 7 elite accessions determined above. The first stage in this process is to establish a set of genes that are differentially expressed during heat and drought stress in a commercial genotype of cotton. DPL-90 has been chosen as this genotype based on anecdotal input from Alabama growers that this genotype is clearly superior to other commercial genotypes in heat and drought tolerance. Subsequent work will then involve examination of the genes that are differentially expressed in the handful of most heat and drought tolerant accessions that we have obtained in this project. This will allow us to compare the gene expression pattern in genotypes that demonstrate greater heat and drought tolerance than does DPL-90.

Eighty-eight sequences that show altered expression during episodes of heat and drought have been identified and cloned using the technique of differential display. However, we concluded that we need a greater number of differentially regulated sequences (genes) than we have found thus far by differential display, and have been using an alternative technique referred to as cDNA AFLP analysis. This has expanded our list of differentially expressed sequences during heat and drought to over 300 sequences. Currently we are analyzing these sequences to determine if any of them are related and to define what they are. Additionally, the sequences are being evaluated by quantitative RT-PCR to validate the expression level of the sequences listed above. Initial data from this RT-PCR show that the majority of the changes in gene expression that were observed have been validated using this technique. The characterization of these sequences will be completed and genes differentially expressed in the 7 elite accessions will also be examined in the near future. From this we will determine those genes that are useful as markers for use in the breeding effort.