

Table 1. Typical high-value substances that have been found in plant cell tissue culture<sup>1-6,12-14</sup>

Types of substances	Products
Agricultural and chemicals	Growth regulators
	Hormones
	Insecticides
	Plant virus inhibitors
Biochemicals	Amino acids
	Enzymes
	Enzyme inhibitors
	Lipids
	Nucleic acids and derivatives
	Peptides
	Proteins
	Vitamins
Food additives and fragrances	Condiments
	Emulsifiers
	Flavanoids
	Flavors
	Fragrances
	Perfumes
	Spices
	Sweeteners
Medicinals and drugs	Alkaloids
	Allergens
	Anticancer agents
	Antimicrobial agents
	Cardioactive substances
	Opiates
	Immunochemicals
	Steroids

## Metabolite production is frequently higher in cell cultures

- Berberine production from *Coptis japonica* is about 5% of dry weight after 5 years of root growth, which equals 0.17 mg/g per week.
- Whereas in selected cell lines it can be 13.2% of the dry weight in cell culture after 3 weeks, which is about 44 mg/g/week or about 250 times higher

## Secondary Metabolites Produced in High Level by Plant Cell Cultures

COMPOUND	PLANT SPECIES	YIELDS (% DRY WT)		CULTURE TYPE*
		CULTURE	PLANT	
Shikonin	<u>Lithospermum erythrorhizon</u>	20	1.5	s
Ginsenoside	<u>Panax ginseng</u>	27	4.5	c
Anthraquinones	<u>Morinda citrifolia</u>	18	0.3	s
Ajmalicine	<u>Catharanthus roseus</u>	1.0	0.3	s
Rosmarinic acid	<u>Coleus blumeii</u>	15	3	s
Ubiquinone-10	<u>Nicotiana tabacum</u>	0.036	0.003	s
Diosgenin	<u>Dioscorea deltoides</u>	2	2	s
Benzylisoquinoline Alkaloids	<u>Coptis japonica</u>	11	5 - 10	s
Berberine	<u>Thalictrum minor</u>	10	0.01	s
Berberine	<u>Coptis japonica</u>	10	2 - 4	s
Anthraquinones	<u>Galium verum</u>	5.4	1.2	s
Anthraquinones	<u>Galium aparine</u>	3.8	0.2	s

- There are **three ways** for the production of secondary metabolite by plant tissue culture which are as follows:-

- i) **Cell suspension culture**
- ii) **Hairy root culture**
- iii) **Immobilized cell culture**

## Root cultures are often better than cell cultures

- Roots often secrete the metabolites into the surrounding medium, making it easy for collection.
- Charcoal can be added to the medium, the metabolites are absorbed by the charcoal, and this stimulates even higher production of the metabolite.

## ELICITOR INDUCED PRODUCTION OF SECONDARY METABOLITES:

Production- very low, demand- not met...☹

Effort : for product formation at molecular level, and exploit the ways for increased production.

Elicitors are the compounds of biological origin which stimulate the production of secondary metabolites, and the phenomenon is called **ELICITATION**.

# ELICITORS

## ENDOGENOUS

Within plant cell:  
pectin, pectic acid,  
cellulose, etc

## EXOGENOUS

Produced by  
microbes. Eg:  
chitin, chitosan,  
glucans.

## BIOTIC

All elicitors  
of biological  
origin

## ABIOTIC

**Physical agent:**  
heat, cold, UV,  
osmotic pressure  
**Chemical agent:**  
antibiotics,  
fungicide, etc..

## METHODOLOGY FOR ELICITATION

- a) Selection of microorganisms
- b) Co-culture

## D) Addition of precursors to the culture medium

- ▶ Tropic acid in the media of *Datura* and *Scopolia* potentiate *tropane* alkaloids
- ▶ O-succinylbenzoic acid to the cultures of *Morinda* resulted in a two folds increase in the accumulation of *anthraquinones*.
- ▶ Addition of phenylalanine to the culture of *Taxus cupsidata* cultures potentiated the levels of *Rosmarinic acid* and *Taxol* respectively.
- ▶ Trp. Is starting material for *indole alkaloids* [ergot alkaloids, curare alkaloids]



# Effect of precursors

- ▢ Addition of precursors to the medium enhances product formation.

