Bharathidasan University

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Botany

Course Title: Plant Biotechnology
Course Code: 22PGBOTCC204

Unit I - PLANT TISSUE CULTURE

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Historical Events

Lord Brahma

↓

Rishi Bharadwaj

↓

Sage Atreya Punarvasu

↓

Agnivesa, Bhela, Jatukarna, Parasara, Harita & Ksarapani

↓

Ved Vyas – 4 Vedas (Rig veda-Veda of Praise; Yajur veda-religious activities; Sama veda-Sacred songs, Atharva veda-medicine & sorcery)

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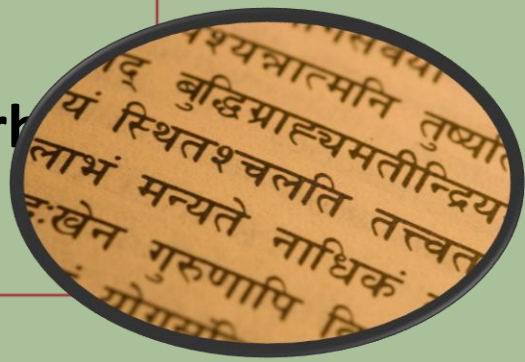
Atharva Veda – Ayurvedic treatment to cure diseases by herb

Atreya Samhita by Sage Atreya (Oldest medicine book)



>1500 BC- Ayurveda

School of Physicians-Atreya

School of Surgeons-Dhanvantri

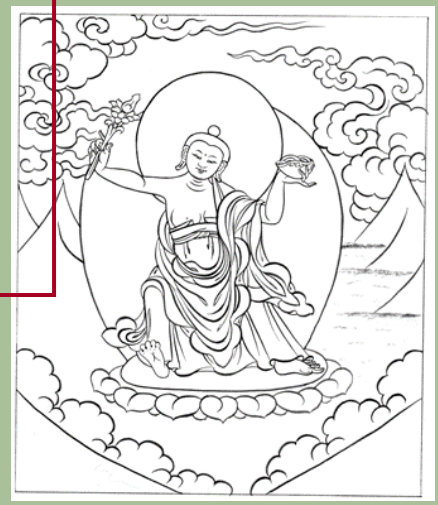
>1000 BC- Charaka- Charaka Samhita (based on Agnivesa book)

600 BC Susruta- Susruta Samhita (Cosmetic surgery–Dhanvantri School)

323BC Buddhist monk-Nagarjuna (Surgery)

500AD Astanga Hridaya Samhita by Vagbhata

500 AD – 1900 AD – 16 books information on Ayurveda-1800 medicinal plants



Takshasila University & Nalanda University

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2 Arabian physicians- Avicenna & Razi Sempion- used Ayurveda knowledge – Beginning of Unani medicine

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16th century –Europe-Parcelsus- Father of Modern Western medicine used Ayurvedic medicine

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Tibet & China – influenced by Ayurveda

↓

Akbar- Unani & Ayurveda side by side

↓

1827- Sanskrit college- Pandit Madhusudan Ojha- Sanskrit course

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1905 – Venkataraman Ayurvedic college – Chennai

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1916- At Ahmednagar

↓

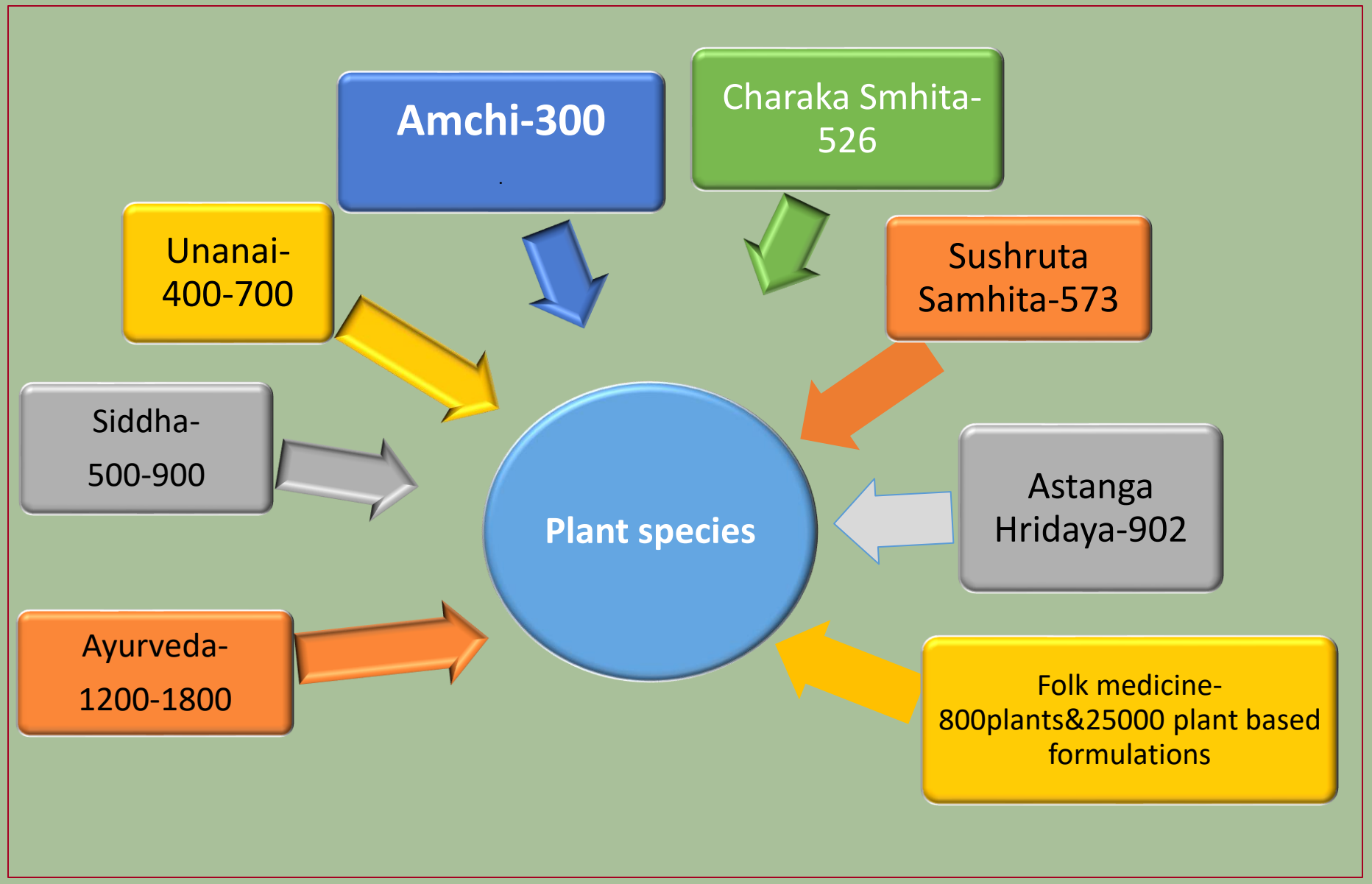
1925 – Government School of Indian Medicine Research in Ayurveda

Siddha	• -Dravidian culture origin- • Cf. Chinese alchemy, Taoism, • 18 Siddhars
Unani	• Egypt, Syria, Iran, Iraq, China, India. Origin- Greece & developed in Arab.
Amchi	• Tibet, Mongolia, Nepal, Bhutan, Himalayan region of India, China & Soviet Russia

Ayurveda

Charaka Samhita	• Kayacikitsa- Internal medicine
Susruta Samhita	• Salya tantra- Surgery
Madhava Nidana	• Diagnosis of disease
Bhava Prakasa	• Related to Plant & diet
Sarangadhara Samhita	• Formulation & dosage form
Salakya	• Disease of supra-clavicular origin
Kaumarabhrtya	• Pediatrics, Obsterics, gynecology
Bhutavidya	• Psychiatry
Agadatantra	• Toxicology
Rasayana tantra	• Rejuvenation & geriatrics
Vajikarana	• Aphrodisology & eugenics

1959	• Govt. of India –ISM-Indian system of Medicine- 25000 plant based formulations
1970	• Central council of Indian Medicine (CCIM)
2013	• WHO-developed & launched “WHO Traditional Medicine Strategy”, by 2014-2023- to integrate traditional & complementary medicine, to promote universal health care & ensure the quality & safety and effectiveness of such medicine.
2014	• AYUSH-Ayruveda, Yoga& Naturopathy, Unani, Siddha, Homeopathy. Separate Ministry.-INR 80-90 billion trade


Amchi-300
Charaka Smhita-526
Unanai-400-700
Sushruta Samhita-573
Siddha-500-900
Plant species
Astanga Hridaya-902
Ayurveda-1200-1800
Folk medicine-800plants&25000 plant based formulations

Major Importers of Medicinal Plants (2002)

Country	US $ 000	% Share
Hong Kong	176720	16.41
USA	147131	13.67
Japan	118994	11.05
Germany	76102	7.07
France	51814	4.81
China	48582	4.51
Korean	43094	4.00
Republic	42839	3.98
Italy	35988	3.34
Canada		
Total above	**741264**	**68.84**
World	1076662	100.00

