
The Tissue Culture Techniques And Their Applications Used in Today's World

Plant tissue culture is a set ,group,or group of system that are performed to grow and generate new plants under germ free and controlled environment.They generate clones and hybrids that have different desirable traits. It helps to quickly produce mature plants with desirable characters. Produces multiple of plants in the absence of seeds. It helps to regenerate whole plant using explant. They produce plants in sterile containers that allows them to eliminate various types of pathogens,bacteria and diseases to get transmitted.

They produce plants from seeds that have very low chances of germinating and growing. Plant tissue culture depends on the fact that cells have the ability to regenerate a whole plant .Totipotency is a mechanism in which a single cell have the ability to divide and transform into a new individual.

APPLICATIONS:

It is widely used in biotechnology, plant science, forest research,etc.

To conserve rare or endangered plant species.

A plant is generate from tissue culture and can generate more hybrid species.

They breed distantly related species by using these techniques.

They culture the final embryo that would have die if not taken care of.

Generate identical sterile hybrid organisms.

CHOICE OF EXPLANT

It is the tissue that is extracted from a plant and further it will be used to culture more species.

It can be obtained from different parts of the plants that is the shoot ,root ,stem. leaves, bud,fruit,and the flowers.

Totipotency is the ability of a cell to divide and divide more to transform into a new individual.

examples; spores and zygotes.

The choice of explant is done whether it is a haploid or a diploid organism.

In case if the explant are not proper then the changes of exposure to germs increases.

TECHNIQUES:

A free from contamination condition is used to culture tissue from plant tissue culture techniques. In laminar air flow cabinets there are HEPA filters presents that allow to provide use the germ free environment. These filters stop the entry of germ by providing the high speed of air into the cabinet and in case if some germs are capable to enter then these are kill by UV radiations.

The explants are sterilised before using them so that no contamination is observed. The media prepared first is in liquid form but this media is solidify by adding gelling agent to it so that is get solidified. The hormones are provided in balanced proportions. The solid media is composed of hormones, nutrients, minerals which are important for growth. For performin tissue culture one should have proper skill and experience. They explants are placed in culture tubes and after few time we can observe the growth of new plants and then we can plant them into the soil.

GREENHOUSE:

A greenhouse is a specialized room or area in which we provide special and controlled conditions to avoid the extreme climatic conditions. So that plants can be provided with appropriate conditions to grow .

USES:

- greenhouses provided a better environment for growth of plants.
- these provide shade in extreme climates.
- these sre used to grow flowers ,vegetables,fruits.
- there are some special greenhouses that are used to grow special varieties of crops.
- there are alpine houses that specially grows the alpine plants.

GREENHOUSE EFFECT:

Greenhouse effect is the trappment of sun radiations and its heat. These are easily trapped by some gases called greenhouse gases.

LABORATORY GLASSWARE:

Glassware are used in wide range now a day because the laboratory work is increasing. The glassware are first sterilized to avoid the germs, therefore when these get germ free then they are appropriate to be used in the labs.

The glassware are mainly made of borosilicated, silanised, or quartz glass. The glass are melt and converted to different shapes and sizes. These used for different works in lab.

Firstly these are made strong and able to with stand the stress of chemicals and heat and chemical composition.

In many labs there are tutorial done for making people understand how to use them more appropriately and with safety, the basically explains the rules and regulations of the glasswares.

LABORATORY GLASSWARE SELECTION:

The selection is done by a incharge of laboratory, they tests the capabilities of the instrument by test them through some challenges.

Laboratory glassware are made from several types of glass, each with different capabilities and used for different purposes.

Borosilicated glassware, quartz glass, fritted glass, Silanized.

GLASSWARE INCLUDES:

Different glassware are used for different purposes

- **BEAKERS**
- **FUNNELS**
- **GRADUATED CYLINDERS**
- **VOLUMETRIC FLASKS**
- **PIPETS**
- **TEST TUBES**
- **BUNSEN BURNER**
- **SLIDES**
- **COVERSLIPS**
- **INOCULATING LOOPS**
- **NEEDLES**

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- WATER BATHS
 - BURETTES
 - DROPPERS
 - SPOT PLATES
 - EVAPORATING DISHES
 - MORTAR AND PESTLE
 - CRUCIBLE
 - AUTOCLAVES BAGS
 - SYRINGES
 - FORCEPS

SOME MAJOR INSTRUMENTS ARE AS FOLLOWS:

