Introduction

Lesson Aim

Explain the nature of plant growth processes, in the tissue culture environment.

STAGES OF PLANT DEVELOPMENT IN TISSUE CULTURE

Plant tissue usually goes through several distinct stages in the tissue culture laboratory:

> The first stage is normally when there is rapid vegetative growth. The aim of this stage is to increase the amount of tissue from which plants can be developed.
>
> The next stage is usually to differentiate the parts of the plant (i.e. stimulate roots etc.).
>
> The final stage is usually a conditioning stage (i.e. hardening up or bringing the tissue into a condition which is better able to be removed from the tissue culture environment.

Each stage will usually require some changes in the growing environment (e.g. a different growing media, light conditions, temperature etc.)

This lesson aims, among other things, to give you sufficient understanding of the way plants grow, so that you can later relate that knowledge to procedures in tissue culture propagation of plants.

HOW PLANTS GROW

Plants are made up of microscopic cells. The cells are able to take in nutrients, water and gases, absorb energy from the sun and store it in chemicals within the cell.

Plants take in food by it soaking (or filtering) through the walls of the cells, and moving in the same way from cell to cell throughout the plant. Air moves into the plant a little differently, through pores (called stomata) which open up on the under surface of the leaves. Dirty or polluted air can clog up these stomata and cause the plant to become starved for air. Once inside a plant air can diffuse or soak into the cells eventually going into solution. Plant takes carbon out of the carbon dioxide in the air (40% of the dry weight of a plant is carbon which comes this way). Some oxygen is used, but most of it is lost back to the outside air, by reversing this whole process.

When rain falls on plants it washes the stomata clean. (Note: this does not occur on indoor or greenhouse plants and they can often benefit from a washing down)

Up to 90% of the normal weight of a plant is water. From this you can see the need for good water supply. Water is normally taken in through the roots, moves up through the plant, some being used, and some being lost through the leaves to the air.

BIOCHEMISTRY

Biochemistry is the chemistry of living organisms. An organism is anything that is alive or was once alive (a '*dead organism*').

What, then, is the condition we call 'life'? We cannot offer a precise definition, but we do know that living things are characterised by metabolism, growth, and reproduction.

Metabolism is the process by which a body introduces into itself ('ingests') various energy-rich materials from its environment ('food'), and transforms these materials, with the release of energy, into other substances, some of which are retained by the body ('growth' or 'repair') and some eliminated.

Reproduction is the process by which one body produces another that is like itself in properties, structure, composition, and function, including metabolism and reproduction.

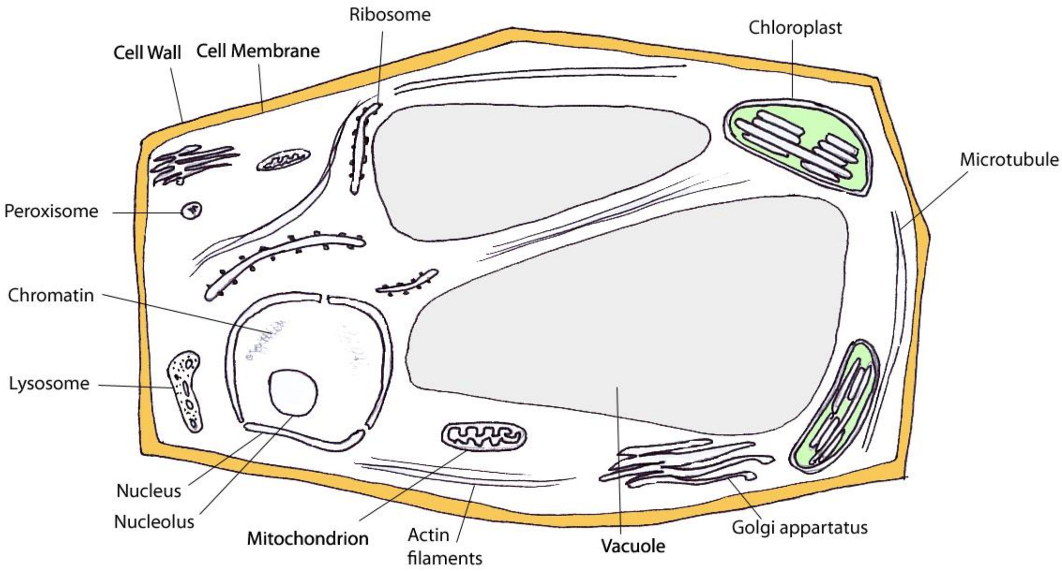
The distinction between an organism and a material is not always clear. A virus consists of particles several hundred angstrom units in length or diameter; these particles can reproduce themselves in a suitable environment but they do not ingest food, or grow, or carry on any other metabolic processes.

