Plant Generation of TM-1 via Tissue Culture

LIU Chuan-liang, LI Fu-guang, XU Leng-chun

(*Cotton Research Institute, Chinese Academy of Agricultural Sciences; Key Laboratory of Cotton Genetic Improvement, Ministry of Agriculture, Anyang, Henan* 455000, *China*)

Plant generation of TM-1 via tissue culture was established. The hypocotyledon sections as explants which were cultured in a series of improved MS media containing 0.05～0.10 mg • L^{-1} IAA, 0.1～0.15 mg • L^{-1} Kt, 0.07～0.14 mg • L^{-1} 2,4-D could produce a large number of calli which were easier to regeneration in this system. The calli, which were subcultured in another MS media containing 0.03～0.05 mg • L^{-1} Kt for 3-4 times produced embryoid callus in a rate of 35%. Fifty-six somatic embryoid calli were subcultured in an improved MS medium containing 0.1 mg • L^{-1} BA and 0.1～0.15 mg • L^{-1} IAA for plant regenerating, and 47 cotton plantlets were regenerated from them. The cycle of plant regeneration of TM-1 was 11～14 months, which should be improved. In the tissue culture system, both hormones and culture procedure were crucial in our practice. All regeneration plants of TM-1 were planted in field to observed their agronomic trait and chromosomes through out the season. Compared with TM-1, some differences were found in 14 cotton plants, mainly in leaf and flower, and these will be tested next year, the others were same as TM-1. No chromosome variation was found in these cotton plants. In order to improve the regeneration rate and decrease the cycle of regeneration, gene-expression in the somatic embryogenesis of TM-1 from callus to somatic embryo was compared using cDNA-AFLP technique. The result showed that 3425 amplified bands, 38 specially displayed in the somatic embryo. These cDNA fragments were further cloned and sequenced. Sequence analysis demonstrated that among 38 sequenced fragments, twelve were sequence-similar to cDNA sequences with the different tissues of *Gossypium raimondii*, *G. hirsutum* and *G. arboretum* in GenBank, their identities were above ninety percent. The results indicated that the gene expression of the whole plant had already finished at the early time of embryogenesis. No similar sequences were found in GenBank for the other 26 fragments. According to these results, a complicated gene network controlled production of somatic embryos of TM-1. So, measurement of the contribution of every gene/Est in the network will be an onerous research.

Key words: TM-1; tissue culture; regeneration; cDNA-AFLP