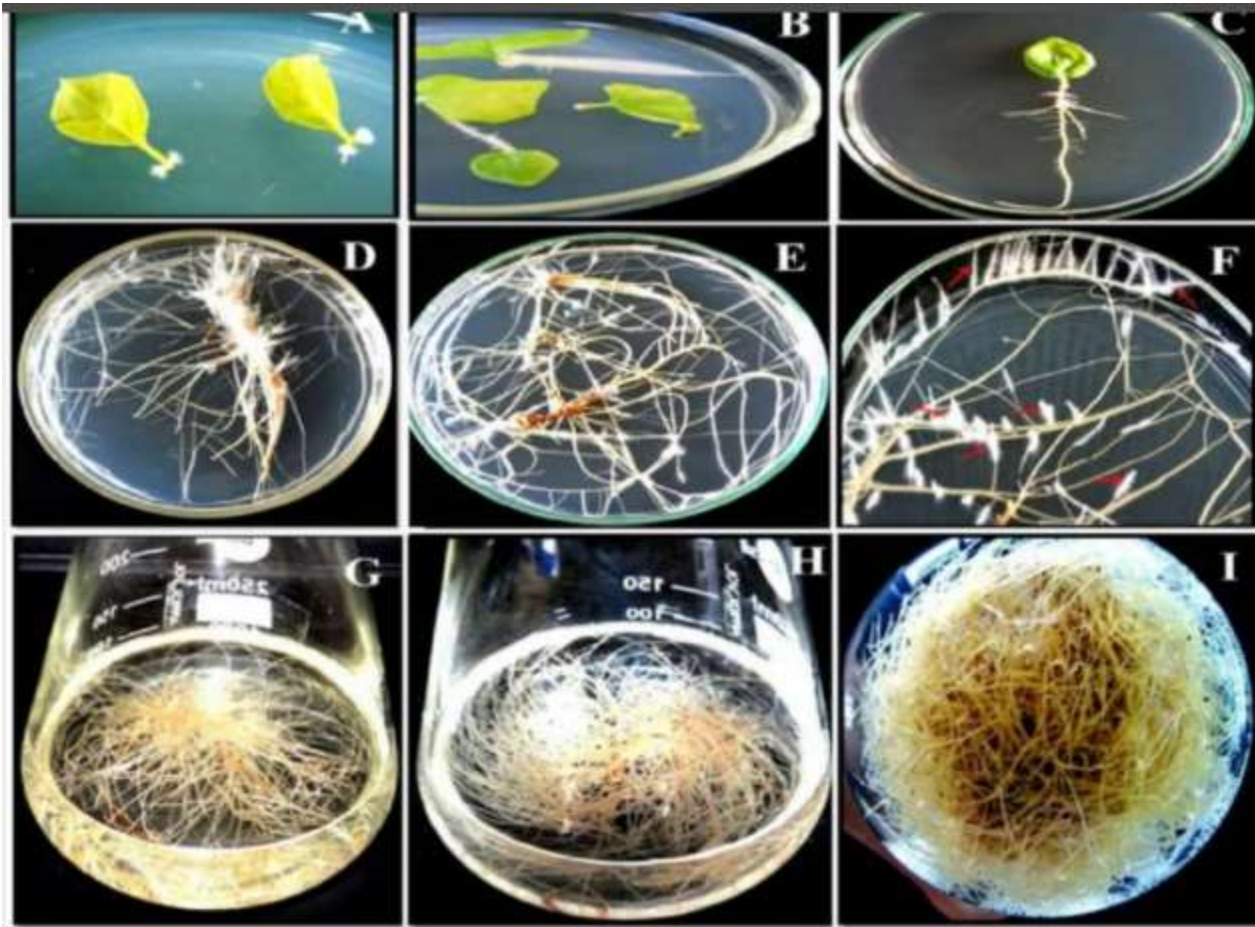
Eg: ornithine, phenylalanine, tyrosine and sodium phenylpyruvate, precursors typtamine and secologanin increase ajmalicine production in *C.roseus* culture.

