# USE OF DIFFERENTIAL DISPLAY TO IDENTIFY COTTON FIBER cDNAs WITH SECONDARY WALL-SPECIFIC AND COOL TEMPERATURE-INDUCED EXPRESSION C.H. Haigler, A.S. Holaday, C.H. Wan<sup>1</sup>, and Shaoyi Zhuang Dept. of Biological Sciences Texas Tech University Lubbock, TX

# Abstract

Cotton fiber genes with cool temperature-induced and secondary wall-specific expression have been identified by use of mRNA differential display.

#### **Introduction**

We hope to improve the efficiency of fiber maturation under cool night temperatures so that producers on the Texas Southern High Plains (and other similar regions) can produce a high quality, high yield cotton crop in the shortest growing season ever likely to occur. The cotton growing season is limited in temperate regions to the months between adequate warming of soil to allow planting and the first killing frost. Furthermore, cool nights (below about  $75^{\circ}F = 24^{\circ}C$ ) during the season greatly limit the time that is truly effective for fiber maturation. To achieve our goal, we must know where cool temperature limited steps occur in cellulose synthesis so that particular genes can be manipulated by genetic engineering. (Cellulose synthesis is required for at least 90% of the secondary wall thickening required to produce a mature fiber.)

We have tried to identify genes that are associated with fiber adaptation to cool temperatures, which can occur on a slow time scale of several days in cool-sensitive Gossypium hirsutum cv Acala SJ-1 (Haigler, 1992). If these or related genes were constitutively expressed or up-regulated by genetic engineering, fiber thickening might become more efficient under cool temperatures so that maturity could be achieved faster. We have used the technique of mRNA differential display (Liang and Pardee, 1992), which allows the identification and cloning of mRNAs that are differentially expressed in two cell types, to analyze differences in mRNA of cultured cotton fibers that had or had not been exposed to cool temperatures (a  $34^{\circ}/15^{\circ}C =$ 94°/60°F 12 hr/12 hr cycle). Previous work indicated that cultured cotton ovules with attached fibers are a valid model for the response of field fibers to cool temperatures (Haigler et al., 1991; Roberts et al., 1992; Wuzi et al., 1993).

# **Discussion**

Reprinted from the Proceedings of the Beltwide Cotton Conference Volume 2:1468-1469 (1997) National Cotton Council, Memphis TN Among many negative results, which indicate that the expression of most genes does not change in response to cool temperatures, we were able to identify and clone one mRNA with an expression pattern in Northern blots indicating that it may be relevant to adaptation to cool temperatures.

We also used differential display to identify an mRNA that is expressed only in the secondary wall stage of cotton fiber development. The promoter of the corresponding gene is expected to allow genetic modifications to enhance rate of fiber maturation under cool temperatures to be made in a tissue- and stage-specific manner, thereby avoiding possible undesirable effects of the same genetic change in other parts of the plant or stages of development.

# **Summary**

Differential display can be a useful tool to identify genes with probable relevance to cotton improvement. However, the number of negative results and false positives encountered means that substantial work will often be needed to identify the target genes.

#### **Acknowledgments**

Financial support for this work was provided by the Texas Tech Institute for Research in Plant Stress, the Texas Advanced Research Program, and Cotton Incorporated. We thank Drs. Linda Koonce, Randy Allen, and Ping Song for assistance with the differential display technique and Dr. Hong Zhang and Ms. Jing Wang for general advice on molecular biology techniques.

<sup>1</sup>Current Location: Clemson SC

#### **References**

Haigler, C.H, N.R. Rao, E.M. Roberts, J.Y. Huang, D.R. Upchurch, and N.L. Trolinder. 1991. Cultured cotton ovules as models for cotton fiber development under low temperatures. *Plant Physiology* 95: 88-96.

Haigler, C.H. 1992. The crystallinity of cotton cellulose in relation to cotton improvement. *Proc. Cotton Fiber Cellulose: Structure, Function, and Utilization Conference,* National Cotton Council of America: Memphis, TN., p. 211-225.

Liang, P. and A.B. Pardee. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 257: 967-971.

Roberts, E.M., R.R. Nunna, J.Y. Huang, N.L. Trolinder, and C.H. Haigler. 1992. Effects of cycling temperatures on fiber metabolism in cultured cotton ovules. *Plant Physiology* 100: 979-986.

Wuzi, X., N.L. Trolinder and C.H. Haigler. 1993. Cool temperature effects on cotton fiber initiation and elongation clarified using *in vitro* cultures. *Crop Science* 33: 1258-1264.