Are viruses, then, living organisms or are they chemical materials that consist of large molecules capable of replicating themselves under suitable conditions? To include viruses among the living the definition of life must be modified. Most broadly, we may consider anything living if it can bring order out of disorder at the expense of energy and has the capability to preserve accidental variations (called mutations) that may occur in the process.

In an organism, the structure called the cell may be considered to be a biochemical reactor. An organism consists of one or more cells, and the various groups of cells in a multicellular organism may be sharply differentiated as to biochemical function. The reactions in the cell are said to occur *in vivo* (Latin, 'in the living organism'); the corresponding reactions outside of the cell are said to occur *in vitro* (Latin: in glass).

The living cell is not merely a tiny membranous beaker with homogeneous contents. It is, rather, an entity of great complexity, not yet completely understood as to structure and function.

There are specific sites within the cell at which specific reacting systems, metabolic or reproductive, operate. The biochemist seeks to identify these sites, and to illuminate the course and mechanism of the reactions that occur there. Sometimes he tries to remove a chemically reacting system from its cellular environment and duplicate it in vitro. He does this because reactions are usually easier to study under the more controllable conditions of laboratory reactors than they are in vivo.



Biochemical Processes in the Cell

Several anatomical features are so small that they can be revealed only with the aid of an electron microscope. Some of these fine structures of the cell are *non-essential* inclusions, like globules of fat or particles of starch. Others, called *organelles*, perform *essential* functions and are reproduced when the cell divides. *Some of these functions are well known; others still elude us.*

The mitochondria are organelles shaped like elongated slippers; their cross-sectional diameters are about 1 micron. The highly differentiated structure of a mitochondrion contains some 40 enzymes, which control a complex series of *redox reactions*, including the conversion of diverse organic substances into ATP. The energy reservoir that is thus stored up is available for biochemical work such as muscle contraction, for electrical work like the action of nerve impulses, and for the activation of other biochemical reactions. Because of these functions, the mitochondria have been called, by an analogy that not all mechanical engineers would accept, the "furnace of the cell".

Chloroplasts are organelles that occur in plant cells and that contain the green pigment chlorophyll. *Chlorophyll* is the catalyst for the *endothermic* process of *photosynthesis*, in which glucose is synthesised from carbon dioxide.

The nucleus is a well-defined structure which contains the genetic material of the cell; the nucleus thus is the site of the reproductive function. Each time a cell divides, it reconstitutes itself. This ability of self-duplication is retained by new cells and is transmitted repeatedly through successive generations of cells.

The reliability of this transmittal accounts for the continuity of species.

TRANSPIRATION

Transpiration is the loss of water vapour through the stomata of the leaves. Stomata are small openings in the leaf surface which have guard cells either side. The guard cells can facilitate the openings to be open or closed controlling the flow of water and gasses into or out of the plant.

The movement of water from the roots through the xylem and up to the leaves is called the 'transpiration stream', because transpiration is the main reason for movement along that pathway. This works as follows:

Water evaporates and is lost through stomata on the leaves.

This creates a change in pressure or a lower pressure in the upper leaves, which is evened out by water under higher pressure (further down the plant) moving upwards.

Water will always diffuse or move from cells that have more water into cells with less water.

A chain reaction set in motion by the evaporation from the leaves thus causes movement right along the transpiration stream, and that result in lower levels of water in the roots - that tends to cause water to be absorbed (or sucked) into the roots.

