Coffea arabica Protoplast Culture: Transformation Assays

J. GREZÈS, B. THOMASSET, D. THOMAS

URA 1442, LTE Université Technologique de Compiègne, BP 649, 60200 Compiègne, France

SUMMARY

The biosynthesis of the secondary metabolites seems to be related to the differentiation of the cells or plant organs such as shoots or roots. Many efforts have been made to improve the productivity of the plant tissue cultures. A recent development to overcome those difficulties is the genetic transformation of plant cells with *Agrobacterium rhizogenes* or *tumefaciens* to introduce useful traits or to establish high-producing lines. The must widely used procedure to obtain high producing plant cell cultures is to prepare single cell clones by preparing protoplasts. The prepared protoplasts are plated at low density ($2\ 10^5$ p/ml) and allowed to grow into callus culture which subsequently are selected for their productivity before their use in bioreactor.

Coffee cells has been chosen as a model cell line because the biosynthesis of the secondary metabolites is well characterized. Moreover, it's possible to isolate biochemically intact protoplasts. However, high rates of callus formation from protoplasts are necessary to allow efficient recovery transformed cell lines. Identification of limiting factors (auxine and cytokinin requirements, sugar, cystein, BSA, light, ...) should facilitate the development of a culture procedure to obtain high rates of callus formation.

If genetically engineered plant cell cultures have to be used for the production of secondary metabolites, the expression of the introduced gene has to be maintained stable over a long period of subculture. In order to obtain functional transgenic cell lines the optimization of the transfer DNA protocol (Agrobacterium strain, explant lines, bacterial concentration, cocultivation period) must be achieved. The expression of a chimeric GUS gene is used as a model to establish conditions for both the transformation and for efficient and stable expression of foreign genes.