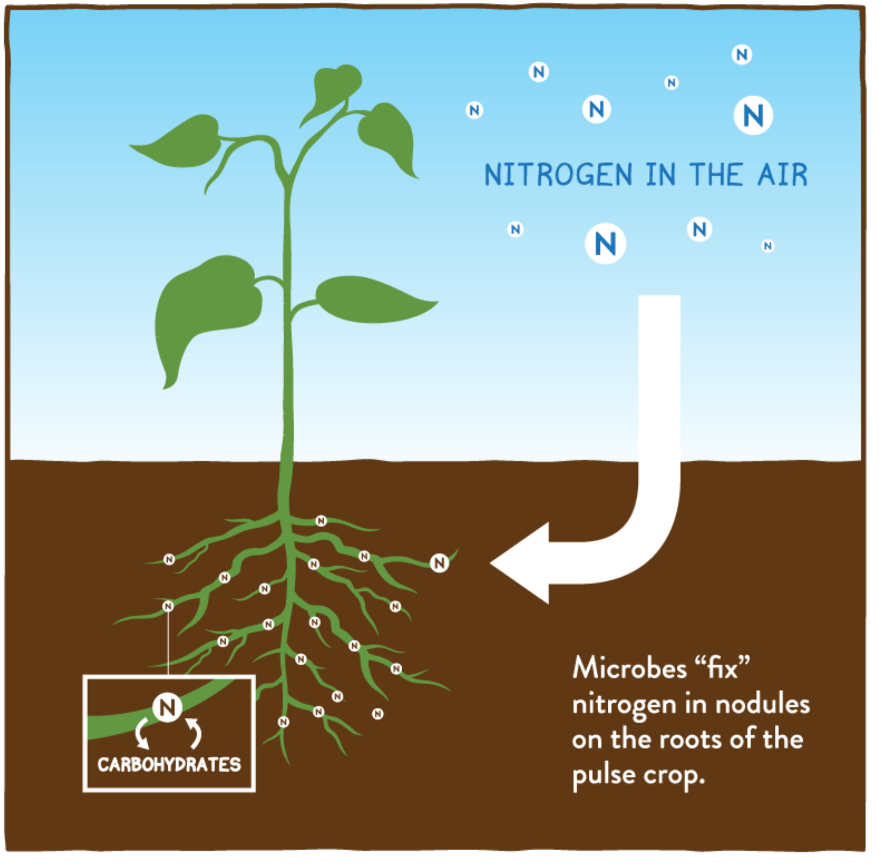
Nitrogen



Dr. Seema Margret Singh

Associate Professor

P.G College, Datia, (M.P)

Role of Nitrogen in Plants

- Major substance in plants next to water
- **Building blocks**
- Constituent elements of

✓ **Chlorophyll**

✓ **Cytochromes**

✓ **Alkaloids**

✓ **Vitamins**

- Play important role in **metabolism, growth, reproduction and heredity.**

Sources of Nitrogen

- Atmospheric nitrogen

✓78% of atmosphere

✓Plants cannot utilize this form

✓Some bacteria, blue green algae, leguminous plants

- Nitrates, nitrites and ammonia

✓Nitrate is chief form

- Ammonia acids in soil

✓Many soil organisms use this form

✓Higher plants can also taken by higher plants

- Organic nitrogenous compounds in insects

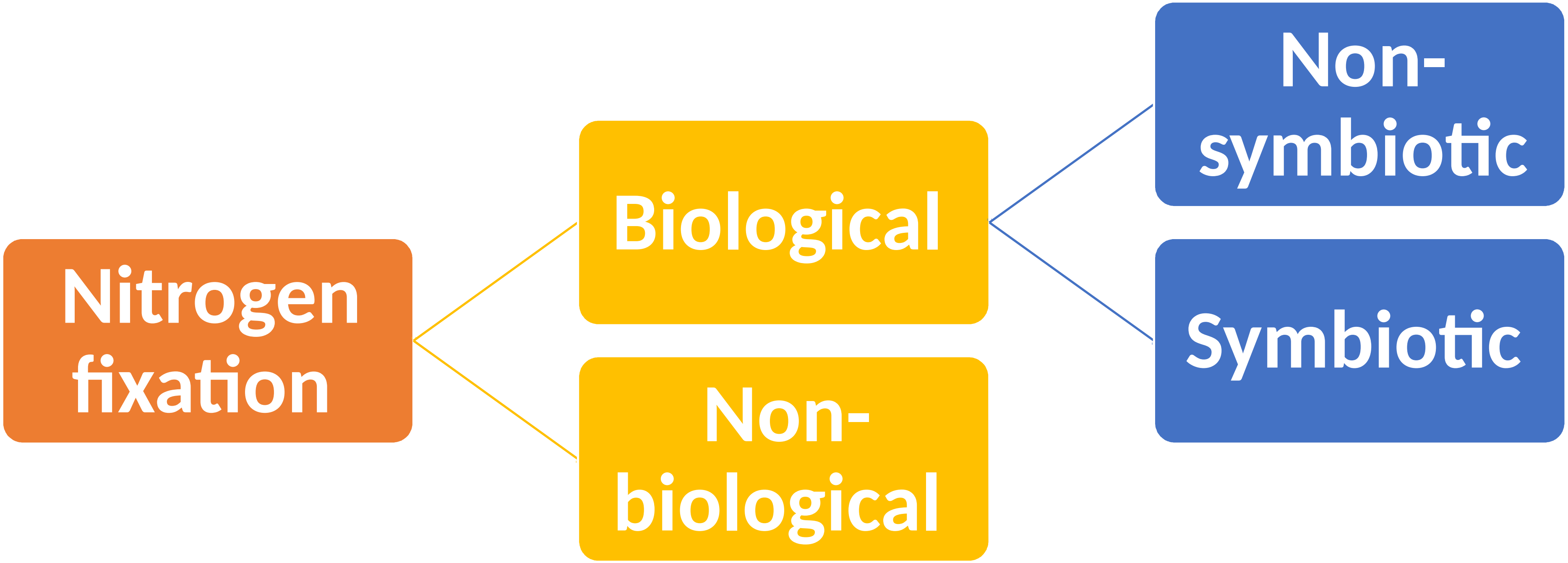
✓Insectivorous plants

Nitrogen Fixation

- The conversion of free nitrogen into nitrogenous salts to make it available for absorption of plants.

- Definition -**The chemical processes by which atmospheric nitrogen is assimilated into organic compounds, especially by certain microorganisms as part of the nitrogen cycle**.

Types Of Nitrogen Fixation



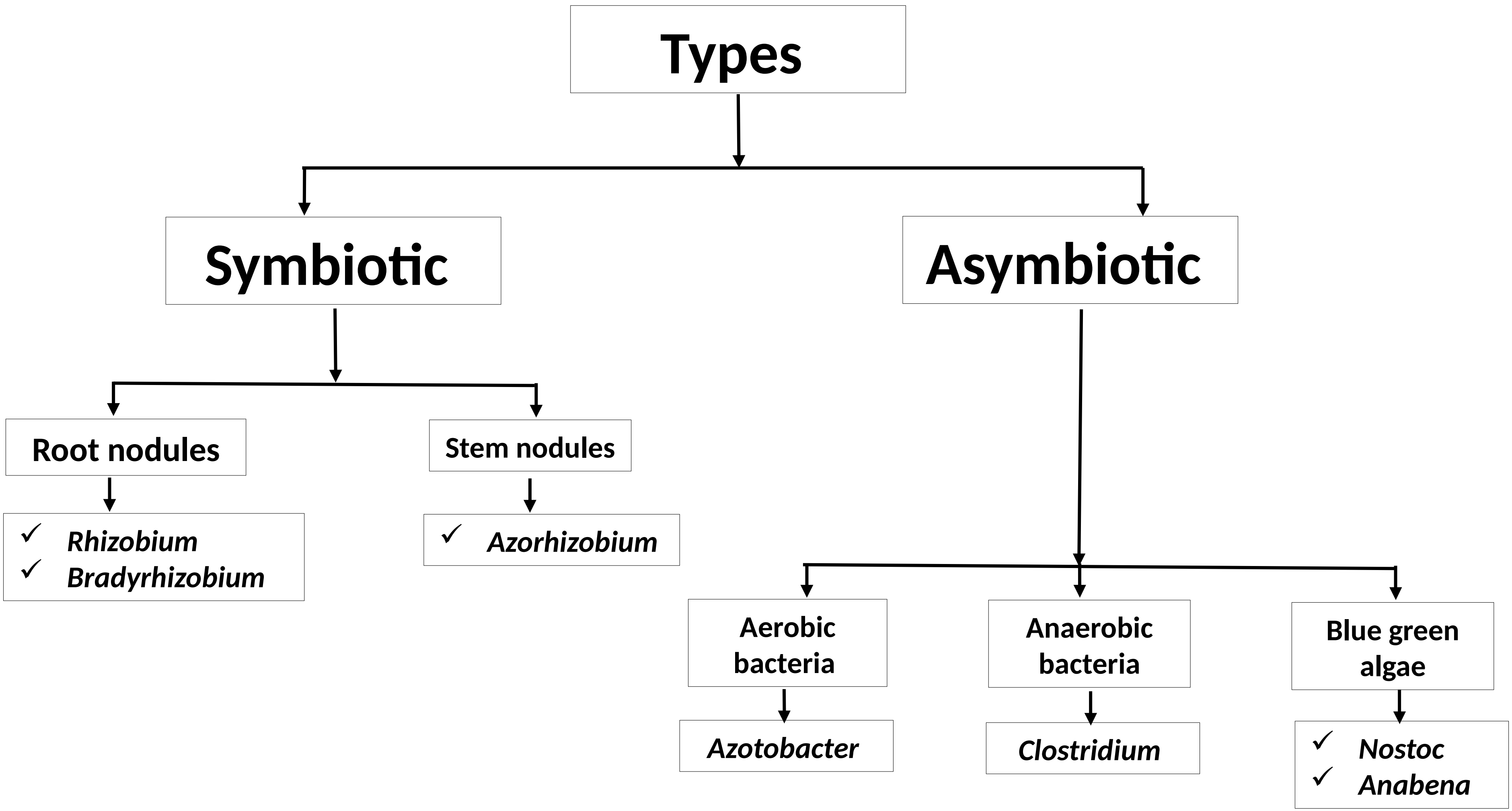
Non-biological Fixation

- The micro-organism do not take part
- Found in rainy season during lightning

1. $N_2 + O_2 \xrightarrow{\text{lightning}} 2\,NO$ (Nitric oxide)
2. $2NO + O_2 \xrightarrow{\text{oxidation}} 2NO_2$ (Nitrogen per oxide)
3. $2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$
4. $4NO_2 + 2H_2O + O_2 \rightarrow 4HNO_3$ (Nitric acid)
5. $CaO + 2HNO_3 \rightarrow Ca\,(NO_3)_2 + H_2O$ (Calcium nitrate)
6. $HNO_3 + NH_3 \rightarrow NH_4NO_3$ (Ammonium nitrate)
7. $HNO_2 + NH_3 \rightarrow NH_4NO_2$ (Ammonium nitrite)

Biological Fixation

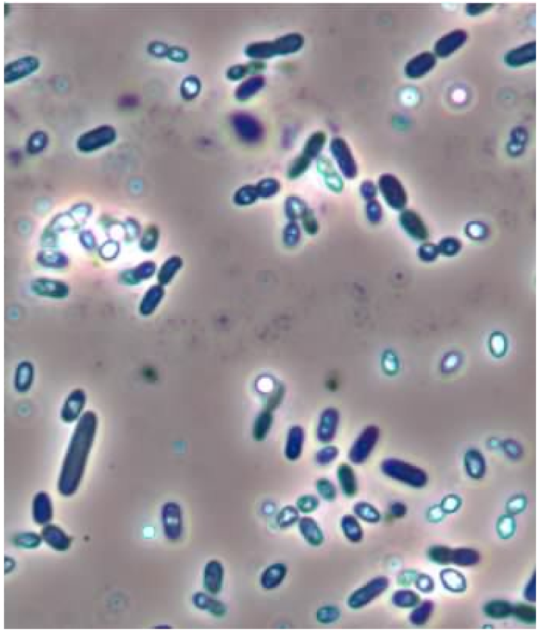
- Fixation of atmospheric nitrogen into nitrogenous salts with the help of micro-organisms.



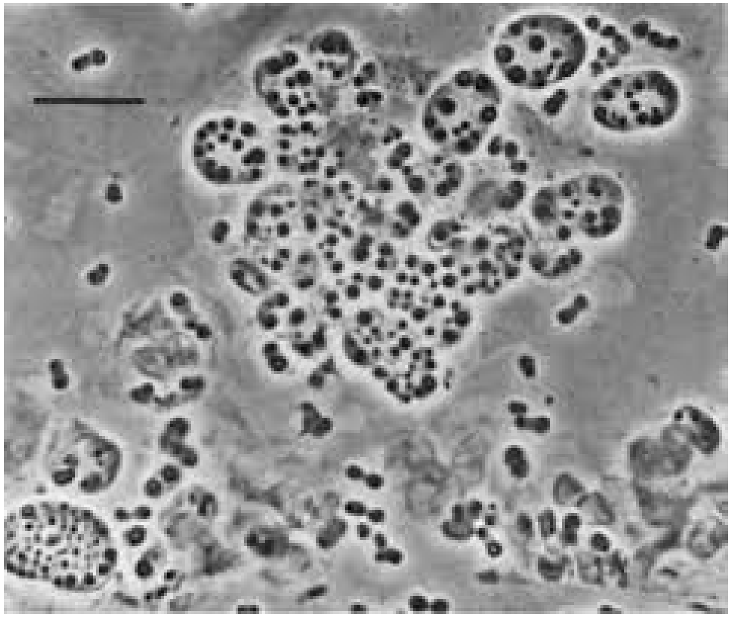
Non-symbiotic

- Fixation carried out by free living micro-organisms
- Aerobic, anaerobic and blue green algae
- Special types of bacteria

✓ Free living aerobic – ***Azotobacter*, *Beijerenckia***

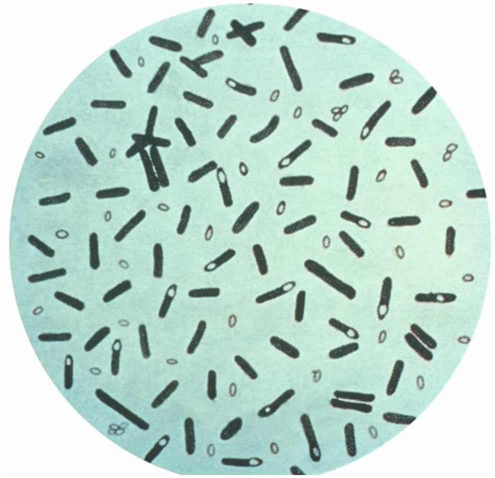


Azotobacter



Beijerenckia

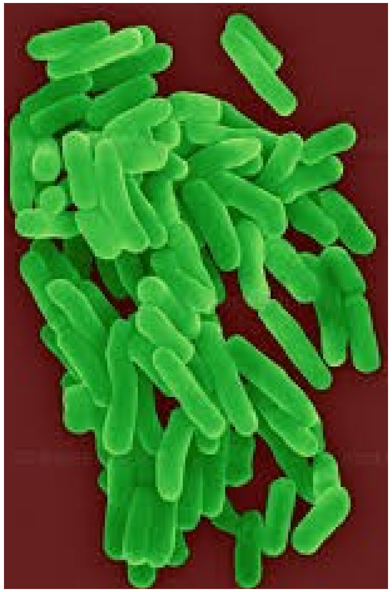
- ✓Free living anaerobic – ***Clostridium***



Clostridium

- ✓Free living photosynthetic – ***Chlorobium*, *Rhodopseudomonas***

- ✓Free living chemosynthetic – ***Desulfovibro*, *Thiobacillus***



Thiobacillus

- Free living fungi : yeasts and *Pillularia*

Pillularia

- Blue green algae :

✓Unicellular – ***Gloeothece*, *Synechococcus***

Oscillatoria

✓Filamentous (non heterocystous)- ***Oscillatoria***

✓Filamentous (heterocystous)- ***Tolypothrix*, *Nostoc*, *anabena***

Symbiotic

- Fixation of free nitrogen by microorganisms in soil living symbiotically inside the plants.

- 'Symbiosis' coined by DeBary

- Three categories

1. Nodule formation in leguminous plants
2. Nodule formation in non-leguminous plants
3. Non-nodulation

Nodule Formation in Leguminous Plants

- 2500 species of leguminosae (*Cicer arientum*, *Pisum*, *Cajanus*, *Arachis*) produce root nodules with *Rhizobium* species.
- They fix nitrogen inside the root nodules.
- Association provides food and shelter to bacteria and in return bacteria supplies fixed nitrogen to the plant.
- Nodule may be buried in soil even after harvesting – continue nitrogen fixation.

Nodule formation in non-leguminous plants

- Some other plants also produce root nodules

✓ ***Causaurina equisetifolia – Frankia***

✓ ***Alnus – Frankia***

✓ ***Myrica gale – Frankia***

✓ ***Parasponia –Rhizobium***

- Leaf nodules are also seen in

✓ ***Dioscorea***

✓ ***Psychotria***

- Gymnosperms – root – ***Podocarpus***

leaves – ***Pavetta zinumermanniana*, *Chomelia***

Non-nodulation

- Lichens – *cyanobacteria*
- *Antheroceros – Nostoc*
- *Azolla – Anabena azollae*
- *Cycas- Nostoc* and *Anabena*
- *Gunnera macrophylla – Nostoc*
- *Digitaria*, Maize and sorghum – *Spirillum notatum*
- *Paspalum notatum – Azotobacter paspali*

Symbiotic Nitrogen Fixation

- Small, knob like protuberances – root nodules
- Size and shape varies
- Spherical, flat, finger-like or elongated
- Named after host plant

✓ Pea – ***Rhizobium leguminosarum***

✓ Beans – ***R. phaseoli***

✓ Soyabeans – ***R. japonicum***

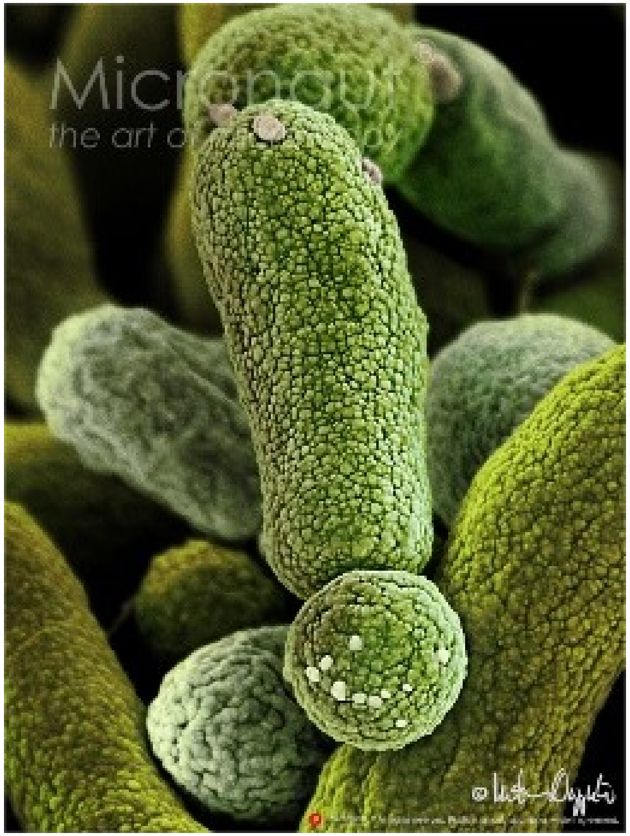
✓ Lupins – ***R. lupini***

- 2 types of *Rhizobium* **:**

✓ ***Bradyrhizobium*** **–** slow growing spp.