Factors that affect Transpiration and water uptake:

Solar energy	Wavelength variations effect photosynthesis, transpiration and morphology. As radiation increases so too does transpiration, limited by irrigation availability. Variations of season and of the plant itself.
Atmosphere humidity	As humidity increases, water uptake and transpiration decreases.
Wind velocity	As wind velocity increases so too does transpiration.
Temperature	Temperature of the plant effects evaporation. Temperature of the atmosphere (if high, so too is water uptake).
Stomatal aperture	Stomatal aperture (size of opening) is dependent on light, plant water status, carbon dioxide concentration and the temperature.
Available soil water	This is affected by soil type. This effects turgidity which controls stomatal aperture thereby effecting photosynthesis and production.
Tenderness of Plant Growth	Tissue which grows fast is tenderer and will lose water easier and faster.
Amount of Wax - Coating on Plant Tissue	Leaves, stems and other plant parts develop a waxy coating which normally reduces loss of water from plant tissue. Tissues grown in tissue culture do not tend to develop the waxy, protective coat. This will only develop gradually as the plant tissue is removed from the conditions of tissue culture. The removal of the specially manipulated conditions must therefore

	be controlled and gradual, allowing the waxy coating to build gradually

PHOTOSYNTHESIS

Photosynthesis is a process whereby a plant is able to take light energy, store it within a chemical (i.e. a phosphorus compound), then release the same energy by way of a chemical reaction, for later use. Photosynthesis is very important to plant growth, and normally there is a direct relationship between the rate of photosynthesis and the rate of growth.

Environmental factors which effect photosynthesis include:

Light	As a general rule, as light increases so too does photosynthesis. In relation to C3 and C4 plants, C4 plants will continue to photosynthesise in higher light intensities whereas C3 plants stop its rate of photosynthesis at its saturation point.
Carbon dioxide concentration	As CO_2 increases, so too does photosynthesis.
Temperature:	Photosynthesis will increase with temperature until a point of equilibrium is met. At this point photosynthesis stays uniform as temperature increases. Growth is defined as photosynthesis minus respiration. As temperature increases, respiration will continue to increase, therefore at some point respiration will exceed photosynthesis resulting in no net growth.
Water balance:	Stomatal aperture is controlled by light energy, turgor pressure of the surrounding guard cells, temperature, CO_2 concentration in the stomatal cavity, and chemicals such as anti-transpirants.
Leaf age	As the leaf becomes older in age, the photosynthetic capacity of the leaf decreases.
Environmental history	Longitude, latitude, and elevation all effect photosynthesis.

RESPIRATION

Respiration in a plant is when:

- Energy stored in plant tissue is released.
- That energy was originally captured and stored through the process of photosynthesis.

- Respiration is therefore the reversing of the photosynthesis process: Carbohydrate (e.g. sugar) reacts with water and oxygen to produce energy plus carbon dioxide and water (NB. The amount of water produced is greater than what is started with)
- Respiration is a relatively slow step by step chemical process where the degradation of carbohydrate occurs slowly, releasing small amounts of energy in each step, slowly over a period of time.

The Rate Of Respiration Is Affected By:

Oxygen Available	At lower oxygen levels, there is a lower rate of respiration. Fruits and vegetables are sometimes stored in environments where the oxygen in the air has been lowered (Through pumping in nitrogen or carbon dioxide). This reduces the respiration rate, hence preserving carbohydrate in the tissue and helping the produce to be kept longer.
Temperature	Respiration is slower at lower temperatures. At 0 Celsius respiration occurs at less than half the rate it does at 10 Celsius. Most plants grow better if night temperatures are lower than day temperatures because: At night respiration slows allowing more of the energy stored by photosynthesis during the day to be retained.
Soil Moisture	If a soil is over wet, the water reduces the oxygen available to plant roots which in turn reduces respiration in the roots and results in poor growth.
Light Intensity	In shaded conditions plants generally have lower respiration rates because: - lack of light reduces photosynthesis, thus reduces the carbohydrate produced - in turn, if there is less carbohydrate, there are less carbohydrates available for respiration

PARTS OF A PLANT

The majority of plants grown in horticulture are flowering plants. These plants have four main parts:

- Stems - the framework.
- Leaves - required for respiration, transpiration and photosynthesis.
- Roots - the parts which grow below the soil.
- Reproductive Parts - flowers and fruits.