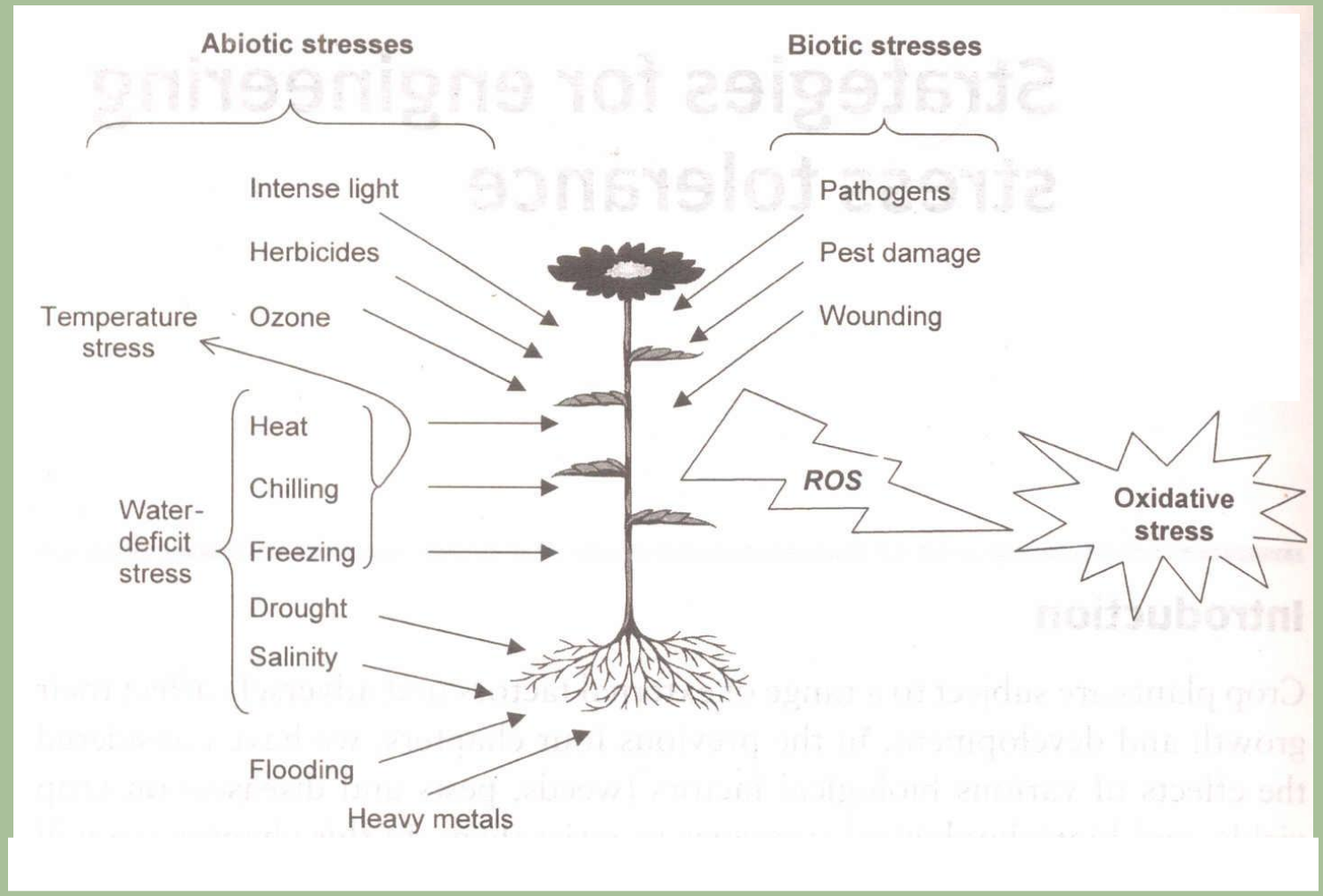
- AYUSH-2012-2013: 24, 741.2 crores INR.
- Global trade –reach USD 7 trillion by 2050.
- 9000 registered licensed manufacturing units, of these 95% are cottage and small-scale sector.
- China & India – 40% global biodiversity.
- Enormous scope for India – a major player in global herbal product based medicines.
- >200 tons of medicinal plant raw material required per year.
- 1500 herbals sold as dietary supplements.
- In trade- 960 spp.

Item	Value (US $ Million)
Psyllium husk (*Plantago* sps)	35.49
Saps & extracts of Opium	18.72
Cambodge extract (*Garcinia cambogia*)	11.23
Other extracts	7.77
Henna Powder (*Lawsonia inermis*)	4.27
Ayurvedic & Unani herbs	5.03
Others Crude drugs	5.82
Senna leaves & pods (*Cassia anguistifolia*)	7.63
Sandalwood chips & dust	6.53
Karaya gum (*Sterculia urens*) (thickener, emulsifier & laxative)	2.73
Total above	105.22
Total export performance	124.85

Primary metabolites

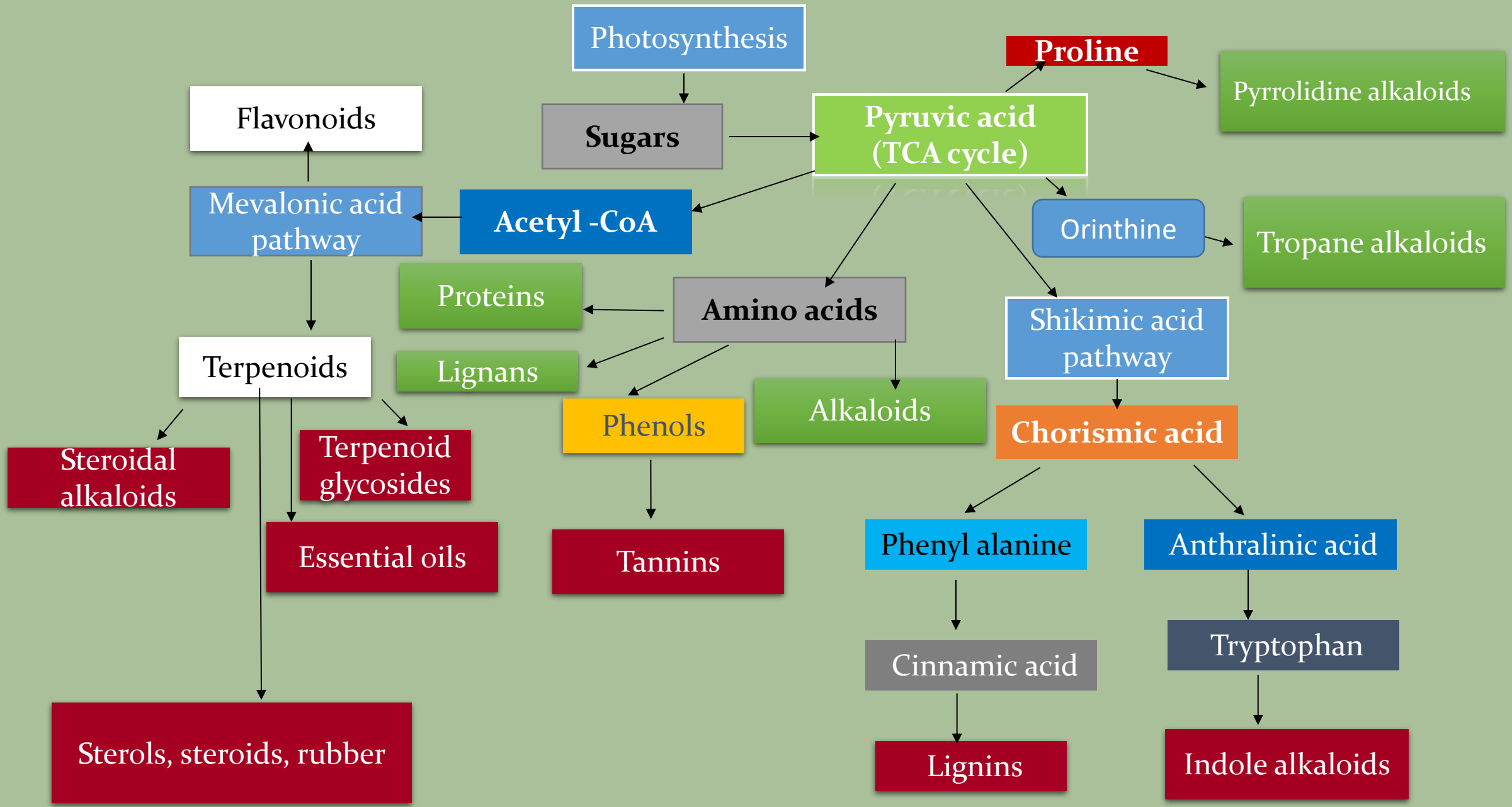
Recognized roles in the processes of assimilation, respiration, transport and differentiation.

- Carbohydrates
- Amino acids
- Proteins
- Nucleotides
- Lipids



Stress factors affect plant growth & development

Secondary metabolites are derived from **primary metabolites**



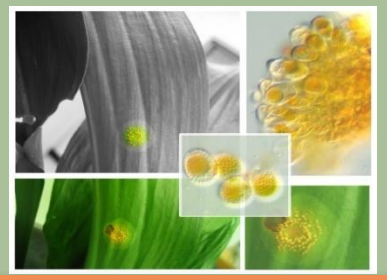
Molecules that are essential for growth and development of an organism.

Recognized roles in the processes of assimilation, respiration, transport and differentiation.

Produced by all plants - directly used in plant growth and development.

Primary metabolites

Carbohydrates, Chlorophyll, amino acids, proteins, lipids, nucleotides



Phenolics Combat infectious diseases

Protect from potential predators

Not directly involved in the normal growth, development or reproduction of an organism

Alkaloids, terpenoids, phenolics, glycosides.

