Production of Secondary Metabolite In vitro for Commercial purpose Dr. Rena

Introduction – Higher plants are ~~evaluable~~ valuable & the most important producers of natural products including food, fiber, wood & oils. However several plants are rich in secondary metabolites including proteins, flavonoids, antimicrobials, fragrances, pigments, food flavours, essential oils, Alkaloids, Steroids, Rotenoids, Glycosides Pyrethrins, pesticides etc.

"Secondary metabolites are the substances which are produced, by the plants but they are of no use for plants. But these substances, may be useful for humans in several ways."

Sec. Meta. extracted from higher plants are valued from several dollars per pound.
eg- The Sedative Codeine or the Insecticide Pyrethrin to several thousand dollars per pound, Jasmine Oil or higher.

The antitumour alkaloids Vinblastine & Vincristine from Catharanthus roseus used in the treatment of leukemia are sold at over $6000 per gram.

In United States alone, the market for plant derived pharmaceuticals is estimated about $ a billion per year. The world markets for flavours, fragrances & agrichemicals are of a similar size.

Two following points have a major impact on idustries based on plant-derived chemicals

① Development of novel technologies for large scale growth & produc^{n} of plant secondary metabolites.

② Discovery of new secondary metabolites from unexplored plant sources.

So there is the urgent need to understand & preserve the chemical inventory of higher plant. Tropical forests are the main source of diff. varieties of plants, but genetic erosion causes glo gradual loss of plant resources. So conservation of biodiversity or PGR is essential before they are lost for ever.

HISTORY :-

In 1950's the potentialities of the isolated cells of higher plants in producing the useful metabolites was recognized. But at that time the technique of tissue culture was not so developed. Later the plant tissue culture techniques have developed which opened the new front for the produc^{n} of secondary metabolites. The plant cell cultures are used to produce sec. metabolites

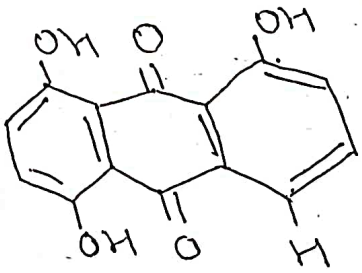
→ A considerable effort in this area was made in laboratories in Japan & Germany & to a lesser extent in U.S.

→ In 1983, the first plant cell culture proces was commercialized in Japan

The Shikonins (a family of Anthraquinones) was produced commercially from the root-derived callus cultures of Lithospermum erythrorhizon in Japan.

These anthroquinones are used for their anti-inflammatory properties, antibacterial activity & as dyes. The company is Mitsui Petrochemical Industries

In India the beginning of this work goes back to 1964 when Nitta & Kaul at NBRI (National Botanical Research Institute, Lucknow) showed the produc^n of Reserpine from Rauwolfia serpentina tissue culture. Later on work on various metabolites was carried out in other laboratories as well in India.



Shikonin

Nicotine

Advantages of Tissue Culture technique for Secondary metabolite produc^n :-

Plant cells in culture offer many advantages over intact plants for Sec. meta. produc^n & their biosynthetic studies. These are as follows:—

1. Plant cells are easy to grow, & rate of cell growth & biosynthesis could be high enough to give a good produc^n of the final product in a short period of time.
2. Cells can be kept under strictly controlled nutritional & environmental conditions. Hence the uncertainties of climate & soil can be avoided.
3. Cells are cultured under aseptic conditions devoid of microorganisms or insects etc.
4. Precursors can be incorporated in suspension cultures which is difficult to administer to the plant growing in nature.

(5) Technology is now available for relatively large scale producⁿ of plant suspensions. Batch cultures, Closed continuous cultures system Open continuous culture system etc. provide an efficient mean of producing commercially imp. plant products.

For industrial applications of plant cell cultures for S.M. producⁿ following requirements should be satisfied —

(1) The rate of cell growth & biosynthesis should be high enough to give good producⁿ of final product in a short period of time
(2) The cultured cells should be genetically stable to give a constant yield of the product.
(3) The metabolites should be accumulated in the cells without being catabolized rapidly, they should be released into the liquid medium.
(4) Producⁿ cost including culture medium, precursor & chemical extracⁿ should be low enough to be profitable to the industries.

Hairy Root Culture for Secon. Metabolite Producⁿ —

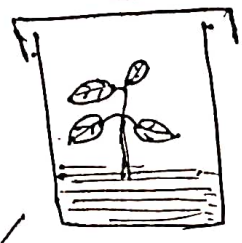
In (1934) White established tomato root cultures which are capable of unlimited growth in medium containing macro & micro nutrients sucrose & yeast extract. The original root clone from White's work were grown for at least 25 years in chemically defined liquid medium & were in fact the first plant organ cultures.

Dawson established root cultures of tobacco & showed that roots have ability to make

Nicotine ⑤

Establishment of Agrobacterium rhizogenes-transformed "hairy Root" cultures

① Seedling grown in sterile conditions is inoculated with an appropriate strain of Agrobacterium



Adventitious "hairy roots" develop at the wound sites

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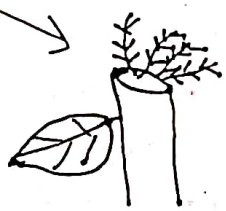
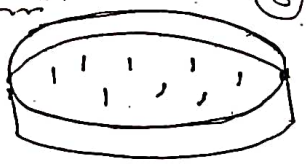


Transfer into antibiotic containing medium



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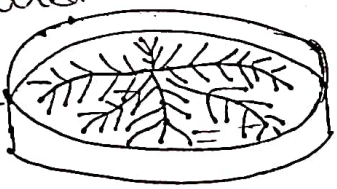
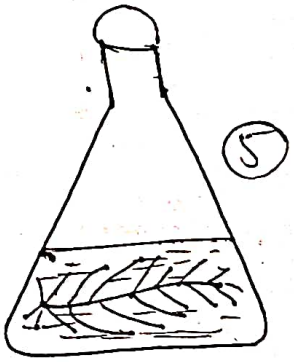
Bacteria-free clone transferred to Agar medium





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Established hairy root clone in shaken culture