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FINAL REPORT

CS36C: COTTON TISSUE-CULTURE AND GENETIC TRANSFORMATION

AIMS:

The first aim of this project was to define a protocol by which regeneration of cotton plants from tissue culture can be consistently and regularly achieved.

The cotton regeneration protocol developed by Trolinder (1985) was successfully followed and eight plantlets were grown through to the large pot stage while more than 50 were potted in vermiculite proparatory to being repotted in large containers.

As other workers have found, genotype is an important factor in cotton regeneration. Thirteen cultivars were used. Of these, Coker 315, Coker 312 and Siokra 1-3 were the most regenerable. Regeneration studies were limited to Siokra 1-3, Siokra 1-4, Sicala 3-2, Coker 315 and Coker 312.

There is a lot of within cultivar variability in regenerability. Coker 315 most reliably produced embryogenic callns. Coker 312 and Siokra 1-3 have much greater variability. A large number of seedlings have to be callused in order to obtain an embryogenic cell line. It takes about two months to identify an embryogenic cell line. This was a major lag step in the regeneration project at Narrabri.

An alternative is to use mature tissues as explants. This has the advantage of being able to screen large numbers of plants and reuse those with the best embryogenic response. The present protocol uses seedling hypocotyl as explant, which doesn't allow reuse of successful material. Gawel *et al* (1986), developed methods of cotton regeneration from leaf and petiole tissue. Coker 315, Siokra 1-3, Siokra 1-4 and Sicala 3-2 varieties were glasshouse grown to gain experience in mature explant regeneration and to identify any highly embryogenic plants. Preliminary attempts at leaf and petiole regeneration proved difficult to callus.

A stock of glasshouse grown seed was collected to establish lines within cultivars by single seed descent. Regenerability studies of this material began in 1988, in an effort to find the most embryogenic seedling tissue and to grow seed from the same plant.

In November 1987 a three week visit to Lubbock, Texas, USA, provided valuable hands-on experience in regeneration techniques and information on protocols and past studies of cotton regeneration. Most importantly an approach to regeneration of 'difficult' cultivars was learned. This approach has been successfully used in Lubbock for Chinese varieties and Paymaster cultivars. It involves selection of the most regenerable plants within a cultivar and using these plants as explant sources for further regeneration or transformation studies. This approach has been used with Coker 315, Siokra 1-3, Siokra 1-4 and Sicala 3-2.

In February 1988 when a nursery of regenerable Australian cultivars was established, work on the project will continue in Canberra, with preliminary studies on cotton transformation.

REFERENCES:

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Trolinder, N.L. (1985). Somatic embryogenesis and plant regeneration in *Gossytpium hirsutum* L. A Dissentation n Biology, Texas Tech. University.