Part B - Summary

Title; *Agrobacterium* mediated genetic transformation of *Mangifera indica, Lycopersicon esculentum* and *Brassica juncea* (species).

Research Institute; Department of Botany, University of Colombo.

Chief Scientific Investigator; Dr. G.A.U. Jayasekera

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Introduction of foreign genes responsible for agronomic traits can be employed to improve the agricultural, horticultural or ornamental value of a particular crop plant. The present study investigates the possibility of introducing genes in to *Mangifera, Brassica* and *Lycopersicon* through the *Agrobacterium* vector system. Specific objectives of the study are assessment of susceptibility to *Agrobacterium* infection, which leads to transformation and subsequent regenerability of infected cells of explants of the three plants *Mangifera*, *Brassica* and *Lycopersicon*.

Agrobacterium tumefaciens strain LBA 4404 harboring the binary plasmid pBI 121 (13 Kb) was used in this study. The vector contained CaMV 35S promoter cloned upstream of the β-glucuronidase gene with NOS terminator as well as a gene conferring kanamycin resistance under the direction of NOS promoter.

In *M.indica* both leaves and fruits were used as the explants, and the hormonal combinations of IAA, NAA, 2,4-D and BAP were tried in order to induce *in vitro* growth or callus by using both the media MS and WPM.

In this case the explants were pre-treated before culturing with MS liquid medium containing either PVP or PVPP with ascorbic acid in order to avoid phenolic exudation and many surface sterilization regimes with clorox, ethanol, benlate and $HgCl_2$ were tried in order to avoid contaminations due to microbes.

Nevertheless in *M. indica* attempts that were made to develop a good regeneration system were unsuccessful.

In *B. juncea*, cotyledonary petioles infected with *Agrobacterium* regenerated shoots with little callus on MS medium containing 20 μM BAP, 500 mg/l carbenicillin/cefotaxime and 15 mg/l kanamycin within ten days. The frequency of shoot regeneration was 8.3% of which 43% were green shoots and therefore were putative transformants.

Of the three varieties, T-245, T-146, KWR tested in *L. esculentum*, the frequencies of shoot regeneration were 2.68%, 4.21% and 0.44% of which 96.2%, 95.6% and 87.5% were green shoots respectively and therefore were considered to be putative transformants.

Although different concentrations of hormones IAA, BAP and GA_3 were tested in combination in order to improve the regeneration potential, no medium was found to be significantly superior to the other.

Of the four different types of explants tested in *Lycopersicon*, ten day old hypocotyls appeared to be more promising (75%) in terms of shoot regenerability than the cotyledons and internodes.

In *Brassica* complete shoot systems were excised and rooted in MS medium containing 2 mg/l IBA and after seven weeks, six putative transformants were transferred to the soil. Fertility of parent plants was almost 100% and subsequently T1 generation was also obtained.

Rooted shoots of three varieties of *Lycopersicon* in MS media containing 1 mg/l IAA were transferred to the soil after 2-3 months of culture. Three rooted plants were obtained from the varieties T-245 and T-146 each and only one rooted plant was obtained from the variety KWR.

The putative transformants of *B.juncea* and *L.esculentum* were analysed by assaying for the GUS expression histochemically. In *Brassica* T1 generation, only two plants of the twelve shoots and one of the five germinating seeds were GUS positive, while in *Lycopersicon*, only one plant (T-146) of parent progeny was GUS positive.

These results demonstrate that, with further optimization of the techniques that have been developed in this study, it is possible to introduce and stably integrate genes in to local varieties of *B. juncea* and *L. esculentum* and which allows the improvement of these varieties by the introduction of agronomically useful genes by this method.

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Title of a Paper ; *Agrobacterium* mediated genetic transformation of Local Mustard (*Brassica juncea*) and Tomato (*Lycopersicon esculentum*) species.