Stems

The main stem (and its branches) is the framework that supports the leaves, flowers and fruits. The leaves, and also green stems, manufacture food via the process known as photosynthesis, which is transported to the flowers, fruits and roots. The vascular system within the stem consists of canals, or vessels, which transfer nutrients and water upwards and downwards through the plant (i.e. this is equivalent to the blood system in animals).

Stems may be modified for a variety of reasons. Some modifications are:

- Tendrils
- Thorns
- Stolon or runners - above ground
- Rhizome - below ground
- Stem tubers - e.g. potato
- Corm - function as a food storage to carry the plant over till next season e.g. gladioli.

Leaves

The primary function of leaves is *photosynthesis*, which is a process in which light energy is caught from the sun and stored via a chemical reaction in the form of carbohydrates such as sugars. The energy can then be retrieved and used at a later date if required in a process known as respiration. Leaves are also the principle plant part involved in the process known as transpiration whereby water evaporating, mainly through the leaf pores (or stomata), sometimes through the leaf cuticle (or surface) as well, passes out of the leaf into a drier external environment.

This evaporating water helps regulate the temperature of the plant.

This process may also operate in the reverse direction whereby water vapour from a humid external environment will pass into the drier leaf. The process of water evaporating from the leaves is very important in that it creates a water gradient or potential between the upper and lower parts of the plant.

As the water evaporates from the plant cells in the leaves, then more water is drawn from neighbouring cells to replace the lost water. Water is then drawn into those neighbouring cells from their neighbours, and from conducting vessels in the stems.

This process continues, eventually drawing water into the roots from the ground until the water gradient has been sufficiently reduced. As the water moves throughout the plant it carries nutrients, hormones, enzymes etc. In effect this passage of water through the plant has a similar effect to a water pump, in this case causing water to be drawn from the ground, through the plant and eventually out into the atmosphere.

Leaf modifications include:

- **Stipules** - at the base of the petiole e.g. peas.
- **Bulbs** - storage tissue e.g. daffodil.
- **Tendrils** - the leaf is modified into a tendril. Identifiable due to the bud at the base of the tendril with frequently large stipules

- **Thorn**
- **Phyllode** - characteristic of Acacias where the lamina is very small and the petiole is enlarged
- **Pulvinus** - swelling at the base of the leaf and leaflets, provides the ability to allow movement by turgidity e.g. *Mimosa pudica*
- **Auricle** - ear like appendage on grasses

Roots

Soil provides the plant with the following things:

- Nutrients
- Water
- Air
- Support

Roots absorb nutrients, water and gasses transmitting these 'chemicals' to feed other parts of the plant. Roots hold the plant in position and stop it from falling over or blowing away.

Plant nutrients can be supplied, broadly speaking, in three different forms:

Water soluble simple chemical compounds **-** Nutrients in these compounds are readily available to plants (i.e. the plant can absorb them quickly and easily)

Less soluble simple chemical compounds - The nutrients in these compounds can be used by plants without needing to undergo any chemical change, but because they don't dissolve so easily in water, they aren't as readily useable as the more soluble compounds. The diminished solubility may be because of the nature of the compound (e.g. superphosphate) or may be due to something else (e.g. Slow release fertilizers such as Osmocote™, which is made by incorporating the simple chemicals inside a semi-permeable bubble -thus nutrients move slowly out of the bubble). This second group of nutrients when placed in soil will last longer than the first group of water soluble nutrients.

Complex chemical compounds - These require chemical changes to occur before the nutrients can be absorbed by plants. They include organic manures and fertilizers which need to be broken down by soil micro-organisms into a form which the plant can use. They also include other complex fertilizers which need to be affected by natural acids in the soil, or heat from the sun, to become simple compounds which the plant roots can use. Complex chemicals release their nutrients gradually over a long period of time, depending on the range of chemical changes needed to take place before the plant can use them.

