A Study of *Agrobacterium* Mediated Genetic Transformation of *Mangifera indica, Brassica juncea* and *Lycopersicon esculentum*

By

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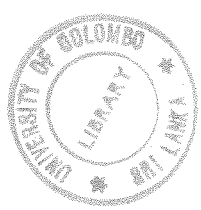

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Abstract

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 indica, Brassica juncea* and *Lycopersicon esculentum* through the *Agrobacterium* vector system. Specific objectives of the study are the assessment of susceptibility to *Agrobacterium* infection and subsequent regenerability of infected cells of explants of the three plants *Mangifera indica*, *Brassica juncea* and *Lycopersicon esculentum*.

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 Murashige & Skoog (MS) medium and woody plant medium (WPM).

In *M. indica* attempts that were made to develop a good *in vitro* 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 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 3.91%, 4.88% and 0.81% of which 100%, 96.8% and 90.9% were green shoots respectively and therefore were putative transformants.

The regeneration medium of *L. esculentum* contained 20 mg/l kanamycin and 300 mg/l cefotaxime in addition to the different concentrations of hormonal combinations.

Of the four different types of explants tested in *Lycopersicon,* 10 day-old hypocotyls appeared to be more promising in terms of shoot regenerability.

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 the 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 variety T-245 and the variety 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, two plants of the twelve shoots were GUS positive, while in *Lycopersicon*, one plant (T-146) of parent progeny was GUS positive.

These results demonstrated 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 the local varieties of *B. juncea* and *L. esculentum* and, which allows improvement of these varieties by the introduction of agronomically useful genes by this method.