Cotton Genome Manipulations: Exploring Smart Tools, Novel Germplasm, and Elite Genes for Super Cotton Design

ZHANG Xian-long

(*National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070, China*)

Plant regeneration is the first step to cotton biotechnology. We screened over 100 genotypes and found two genotypes, YZ-1 and Y668, which are very easy to regenerate. It takes about 5 to 6 months for the two genotypes from explant inoculation to plant regeneration. Meanwhile, we investigated the gene expression patterns during somatic embryogenesis (SE) in cotton. The results suggested that a complicated and concerted mechanism involving multiple pathways is responsible for cotton SE. We constructed a network to show the relationship between genes during SE. Some candidate genes related to SE are under going functional analysis. Genetic transformation and protoplast fusion were used to generate novel germplasms. We developed a fast procedure to produce transformants via transforming embryogenic callus. Transgenic plants were obtained in five months. During our transformation work, we found that the transgenic shoots developed few roots, which made the transplanting difficult. It was found that after grafting the transgenic shoots to hypocotyls of five-day old seedlings, it was very easy to get transgenic plants with well-developed roots. Based on this technique, 90 percent of the transgenic cotton plants survived when they were transplanted to soil. Based on the transformation system, we developed pest-resistance, disease-resistance, and high-fiber cottons, which would be used as novel germplasm for breeding. An efficient system for protoplast culture and protoplast fusion in cotton was established in our laboratory. Five cotton species regenerated plants from protoplasts, and efforts have been made to improve the protoplast yield, viability, and plating efficiency in protoplast culture. The receptor and the donor in protoplast fusion were treated to produce asymmetric hybrids for special breeding programs. Four interspecific symmetric somatic hybrids and two interspecific asymmetric somatic hybrids were obtained in this program. The hybrids were confirmed by morphological, cytological, and molecular analysis, and they could be used in further investigations and breeding programs. To explore the elite genes regulating fiber quality for future cotton improvement, we compared genes expression during fiber development at 15 DPA and 25 DPA by constructing two SSH libraries. By subtraction between fibers and five none-fiber tissues, two sets of genes have been identified, and their fiber-specific or fiber-preferential expression indicates that they are involved in the network controlling cotton fiber development. Some full-length cDNAs contributing to fiber development were recovered by RACE, and they also were cloned to a plant expression vector. R0 and R1 lines were obtained for functional analysis. In order to investigate fiber development in large scale, we constructed a normalized cDNA library. For mining the novel genes included in the fiber development, we performed a screen with the 0, 5, 10, 15, and 20 DPA RNA of cv. 3-79 fiber to a cDNA array that included about 10000 plasmids randomly selected from the library. In total about 1000 clones were selected to sequence. About 20 genes were introduced to cotton genome for function analysis. We believe that more and more targeted cDNAs will be characterized functionally through our fast transformation approaches.