✓ ***Rhizobium*** – fast growing spp.

Rhizobium

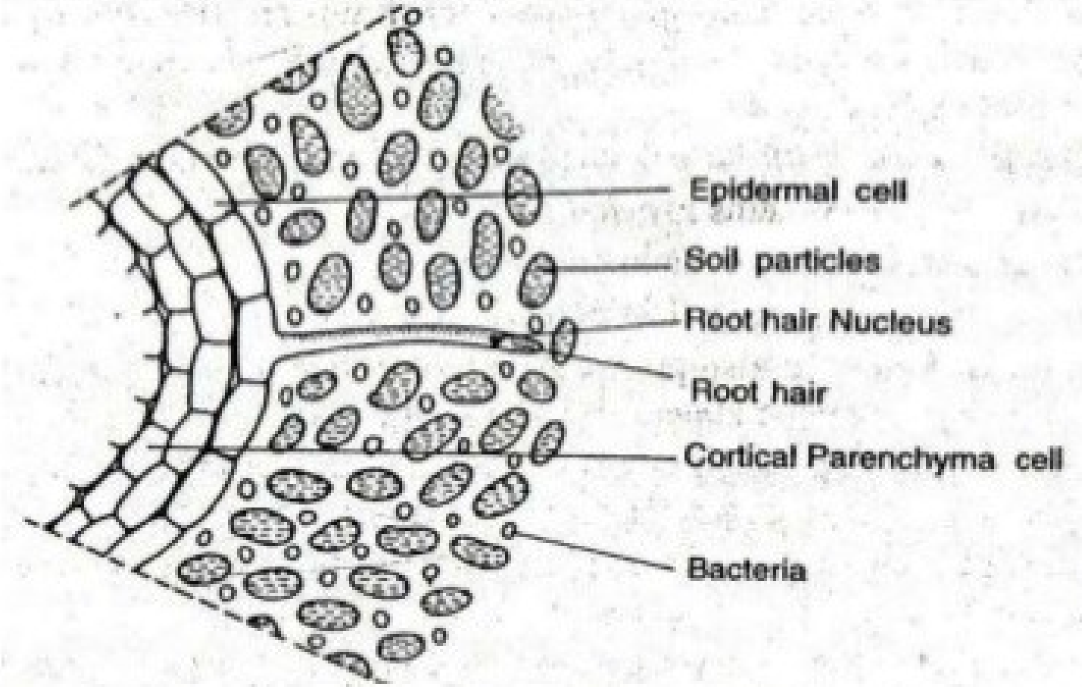


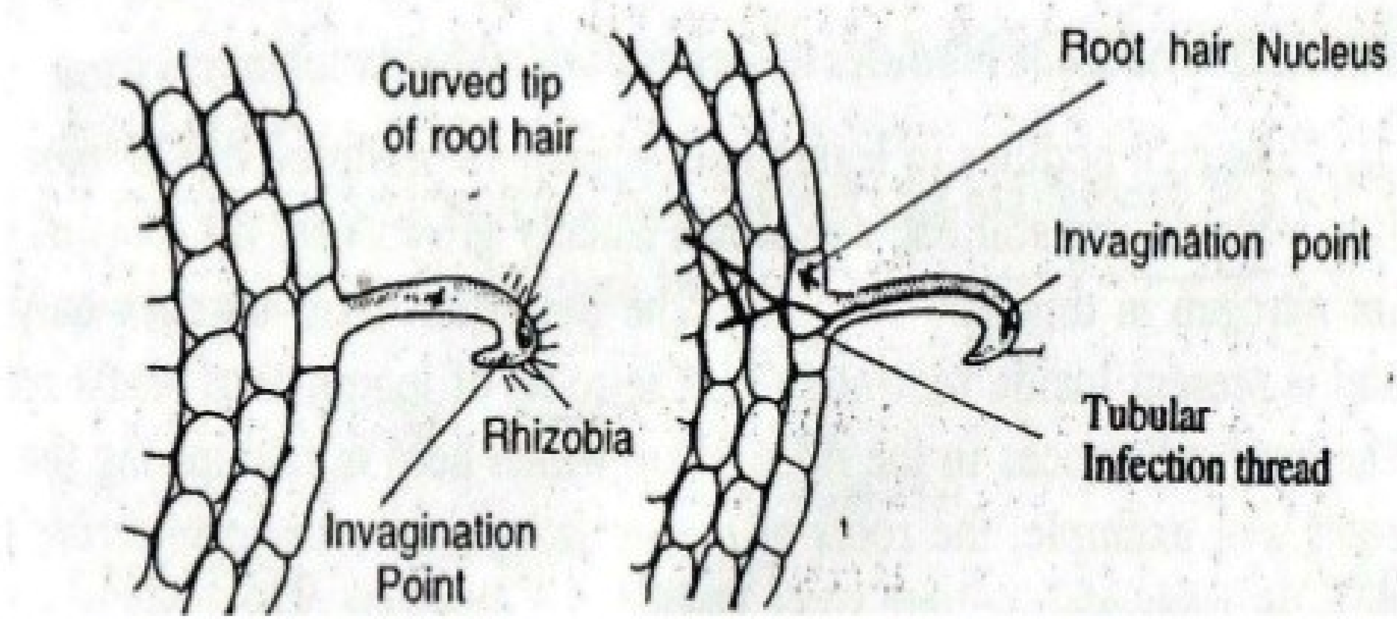
- Gram negative
- Non-spore forming
- Micro-aerobic
- Shows a degree of specificity
- 2 partners (bacteria and host) recognized by chemical substances **LECTINS** which is a phytoagglutinins (carbohydrate containing plant protein).

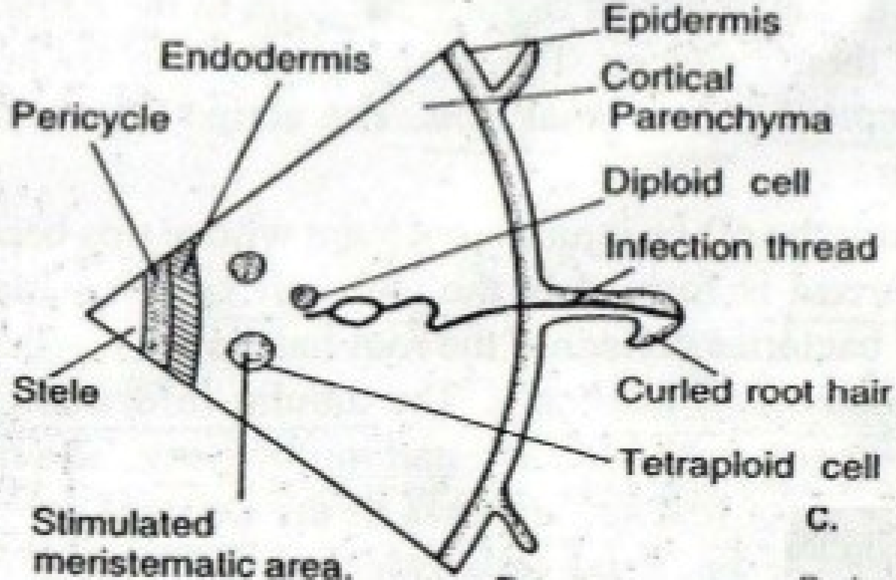
Formation of Root Nodules in Legumes

- Root nodules formed due to infection of Rhizobium
- Free living bacteria growing near roots of legumes to fix nitrogen in free condition.
- Roots of legumes secrete growth factors which helps in fats multiplication of bacteria
- ***Pisum sativum*** secretes **homo serine** over their surface
- This helps in recognition and attachment of rhizobial cells.

- Rhizobial cells have carbohydrate receptor on their surface.
- Lectins interact with the carbohydrate receptor of rhizobial cells
- This occurs between root hairs and young root hairs.
- Bacteria enters the roots through soft infected root hairs.
- Tips are deformed and curved.
- Tubular infection thread is formed in the root hair cell and bacteria enters into it.

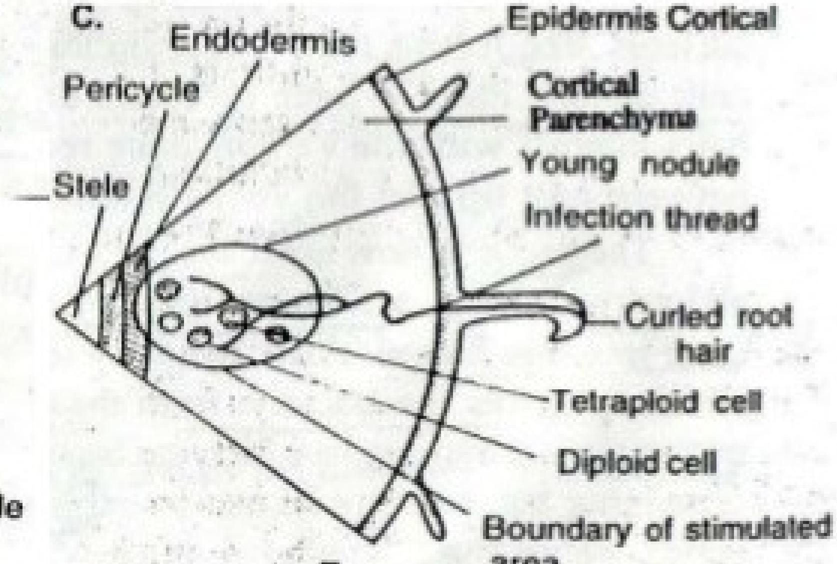

Epidermal cell
Soil particles
Root hair Nucleus
Root hair
Cortical Parenchyma cell
Bacteria


Curved tip
of root hair
Rhizobia
Invagination
Point
Root hair Nucleus
Invagination point
Tubular
Infection thread

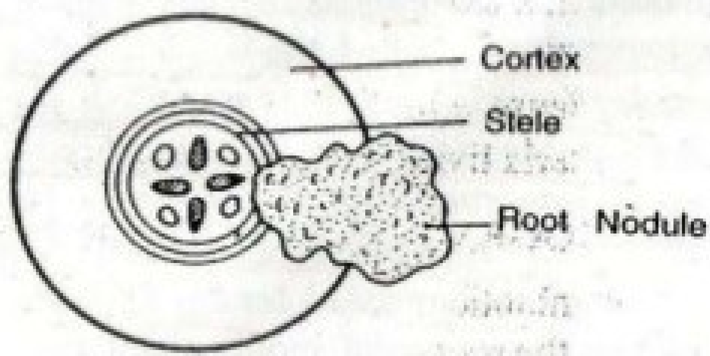

Epidermis
Endodermis
Cortical
Parenchyma
Pericycle
Diploid cell
Infection thread
Stele
Curled root hair
Tetraploid cell
Stimulated
meristematic area.

C.

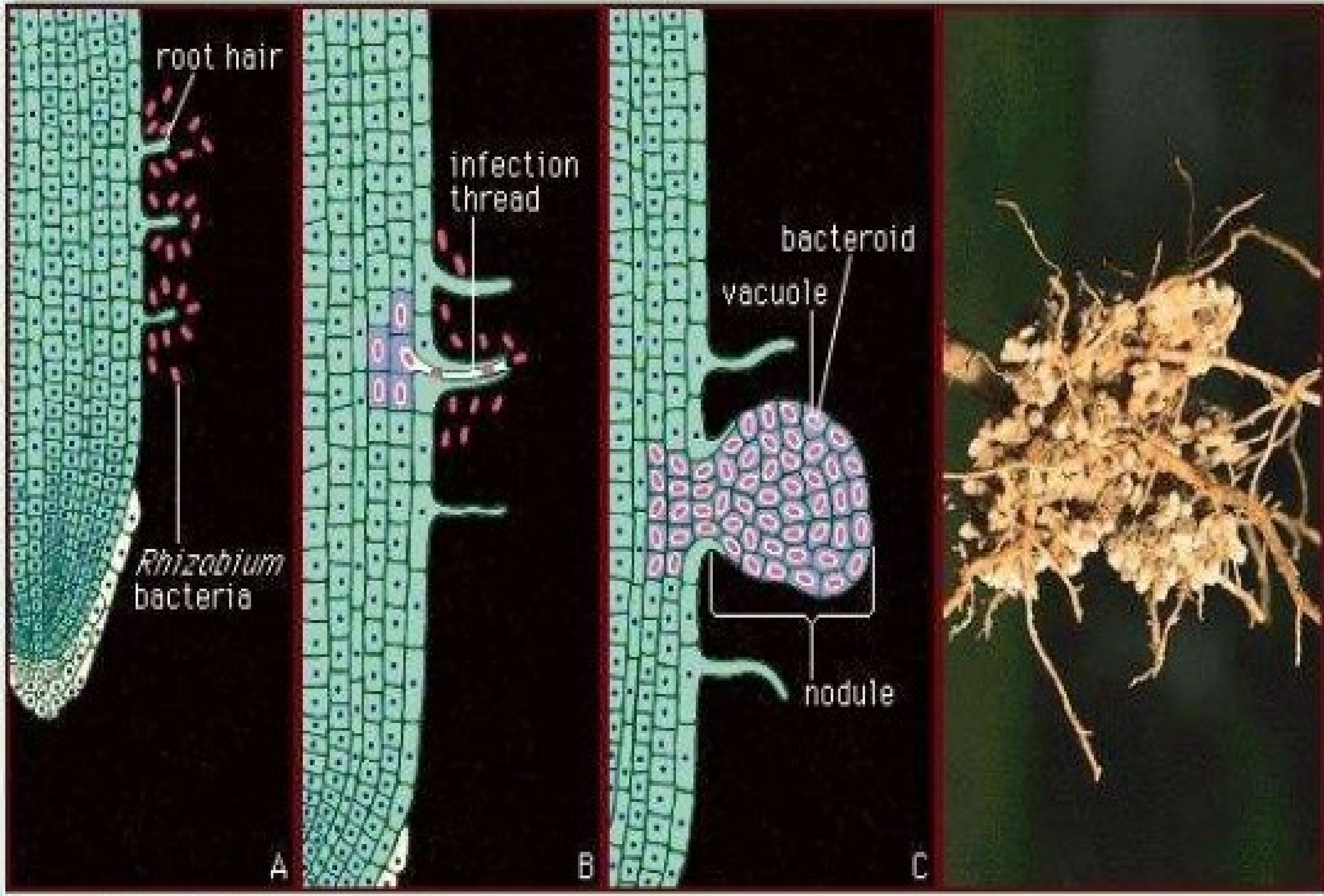
D.


Epidermis Cortical
Endodermis
Pericycle
Cortical
Parenchyma
Young nodule
Stele
Infection thread
Curled root
hair
Tetraploid cell
Diploid cell
Boundary of stimulated
area

E.


Cortex
Stele
Root Nodule

F.


root hair
Rhizobium bacteria
A
infection thread
B
bacteroid
vacuole
nodule
C

- After entry, new cell wall is formed
- Tubular infection contains mucopolysaccharides where bacteria embedded and start multiplication.
- It grows and reaches the inner layers of cortex and the bacteria is released.
- It induces the cortical cells to multiply which results in the formation of nodule on the surface
- The bacterial cells multiplies and colonize in the multiplying host cells.

- After host cells are completely filled, bacterial cells become dormant – bacteriods
- Float in leghaemoglobin – reddish pigment in cytoplasm of host cells
- Nitrogenous compounds synthesized is translocated through vascular tissues
- Groups of rhizobia surrounded by double membrane originated from host cell wall
- Bacteroids lack firm wall (osmotically liable).

