Application of Biotechnological Methods for the *in vitro* Biosynthesis of Bioactive Saponins and Propagation of Endod

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Abstract

There has been considerable interest, in recent years, in examining the potential of plant cell-cultures as an alternative to traditional agriculture for the industrial production of biologically active compounds.

Studies with callus and cell suspension cultures of *Phytolacca dodecandra* (Endod) have established that significant amounts of hemolytic triterpenoid saponins are produced and retained intracellularly by these cultures. Although at present, saponin production from cell suspension systems would have to be accomplished using batch culture techniques, it may be possible to develop Endod cell suspension systems in the future that excrete saponins into the culture medium, making continuous fermentation processes feasible.

Tissue culture is a viable technology that can be used for the genetic improvement of Endod. Tissue culture methods can be employed as micropropagative tools for clonal multiplication of Endod plants, both for cultivation and for selection and breeding programmes. Clonally propagated plants would likely guarantee genetic uniformity in the pattern of growth and development, simplifying the cultivation and harvest of Endod plants for biologically active compounds.

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INTRODUCTION

Since 1964, phytochemical and pharmacological investigations conducted on extracts from the Endod plant, *P. dodecandra,* have established that the plant produces a series of structurally related triterpenoid saponins with molluscicidal (antischistosomal) activity (Lemma *et al.*, 1979). The saponin with the most potent molluscicidal activity has been identified as lemmatoxin, an oleanolic acid glycoside.

The need to produce strains of Endod with high yields of these saponins and the possibilities of extracting the biologically active compounds (including lemmatoxin) from callus tissues and batch cell cultures provide unique opportunities for the application of plant tissue culture and related biological technologies.

The potential of plant cell cultures for the production of biologically active compounds

Many bioactive compounds derived from higher plants possess highly complex chemical structures for which no practical commercial syntheses are available. These substances must therefore continue to be extracted from their natural sources (Farnsworth and Bingel, 1977; Nakanishi, 1982). For this reason, there has been considerable interest, in recent years, in examining the potential of plant cell cultures as an alternative to agriculture for the industrial production of biologically active compounds (Carew and Staba, 1965; Staba, 1969; Steck and Constabel, 1974; Barz *et al.*, 1977; Barz and Ellis, 1981; Shuler, 1981).

Much recent work has been directed toward the biotechnological application of static tissue (callus) and cell suspension cultures for the commercial production of substances, such as the cardiac glycosides and the opium, belladonna, and anti-leukemic *Catharanthus* alkaloids. A number of workers have also investigated such culture systems for the commercial production of steroidal saponins (e.g., Kaul and Staba, 1968; Stohs *et al.*, 1974; Stohs and Rosenberg, 1975; Sharma and Khanna, 1980; Jain *et al.*, 1981).

Tissue culture is a viable technology that can be used for genetic improvement of Endod, and for developing callus tissue and batch cell culture systems of the plant for purposes of *in vitro* biosynthesis of lemmatoxin and other saponins with molluscicidal and/or other biological activities. The use of tissue culture in genetic improvement is feasible, both by its integration into a conventional breeding programme via rapid clonal multiplication of desired genotypes, and by using it to select out somatic cell variants. Cell suspension systems offer the potential of large-scale culturing of cells from which saponins with biological activity can be extracted. In addition, experiments to alter the chemical composition of the culture

medium or the culture environment are being conducted in an attempt to increase production. The advantage of this method is that it will provide a continual, predictable source of the saponins and eliminate the inconsistencies in production from whole plants which occur due to environmental influences.

Plants, with as much as four times greater biological activity than usual, have already been selected (Lugt, 1978), and tissue culture can be employed as a micropropagative tool for clonal multiplication of Endod plants, both for cultivation and for breeding programmes. In addition, clonally propagated plants would likely guarantee genetic uniformity in the pattern of growth and development. Since the highest saponin content occurs when the berries are green (Lemma and Wolde-Yohannes, 1979), the uniformity of growth pattern should simplify the cultivation and harvest of the Endod plants for compounds with biological activity.

The most immediate research interests at PRI are mass *in vitro* clonal multiplication of selections of Endod, which produce fruits with high levels of the desired bioactive saponins or which produce a large number of berries per plant, and the direct *in vitro* biosynthesis of Endod saponins via callus tissue and/or cell suspension cultures. Quantitative saponin yields from callus tissues and cell suspension systems (both cells and media) are currently being determined in order to assess the economic potential of *in vitro* production and extraction. The PRI has already established Endod callus and cell suspension cultures which produce substantial quantities of saponins, as shown by chromatographic procedures and hemolytic *in vitro* bioassay. Cold aqueous suspensions of the callus cultures have molluscicidal LD_{100} values of 40 ppm (Dr. Lemma, personal communication). These early results are very encouraging and serve to illustrate the significant industrial potential of Endod plant cell cultures.

Our studies have established that callus and cell suspension cultures of Endod produce hemolytic triterpenoid saponins that are retained intracellularly by the cultured cells. Only very slight traces of saponins can be detected in culture media, probably due to instances of cell rupture (especially during cell filtration to obtain pure media). At present, it is necessary to grow batches of cells, harvest, dry, and then powder them in order to obtain (extract) significant amounts of saponins. Callus tissue and suspension cells have the advantage, however, that they are essentially pigment-free, making saponin extraction and purification relatively easy compared to green plant parts. The intracellular retention of saponins has been observed previously with tissue cultures of *Agave wightii, Yucca glauca, Dioscorea deltoidea,* and *Solanum jasminoides.* Saponins appear to be stored in subcellular compartments and apparently are not readily excreted from callus tissues or cells in suspension.

It is hoped that in the future, cell suspension systems that actively excrete bioactive saponins into the culture medium may be produced, so

that continuous fermentation, analogous to present-day antibiotic production methods, will prove feasible. However, before this becomes a reality, a number of questions must be answered, not the least of which is the question of Endod cell membrane autotoxicity due to the "leached" saponins. There may be a physiological limit as to the saponin concentration that can exist in a cell suspension medium before the system "crashes" due to extensive cell lysis.

Many plants are known to produce molluscicides (Kloos and McCullough, 1982). The Plant Resources Institute is also examining the feasibility of producing biologically active saponins from other plant species which have surfaced in our bioscreening programme, such as *Saponaria officinalis*, *Atriplex* spp., *Agave* spp., and *Yucca* spp.

Although cell suspension cultures offer considerable promise, the main use of tissue culture selection for high saponin producers may be to develop new lines for traditional breeding. The high yielding strains could then be used in a self-help system.

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