Secondary

Secondary metabolites

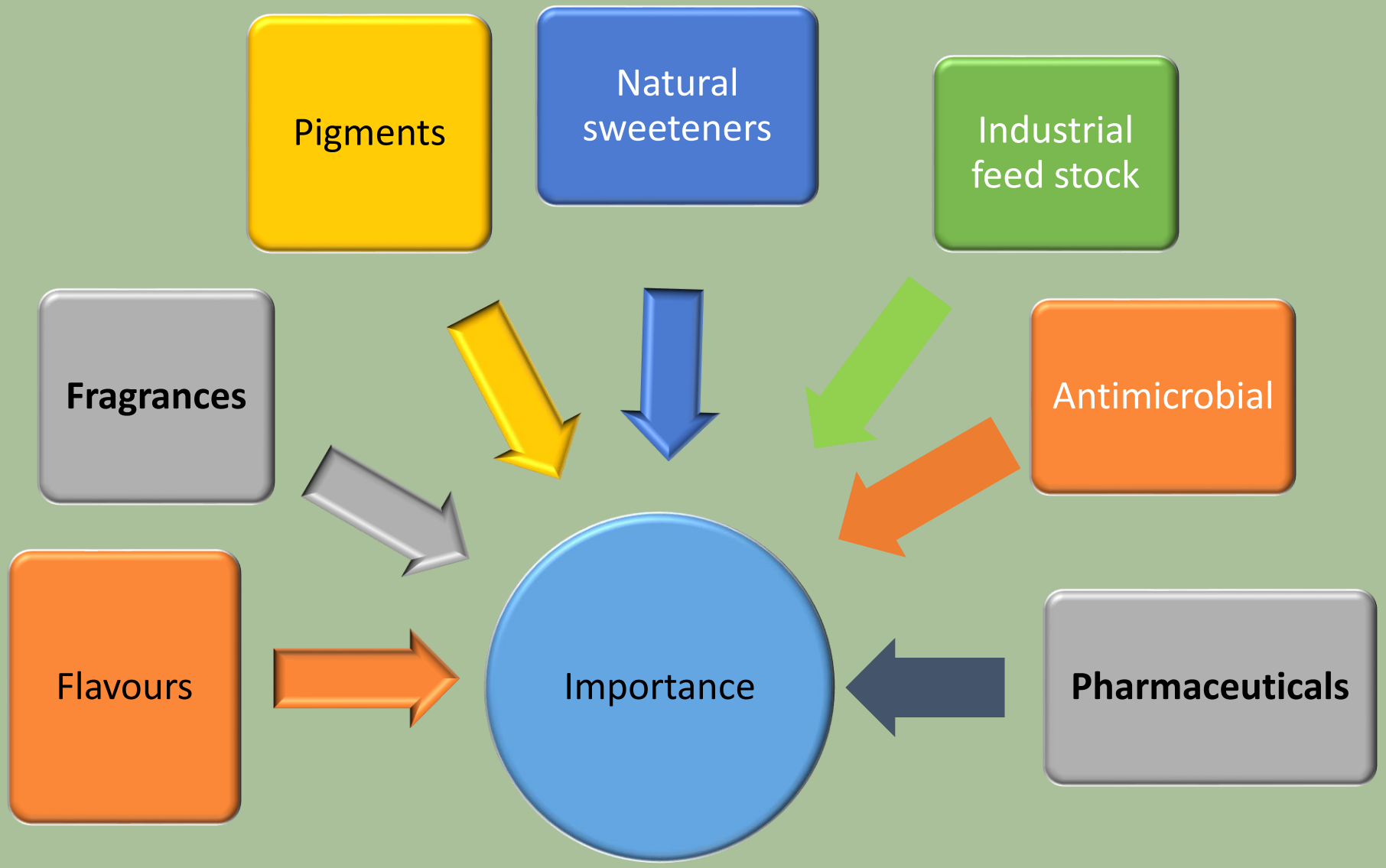
Ecological function : Protect from against herbivores or pathogens, Attractants for pollinators and seed-dispersing animals, Agents of plant-plant competition and plant-microbe symbiosis

Restricted distribution

Drugs, poisons, flavors & industrial materials

Tremendous resource for scientific, clinical research and new drug development.

Accumulate specific cell &/ or organs , vacuoles etc.


Pigments
Natural sweeteners
Industrial feed stock
Fragrances
Antimicrobial
Flavours
Importance
Pharmaceuticals

Groups of secondary metabolites

Phenolics

- Aromatic substances from shikimic acid or mevalonic acid pathway

Terpenes

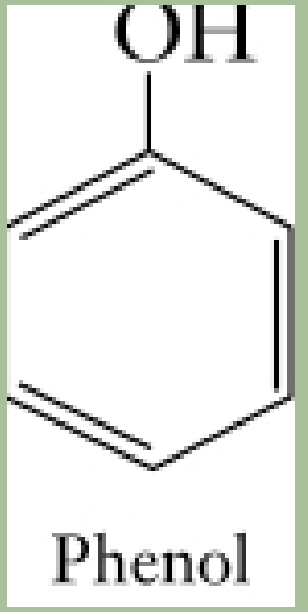
- From mevalonic acid pathway

Alkaloids

- Nitrogen containing secondary metabolites derived from amino acids

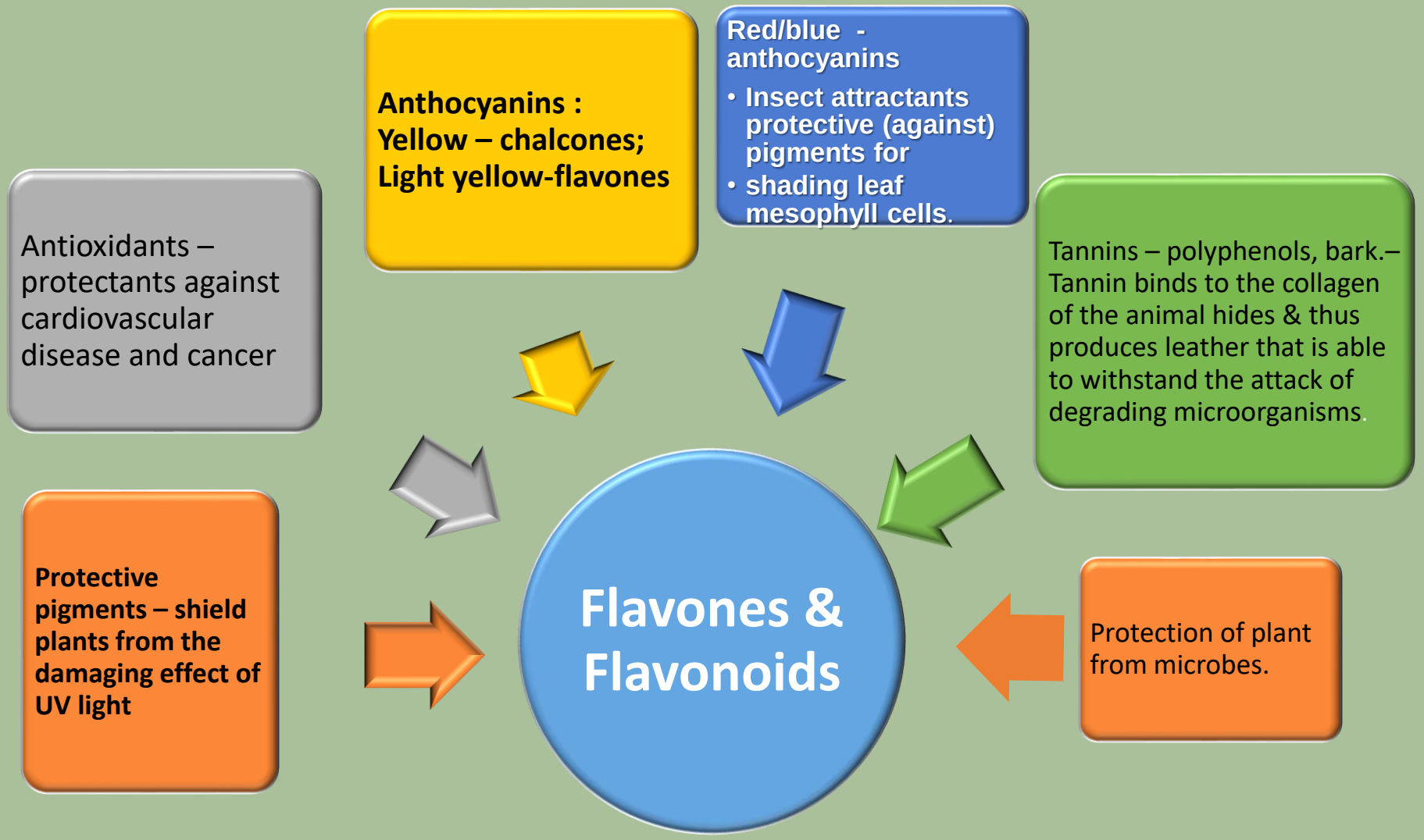
Phenolics

Phenols	• 8000
Flavonoids	• 2000
Polyacetylens	• 1000
Polypeptides	• 750
Phenylpropanoids	• 500



Phenol

Role- Protect against stress


Anthocyanins : Yellow – chalcones; Light yellow-flavones
Red/blue - anthocyanins
• Insect attractants protective (against) pigments for
• shading leaf mesophyll cells.
Antioxidants – protectants against cardiovascular disease and cancer
Tannins – polyphenols, bark.– Tannin binds to the collagen of the animal hides & thus produces leather that is able to withstand the attack of degrading microorganisms.
Protective pigments – shield plants from the damaging effect of UV light
Flavones & Flavonoids
Protection of plant from microbes.

Terpenoids

Hemiterpinoids (C^5H^8)	
Monoterpenoids ($C^{10}H^{16}$)	• 1000
Sesquiterpenoinds($C^{15}H^{24}$)	• 3000
Diterpenoids ($C^{20}H^{32}$)	• 1000
Sesterpenoids ($C^{25}H^{40}$)	• 4000
Triterpenoids ($C^{30}H^{48}$)	
Tetraterpenoids ($C^{40}H^{64}$)	
Polyterpenoids (C^5H^8)n	

Role-Growth & development, protection against abiotic & biotic stress

Isoprene

Alkaloids

Analgesics/ narcotics	• Morphine • (Used on trauma and shock patents)
Mydriatics	• Atropine
Miotics	• Pilocarpine
Hypertensives	• Ephedrine (Used by Sinus patents)
Hypotensives	• Reserpine
Bronchodilators	• Lobeline
Antimicrobials	• Berberine
Antileukemic	• Vinblastin (Against blood cancer)

Cell Theory

Matthias Schleiden

1. All living things are made up of cells.
2. Cells are the basic units of structure and function in living things.
3. Living cells come only from other living cells.
4. The cell contains hereditary information which is passed on from cell to cell during cell division.
5. All cells are basically the same in chemical composition and metabolic activities.