Biochemistry of Nitrogen Fixation

- Basic requirements for nitrogen fixation
- Nitrogenase and hydrogenase
- Protective mechanism against oxygen
- Ferrodoxin
- Hydrogen releasing system or electron donor (Pyruvic acid/ sucrose)
- Constant supply of ATP
- Coenzymes and cofactors TPP, CoA, inorganic phosphate and Mg^{2+}
- Cobalt and molybdenum
- Carbon compound

Nitrogenase enzyme

- Active in anaerobic condition
- Made up of protein subunits
- Non-haem iron particles (Fe- protein or dinitrogen reductase)
- Iron molybdenum protein (Mo Fe protein or dinitrogenase)
- Fe protein reacts with ATP and reduces second subunit which ultimately reduces N_2 into ammonia

$$N_2 + 6H^+ + 6e^- \longrightarrow 2NH_3$$

- The reduction of N_2 into NH_3 requires 6 protons and 6 electrons
- 12 mols of ATP required
- One pair of electron requires 4 ATP

- The modified equation

$$N_2 + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2$$

- Hydrogen produced is catalyzed into protons and electrons by hydrogenase

$$H_2 \underset{}{\overset{\text{Hydrogenase}}{\rightleftharpoons}} 2H^+ + 2e^-$$

Pathway of Nitrogen Fixation in Root Nodules

- Glucose-6- phosphate acts as an electron donor

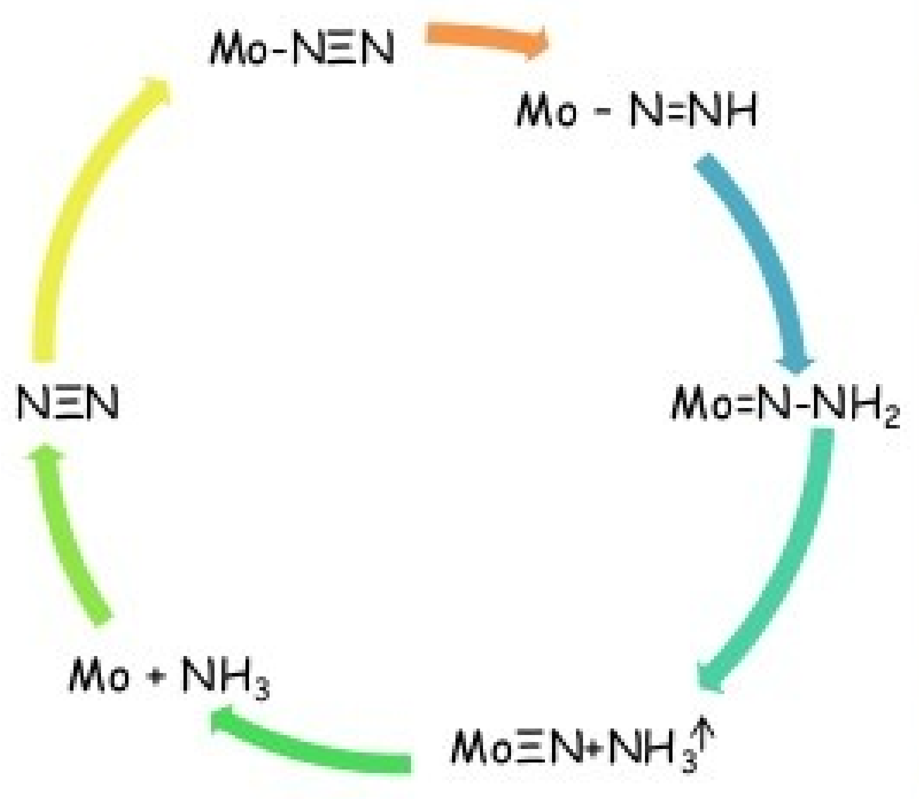
Sucrose synthesized in leaves → Sucrose in roots → Glucose and fructose → Glucose-6-phosphate

- Glucose-6-phosphate is converted to phosphoglyconic acid

Glucose-6-phosphate + NADP + H_2O $\longrightarrow$ 6-phosphoglyconic acid + NADPH + H^+

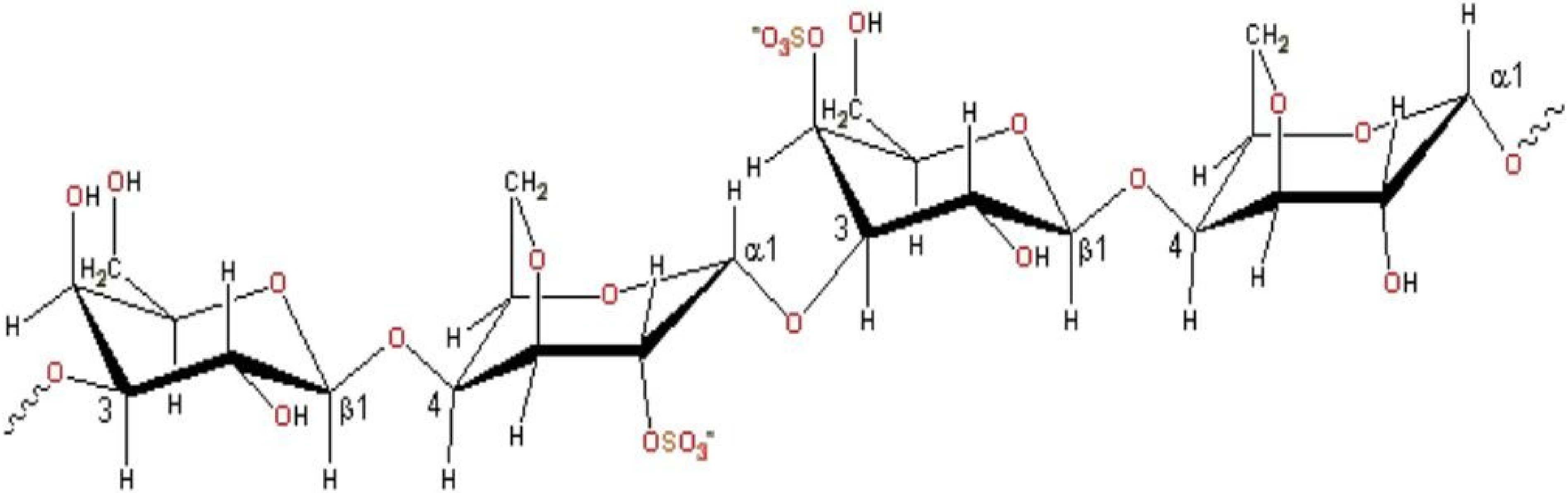
- NADPH donates electrons to ferrodoxin.
- Protons released and ferrodoxin is reduced
- Reduced ferrodoxin acts as electron carrier.
- Donates electron to Fe- protein to reduce it
- Electrons released from ferrodoxin thus oxidized

- Reduced Fe protein combines with ATP in the presence of Mg^{2+}
- Second subunit is activated and released
- It donates electrons to N_2 and NH_3
- Enzyme set free after complete reduction of N_2 to NH_3



Thank you

Polysaccharides : Structure & Functions



Dr. Seema Margret Singh

Associate Professor

Department of Botany

P.G College, Datia (M.P)

Introduction

- Polysaccharides are complex biomacromolecules that are made up chains of monosaccharides. The bonds that form these chains are glycosidic bonds.

- Commonly found monomer units in polysaccharides are :

✓ Glucose

✓ Fructose

✓ Mannose

✓ Galactose which are simple sugars.

Types

Homo-polysaccharides

- Made up of one type of monosaccharide units
- Eg: cellulose, starch, glucose

Hetero-polysaccharides

- Made up of two or more types of monosaccharide units
- Eg: hyaluronic acid

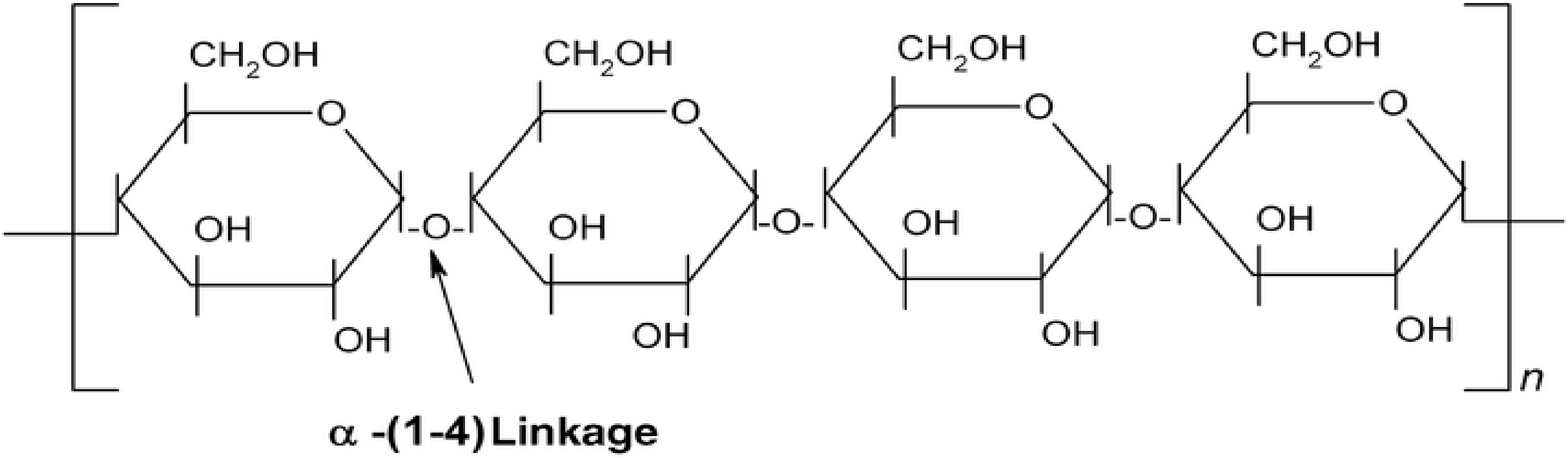
Starch

- Storage polysaccharide found in plant cells
- Exists in two forms:

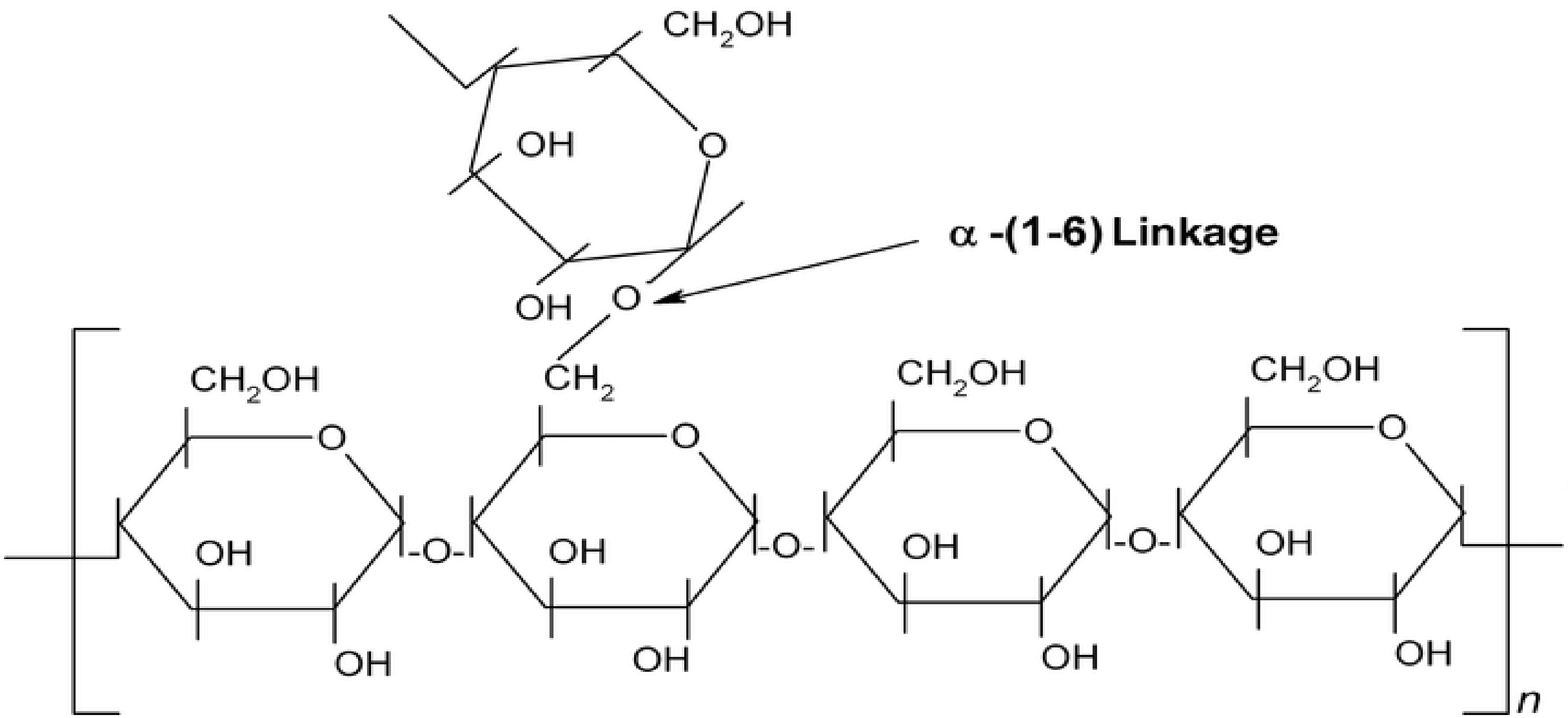
✓ Amylose

✓ Amylopectin

- Amylose – helical form of starch comprised of alpha-1,4 linkages
- Amylopectin - as a structure like glycogen except that the branched alpha-1,6 linkages are present on only about one in 30 monomers.



Amylose



Amylopectin

- Starch or amylum is a polymeric carbohydrate consisting of numerous glucose units joined by glycosidic bonds.

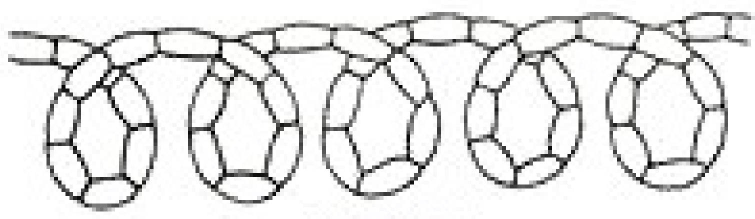
- This polysaccharide is produced by most green plants as energy storage.

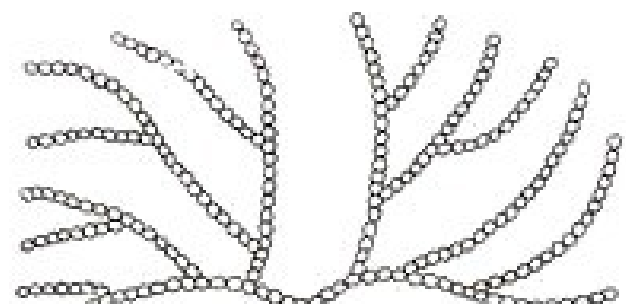
- It is the most common carbohydrate in human diets and is contained in large amounts in staple foods like potatoes, wheat, maize (corn), rice, and cassava.

- Depending on the plant, starch generally contains 20 -25% amylose and 75 to 80% amylopectin by weight.

- Both amylose and amylopectin are formed by the condensation of α -D-glucose (pyranose forms).

- Amylose is in the form of a continuous straight but helically arranged chain where each turn contains about six glucose units.





- The successive glucose units are linked together by 1-4 α-linkages, that is, the link is between carbon atom 1 of one and carbon atom 4 of the other.

- A molecule of water is lost during the formation of the linkage.

- A straight chain of amylose consists of 200-1000 glucose units

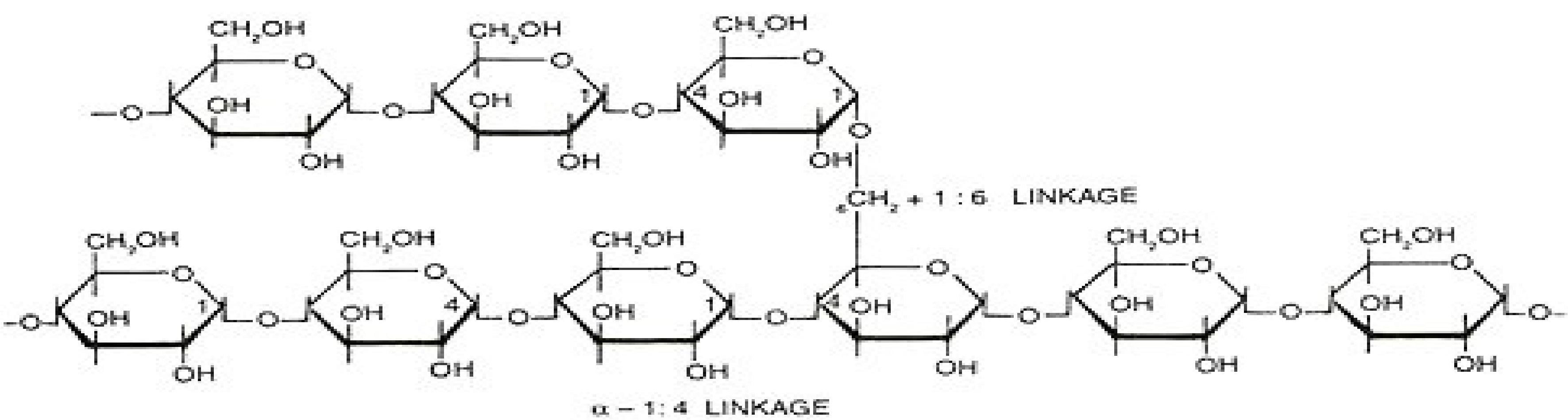


Fig. 9.8. Structure of starch.

Cellulose

- A structural polysaccharide that is found in the cell wall of plants and when consumed, it acts as a dietary fibre.

- Cellulose is said to be the most abundant organic molecule on earth.

- Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$

- Consists of a linear chain of several hundred to many thousands of $\beta(1\rightarrow4)$ linked D-glucose units

- Common forms of cellulose and % of cellulose are :

✓ Wood (25-50%)

✓ Paper

✓ Cotton (90%)

- It is fibrous homopolysaccharide of high tensile strength which forms a structural element of cell wall in all plants, some fungi and protists.

- Cellulose is the most abundant organic substance of the biosphere forming 50% of carbon found in plants.

- A chain of cellulose molecule contains 6000 or more glucose residues.
- The successive glucose residues are joined together by 1-4 β-linkages
- Consequently alternate glucose molecules lie at 180° to each other
- Hydroxyl groups of glucose residues, therefore, project in all directions.
- The molecular weight of cellulose ranges between 0.5 to 2.5 millions.

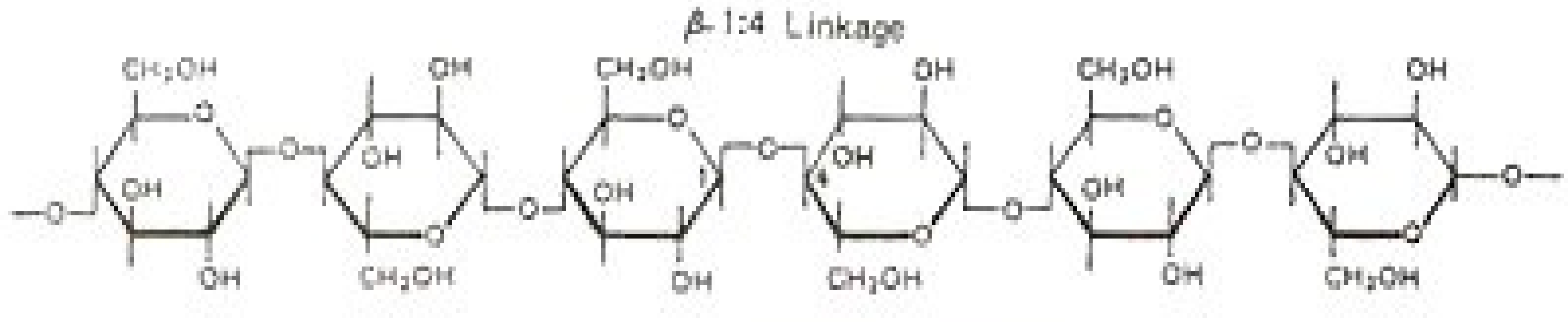


Fig. 9.10. Structure of cellulose.

- Cellulose molecules do not occur singly. Instead a number of chains are arranged in **close antiparallel** fashion.

- The molecules are held together by **intermolecular hydrogen bonds** between hydroxyl group at position 6 of glucose residues of one molecule and glycosidic oxygen between two glucose residues of the adjacent molecule.

- There is also intermolecular strengthening of the chain by the formation of **hydrogen bonds** between hydroxyl group at position three and oxygen atom of the next residue.

- About **2000 cellulose chains** or molecules are packed together to form a micro fibril visible under the electron microscope.