Plants grown in a soil derive their nutrients from all three types of compounds. The availability of these compounds varies according to not only the group they come from but also according to factors such as heat, water, soil acids and microorganisms present. As such, it is impossible to control the availability of nutrients in soil to any great degree.

Reproductive Parts

Flowering plants reproduce by pollen (i.e. male parts) fertilizing an egg (i.e. female part found in the ovary of a flower). The ovary then grows to produce a fruit and the fertilized egg(s) grow to produce seed.

Buds

Buds are swellings from which vegetative growth (shoots of stems and leaves), or sexual growth (flowers) emerge. Some buds contain develop both flowers and shoots from the same bud. These are called mixed buds.

A bud may be positioned *apically*, *axillary, embryonic, adventitious* or *auxiliary/epicormic* (such as with Eucalypts).

When cut open and examined under a magnifying glass or microscope the "premature growths" of new shoots or flowers can be seen inside a bud.

Typical Angiosperm Buds

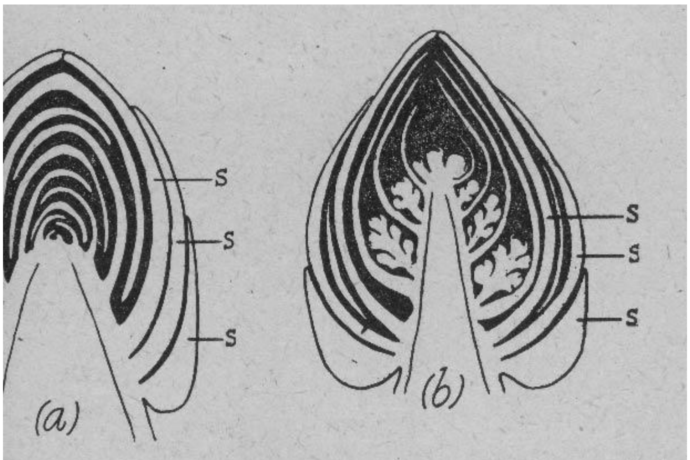


FIG. 27
ds of lilac: *a*, a leaf bud; *b*, a flower bud. S = scales. (x 7·5)

a – Leaf Bud   b – Flower Bud s- Bud Scales

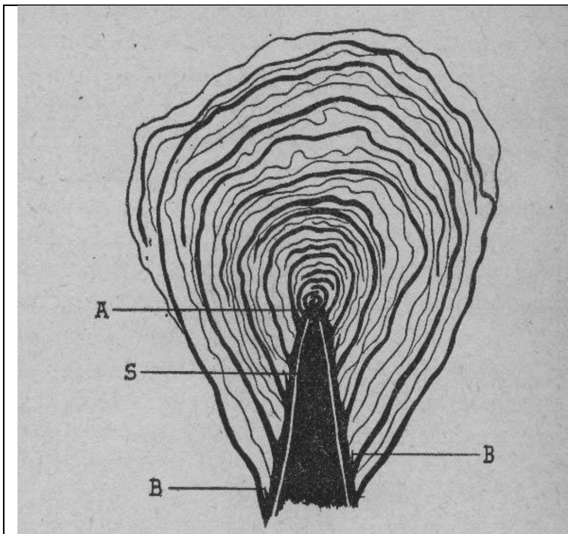


FIG. 21
A lettuce bud. A = apex; B = axillary bud; S = stem. (x 0·5)

Lettuce Bud

WHAT HAPPENS AS PLANT TISSUE MATURES?