Theodor Schwann

Totipotency

Gottlieb Haberlandt

"The ability for a differentiated cell to retain all the genetic material in a form required to form an entire organism".

Tissue Culture

Need – Rare & Vulnerable

- Indiscriminate collection & exploitation of natural resources for commercial purposes (Pharmaceutical industry) -
- Natural strands - fast disappearing , threatened & extinction

Non-conventional approaches

- ex situ conservation – Biotechnology
- Rapid cloning elite germplasm,
- plant improvement – genetic transformation

Conventional methods

- Seeds, layering, stem cuttings

Seeds

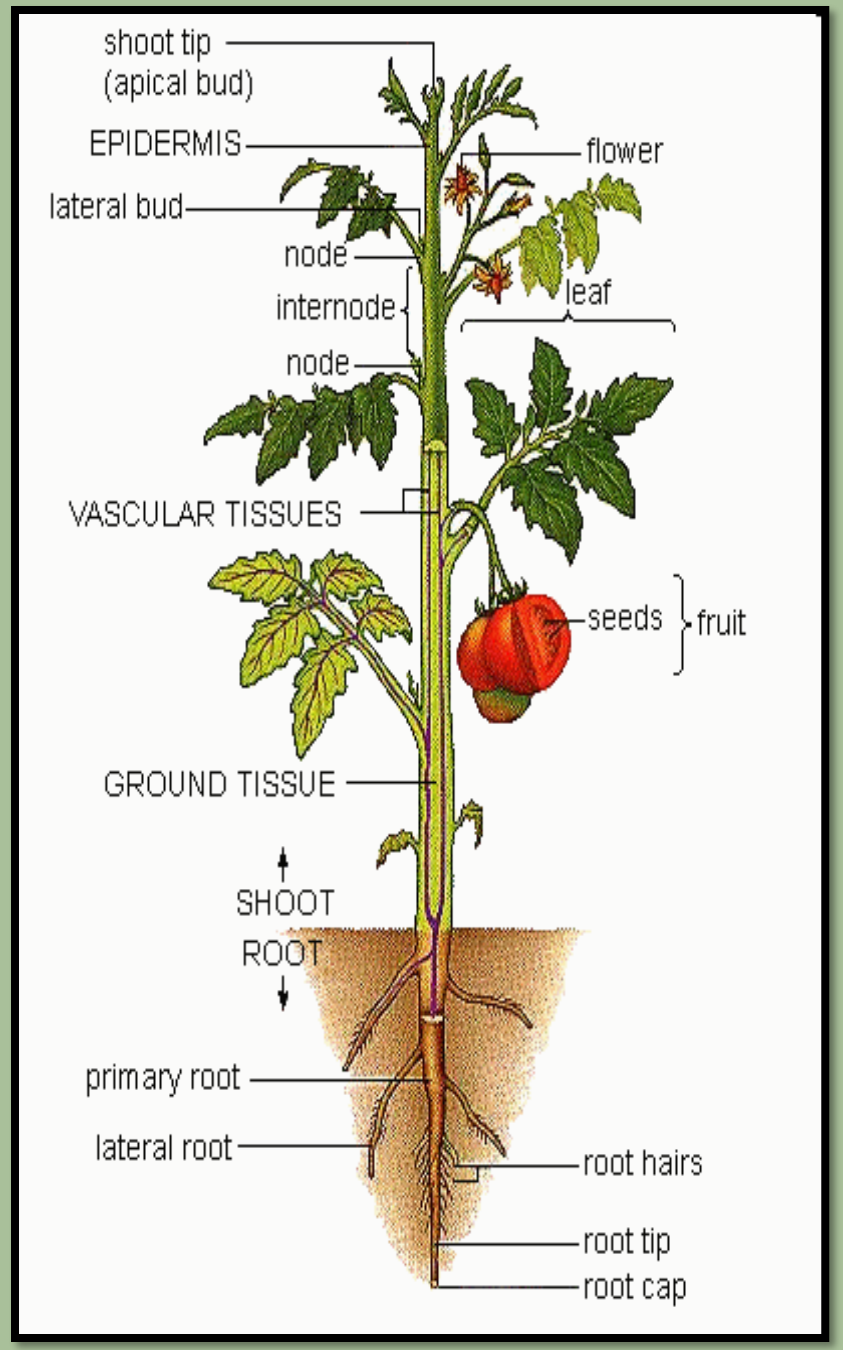
- Cross pollinated, heterozygosity, poor seed viability, low ratio of germination

Stem cuttings

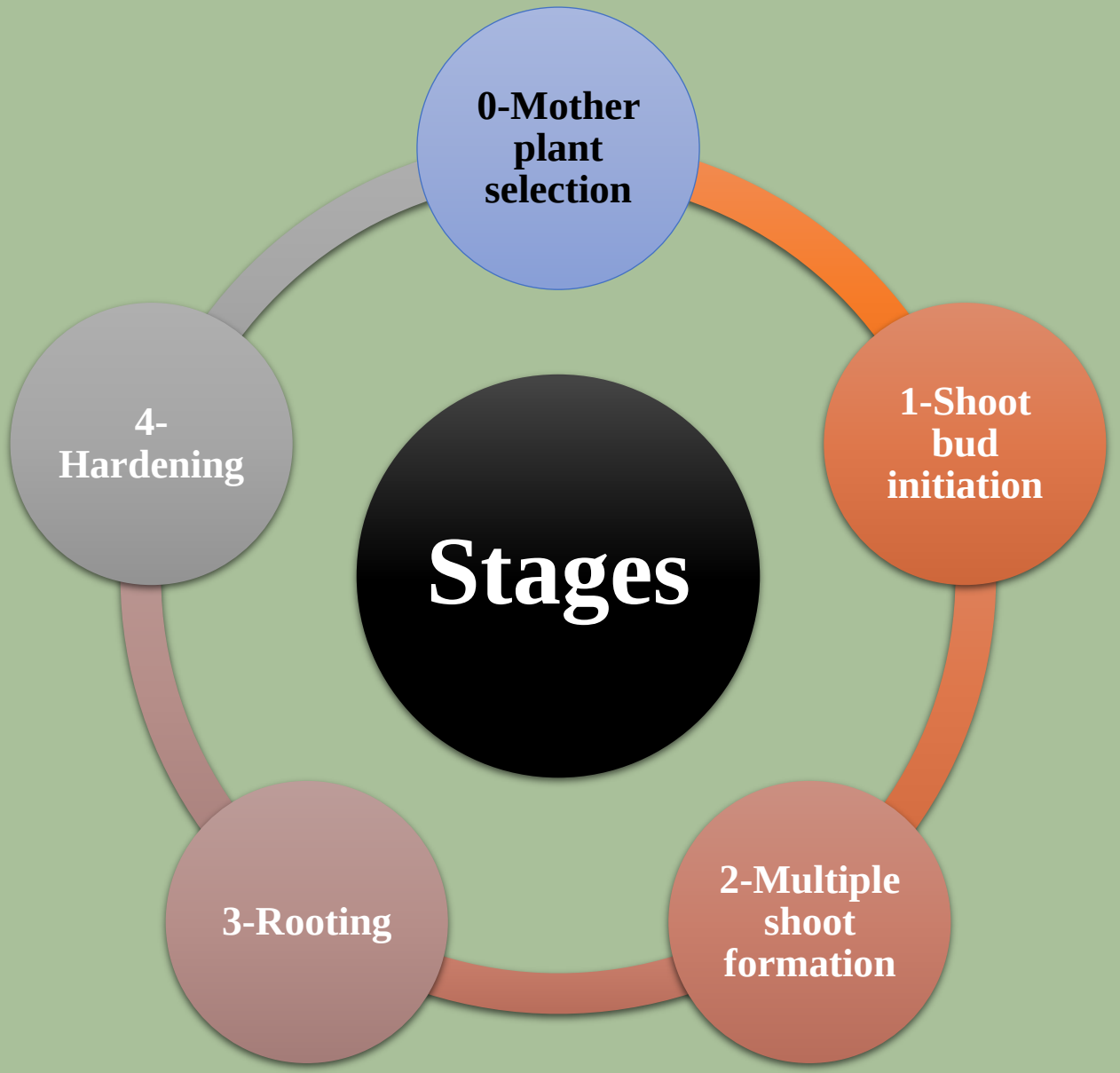
- Poor rooting ability

Plant Tissue Culture

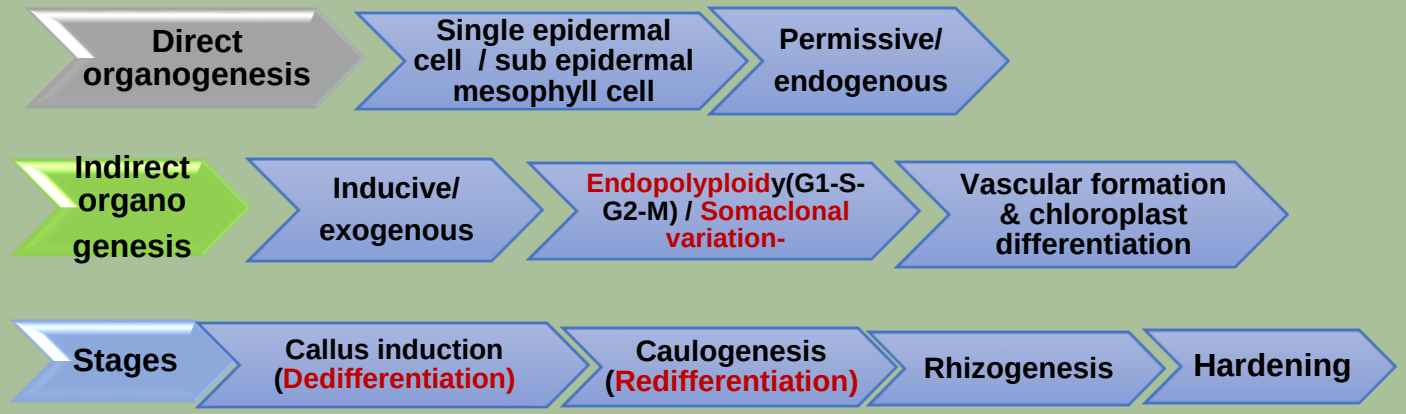
- Micro propagation
  - Horticultural crop
  - Rare Plant Sp
  - Agricultural crop
  - Plantation crop
- Cryo-preservation
  - DNA Lib
  - Genomic Bank(Germ Plasm Storage)
- Extraction of Secondary Metabolites
  - Pesticides
  - Antibiotics
  - Alkaloids
  - Hormones
- **Crop Improvement**
  - Pesticide Resistance
  - Reduction in fertilizer application
  - Short Season yield
  - High Quality
  - High yield
  - Drought Resistance
  - Disease Resistance


shoot tip
(apical bud)
EPIDERMIS
lateral bud
node
internode
node
VASCULAR TISSUES
GROUND TISSUE
SHOOT
ROOT
primary root
lateral root
flower
leaf
seeds
fruit
root hairs
root tip
root cap