Importance of cellulose :

- ✓ Cellulose constitutes the bulk of human food. However, due to being polymer of β-glucose, cellulose is not acted upon by amylases present in human digestive juices. In humans, cellulose has a roughage value which keeps the digestive tract in functional fitness

- ✓ Cellulose is an important constituent of diet for ruminants like cows and buffaloes. The stomach of ruminants contain micro-organisms capable of digesting or breaking down cellulose. Termites and snails also possess micro-organisms in their gut for this purpose.

- ✓ Cellulose rich wood is employed in building furniture, tools, sports articles, paper etc.

- ✓ Depending upon the percentage of cellulose present in the fibers, the latter are used in textiles (e.g., Cotton, Linen), preparation of sacs (e.g., Jute) or ropes (e.g., Hemp, China Jute, Deccan Hemp).

- ✓ Cellulose nitrate is used in propellant explosives.

- ✓ Cellulose acetate is obtained by treating wood pulp with acetic acid, acetic anhydride and a catalyst.

- ✓ Carboxy methyl cellulose is used as emulsifier and smoothening reagent of ice creams, cosmetics and medicines.

- ✓ Cellulose acetates are used in preparing fibers for double knits, tricot, wrinkle proof, and moth proof clothing.

- ✓ Cigarette filters are also prepared from these fibers. Other uses of cellulose acetates include preparation of plastics and shatter proof glass.

Structure of Polysaccharides

- All polysaccharides are formed by the same basic process where monosaccharides are connected via glycosidic bonds.
- These glycosidic bonds consist of an oxygen molecule bridging two carbon rings.
- The bond is formed when a hydroxyl group is lost from the carbon of one molecule, while the hydrogen is lost by the hydroxyl group of another monosaccharide.

- Because two molecules of hydrogen and one of oxygen are expelled, the reaction is a dehydration reaction.
- The structure of the molecules being combined determines the structures and properties of the resulting polysaccharide.
- A polysaccharide used for energy storage will give easy access to the constituent monosaccharides whereas a polysaccharide used for support is usually a long chain of monosaccharides that form fibrous structures.

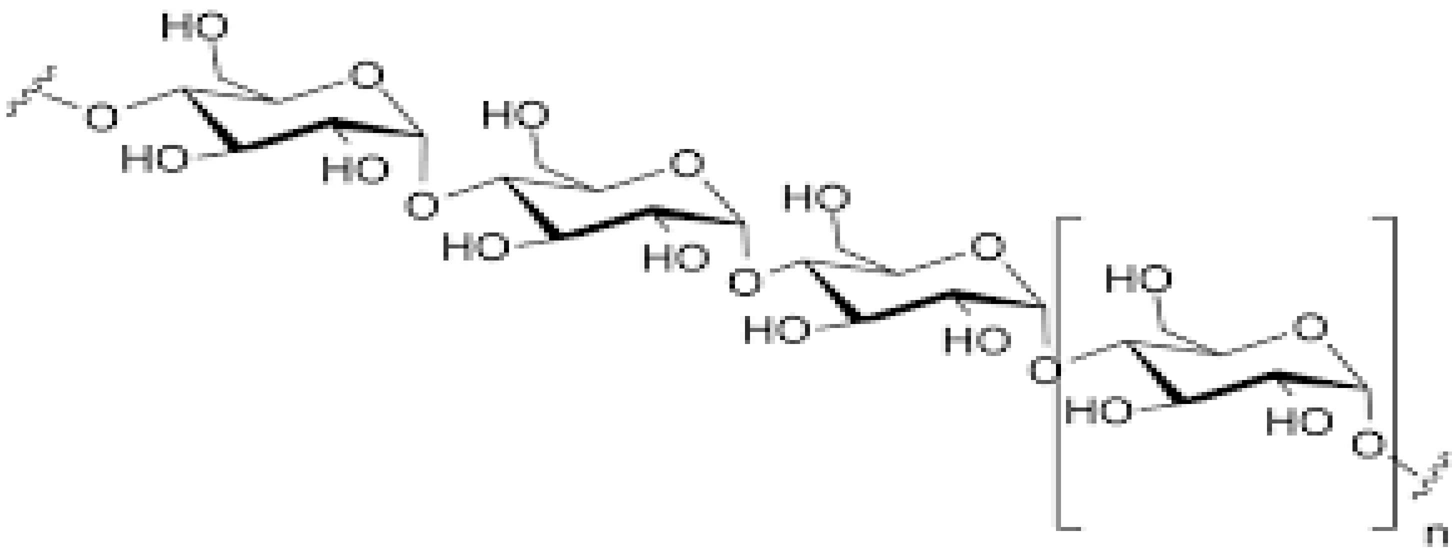
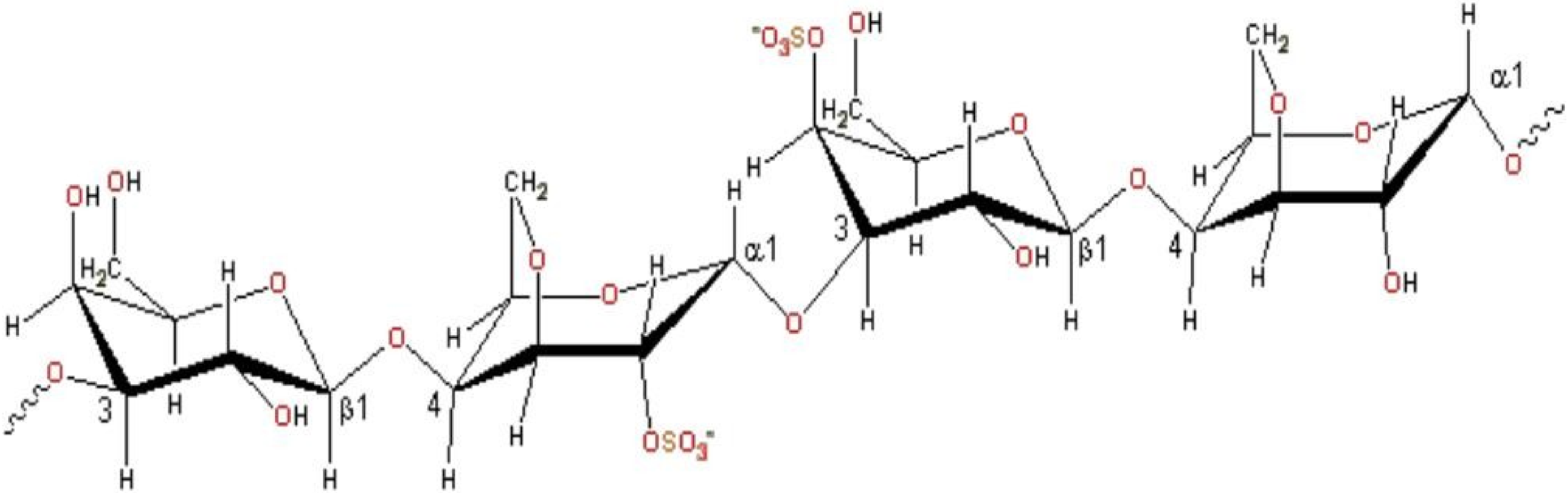


TABLE 5–2 POLYSACCHARIDES

Class	Name	Source	Composition	Linkages
Structural polysaccharides	Cellulose	Plant cell walls	Glucose (beta linkage)	Unbranched 1→4
	Mannan	Yeast cell walls	Mannose (beta linkage)	Branched 1→2, 1→3, and 1→6
	Chitin	Arthropod shells, fungal cell walls	Acetylglucosamine and glucuronic acid (beta linkage)	Unbranched 1→4
	Hyaluronic acid	Synovial fluid (joints), subcutaneous tissue	Acetylglucosamine and glucuronic acid (beta linkage)	Unbranched 1→3 and 1→4
	Peptidoglycans	Bacterial cell walls	Acetylglucosamine and acetylmuramic acid	Unbranched 1→4
Nutrient polysaccharides	Inulin	Artichokes, dandelions	Fructose (beta linkage)	Unbranched 2→1
	Paramylum	Certain protozoa (e.g., *Euglena*)	Glucose (beta linkage)	Unbranched 1→3
	Glycogen	Certain protozoa (e.g., *Tetrahymena*) and most animals	Glucose (alpha linkage)	Branched 1→4 and 1→6
	Starch:			
	Amylopectin	Plant cells and some protozoa (e.g., *Polytomella*)	Glucose (alpha linkage)	Branched 1→4 and 1→6
	Amylose		Glucose (alpha linkage)	Unbranched 1→4

Polysaccharides : Structure & Functions



Dr. Seema Margret Singh

Associate Professor

Department of Botany

P.G College, Datia (M.P)

Introduction

- Polysaccharides are complex biomacromolecules that are made up chains of monosaccharides. The bonds that form these chains are glycosidic bonds.

- Commonly found monomer units in polysaccharides are :

✓ Glucose

✓ Fructose

✓ Mannose

✓ Galactose which are simple sugars.

Types

Homo-polysaccharides

- Made up of one type of monosaccharide units
- Eg: cellulose, starch, glucose

Hetero-polysaccharides

- Made up of two or more types of monosaccharide units
- Eg: hyaluronic acid

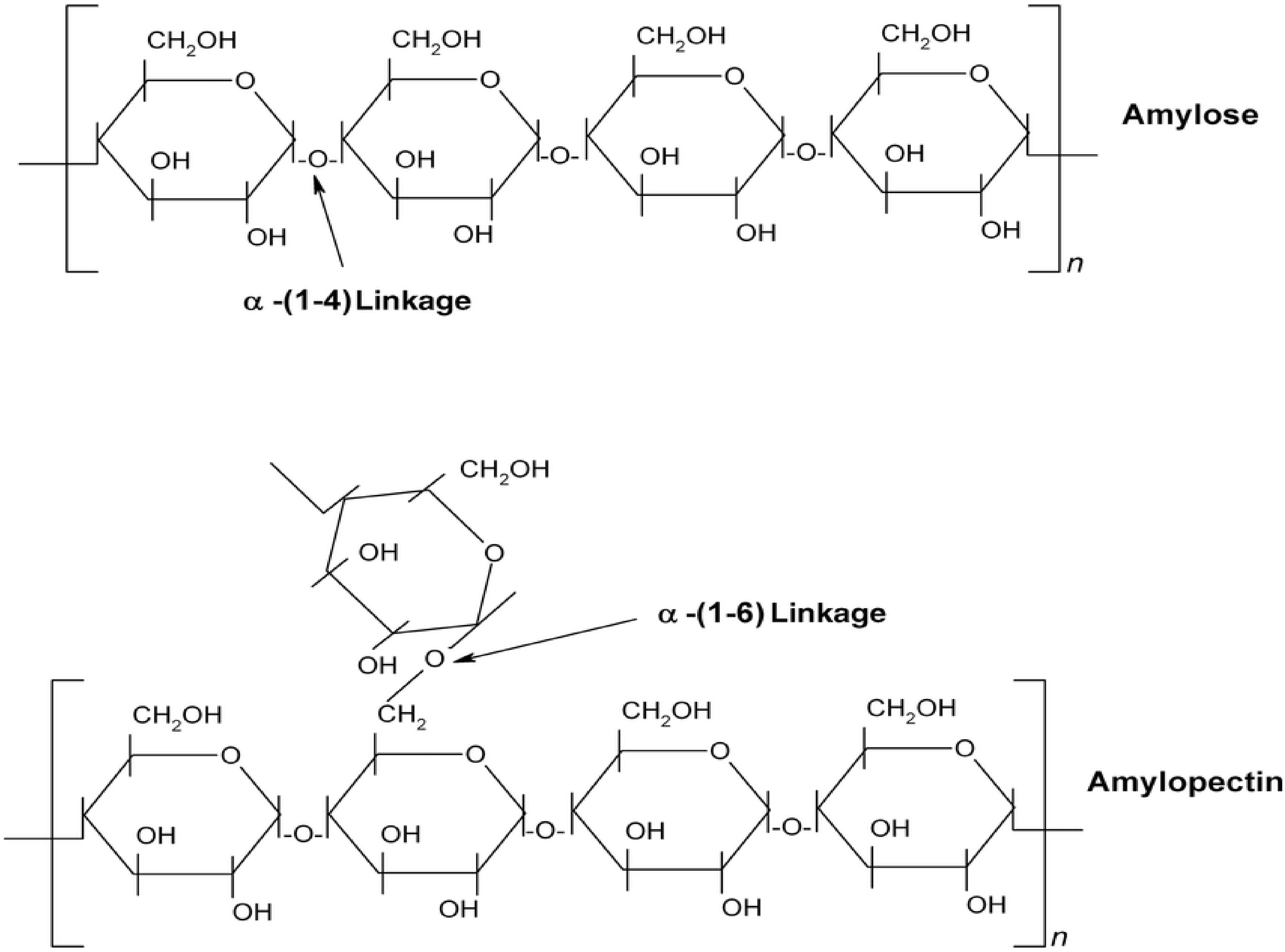
Starch

- Storage polysaccharide found in plant cells
- Exists in two forms:

✓ Amylose

✓ Amylopectin

- Amylose – helical form of starch comprised of alpha-1,4 linkages
- Amylopectin - as a structure like glycogen except that the branched alpha-1,6 linkages are present on only about one in 30 monomers.

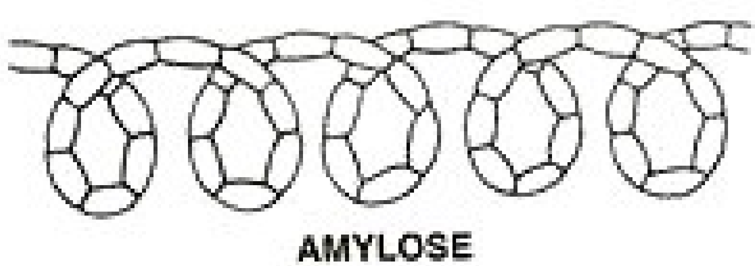

CH2OH
O
OH
-O-
n
Amylose
α -(1-4) Linkage
α -(1-6) Linkage
CH2
Amylopectin

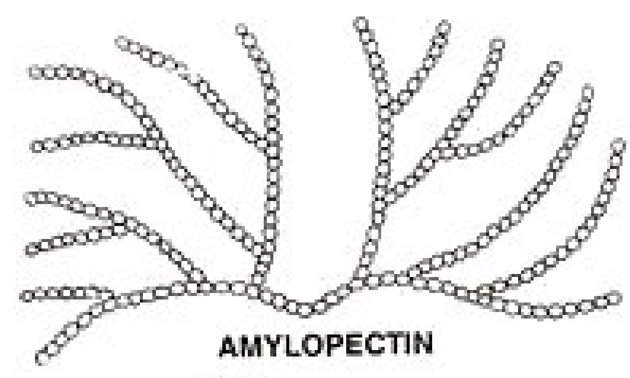
- Starch or amylum is a polymeric carbohydrate consisting of numerous glucose units joined by glycosidic bonds.

- This polysaccharide is produced by most green plants as energy storage.

- It is the most common carbohydrate in human diets and is contained in large amounts in staple foods like potatoes, wheat, maize (corn), rice, and cassava.

- Depending on the plant, starch generally contains 20 -25% amylose and 75 to 80% amylopectin by weight.
- Both amylose and amylopectin are formed by the condensation of α -D-glucose (pyranose forms).
- Amylose is in the form of a continuous straight but helically arranged chain where each turn contains about six glucose units.





- The successive glucose units are linked together by 1-4 α-linkages, that is, the link is between carbon atom 1 of one and carbon atom 4 of the other.

- A molecule of water is lost during the formation of the linkage.

- A straight chain of amylose consists of 200-1000 glucose units

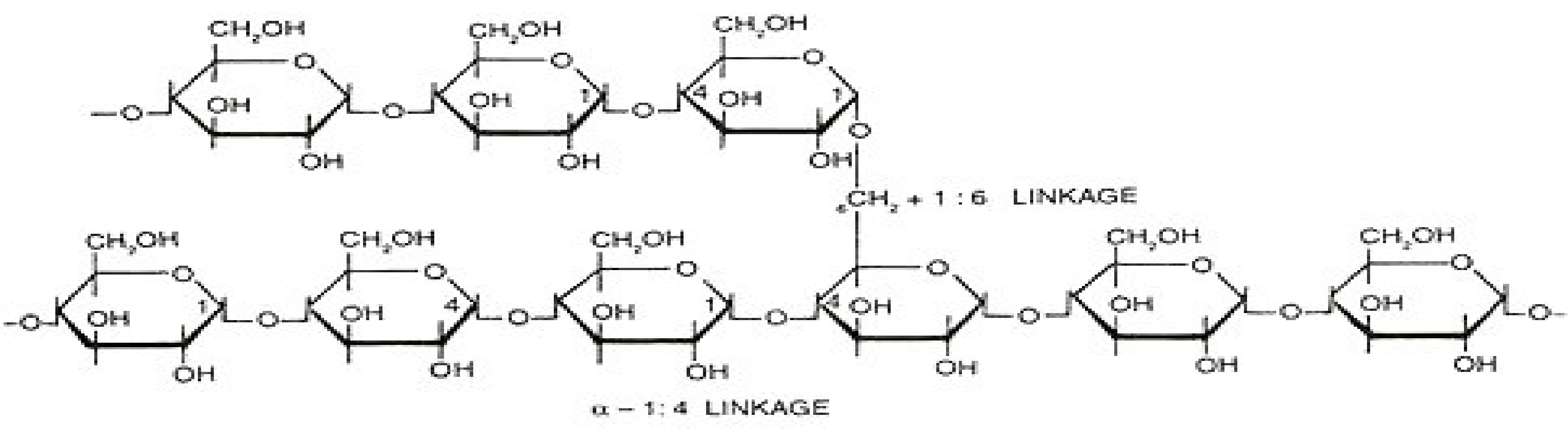


Fig. 9.8. Structure of starch.

Cellulose

- A structural polysaccharide that is found in the cell wall of plants and when consumed, it acts as a dietary fibre.
- Cellulose is said to be the most abundant organic molecule on earth.
- Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$
- Consists of a linear chain of several hundred to many thousands of $\beta(1\rightarrow4)$ linked D-glucose units

- Common forms of cellulose and % of cellulose are :

✓ Wood (25-50%)

✓ Paper

✓ Cotton (90%)

- It is fibrous homopolysaccharide of high tensile strength which forms a structural element of cell wall in all plants, some fungi and protists.

- Cellulose is the most abundant organic substance of the biosphere forming 50% of carbon found in plants.

- A chain of cellulose molecule contains 6000 or more glucose residues.
- The successive glucose residues are joined together by 1-4 β-linkages
- Consequently alternate glucose molecules lie at 180° to each other
- Hydroxyl groups of glucose residues, therefore, project in all directions.
- The molecular weight of cellulose ranges between 0.5 to 2.5 millions.