## Many secondary metabolites are produced in roots

- Scientists have developed a form of root culture using *Agrobacterium rhizogenes*, the cause of hairy root disease. (**Show Fig 14.3**)
- | Cells transformed with some of the bacteria's DNA, causes the cells to be more sensitive to the hormones they produce. The cells form into roots. These roots grow very fast and produce the secondary metabolites that ordinary roots produce.

i) **Hairy root culture**: plant tissue culture that is used to study **plant metabolic processes** or to produce secondary metabolites or recombinant proteins, often with plant genetic engineering. culture produced after the infection of the explants or cultures by the gram negative soil bacterium ***Agrobacterium rhizogenes* (contain Ri plasmids)** can infect plant roots and cause them to produce a food source for the bacterium, **opines** and to grow abnormally.

- Explants are wounded and then inoculated with *Agrobacterium rhizogenes*.
- After 2 or 3 days, the explants can be transferred into solid media with antibiotics such as ceftioaxime, vancomycin etc to kill or eliminate redundant (no longer needed) bacteria.
- The hairy roots will be induced within a short period of time which varies from 1 week to over a month varying on different plant species.
- The decontaminated hairy root cultures can be sub-cultured on phyto-hormone free medium.





## Advantages of Hairy Root Culture Over Plant Cell Suspension Culture

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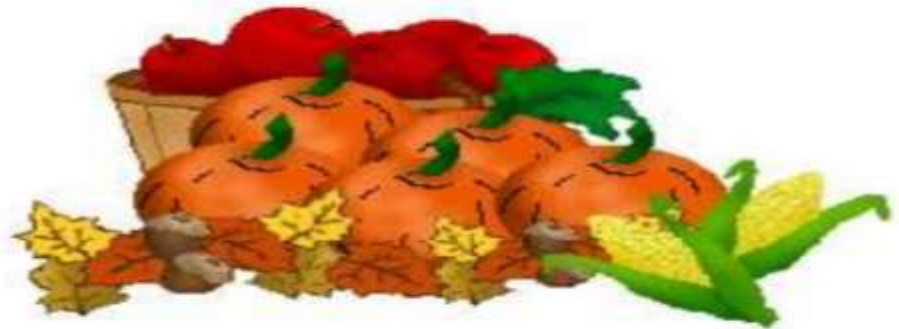
- **Fast growth**
- **Low doubling time**
- **Genetic and biochemical stability**
- **Growth in hormone free media.**

*These fast growing hairy roots can be used as a continuous source for the production of valuable secondary metabolites.*

**FOOD ADDITIVES-  
FLAVOR AND  
COLOUR AGENTS**

## Natural Colors

- saffron
- Anthocyanin
- Carotenoids
- Carotene
- Chlorophyll
- Curcumin
- Iron oxides
- Riboflavin
- Titanium dioxides



## Carotenoids

- **Yellow & Red colors.**
- Sources- Sweet potatoes, spinach and tomatoes.
- Antioxidant - Cancer research.



PRODUCTION OF A FOOD ADDITIVE  
THROUGH TISSUE CULTURE-

**SHIKONIN**- the first  
phytochemical to be produced on  
commercial scale

## Shikonin & its production

- Shikonin, a **red naphthoquinone pigment** or derivative extracted from a traditional Japanese or Chinese medical **perennial herb** (mainly from **root part**), *Lithospermum erythrorhizon* (family- **Boraginaceae**).
- It has been found to possess a variety of biological activities including strong **wound healing, antibacterial, anti-inflammatory, and anti-tumor effects**.
- Production of a **red pigment- (Shikonin)** used for **flavors, fragrances, pigments, dyes, cosmetics and food additives**.