MICROPROPAGATION



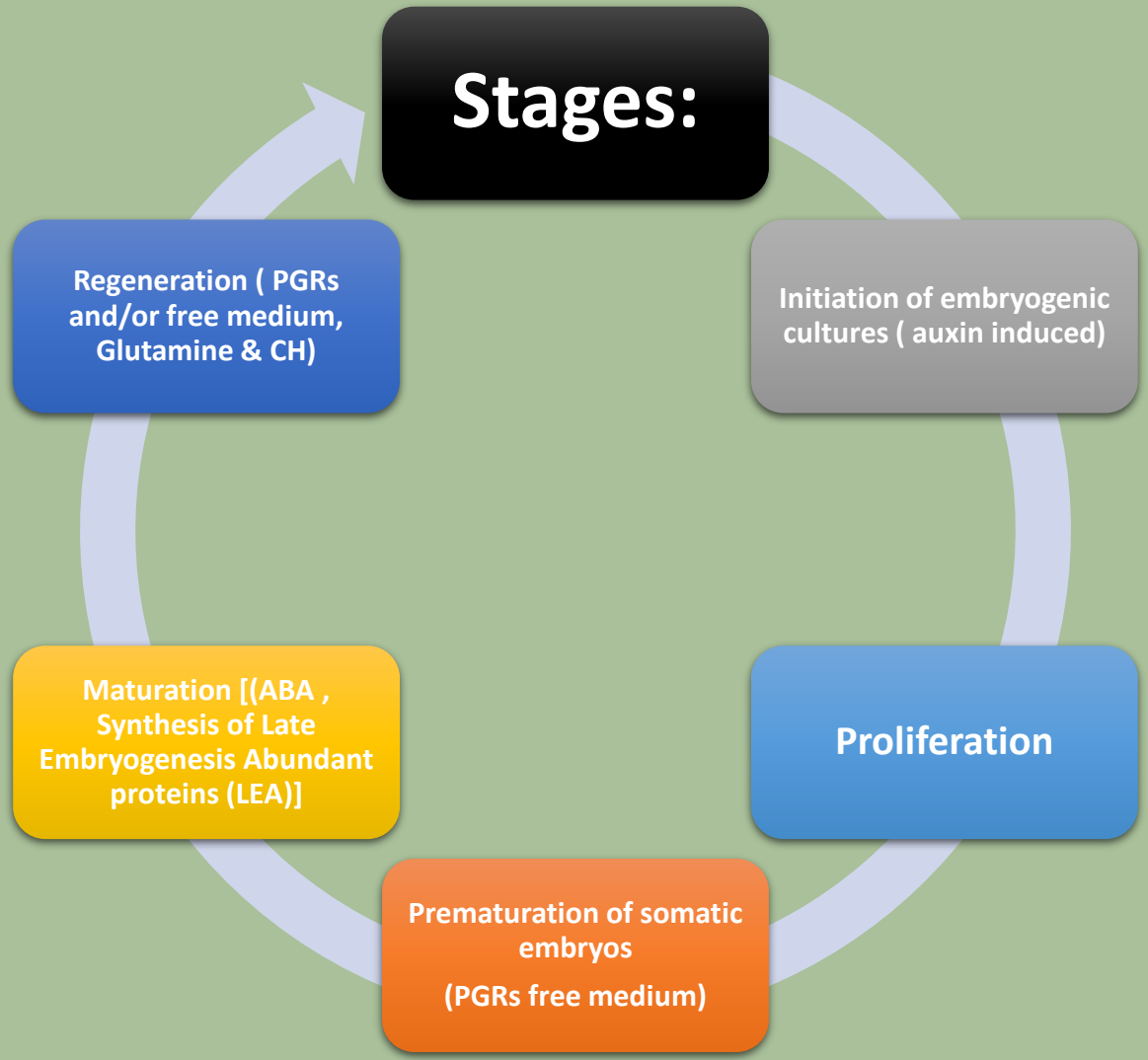
ORGANOGENESIS



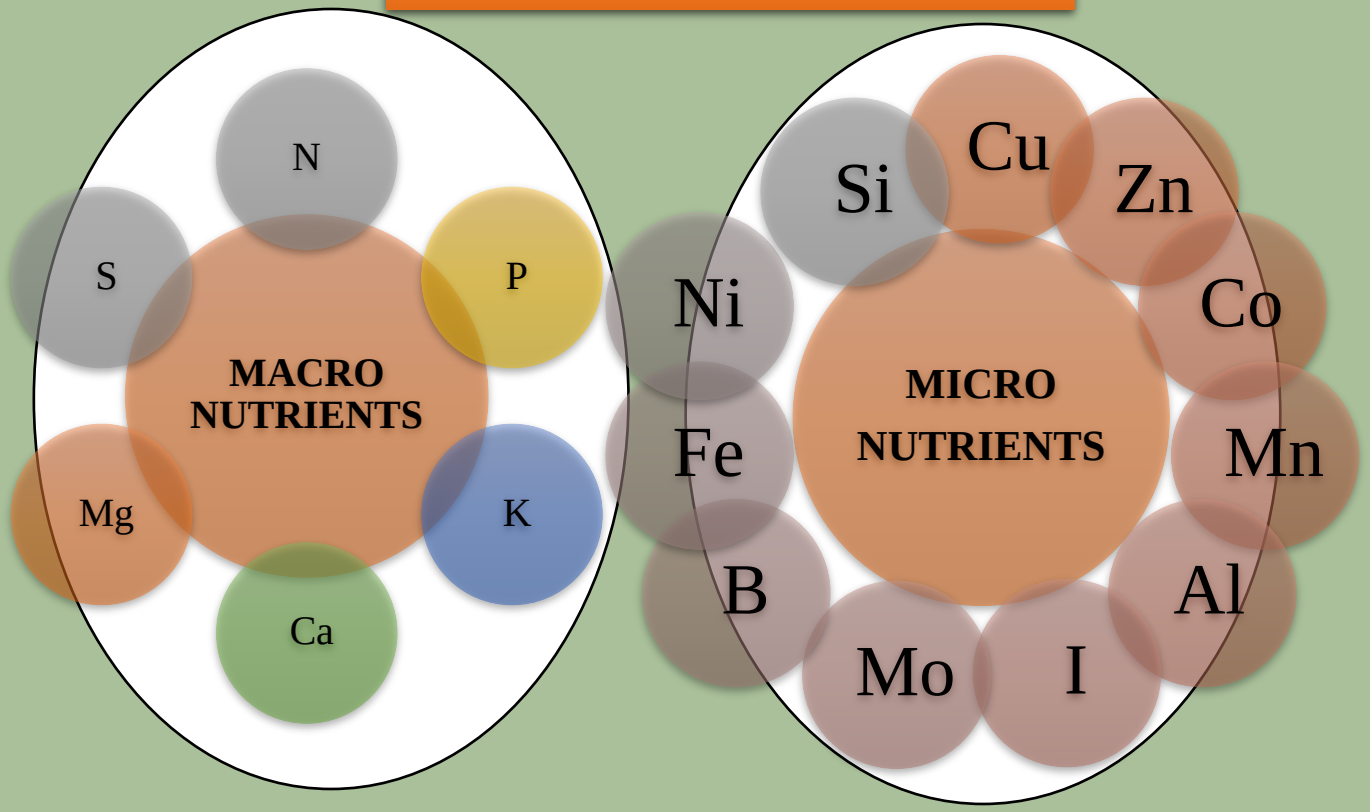
SOMATIC EMBRYOGENESIS

Direct –**Pre determined embryogenic cells**

Indirect - Inducive embryogenic determined cells



MEDIUM COMPONENTS



VITAMINS– Thiamine, Nicotinic acid, Pyridoxine, Myoinositol, Biotin, Pantothenic acid, Folic acid, Riboflavin, Ascorbic acid

ORGANIC ACIDS – Malic acid, Citric acid, Succinic acid, Shikimic acid, Pyrrolidinic acid

Types of Culture media

- MS medium (Murashige & Skoog, 1962)
- Schenk & Hildebrandt (SH) medium (1972)
- Woody plant medium (WPM) (Llyod &McCown, 1980)
- Lisnmaier & Skoog (LS) (1965)
- Gamborg (B5) medium (Gamborg et al., 1968)
- Whites medium (White, 1963)
- Modified MS medium (MS salts+ B5 vitamins)

Explants

-Age of the organ/whole plant

-Physiological age

-Phase of the growth (young or old, juvenile, adult phase)

-Period of culture (no. of cultures, habituation)

-Ontogenetic age

Carbon source - Sucrose

- Inhibit chlorophyll synthesis & photosynthesis
- Effective translocation
- Hydrolysis of sucrose is negligible at pH 5.6-5.8

Gelling agent - Agar

- Not digested by plant enzymes
- Solidify at 45^{o}C and stable at 25$\pm$2^{o}C
- Does not strongly react with medium constituents
- Adsorptive capacity

PLANT GROWTH REGULATORS

Auxins (Gr = To enlarge/to grow)

- IAA
- IBA
- NAA
- 2,4-D
- dicamba
- picloram

Cell expansion & loosening cell wall (cell wall acidification), DNA replication

Cell division & Cell elongation

Callus,

Organized defined organs (polarity with apical dominance)

Cyto-differentiation buds, roots, etc

Cell dispersion in suspension cultures, prevent morphogenesis, induce embryogenesis, Inhibit chlorophyll synthesis.