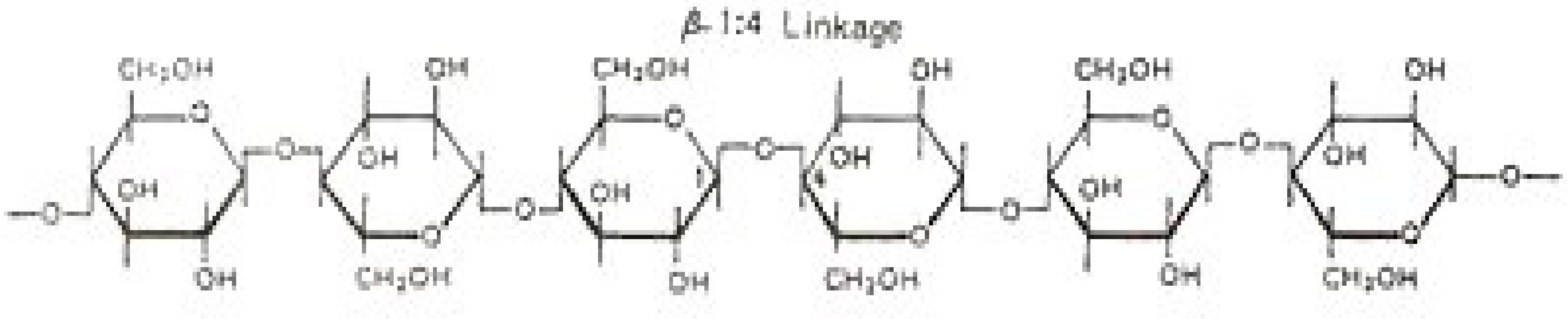


Fig. 9.10. Structure of cellulose.

- Cellulose molecules do not occur singly. Instead a number of chains are arranged in **close antiparallel** fashion.

- The molecules are held together by **intermolecular hydrogen bonds** between hydroxyl group at position 6 of glucose residues of one molecule and glycosidic oxygen between two glucose residues of the adjacent molecule.

- There is also intermolecular strengthening of the chain by the formation of **hydrogen bonds** between hydroxyl group at position three and oxygen atom of the next residue.

- About **2000 cellulose chains** or molecules are packed together to form a micro fibril visible under the electron microscope.

Importance of cellulose :

- ✓ Cellulose constitutes the bulk of human food. However, due to being polymer of β-glucose, cellulose is not acted upon by amylases present in human digestive juices. In humans, cellulose has a roughage value which keeps the digestive tract in functional fitness

- ✓ Cellulose is an important constituent of diet for ruminants like cows and buffaloes. The stomach of ruminants contain micro-organisms capable of digesting or breaking down cellulose. Termites and snails also possess micro-organisms in their gut for this purpose.

- ✓ Cellulose rich wood is employed in building furniture, tools, sports articles, paper etc.

- ✓ Depending upon the percentage of cellulose present in the fibers, the latter are used in textiles (e.g., Cotton, Linen), preparation of sacs (e.g., Jute) or ropes (e.g., Hemp, China Jute, Deccan Hemp).

- ✓ Cellulose nitrate is used in propellant explosives.
- ✓ Cellulose acetate is obtained by treating wood pulp with acetic acid, acetic anhydride and a catalyst.
- ✓ Carboxy methyl cellulose is used as emulsifier and smoothening reagent of ice creams, cosmetics and medicines.
- ✓ Cellulose acetates are used in preparing fibers for double knits, tricot, wrinkle proof, and moth proof clothing.
- ✓ Cigarette filters are also prepared from these fibers. Other uses of cellulose acetates include preparation of plastics and shatter proof glass.

Structure of Polysaccharides

- All polysaccharides are formed by the same basic process where monosaccharides are connected via glycosidic bonds.
- These glycosidic bonds consist of an oxygen molecule bridging two carbon rings.
- The bond is formed when a hydroxyl group is lost from the carbon of one molecule, while the hydrogen is lost by the hydroxyl group of another monosaccharide.

- Because two molecules of hydrogen and one of oxygen are expelled, the reaction is a dehydration reaction.
- The structure of the molecules being combined determines the structures and properties of the resulting polysaccharide.
- A polysaccharide used for energy storage will give easy access to the constituent monosaccharides whereas a polysaccharide used for support is usually a long chain of monosaccharides that form fibrous structures.

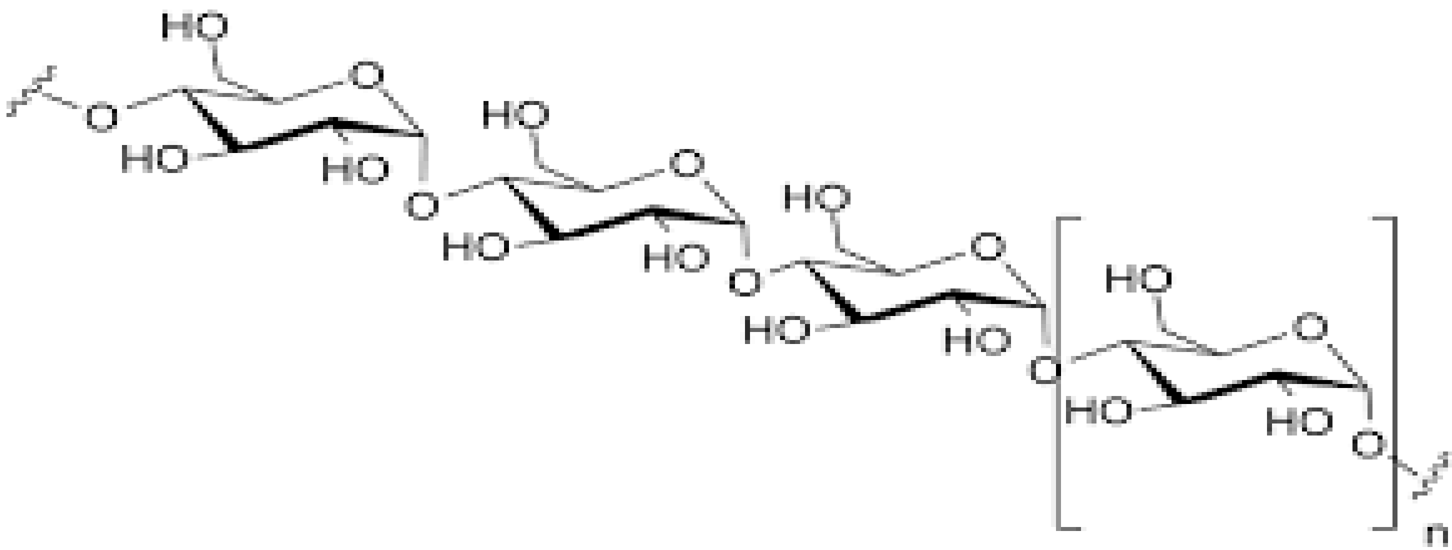
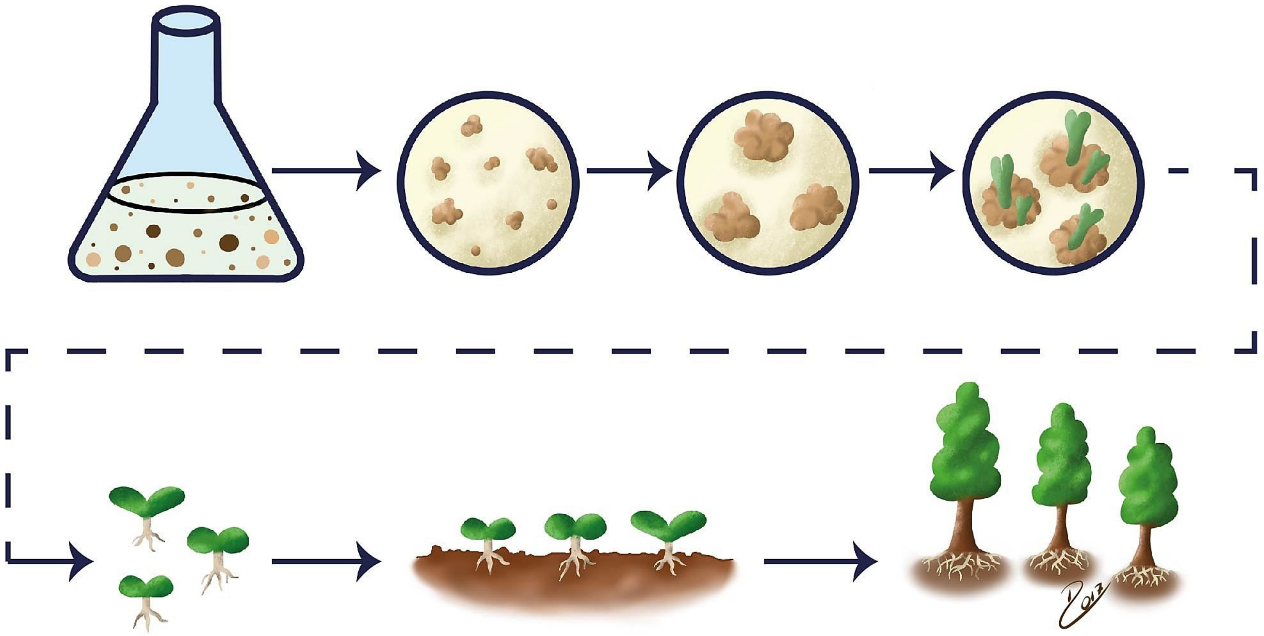


TABLE 5–2 POLYSACCHARIDES

Class	Name	Source	Composition	Linkages
Structural polysaccharides	Cellulose	Plant cell walls	Glucose (beta linkage)	Unbranched 1→4
	Mannan	Yeast cell walls	Mannose (beta linkage)	Branched 1→2, 1→3, and 1→6
	Chitin	Arthropod shells, fungal cell walls	Acetylglucosamine and glucuronic acid (beta linkage)	Unbranched 1→4
	Hyaluronic acid	Synovial fluid (joints), subcutaneous tissue	Acetylglucosamine and glucuronic acid (beta linkage)	Unbranched 1→3 and 1→4
	Peptidoglycans	Bacterial cell walls	Acetylglucosamine and acetylmuramic acid	Unbranched 1→4
Nutrient polysaccharides	Inulin	Artichokes, dandelions	Fructose (beta linkage)	Unbranched 2→1
	Paramylum	Certain protozoa (e.g., *Euglena*)	Glucose (beta linkage)	Unbranched 1→3
	Glycogen	Certain protozoa (e.g., *Tetrahymena*) and most animals	Glucose (alpha linkage)	Branched 1→4 and 1→6
	Starch:			
	Amylopectin	Plant cells and some protozoa (e.g., *Polytomella*)	Glucose (alpha linkage)	Branched 1→4 and 1→6
	Amylose		Glucose (alpha linkage)	Unbranched 1→4

Somatic Embryogenesis



Dr. Seema Margret Singh
Associate Professor
Department of Botany
P.G College, Datia (M.P)

Contents

Introduction

Embryogenesis

- Sexual reproduction
- Formation and development of embryo

Somatic Embryogenesis

- *De novo* process
- Formation of an embryo from **somatic cells**

Introduction

- **Definition :** The process of a single cell or a group of cells initiating the developmental pathway that leads to the reproducible regeneration of **non-zygotic embryos** capable of germinating to form complete plantlets.

- **Reinert, Steward, Mapes and Mears** – 1958, discovered somatic embryogenesis in culture of ***Daucus carota***

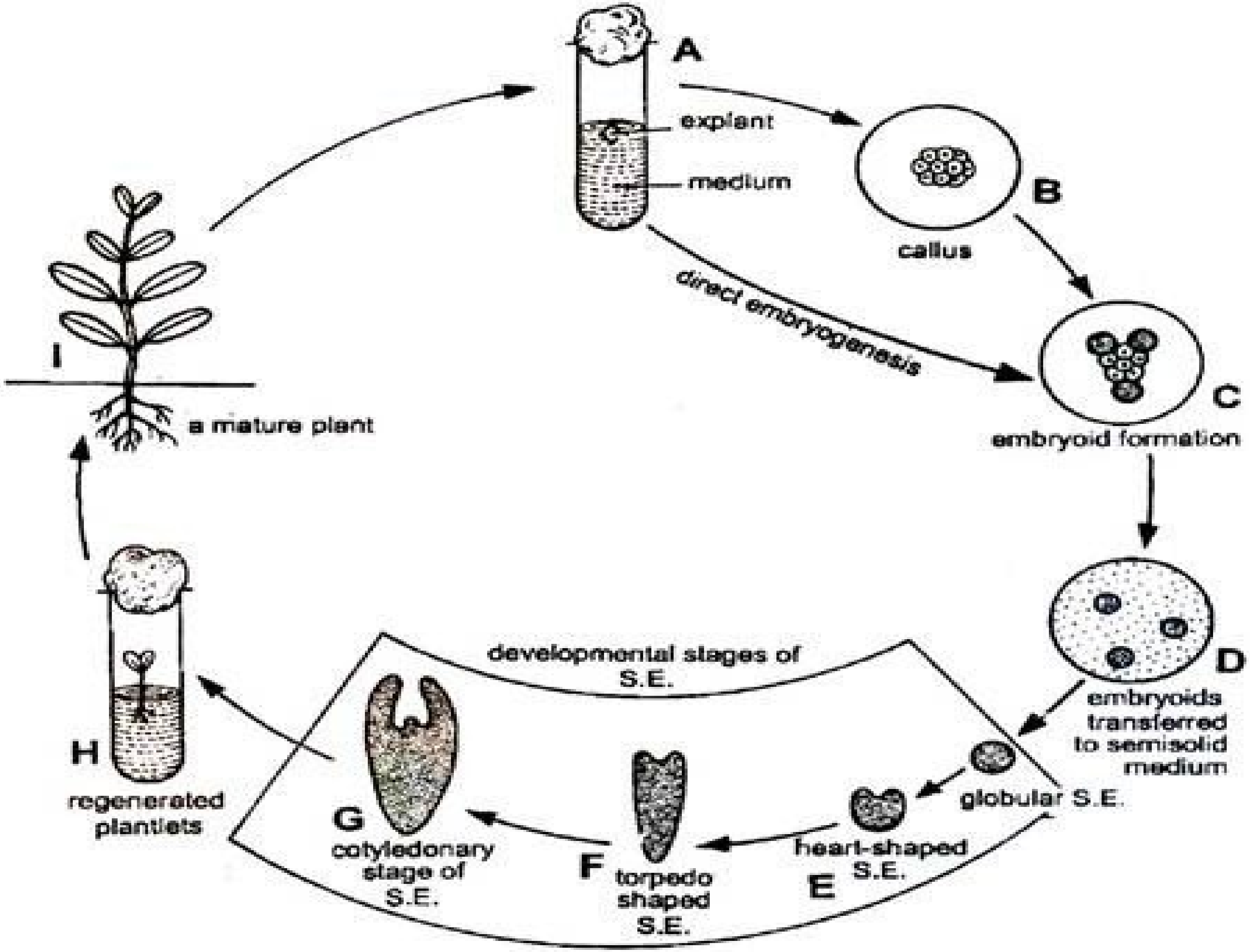


Fig. 5. Somatic Embryo (S.E.) Differentiation

Plants in which SE has been studied

Ranunculus scleratus

Celery leaf buttercup

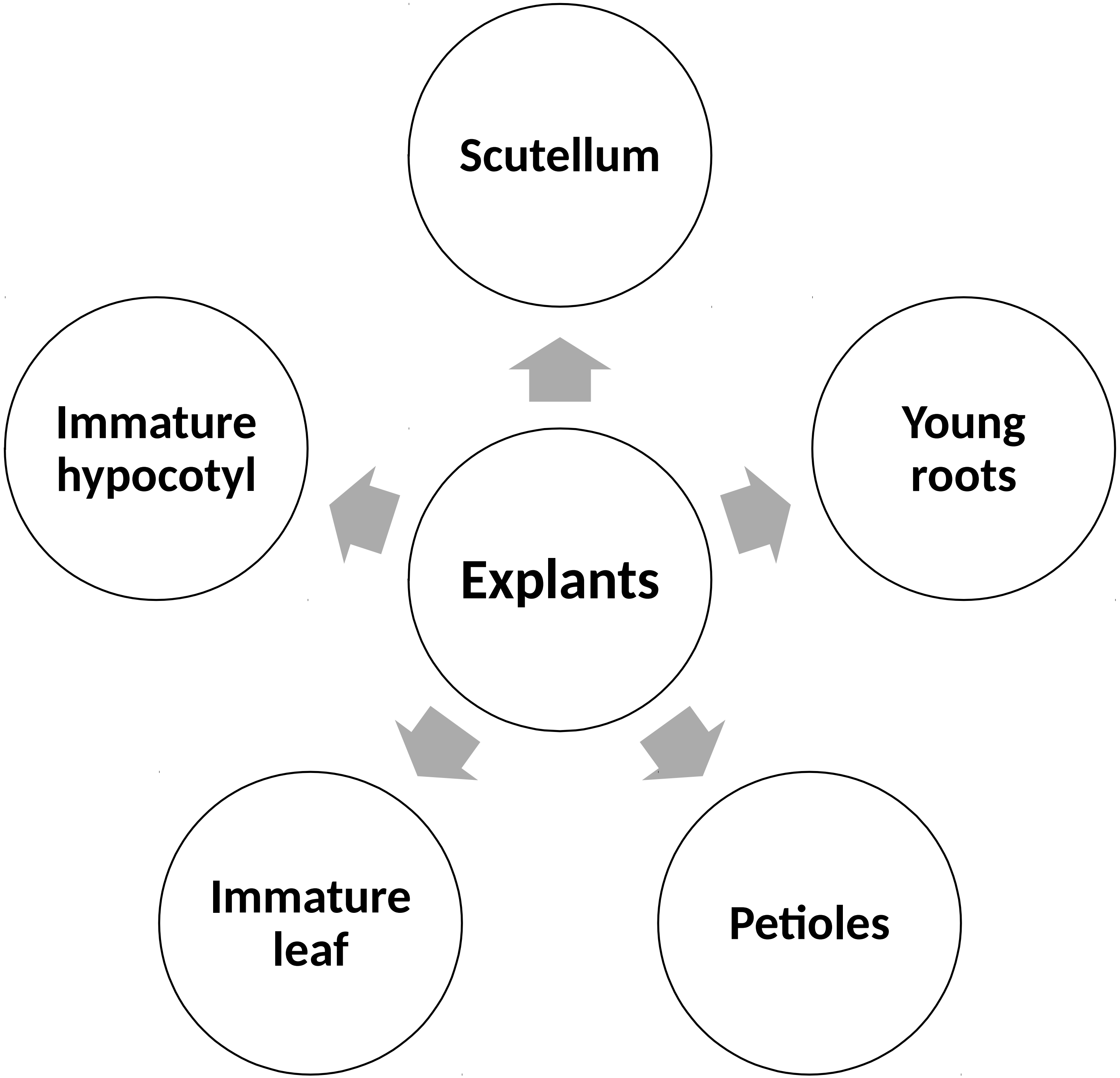
Macleaya cordata

Plume poppy

Citrus spp.