## Production of shikonin

- In *L. erythrorhizon*, it is well documented that shikonin is derived from two precursors originating from different pathways.
- The aromatic precursor 4-hydroxybenzoic acid (4HB) formed via the shikimate and the phenylpropanoid pathway, while the isoprenoid precursor, geranyldiphosphate (GPP) is derived from the mevalonate pathway (Li et al., 1998).
- One of the key enzymes for the shikonin biosynthesis of cells, *p*-hydroxybenzoic acid (PHB) geranyltransferase.

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## Factors effecting the production of Shikonin

### 1. Optimization of Cultural Conditions:

**1.1 Medium:** These factors include media components, phytohormones (growth regulators), pH, temperature, aeration, agitation, light, etc.

**Blaydes**  
**Gamborg - B5; + 2,4-D: 1 mg/l**  
**Gamborg + 2,4 D: 2 mg/l**  
**Gamborg + NAA : 1.86 mg/l**  
**Gamborg**  
**Heller + IAA:0.175; BA: 1.13**  
**mg/l**  
**Linsmaier and Skoog**  
**Murashige and Skoog**  
**Nitsch and Nitsch**  
**Velicky and Martin**  
**White**

- IAA = Indole-3-acetic acid  
NAA = 1-Naphthalene acetic acid  
2,4-D = 2,4-Dichlorophenoxy acetic acid  
Kin = Kinetin  
BA = Benzyladenine

1. Growth medium
2. Production medium with precursors and elicitors  
many elicitors have been used to enhance the production of shikonin, like calcium ions, a crude elicitor extracted from *Aspergillus oryzae*

Fungal elicitation provides a rather specific technique for stimulating secondary metabolism that is not dependent upon reduction of the culture growth rate. By adding elicitors, such as cell wall constituents of microorganisms, enzymes, or heavy metals, certain biosynthetic pathways are induced that enhance the yield of the metabolite

## 1.2 Temperature, pH, Light and Oxygen:

- The effects of temperature, pH, light and oxygen are all parameters that must be examined in the studies secondary metabolites production.
- A temperature of 17- 25° C is normally used for induction of callus tissues and growth of cultured cells.
- The medium pH is usually adjusted to between 5 and 6 before autoclaving and extremes of pH are avoided.

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### 1.3 High Cell Density Culture:

- To increase the productivity of secondary metabolites, high cell density cultures have been investigated. Using a newly designed fermentor and optimized culture medium, cells were grown up to 75 g/L of cell mass.

### 1.4 Absorption of Products:

addition of active charcoal in the medium stimulated the yield, XAD-7.

### 1.5 Selection of High-Producing Strains:

The physiological characteristics of individual plant cells are not always uniform. For example, pigment producing cell aggregates typically consist of producing cells and non-producing cells.

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## 1.6 Addition of Precursors:

- Addition to the culture media of appropriate precursors or related compounds sometimes stimulates secondary metabolite production.
- Phenylalanine is one of the biosynthetic precursors of rosmarinic acid (67).
- Addition of this amino acid to Salvia officialis suspension cultures stimulated the production of rosmarinic acid and shortened the production time as well.