Meristems

Factors involved in the effect of auxins:

Type of growth and /or dominance development required

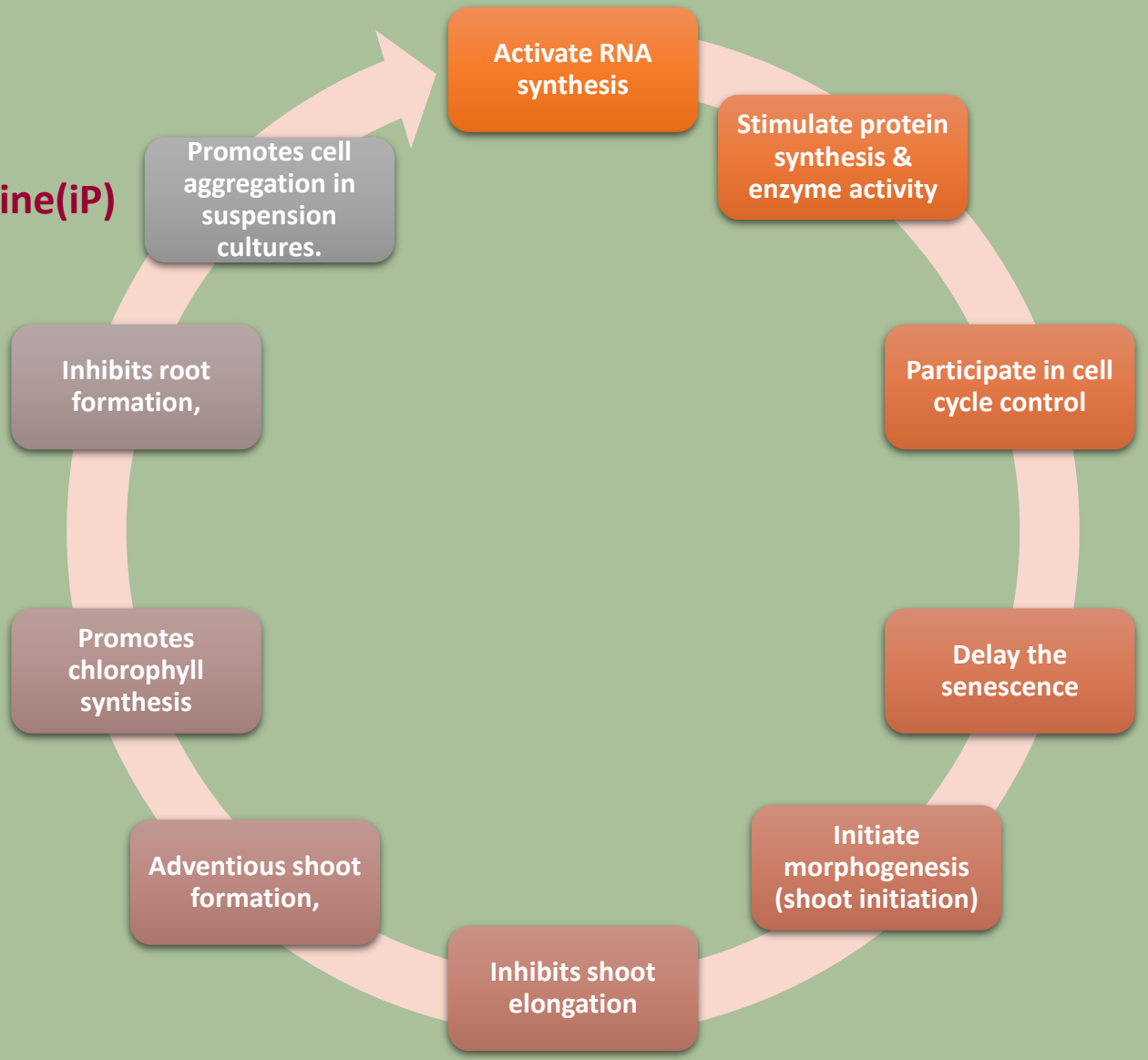
Rate of uptake of & transport of the applied auxin to the target tissue

Inactivation of (oxidation/conjugation) of auxin in the medium & within the plant

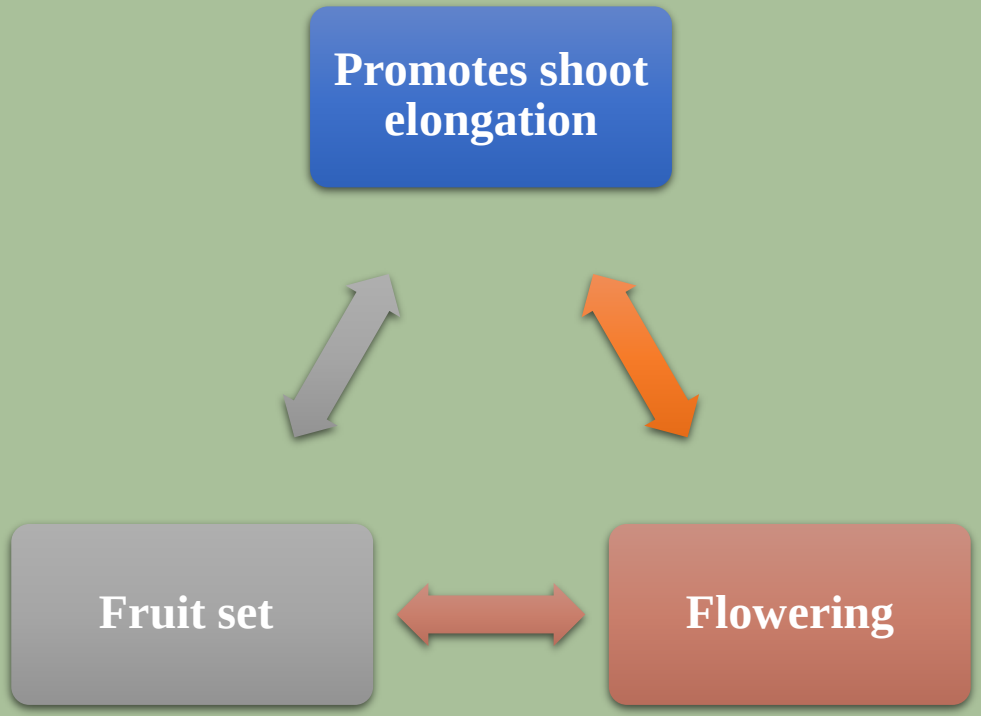
Endogenous levels of auxins

Cytokinins

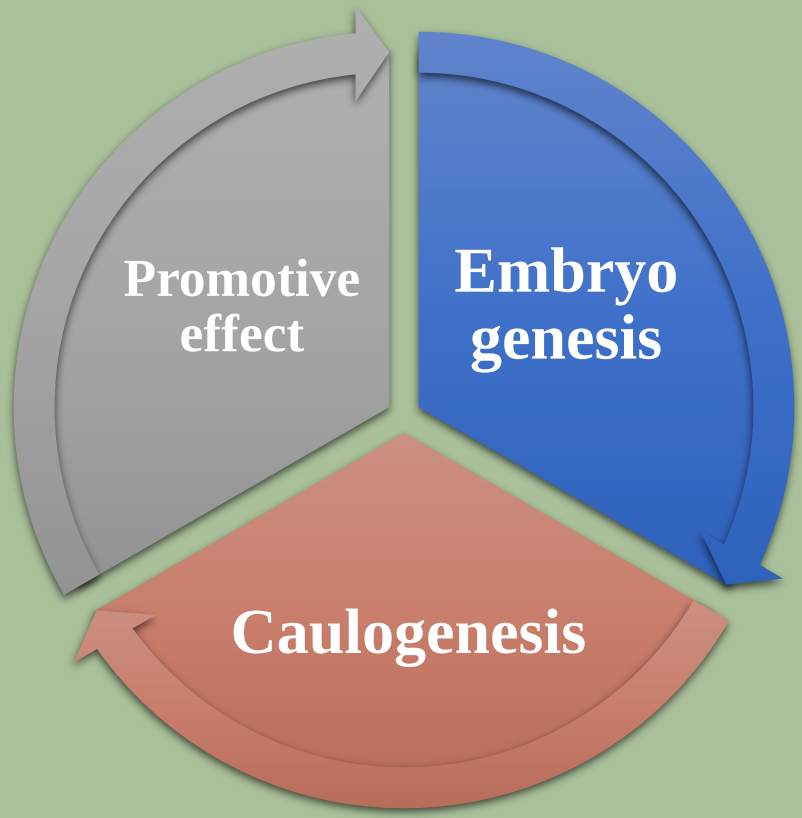
- Zeatin,
- Kinetin
- isopentenyl adenine(iP)
- BAP
- Thiadiazuron