Coffea spp.

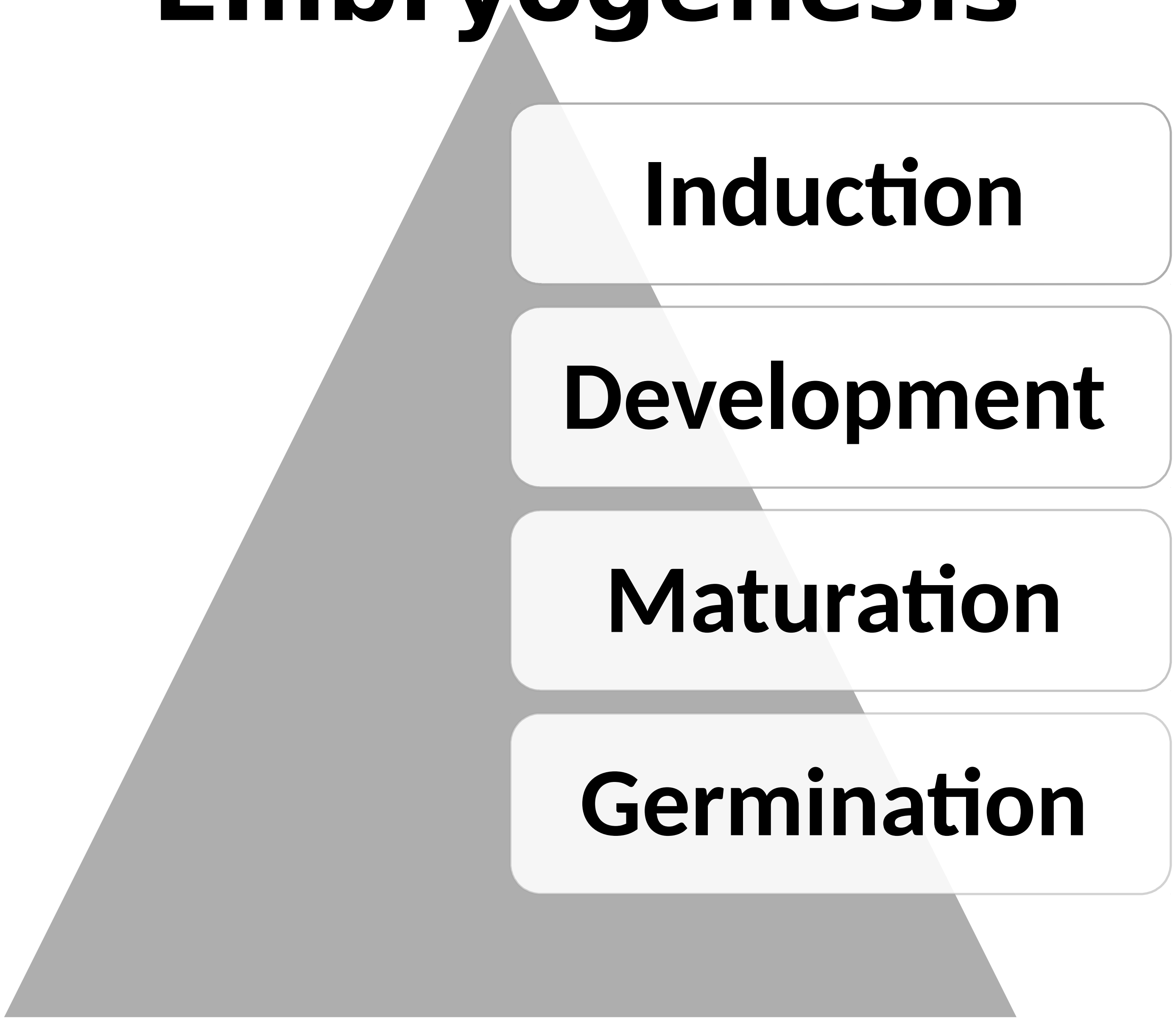
Coffee

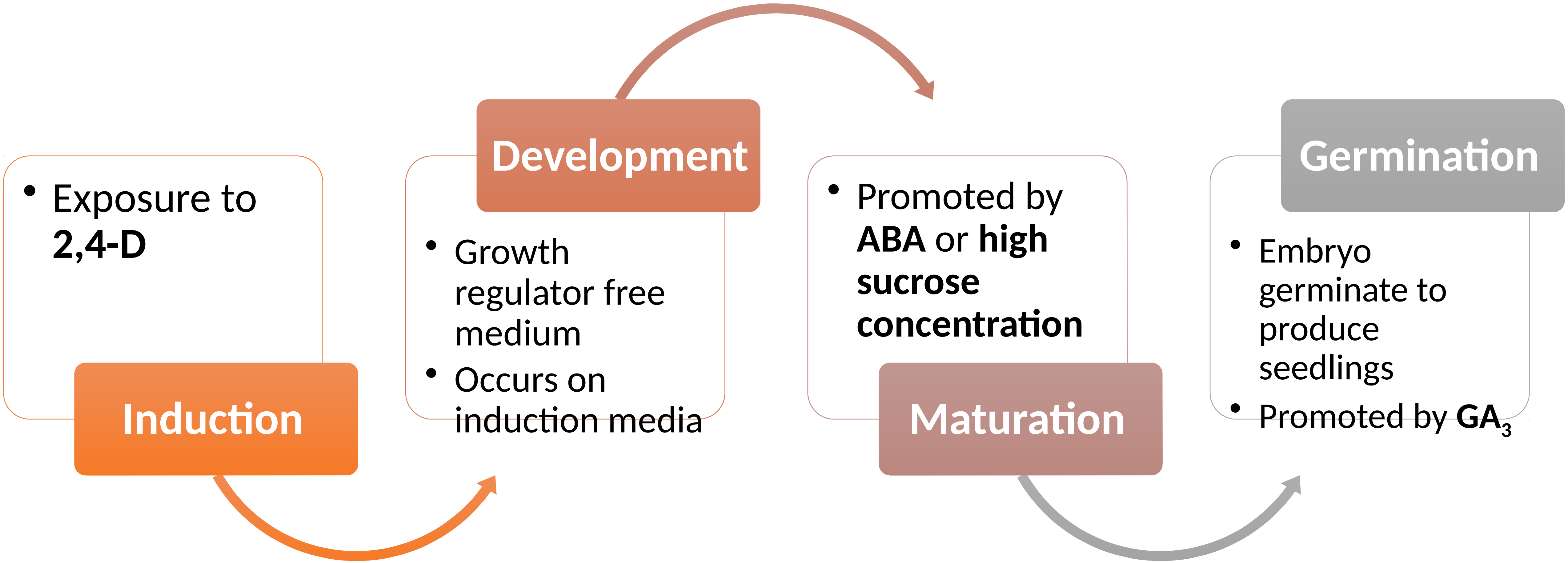

Scutellum
Immature hypocotyl
Young roots
Explants
Immature leaf
Petioles

Factors

- Temperature = 22-25°C
- Low irradiance light
- Growth regulators
- Charcoal
- Physiological condition of explant
- Reduced nitrogen

Phases In Somatic Embryogenesis

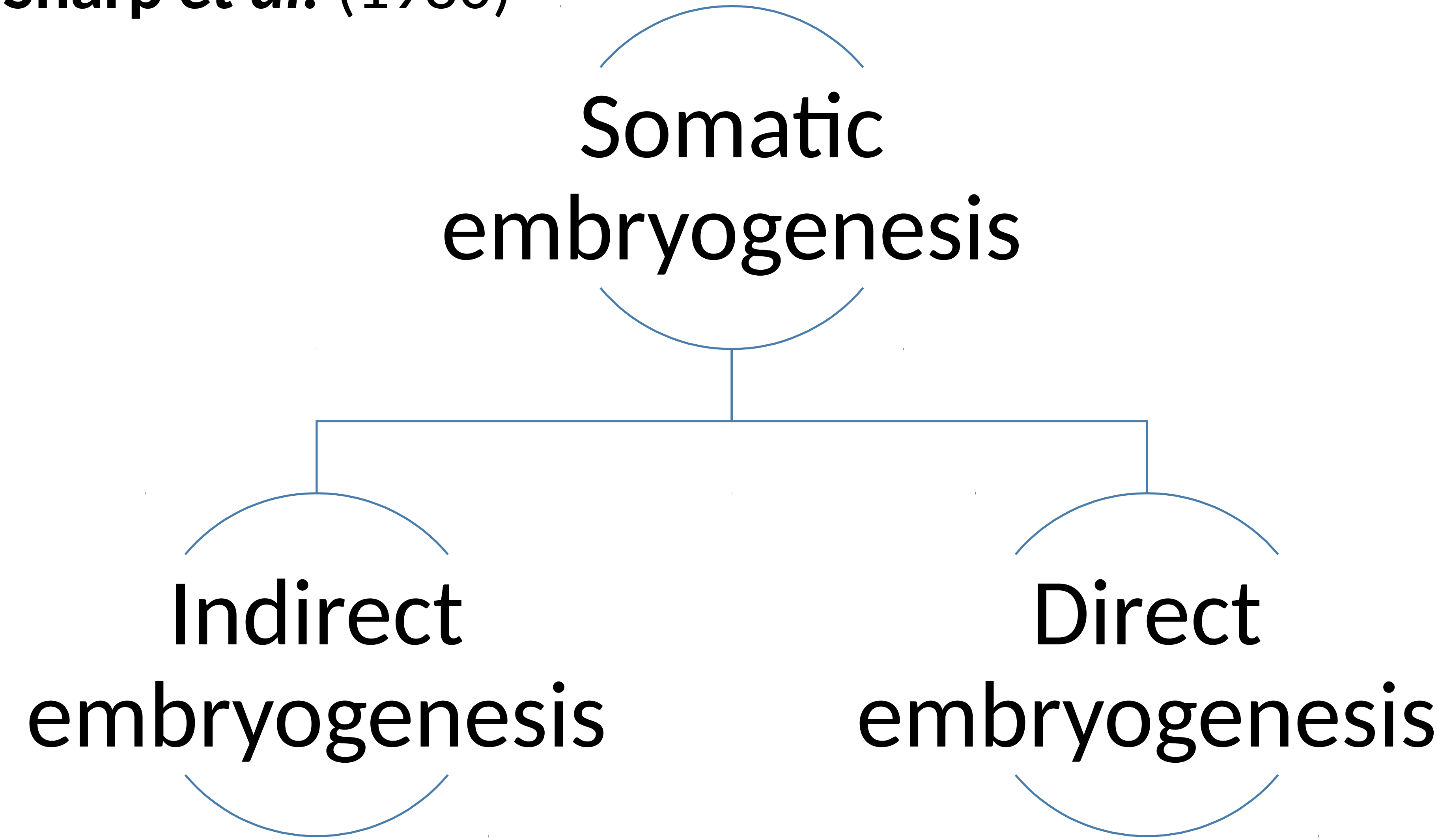




Induction
• Exposure to **2,4-D**
Development
• Growth regulator free medium
• Occurs on induction media
Maturation
• Promoted by **ABA** or **high sucrose concentration**
Germination
• Embryo germinate to produce seedlings
• Promoted by **GA_3**

Routes In Somatic Embryogenesis

Given by **Sharp *et al.*** (1980)



Direct Embryogenesis

- Predetermined explants are cultured on media in the **absence or low concentration of auxin.**

- Embryos initiate **directly from the explant** tissue in the **absence of callus proliferation.**

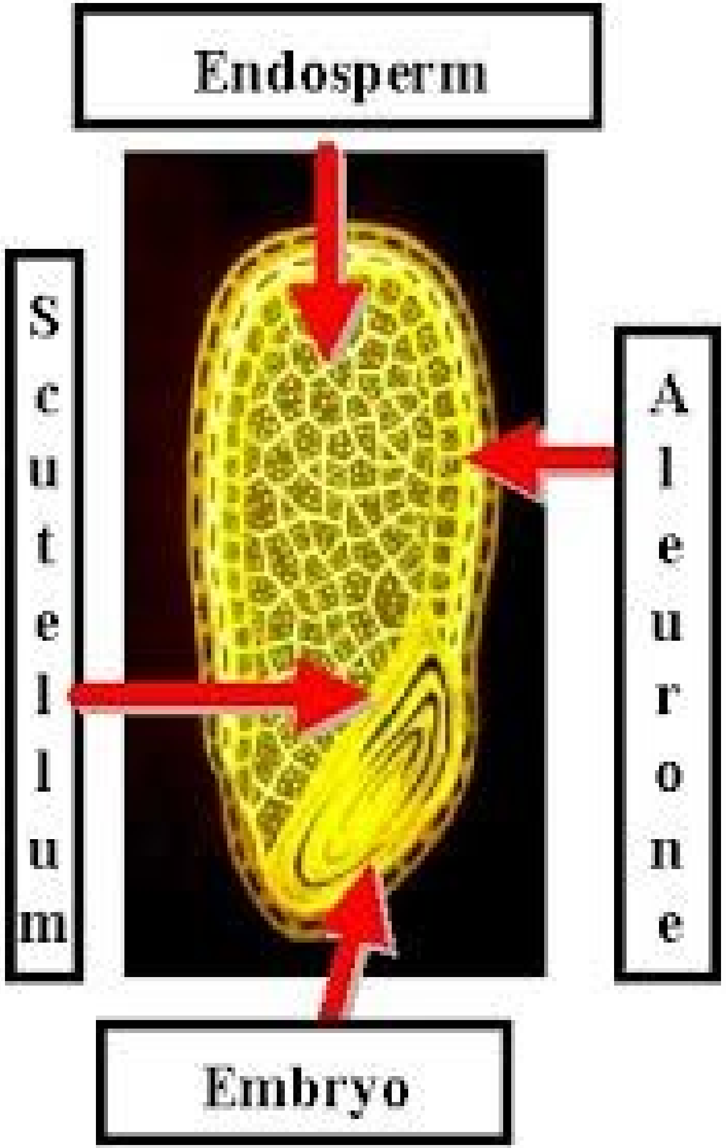
- Occurs through '**Pre-embryogenic determined cells**' (PEDC)

Pre-embryogenic Determined Cells (PEDC)

- Cells are committed to embryonic development.

- Occurrence :

a. Embryonic tissue (eg. Scutellum of cereals)



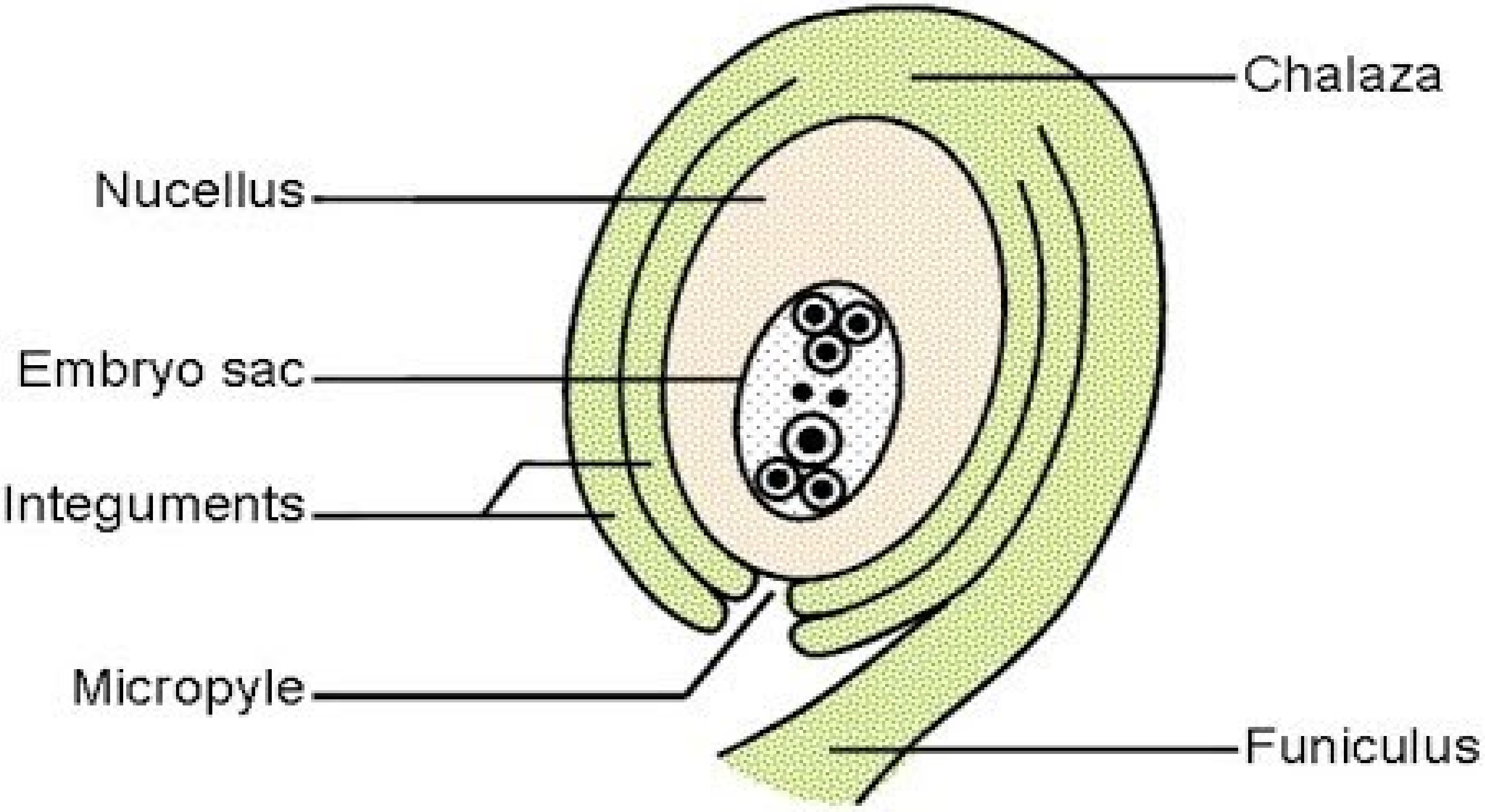
b. Tissues of young in vitro grown plantlets

Brassica napus

Dacus carota

Ranunculus scleratus

c. Nucellus and embryo sac (within ovules of mature plants)



Cross-section through Ovule

Effect Of Auxin On Direct SE

- Addition of growth regulators is **unnecessary**

- Explants are already **embryogenically determined**

- **Low concentration** of auxin is used to **increase the competence** or **no. of cells** which are determined.

Effect Of Auxin On Direct SE

- Less determined tissues – more exposure to auxin

- Embryogenesis was improved by the addition of **20% coconut water**.