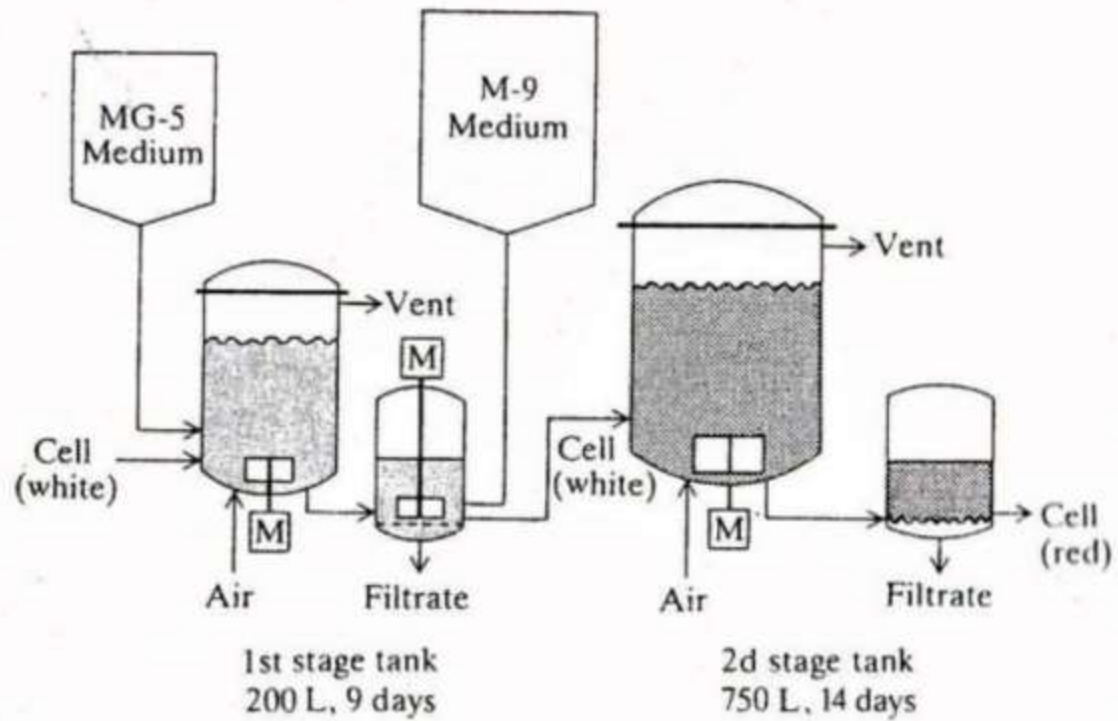
## **1.7 Biotransformation:**

- This approach has been extensively applied in the fermentation industry using microorganisms and their enzymes. For example, L-aspartic acid and L-malic acid are being manufactured commercially from fumaric acid, respectively using microorganisms.
- And various steroids are also produced by microbial biotransformations.

## Protocol

- By systematically accessing of all the component medium, two stages culture systems are involved.
- 1. In first stage:** Cells of high producing strain M-18 were propagated in a growth medium MG-5 (Modified LS Medium) without producing shikonin derivatives. The volume of the culture will be 200L in small tanks and culture for 9 days.
  - 2. In 2nd Stage:-** The cells from the growth medium are transferred to M-9 medium for shikonin production. The components of LS & M-9 medium are more suitable for shikonin production. The shikonin yield also increased when both the initial cell density and medium concentration were doubled. The volume of the culture will be 750L in large tanks and culture for 14 days.

- The **concentration of dissolved oxygen** is also vital. It should be **adjusted to 6.4 and 6.0 ppm for the first and the second culture stage.**
- Cells of the stock culture are grown in a jar fermenters are inoculated in the first stage tank (200 L) with MG-5 medium for cell growth and incubated for **9 days and subsequently transferred to filtering apparatus.**
- The production medium **M-9 is poured into the tank** and cell in this medium are **pumped into 2nd tank (750 L) for shikonin production.**
- After incubating the cells for **14 days in the 2nd tank,** the **pigmented cells** are harvested by filtering off the medium.
- The extracted and purification can be carried by treating the **cells** with **n-hexane** from the cultured cells and subsequently **hydrolysis with 2% KOH** and **recrystallized** to give a pure shikonin.



Two-stage culture for shikonin production

# ALKALOIDS



## WHAT ARE ALKALOIDS?

- These are commonly applied to basic nitrogenous compounds of plant origin that are physiologically active.
- Organic nitrogenous compounds with a limited distribution in native nature.