Gibberellins



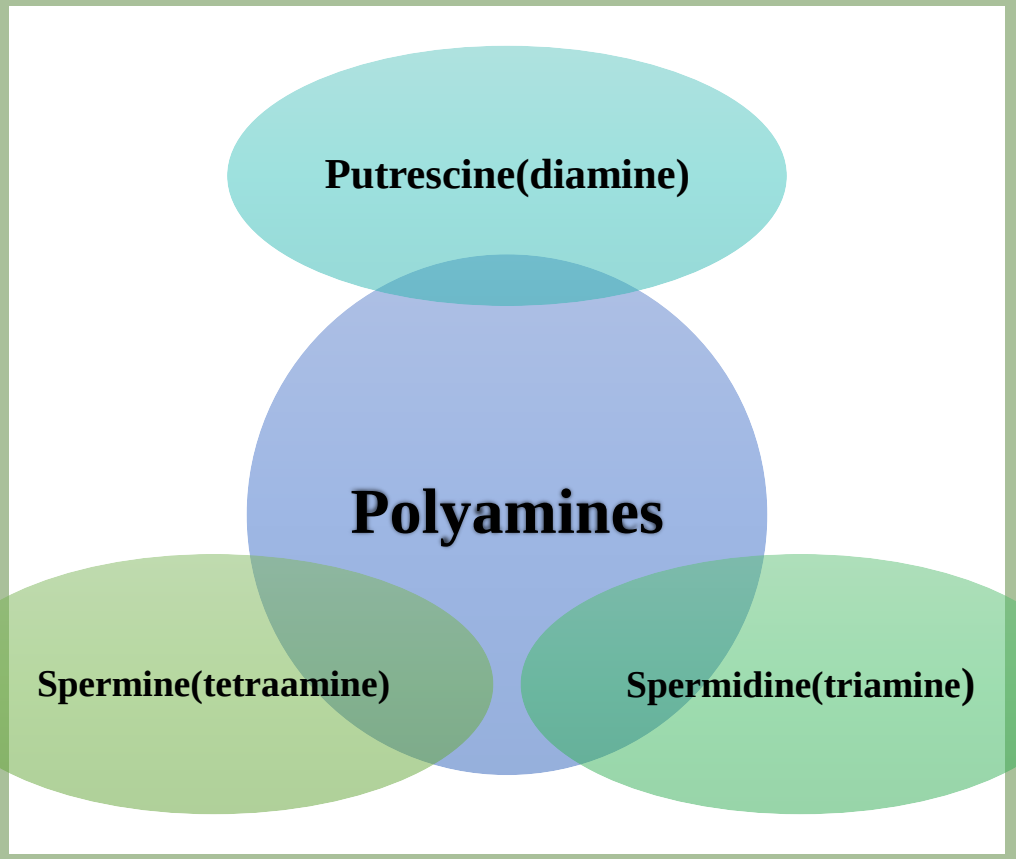
Adenine Sulphate



Abscissic Acid

- Regulate stomata closure
- Control water & ion uptake by roots
- Control leaf abscission & senescence
- Inhibit callus growth
- Promotes adventious shoots
- Promote normal growth of somatic embryos
- Prevent phenolics

Polyamines



- Substitute for auxins, cytokinins, Jasmonate.
- Enhance somatic embryogenesis, adventious roots, shoots, flowering.

Others

Activated Charcoal

- Absorption of compounds
- Prevent unwanted callus growth
- Promote morphogenesis
- Promote rhizogenesis

Brassinosteriods

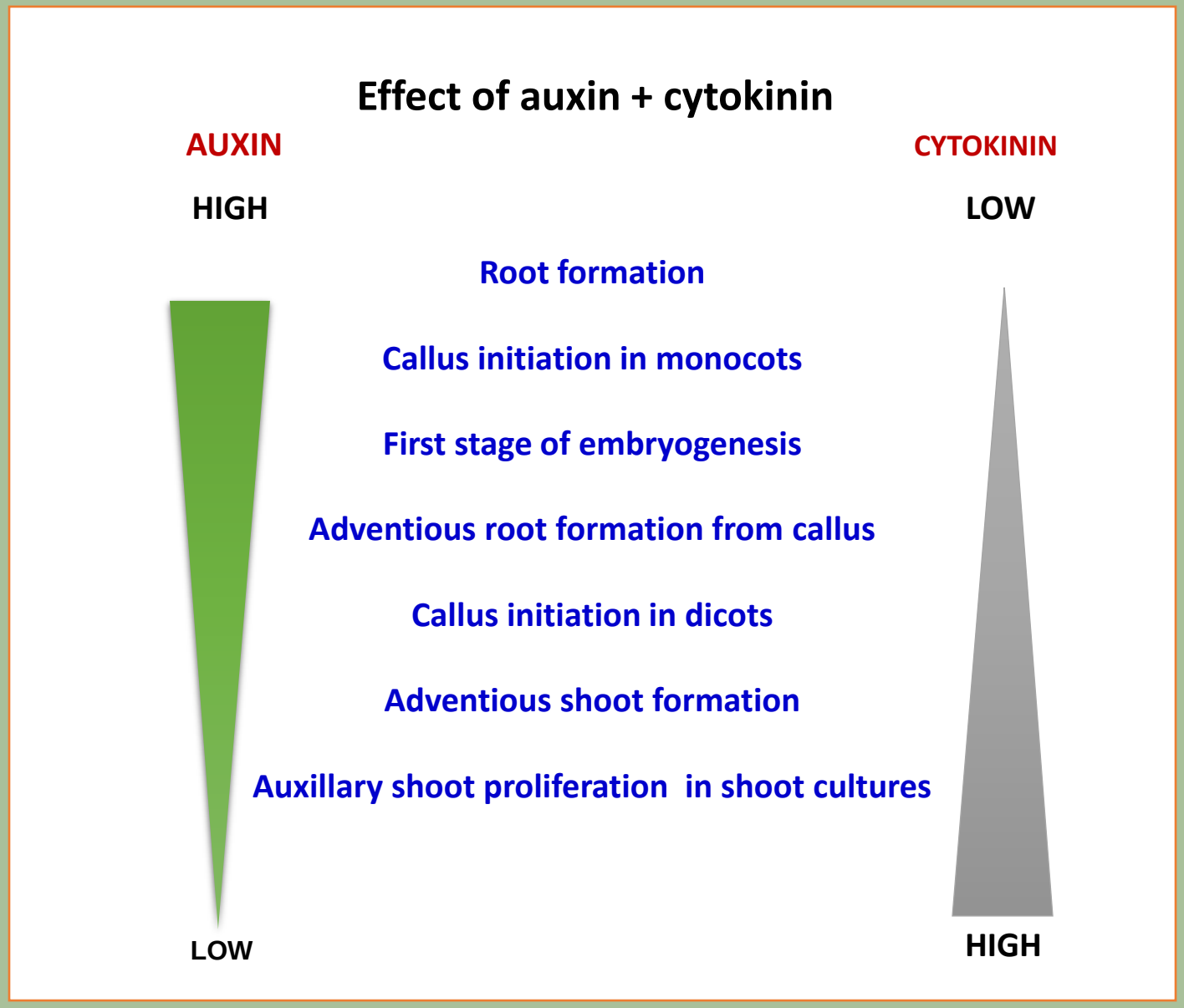
Jasmonic acid

Oligosaccharides

Undefined Supplements

- Yeast extract,
- Malt extract,
- C H,
- Potato extract,
- Banana homogenate,
- C M

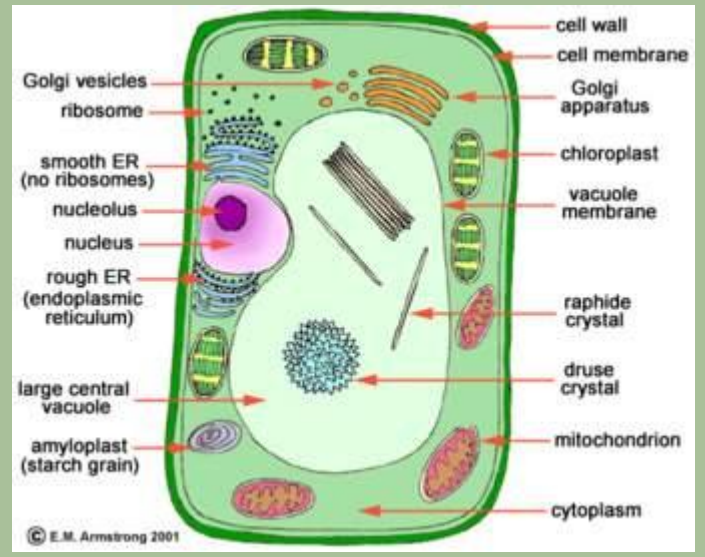
The relative concentrations of Auxin and Cytokinin required for growth and morphogenesis



Suspension culture
&
Secondary Metabolites

- Each individual cell contains many enzymes, which can display different catalytic properties depending on the conditions to which they are exposed.

