PLANT GENETIC MANIPULATION TECHNIQUES FOR THE PRODUCTION OF HUMAN PHARMACEUTICALS

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Introduction

The production of most pharmaceuticals compound was extracted directly from the plant. However, the yield and the productivity were not constant due to uncertainty of supply and instability of raw material. *In vitro* techniques can be an alternative approach for the production of pharmaceuticals. However, undifferentiated plant cells tend to be genetically unstable in culture and the compound products are sometimes chemically different from those produced in whole plant. Consequently, the *in vitro* culture of transformed hairy root appears as an interesting biotechnological system of choice for the production of tropane alkaloids. They offer numerous advantages because their fast growth rate in free phytohormone media, genetically stability and their morphological differentiation and similar pattern of alkaloid production compared to the parent plants (Parr and Hamill, 1987; Aird et al. 1989) Recently, several plants have been transformed, and the established root cultures have shown to produce target secondary compounds (Ionkova et al. 1994; Zehra et al. 1998). Therefore, the objectives of the study were: 1) To produce pharmaceutical compounds via plant cell culture; 2) to genetically improve the explant cell lines via gene transformation and gene manipulation; and 3) to optimise conditions for synthesis suitable for bioreactor systems.

Materials and Methods

In vitro culture of plant was infected with *Agrobacterium rhizogenes,* a phytogenic bacterial to induce the "hairy root disease". Excised hairy roots were then transferred to the free phytohormone media supplemented with antibiotic to eliminate the bacterial. The cultures were maintained on gyratory shaker at 90 rev/min in the dark. Roots were cultured in different media to enhance scopolamine and hyoscyamine production. Studies examined the type of media, effect of carbon sources and ionic strength, nutrient and precursor. Alkaloids were analysed using HPLC methods (Fliniaux et al. 1993).

Results and Discussion

Hairy root culture of *Datura metel,* producing considerable amount of tropane alkaloids (scopolamine and hyoscyamine), were successfully established by using *Agrobacterium rhizogenes* LBA 9402. The production of scopolamine and hyoscyamine was investigated under various culture conditions. Growth was measured by changes in fresh and dry weight over a period of 35 days, by which time all cultures were at the stationary phase. There was an 8-fold increase in fresh weight of roots cultured in B5 media at 25 days of culture. Similarly, at this stage, the alkaloid production was higher than that obtained at other stages of culture. The production of the alkaloids rose steadily during the first 5-10 days of culture and peaked at 0.04 % of total dry weight. Its content appeared to be directly related to the root biomass obtained. Three basal media culture were tested and Gamborg B5 media was the best for growth of hairy roots (3-fold higher) and alkaloid production (78 % higher than that of White's media and 35 5 than that of Ms media). Both alkaloids were detected in the culture media. The release of scopolamine and hyoscyamine from root cultures into the media was highest in White's media (9.4 %) followed by B5 (3.5 %) and MS (2.5 %) media, respectively. The effect of B5 media ionic strength on sucrose concentration was investigated. It was observed that roots cultured in full strength B5 media supplied with 4 % sucrose produced the highest scopolamine and hyoscyamine content. The addition of precursor (putrescine, arginine, ornithine) into root cultures at lower levels enhanced the accumulation of scopolamine and hyoscyamine in roots.

Conclusions

Studies were successful in establishing transformed root cultures of *Datura metel* using *Agrobacterium rhizogenes.* The root cultures produced a spectrum of alkaloid higher than in intact plants. Media manipulation could enhance scopolamine and hyoscyamine production *per se.* B5 media with 4 % of sucrose was the best media for alkaloid productions. The addition of precursor was able to enhance the accumulation of the scopolamine and hyoscyamine.

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