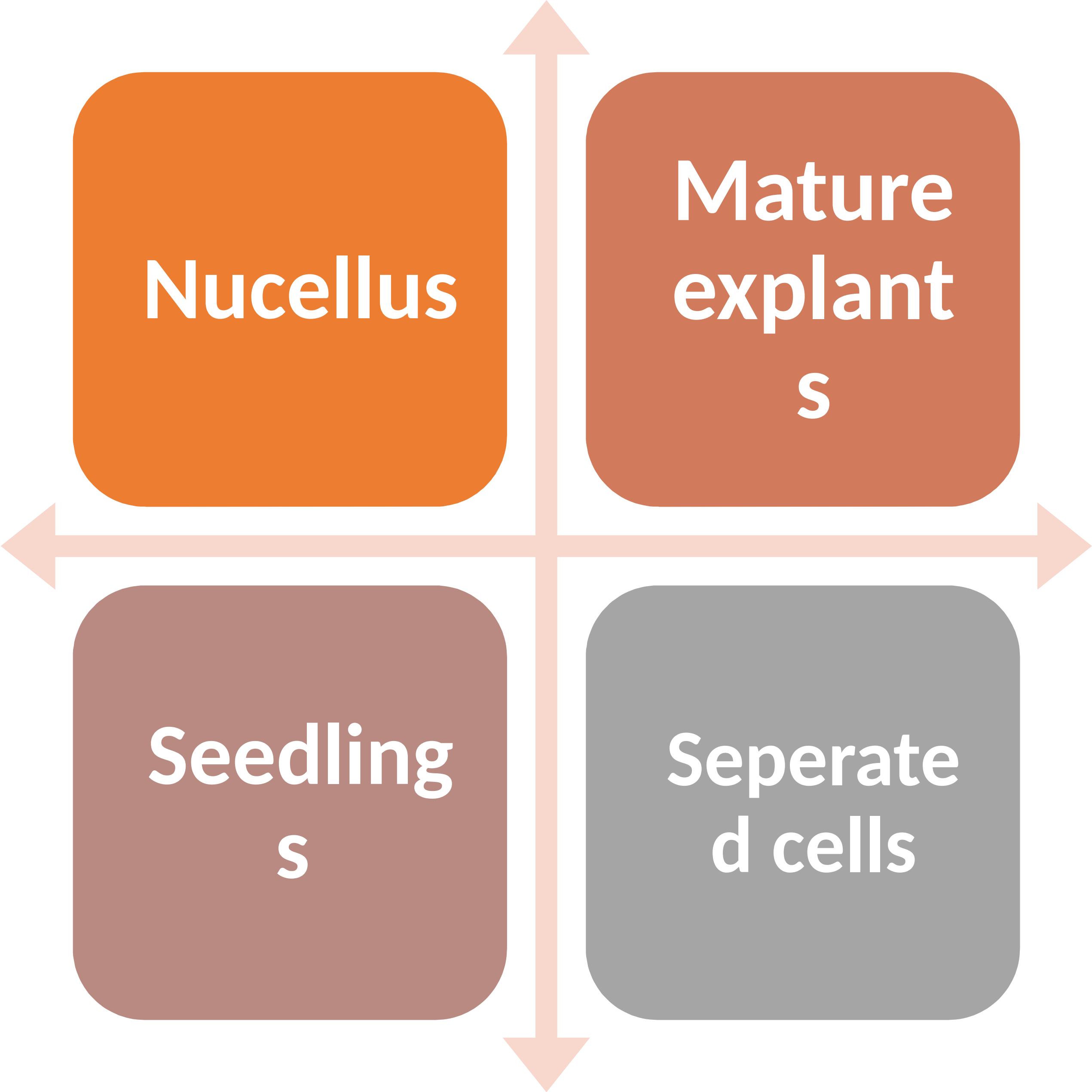
- Advantages of coconut water :

✓ Stimulates **cell division**

✓ Stimulates **morphogenesis**

✓ Suitable p^H ranging between **4.6 – 5.9**

Explants Used In Direct SE



- Plants in which direct SE was done through nucellus are:

Mangifera indica

Pyrus communis

Examples

Plant	Explant	Medium	Sucrose %	Regulants (mg/L)	References
Apium graveolens	Petioles	MS	3	0.5 2,4-D	Zee and Wu (1979)
Brassica alboglabra	Petiole section	MS	3	0.5 2,4-D	Zee and Wu (1979)
Manihot esculenta	Cotyledon, apex, young leaves	MS	2	2-12 2,4-D for 20 days	Stamp (1987)
Solanum melongena	Seedling cotyledons	Kartha et al. (1974)	3	2.5-5 NAA	Fobert and Webb (1988)

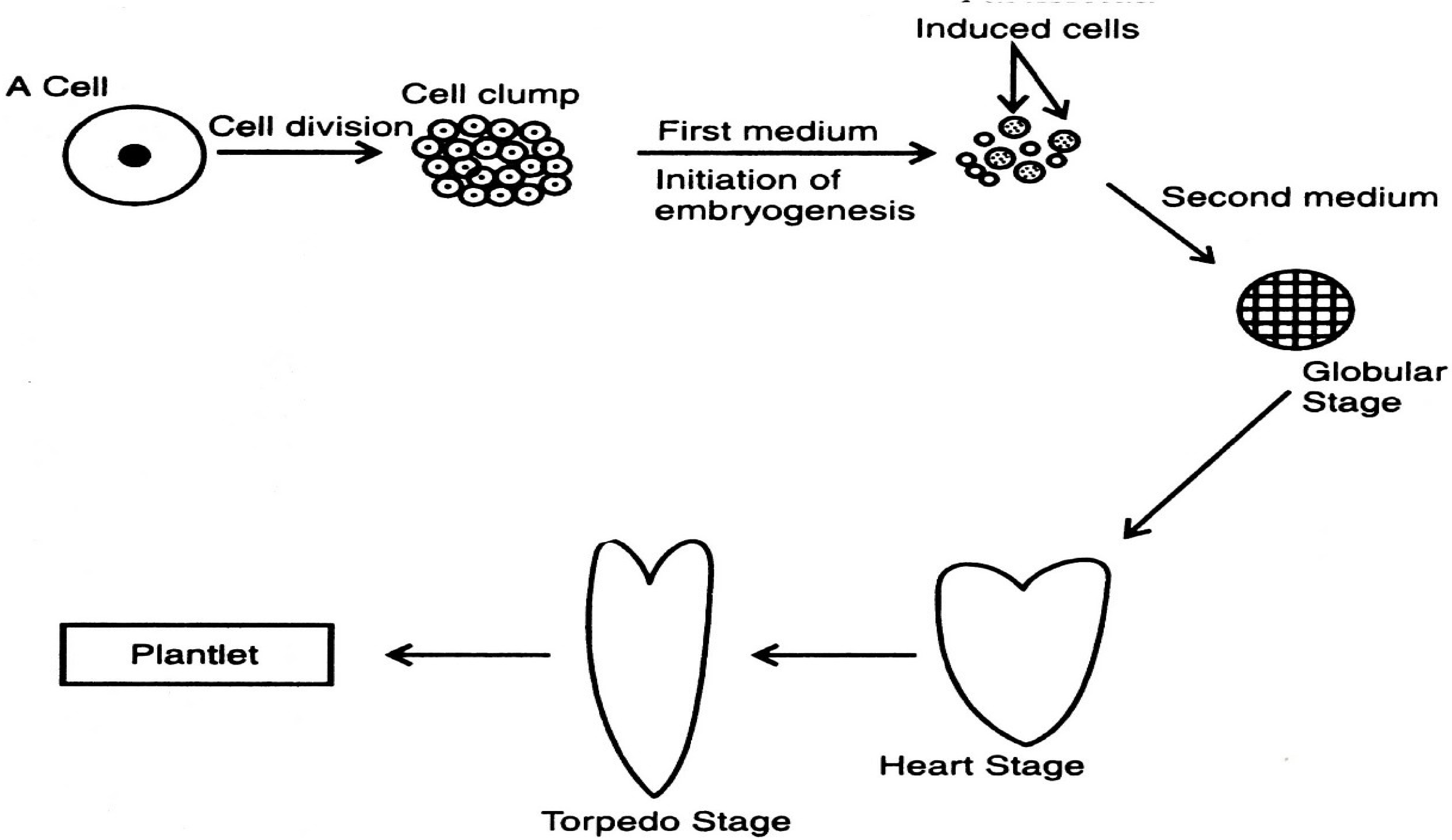
Examples

Plant	Explant	Medium	Sucrose %	Regulants (mg/L)	References
Amaranthus hypochondriacus	Leaf discs	B5	3	2,4-D	Flores *et al*. (1981)
Manihot esculenta	Lobes of young leaves	MS	2	2-12 2,4-D for 20 days	Stamp & Henshaw (1987)
Smilax oldhami	Leaf segments from *In vitro* plants	½ MS	3	1 BAP then 2 2,4-D	Yamamoto & Oda (1992)
Chrysanthemum morifolium	Leaf midribs	Heinz and Mee(1969)	9-18	1 2,4-D + 0.2 BAP	May & Trigiano (1991)

Indirect Embryogenesis

- ***de novo*** formation of embryogenic callus.
- **Induction of embryogenic determination** in previously uncommitted tissue

- Embryo arises from the **callus** of explant
- **Growth regulators** are required for the initiation of callus.



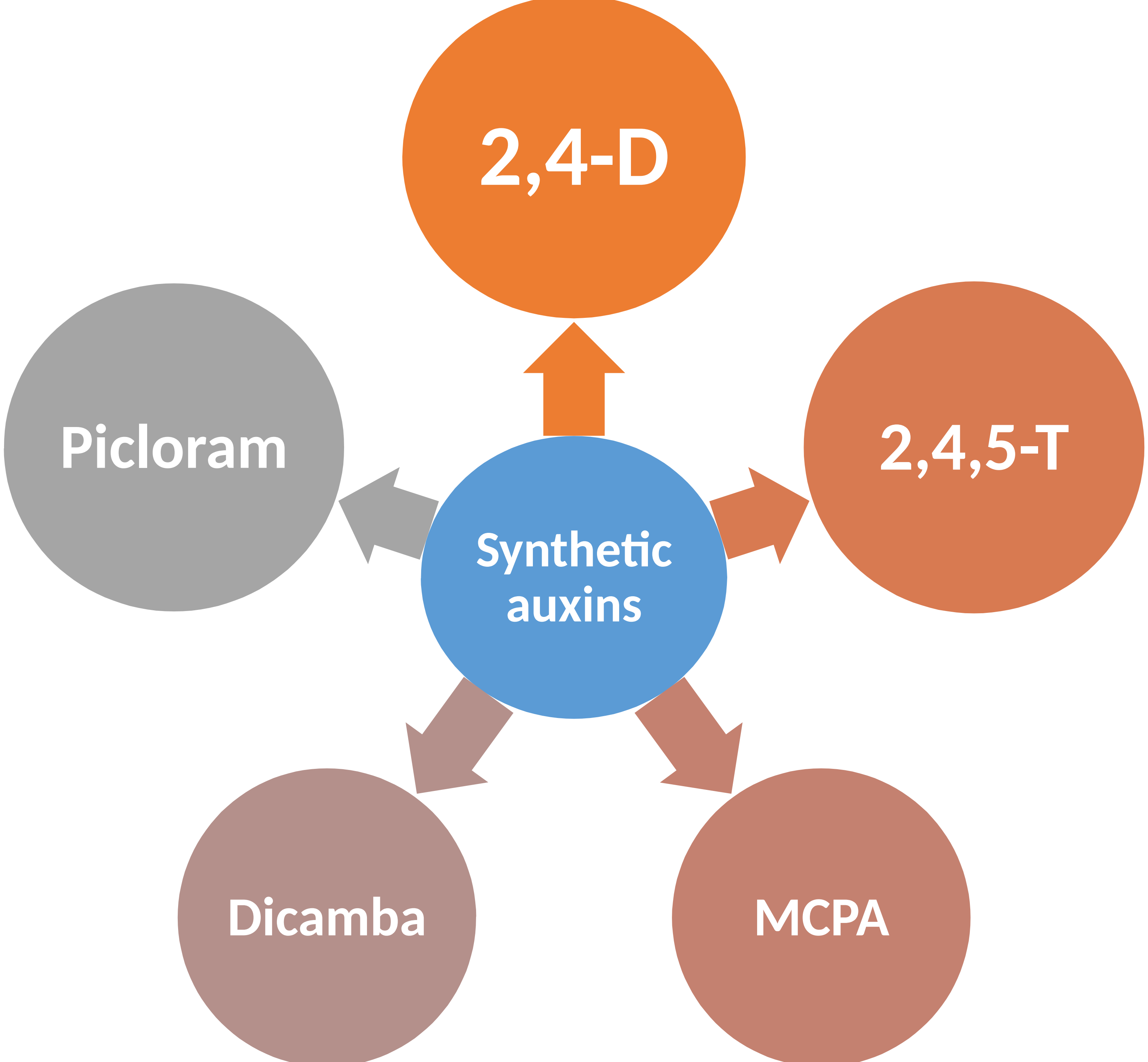
Induced cells
A Cell
Cell clump
Cell division
First medium
Initiation of
embryogenesis
Second medium
Globular
Stage
Plantlet
Torpedo Stage
Heart Stage

Induction Of Embryogenesis

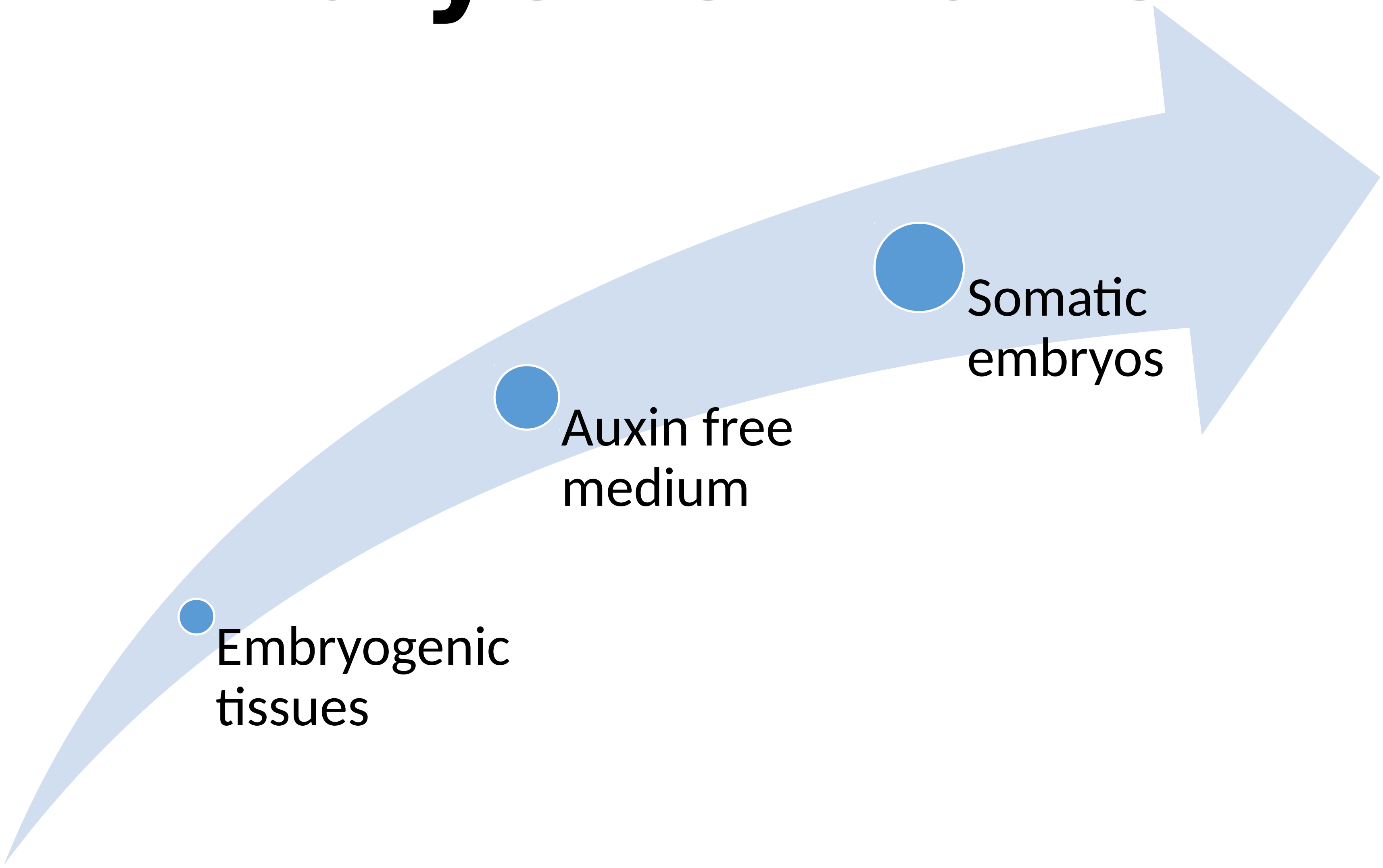
- Controlled by **auxin**

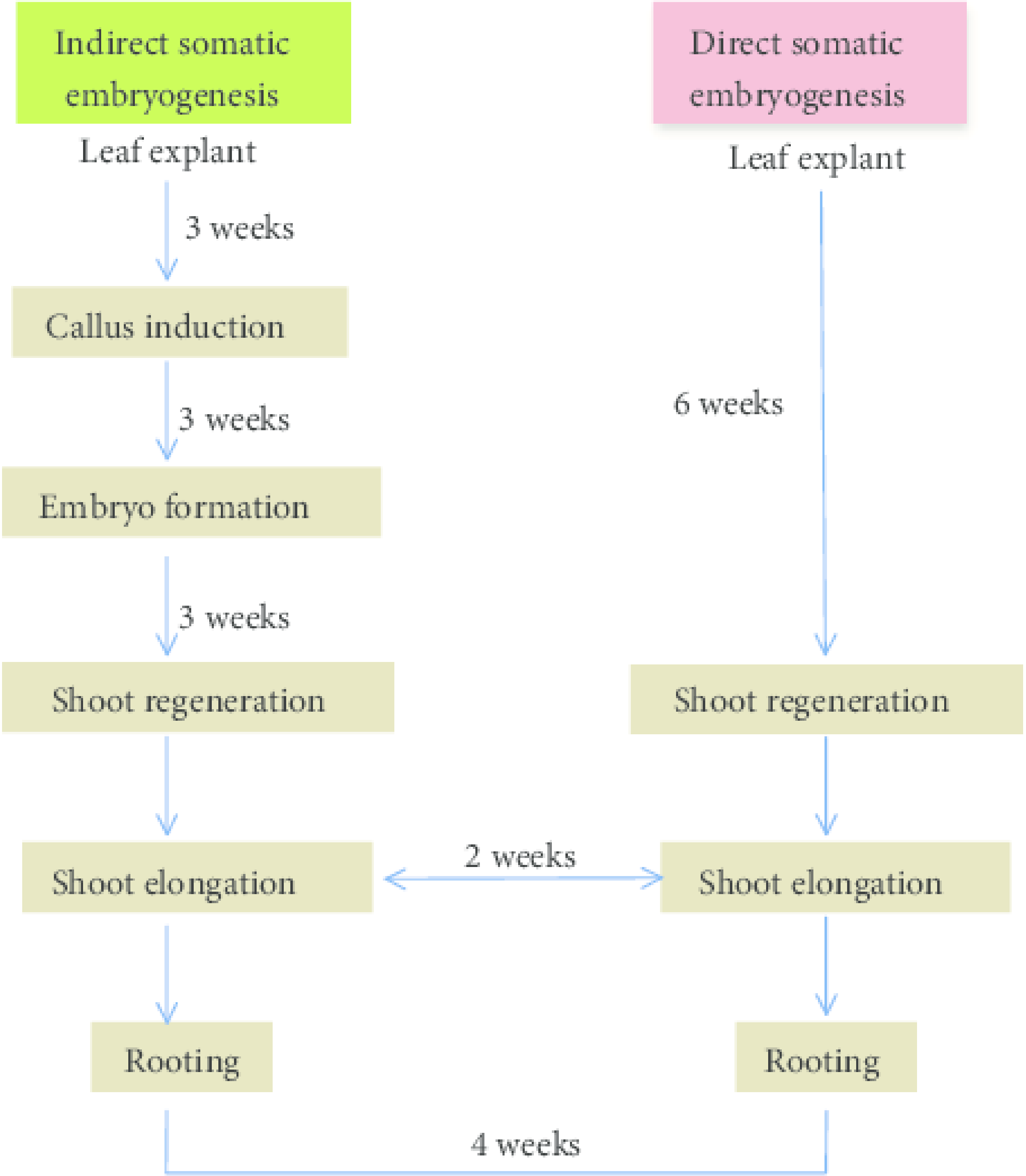
2,4-D

- Formation of proliferating cell clusters
- Possess capacity to form embryos
- Embryogenesis cannot progress until auxin concentration is reduced