Structural Characteristics occurring as a plant matures include:

- Cuticle becomes thicker on leaves.
- Bark may peel or split off.
- Leaf shape and thickness - Heterophylly is where younger leaves are different shapes to the mature leaves.
- Phyllotaxis - this is the arrangement of leaves along the stem. Many plants vary this feature as the plant grows and matures.
- Thorniness and shoot orientation - thorns on juvenile citrus which become more upright when mature plants.
- Branch number and pattern.
- Shoot growth and vigor - some plants are very vigorous when they are juveniles but are less vigorous when mature or reproductive.
- Seasonal leaf retention and pigmentation – e.g. copper beeches - top branches lose leaves while juvenile lower leaves are retained.
- Ability to form adventitious roots and buds.
- Partitioning of photosynthesis.
- Cold resistance - young trees and woods do not have high cold tolerance.

TYPES OF PLANT TISSUE

Parenchyma: forms the ground tissue of plant bodies. The cells are closely or loosely packed together and is permeated by extensive systems of inter air spaces. It is capable of reverting to *meristematic* tissue if wounded. Some specialisation can occur for photosynthesis, aeration, food storage or even water storage.

Mechanical Tissues: provides the support, strength and protection for tissues. There are two main tissues:

1. Collenchyma - functions as a support in young plants and capable of resuming meristematic activity. Three types: angular, lacunar and lamella.
2. Sclerenchyma - rigidity, support and sometimes protection. Two types are:
   Sclereids (stone cells) = short cells, for protection.

   Fibres = very elongated cells, for support in mature regions.

   * Xylary - found in xylem only.

   * Extraxylary - found anywhere except xylem. Occur in groups called 'caps' near vascular bundles. Also occurs in phloem.

Vascular Tissues: for the carrying of nutrients and water within the plant. Two main tissues:

1. Xylem - conducts water and inorganic salts in a continuous upstream from roots to leaves. There are four types of tissues: tracheids, vessel elements, xylary fibres, and parenchyma. The first two function for the translocation process.

2. Phloem - conducts organic solutes around the plant. This is a compound tissue having four types: 1. Sieve tube element; 2. companion cells; 3. fibres / sclerieds; 4. parenchyma.

Meristematic Tissues: is a specific region where active cell division continually occurs from which a permanent tissue is developed. It is commonly referred to as the growth region. There are three regional meristems:

1. Apical - at the apex of stems or roots, provides growth in length in height. This is present in all plants.
2. Lateral - lies parallel with the surface of roots or stems, provides growth in the width and girth of plants. Not present in all plants.
3. Intercalary - found in grasses, results in grasses capable of re-growth when cut.

METHODS OF SHOOT INDUCTION AND PROLIFERATION

Vegetative propagation is achieved through the formation and multiplication of shoot meristems, each meristem being a potential plant. *In vitro* cultures used for propagation may be started from either:

(a) Existing meristems - the main shoot, the embryo, or subsequently formed axillary shoots, or

(b) Organ explants suitable for the induction of adventitious meristems.

When shoot tips are cultured on medium containing cytokinin, axillary shoots often develop prematurely and are followed by secondary then tertiary etc. shoots in a proliferating cluster. Once such a cluster has developed sufficiently, it may be divided up into smaller clumps of shoots or separate shoots which will form similar clusters when subcultured on fresh medium. Provided the basic nutrient formulation is adequate for normal growth, this process may apparently be contained indefinitely.

Within limits, the rate of proliferation may be controlled by the type and concentration of cytokinin used. Cytokinin almost always inhibits root formation, thereby making the shoot clusters more easily separable at each subculture. Rooting often occurs spontaneously on transfer to medium lacking cytokinin.

Multiplication by adventitious roots

In a number of species, shoots arise naturally from mature organs, particularly on leaves, stems and roots. Almost every type of organ can be used as a cutting. Tissue culture has enabled this to occur in many more species.

An in vitro callus is an unorganised mass of proliferation cells and may be obtained from almost any type of plant. Callus formation from explants is occasionally spontaneous (like a wound reaction) but generally requires auxin in the medium, often in combination with cytokinin.

The concentrations of the hormones used vary with the species and organ used, but tends to be higher than those needed to induce shoots directly from the explant where this is possible.

On lowering the hormone levels or adjusting the auxin: cytokinin ratio, some calluses will regenerate shoots or embryos. This method, however, cannot be repeated indefinitely because with repeated subculture, the capacity of many calluses to regenerate shoots is diminished or lost.