## CHARACTERISTICS:

- They are bitter in taste.
  - Derived from amino acids. The amino acids that are most often serve as alkaloidal precursors are: **phenylalanine, tyrosine, tryptophan, histidine, anthranilic acid, lysine and ornithine.**
  - Alkaloids form double salts with compounds of mercury, gold, platinum and other heavy metals. These salts are obtained as precipitates which are microcrystals.
- 
- Insoluble or sparingly soluble in water, but the salts formed on reaction with acids are usually freely soluble.
  - Most are crystalline solids although a few are amorphous.
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- Free alkaloids are usually soluble in polar solvents like ether, chloroform
  - Some alkaloids are liquid because of lacking of oxygen in their molecules. (e.g. coniine, nicotine, sparteine)



## SOURCES AND OCCURRENCE OF ALKALOIDS

- Alkaloids can occur in plant kingdoms; among the angiosperms,
- **Leguminosae,**
- **Papaveraceae,**
- **Ranunculaceae,**
- **Rubiaceae,**
- **Solanaceae,**
- **Berberidaceae** are outstanding alkaloid-yielding plants.

## USES OF ALKALOIDS IN PLANTS:

- Poisonous agents which protect plants against insects and herbivores
- End products of detoxification reactions representing a metabolic locking-up of compounds otherwise harmful to the plants
- For regulatory growth factors
- Reserve substance capable of supplying nitrogen or other elements necessary to the plant's economy

## PHARMACOLOGIC ACTION OF ALKALOIDS:

- Analgesic (morphine, codeine)
- Narcotics (strychnine, brucine which are central stimulant)
- Anti malarial (quinine)
- Anti pyretic
- Anti cancer (vincristine)
- Mydriatics (atropine)
- Anti inflammatory
- Miotics (physostigmine, pilocarpine)
- Ephedrine (rises in blood pressure, bronchodilator)
- Reserpine (produce fall in excessive hypertension)

# INSECTICIDES

# Introduction

- Many insecticidal compounds are known from plants. Most plants make defensive compounds called allelochemicals. Only a few are important commercially.
- Plant-derived insecticides have largely been replaced by synthetic materials, but there are some advantages to the naturally occurring materials. For example, these substances are biodegradable.
- Selectivity is needed. Compounds that are toxic to insects, but not toxic to mammals, are preferable, of course.



Secondary metabolites (1)  
PRIYA KUMARI



Plant Secondary Metabolites  
Ahmed Fathy



PRODUCTION OF PHARMACEUTICALS BY GENETICALLY ENGINEERED CELLS (HORMONES AND I...  
Prabhu Thirusangu



Terpenoids Biosynthesis  
Mona Ismail

## Rotenoids

- A series of compounds found in members of the genera *Derris*, *Lonchocarpus*, *Tephrosia* are known as rotenones.
- Commercially, rotenoids are isolated mostly from the roots of *Derris elliptica* in Indonesia and from *Lonchocarpus*
- These compounds are isolated by grinding the plant and extracting with solvents such as hexane or petroleum ether or chloroform.
- The compounds are oil soluble or lipids. They make up 1-20% of the dry weight of the roots.



False indigo bush,  
*Amorpha fruticosa*,  
Fabaceae

## Pyrethrins

- Another major series of compounds, the pyrethrins, come from species of the genus *Chrysanthemum* (some people put these species in *Pyrethrum*) (Asteraceae or Compositae).
- These were used as far back as the 1st century B. C. by the Chinese. Insecticidal plants mostly are grown in countries with inexpensive labor and high elevations such as Kenya and New Guinea.

Pyrethrum, *Chrysanthemum cinerariifolium*,  
Asteraceae



## Tobacco, *Nicotiana tabacum*, Solanaceae

- Tobacco (which contains nicotine) is another major source of insecticides. Tobacco wastes are often extracted and used as a source of nicotine. Nicotine especially effective against aphids.



## Antifeedants

- Antifeedants are compounds that prevent insect feeding. Although many are toxic, the insects usually don't consume enough to be poisoned.
- Only one of these, neem, *Azadirachta indica*, Meliaceae, is commercially available. The active compound, azadirachtin, is a structurally modified triterpene.