2,4-D
Picloram
2,4,5-T
Synthetic auxins
Dicamba
MCPA

Embryo formation



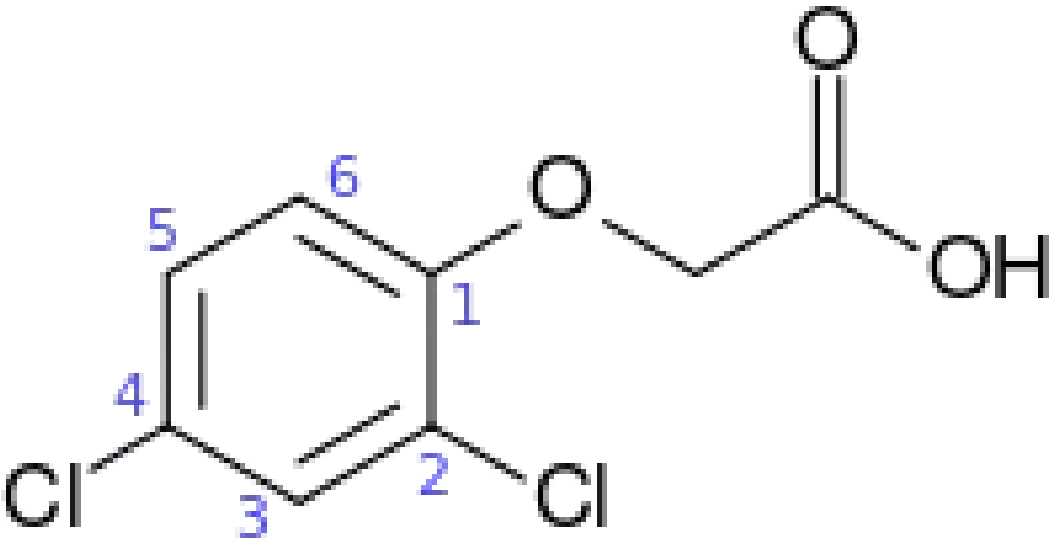


Indirect somatic embryogenesis
Leaf explant
3 weeks
Callus induction
3 weeks
Embryo formation
3 weeks
Shoot regeneration
Shoot elongation
Rooting
Direct somatic embryogenesis
Leaf explant
6 weeks
Shoot regeneration
Shoot elongation
Rooting
2 weeks
4 weeks

Growth Regulators

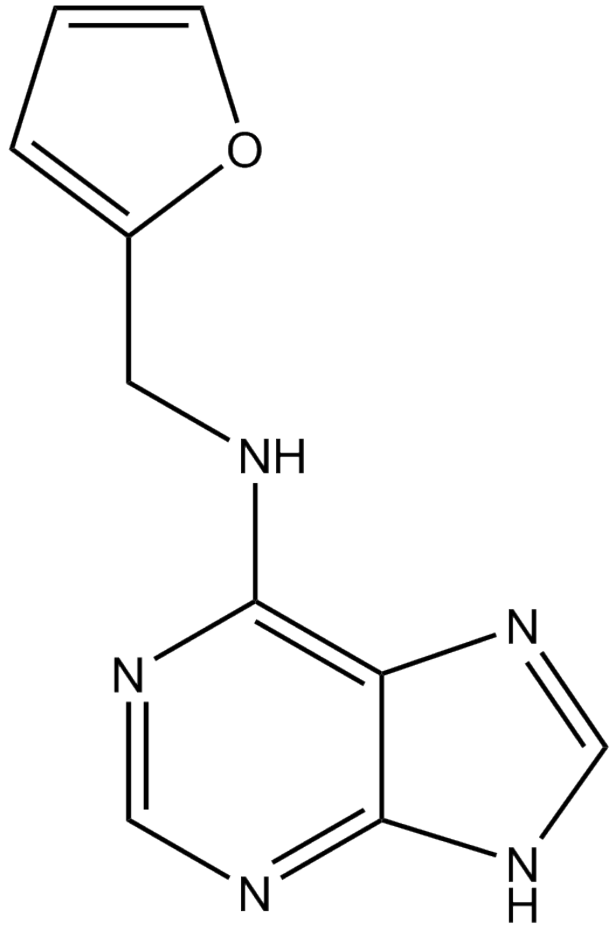
1. Auxin

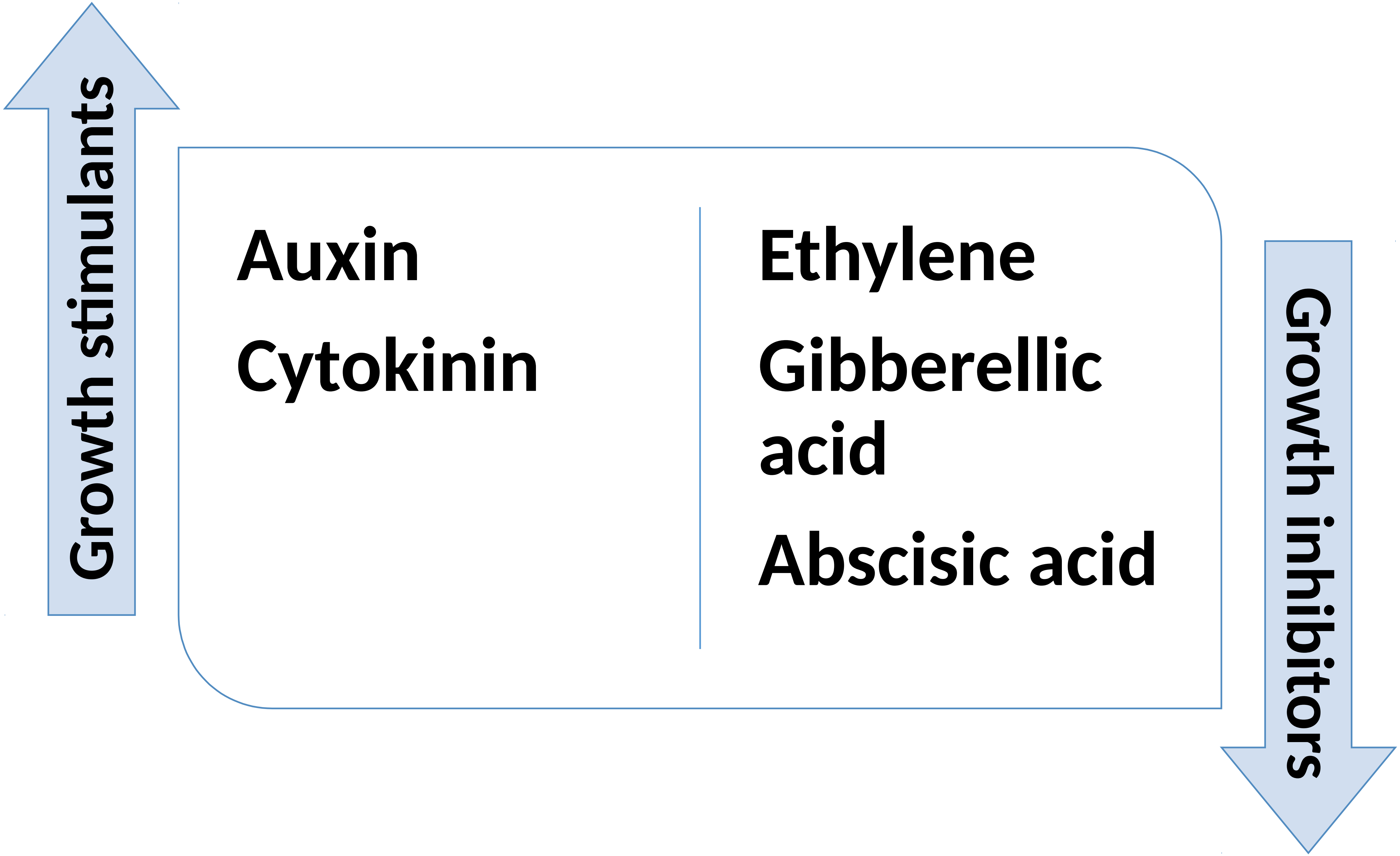
- Onset of growth
- Embryo initiation
- Induces differentiation of embryogenic clumps
- 2,4-D – 0.5-27.6µM
- NAA – 0.5-10.7µM

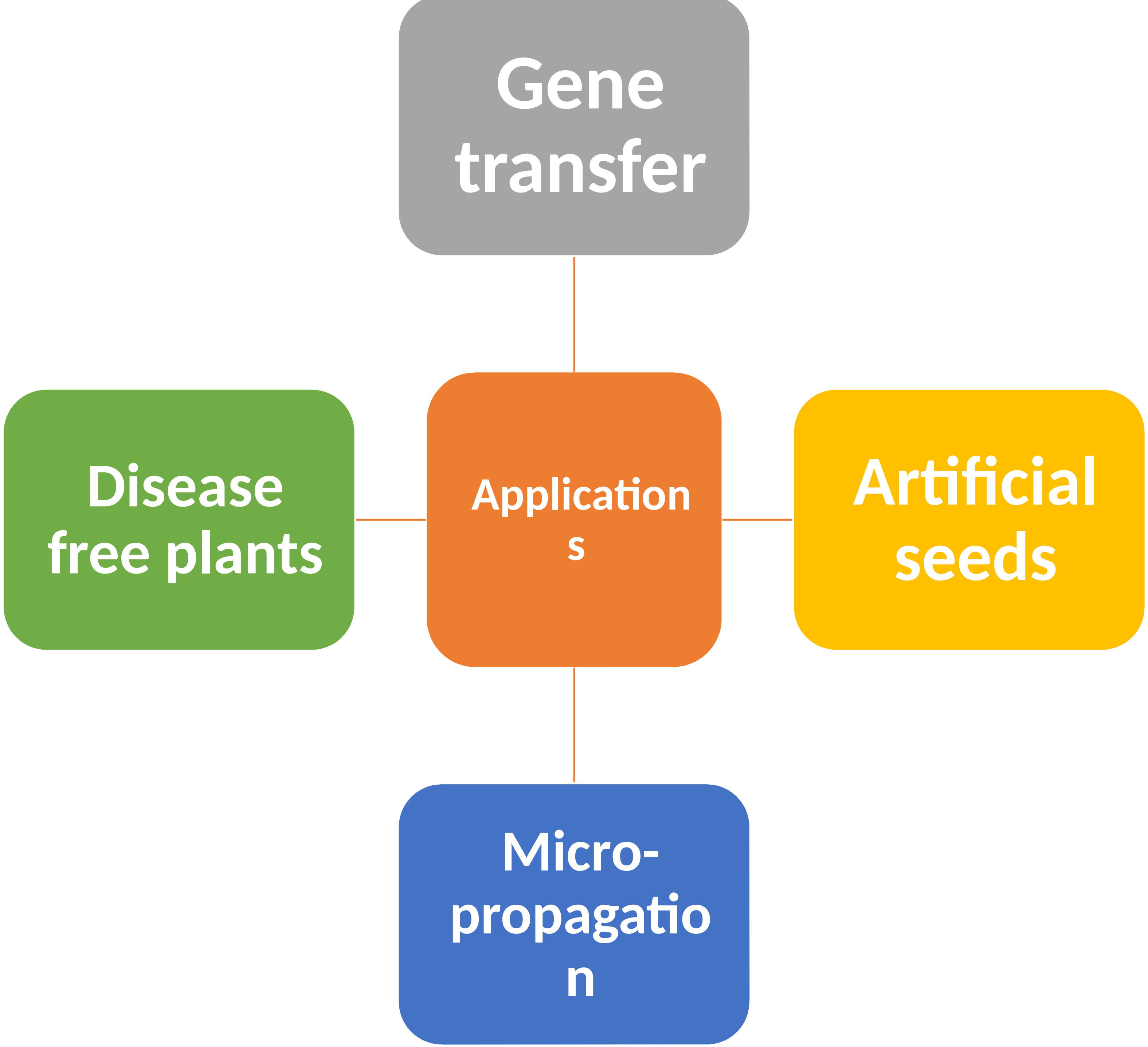


2. Cytokinin

- Used in primary medium in combination with **auxin**
- Fosters **somatic embryo maturation**
- Fosters **cotyledon development**
- Growth of embryos into plantlets
- Eg : BAP, 2iP, kinetin, zeatin




Growth stimulants
Auxin
Cytokinin
Ethylene
Gibberellic acid
Abscisic acid
Growth inhibitors


Gene transfer
Disease free plants
Application s
Artificial seeds
Micro-propagatio n

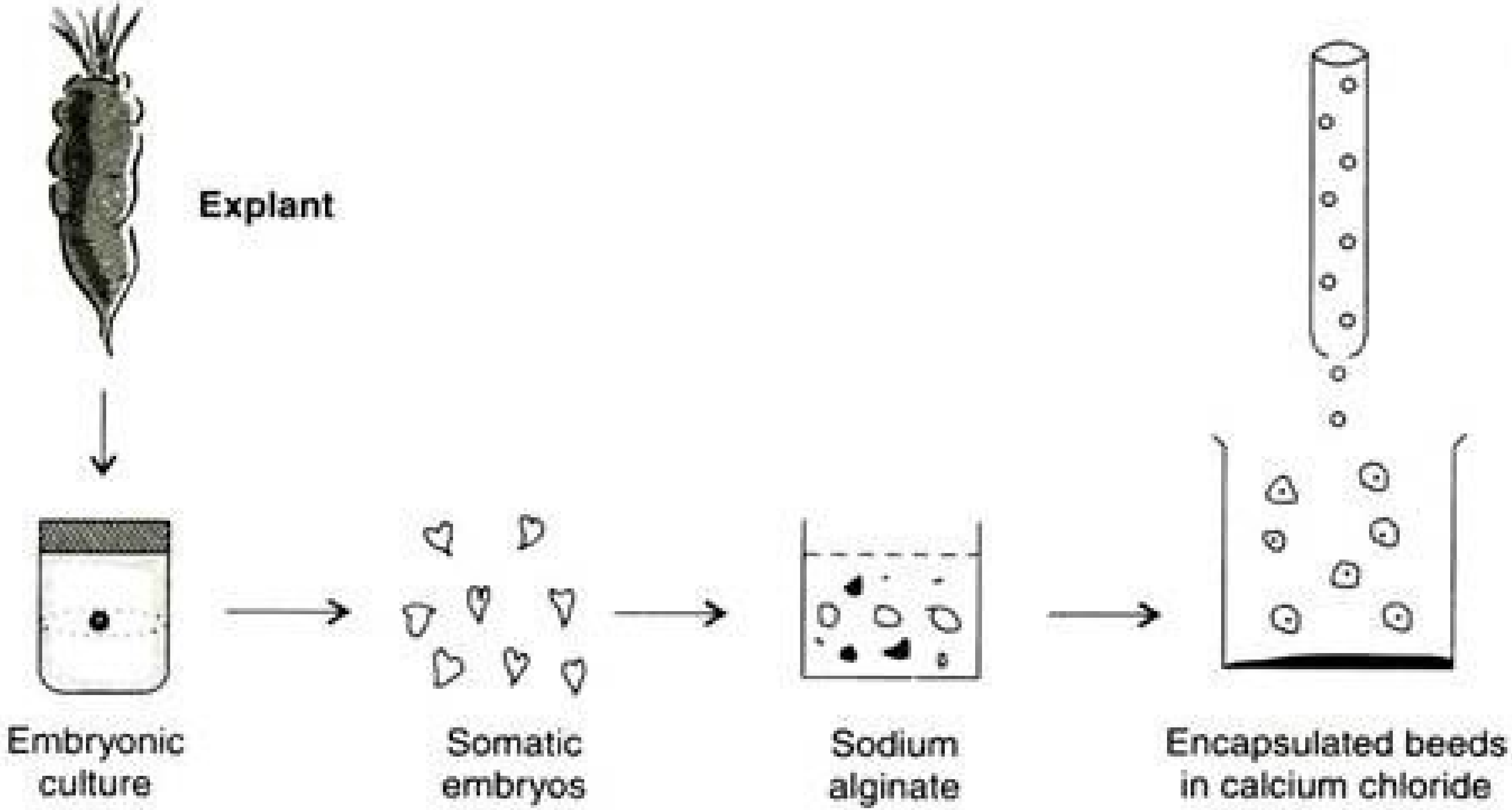


Fig. 8.2 Synthetic seed production

Conclusion

The rapid increase in human population size, environmental pollution, and demand for timber products has put enormous pressure on trees. Development of new technologies for tree propagation, improvement, and breeding can help to solve these problems. This has been achieved in part using biotechnology methodologies like somatic embryogenesis. It can be used for the propagation of various woody plants as well as economically important plant species which in turn will conserve the diminishing biodiversity of flora.