RESOURCE FILE

As you progress through this course of study you are required collect and collate information on the subject into a resource file.

This file will be submitted for assessment with your lesson 9 assignment.

The submitted file must include a profile of at least twenty (20) suppliers of environmental control equipment for use in tissue culture production. The profile of each supplier should ideally be kept on standard card file cards (126 x 76 mm) for ease of handling or may be submitted as a printout of a database file.

Each card should contain the following information:

Name of the supplier.

Address, phone number and contact person.

Outline of what the supplier does/sells.

Any other comments which may be relevant.

The purpose of the resource file is two- fold:

1. To develop your awareness of the industry and different types of environmental control equipment.
2. To develop contacts that might help you with working in the tissue culture industry. You may develop contacts which could eventually lead to work opportunities.

You will also build a file which can help you find information, supplies and facilities quickly and easily when they are needed in your day to day work.

Begin compiling this file now.

Submit at least 3 cards or sheets with your lesson 1 assignment so that the tutor can verify you are compiling the information correctly.

Submit the completed collection of cards/sheets with your lesson 9 assignment.

GLOSSARY

Abscission - Normal separation of leaves or fruit from a plant using a thin walled layer of cells.

Adventitious - Growth from unusual locations (e.g. aerial roots)

Agar - A polysaccharide gel, obtained from some types of seaweed.

Amino Acids - the structural units of proteins

Anther Culture (or Pollen Culture) - tissue culture of anthers to obtain haploid clones

Apical - tip or apex

Apical Meristem - Meristem located at the tip of main or side shoots.

Aseptic - Free from any disease or other microorganisms.

Asexual - without sex, vegetative

Autoclave - A pressurized container, used to sterilize equipment

Auxins - Hormones which cause cell division & enlargement and particularly, root initiation.

Callus - An unorganised growing mass of cells; not differentiated to be any particular type of cell or organ.

Clone - plants produced asexually from a single plant

Culture - A plant that is growing in vitro

Contaminants - Any type of microorganism

Cytokinins - Hormones which induce bud formation & cell multiplication.

Deionise - to remove ions from water by the use of ion exchange resins

Differentiate - the modification of new cells to form tissues

Diploid - having two sets of chromosomes which is typical of vegetative (somatic) cells

Embryo Culture - culture of embryos excised from immature or mature seeds

Embryogenesis - formation of the embryo

Embryoids - embryo-like vegetative structures developing in some cell and callus cultures sometimes capable of developing into embryos

Excise - to remove by cutting

Explant - the part of the plant used to start a culture

Gibberellins - a group of growth regulators influencing cell enlargement

Haploid - having half the normal number of chromosomes in vegetative cells

Hormones - natural or synthetic chemicals that strongly affect growth

Hybrid - the plant from a cross between two different cultivars

Juvenility - Growth stage of a seedling before it becomes able to produce a flower.

Laminar Air Flow - Controlled, regular flow of air in one direction, with no turbulence.

Medium - Agar gel or water, containing nutrient & hormone, in which the tissue culture grows.

Meristem - Undifferentiated types of cells found at the growing tips of roots or shoots (also found in the cambium).

Micropropagation - multiplication in vitro, used interchangeably with tissue culture

Polysaccharide - A group of carbohydrates that comprise a number of different sugars.

Primordia - (pl. of primordium) plant organs in their earliest stages of differentiation

Protoplast - a cell without a cell wall but with a membrane

Protoplast Fusion - the uniting of two protoplasts

Regeneration - the production of new plants or parts of plants

Somatic - vegetative, as opposed to sexual

Somatic Hybridisation - the creation of hybrids by vegetative means i.e. protoplast fusion

Totipotence - capable of developing into a whole plant. Undifferentiated cells or tissues that are not yet modified for their ultimate role (not differentiated)

SET TASK

Cut open a few buds off plants. Look for large buds. Use a razor blade. Look at these buds either under a microscope or a magnifying glass. Draw what you see.