## Biotechnological applications of hairy root research

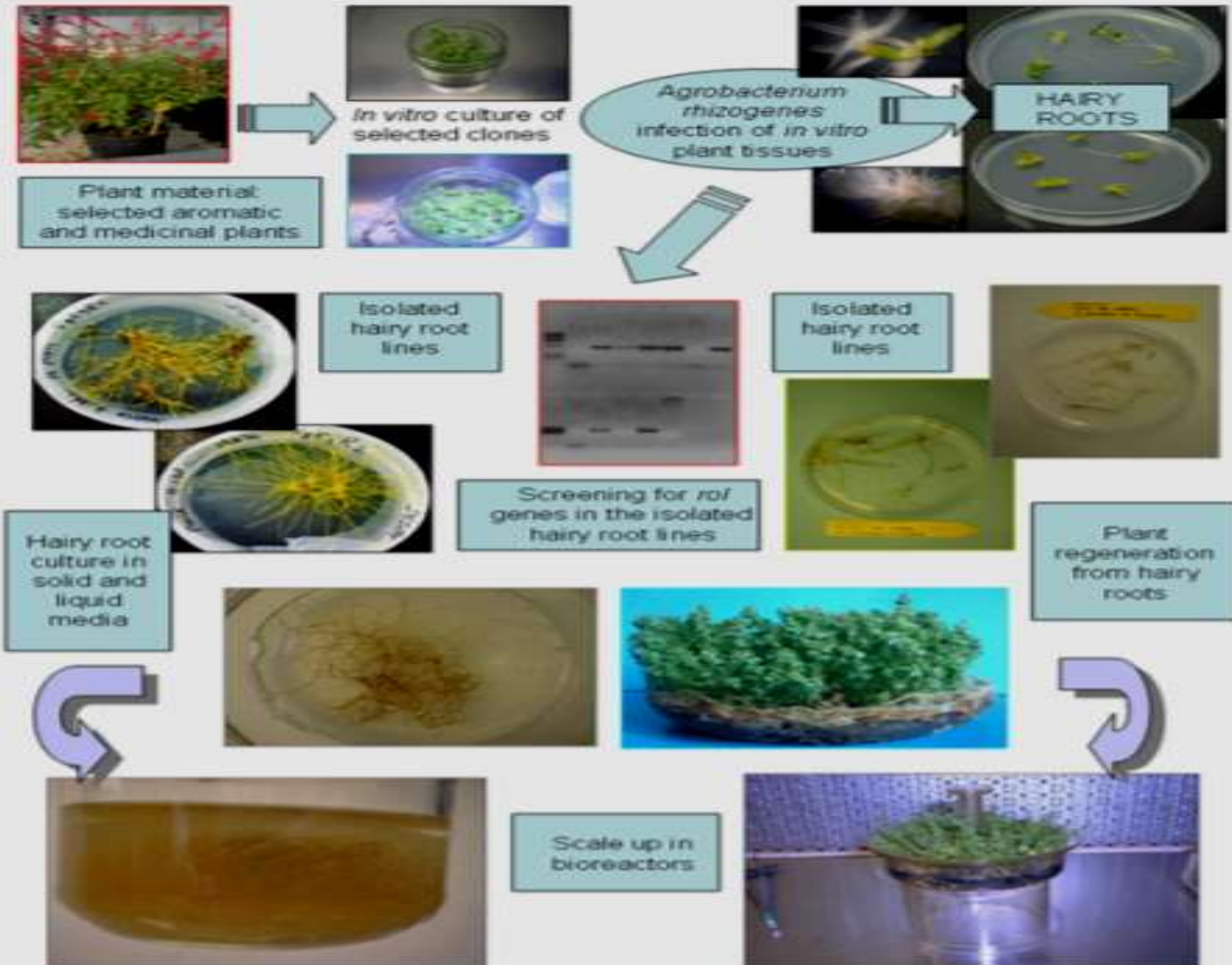


Figure 1: Biotechnological applications of hairy roots research