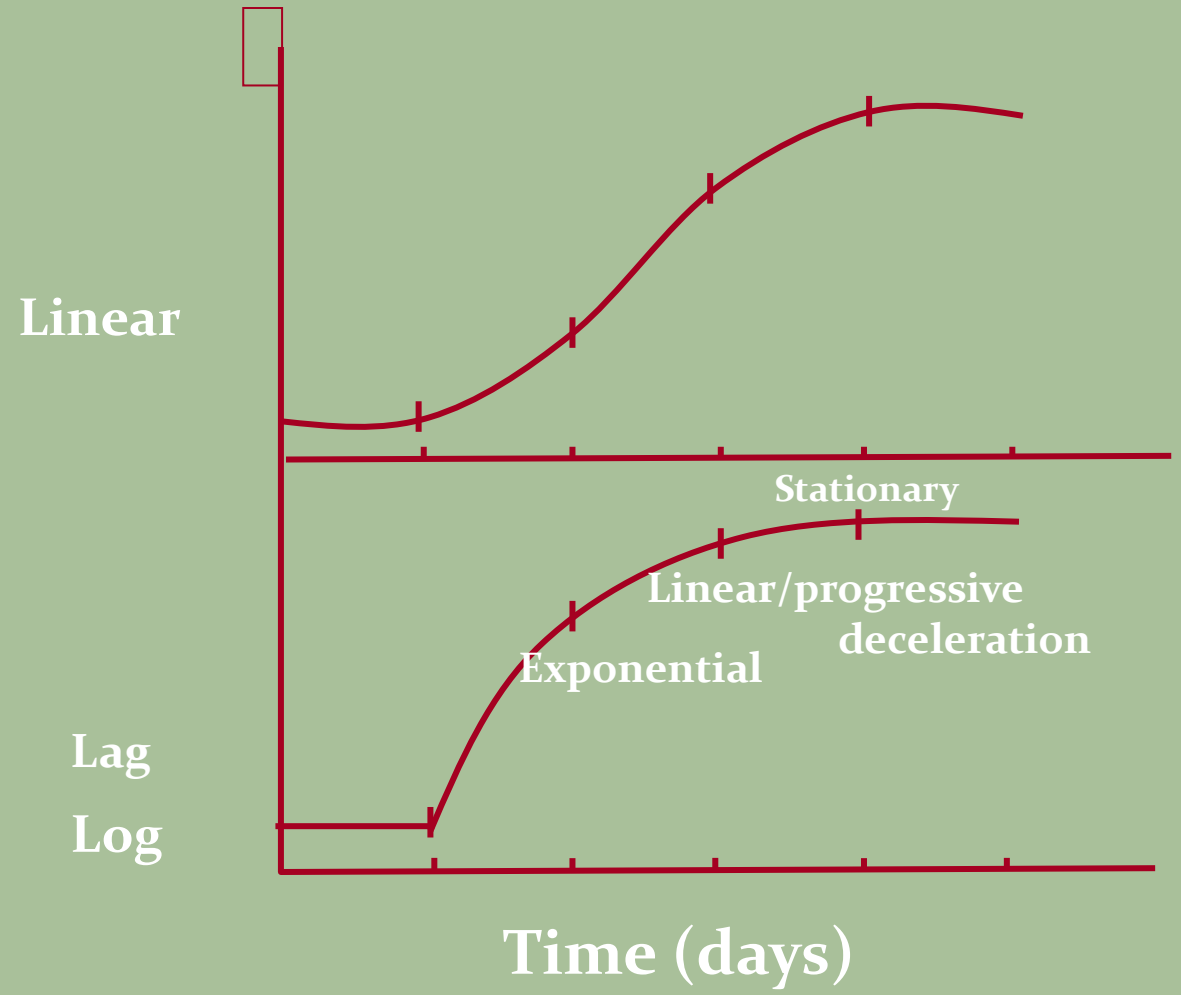
- A **Plant cell** contains 800-1000 different enzymes belonging to primary and secondary metabolism.



Advantage of callus and cell suspension culture

- Relevance to the industry
- Independence from environmental factors
- Not limited by seasonal consideration
- Consistent product quality and yield
- Free from microbes
- The synthesis of novel natural products, which are not normally produced in normal plants
- A means of synthesizing novel natural product where the source plant is difficult to grow

Batch Culture Growth Cycle (Passage)



Requirements

- Culture Medium : Simple nutrient medium – MS, B5, Mineral Salts - Macro and Micro.
- Carbon Source – Sucrose, glucose, fructose, maltose.
- GRs- Auxins, Cytokinins, GA_3
- Amino acids- glycine, glutamine, proline, phenyl alanine, arginine
- Vitamins – nicotinic acid, Pyriodoxine Hcl, thiamine HCl, biotin,
- folic acid, cyanocobalamine
- Organic supplements: Yeast extract, malt extract, casein hydrolysate, coconut water.
- Gelling agents : Agar – Agar

Physical Conditions

- Temperature - 22 -28 ° C
- Illumination : 0- 5000 lux
- photoperiod : 8-16 hrs
- Light – UV, Blue, White
- Subculture of tissues : 2-8 weeks for Static cultures & 1-2 weeks for cell suspensions.

Precursors

Precursors molecules which are directly incorporated into synthesis of secondary metabolites

Ruta graveolens	4 – OH 2 – **Quinoline**	Dictamine – 0.6%
Cinchona ledgerianer	**Tryptophane**	Quinolinines – 0.9%
Lithospermum erythrorhizon	**Phenylalanine**	Shikonin 37- 126 µg -1
Ephedra gerardiana	**Phenylalanine**	Ephedrine 0.17 – 0.5 %

Need to optimize the growth and production conditions for each species and strain, and also for each metabolite.

Biotransformation

(i) Transformation of low cost precursors into valuable product or conservation of racemic /inactive compounds into active forms

Eg. Conversion of D- menthol to L – menthol

(ii) Transformation with the help of Agrobacterium

Elicitors

Keen – Coworkers (1972) – elicitation response.

Fungal cultures – fresh cultures homogenized, autoclaved at 121° C for 20 min, and suitably diluted fungal preparations or chemicals are used to evaluate the elicitation effect.

Eg. *Pythium, Fusarium, Phytopthora, Alternaria, Penicillium* etc.

Bioreactors

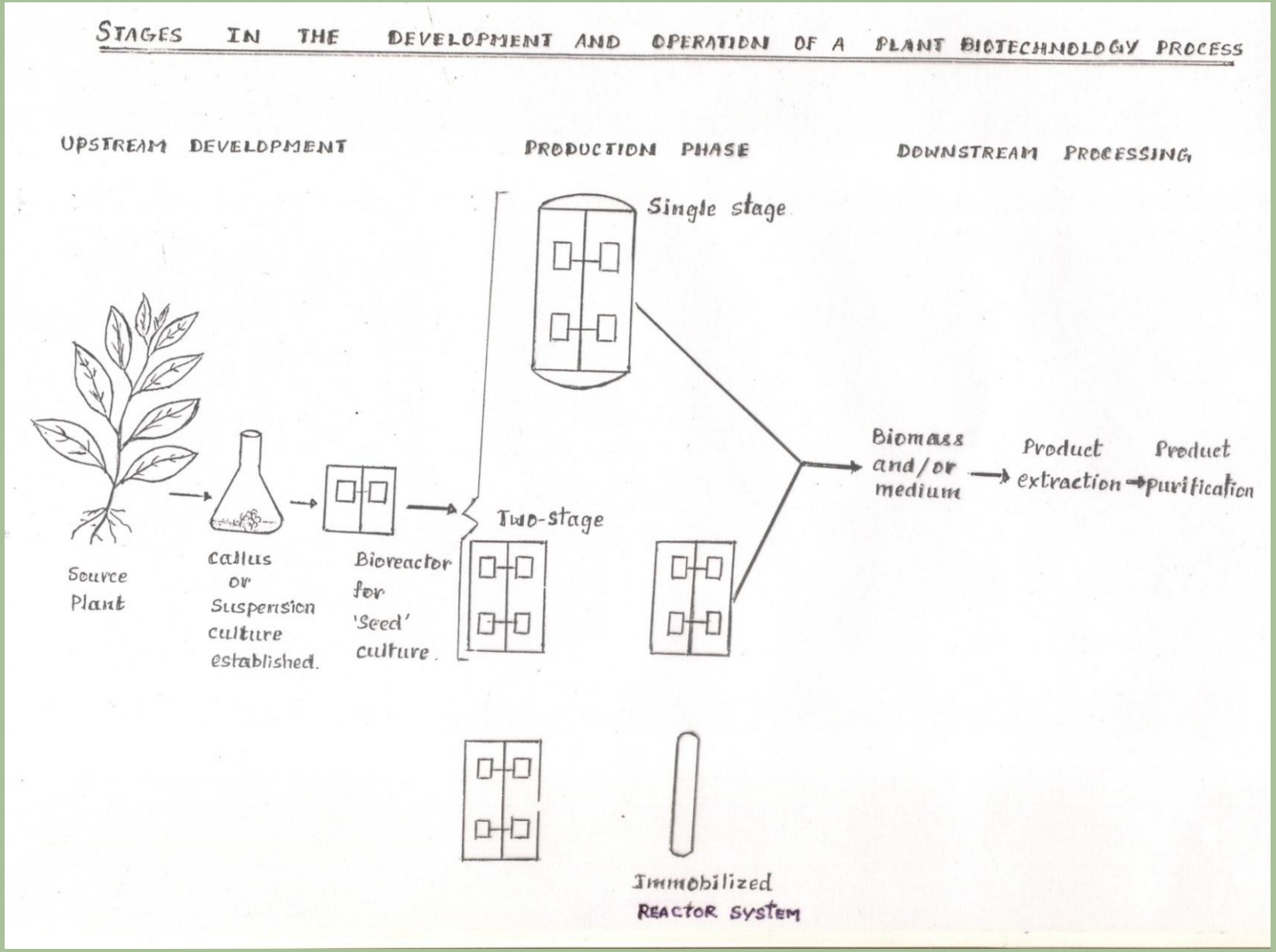
Optimization of secondary metabolite production in plant cells

- ***Lithospermum erythrorhizon*** **cells in Japan – 750L bioreactor for shikonin (a dye & chemical compound) –(1984)**
- **Sanguinarine –** ***Papaver somniferum*** **cells (USA)**
- **Vanilla flavour (USA)**
- **Taxol -** ***Taxus baccata*** **cell cultures 75 m^3. (Germany Co.)**

Hairy root – *Agrobacterium rhizogenes*

- *Atropa belladonna* – Atropine
- *Datura stramonium* – Hyoscamine
- *Hyscyamus multicus* – Hyoscamine
- *Catharanthus roseus* – Ajmaline, Serpentine, Catharanthine
- *Lithospermum erythrorhizon* – Shikonin
- *Cinchona ledgeriana* - Quinolinines
- *A. rhizogenes* is limited to dicotyledonous species only.
- Restricted to species in which the products are synthesized in roots of intact plants.

Steps of large scale secondary metabolites production



Thank you