- **BEAKERS:**These are glass cantainers that comes in a variety of sizes and can be used for mixing and transporting chemicals during a reaction.They are made up of different types of glasses.(borosilicated,siliconized)
- **FUNNELS:**Glass funnels are used against spillage when pouring chemicals to large instruments.These also available in plastic.Plastic funnels are used for light chemicals or used to pour water,cause highly acidic chemcals can burn the plastic.
- **GRADUATED CYLINDERS:**These are elongated and narrow containers used for measuring volume and transferring the chemicals. They are more accurate than beakers, as they easily measure the amount .They have a bumper ring at it bottom to protect, balance,and support the cylinder while pouring in it.
- **PIPETS:**These are long narrow tube like structure mainly used to measure small amount of chemicals.These are available in plastic and glasses.The big pipets are mostly mae up of glass and small pipets are made up of plastic.
- **VOLUMETRIC FLASKS:**These are used to prepare limited quanties of solutions.They made up of glass and measurements are marked on them according to standard measurements.
- **TEST TUBE:**This is also called as culture tube.It is glass tube and has a U shape bottom to allow proper mxing of chemicals.They have special stands called test tube stands. Before using it one should how to handle it ,to avoid breakage,as it is a very delicate instument.
- **BUNSEN BURNER:**Robert Bunsen ,introduced the burner therefore it was naed after him.It is a single flame burner mainly burns due to gas.It has a small switch like stucture that allows it to switch on and off.
- **INOCULATING LOOPS:**Also known as smearing loop ,it is to spread

the inoculum on a desired surface .It is long stick like structure at top with a round structure that helps to inoculate and it axis is a little thicker from a good grip.

- **WATERBATH:**It is a equipment that provide the heat.It is used to provide an accurate temperature to some chemical so that they react and a final conclusion is obtained. These are also used to melt the substances and also to incubate them. It works as a heat source ,it is made up of stainless steel and hot water is collected in it ,in which test tube stands are placed to support the tubes at the of heating .Water have two bulbs one is green and another is red that is basically used for indication purposes.

LABORATORY INSTRUMENTS:

These instruments are known as scientific instruments.A device or tool used for scientific purposes.These are used for research and lab work.

There are different types of instruments of different shapes and sizes, and are made of different materials.

SOME EXAMPLES ARE:

- Autoclave
- Sterilizers
- Balancers and moisture analysers
- Bath and chillers
- Calorimeter
- Centrifuges
- Chromatography
- Electrophoresis
- Hot plates
- Incubators
- Ovens and furnaces
- Water purification

AUTOCLAVE:

An autoclave is chamber full of pressure.This is made up of stainless steel . They are mainly used for sterilisation purpose and works on controlled pressure and temperature.It is a cylinder like structure with nozzles and meters .In side an autoclave there is a chamber like structure that can be separated that is is not fused with ot ,it is the part where sterilisation

OCCURS.

USES:

- used for sterilisation purposes.
- widely used to cure composites, vulcanization of rubber.(converting polymers to duable crosslinked structures.
- It allows the best sterilisation instruments.
- Other are used to generate crystals under high temperature.

STERILISERS:

Sterilization is a process of removing the contamination ,bacteria germs and virus etc from a substance material or medium.The sterilisers can use heat, UV,chemical etc for removing germs and making it germ free .

BALANCERS AND MOISTURE ANALYSERS:

Moisture analysers are instruments used to measure the moisture content present in a substance.The material can be solid liquid or semisolid in nature.The moisture content is analyzed and according to standard values they are monitored.

The traces of moisture need to be controlled as moisture can be dangerous to the material .The high moisture can destroy the composition and can change the properties of a material basically it is harmful.For example in rainy season the moisture content that is humidity in the atmosphere increase and due to this the food stuffs are destroyed.

BATH AND CHILLERS:

Chiller is an equipment that helps to reduce the temperature of a substance by cooling down it.It has a heat exchanger that circulates the material or liquid through it and cools it down.

CALORIMETER:

An equipment used to measure the amount of heat involved in reaction.there is thermometer that measures the temperature of the process. It is used in thermodynamics, chemical processes,biochemical processes.

CENTRIFUGES:

It is an equipment that works on the principle of sedimentation, and work on the centrifugal force and centripetal force it has flip top head then substance ,then kept in the machine and rotated on a high speed so that the constituent of material rotates fast and get separated.

These are available in different shapes and sizes depending upon the requirements.

CHROMATOGRAPHY:

Is a process that helps in separating the components of a mixture. The basic type of chromatography is done as a drop of substance kept on the chromatography paper and putted in alcohol slowly depending on the constituents they move upward and disintergrate.

ELECTROPHORESIS:

It is used to separate the macromolecules of substances, it work on the principle of negative and positive charge. It separates the dna fragment based on their lengths.

It is used in analysis of dna and rna.

HOT PLATE:

A device that helps heat up the substance it is small plate like platform on which the substances are kept on heated up. Hot plate are also used in absences of electricity . These are used in labs to melt agar and to heat them

INCUBATORS:

An equipment that is used to maintain and grow of microbial cultures in controlled conditions like humidity , temperature, oxygen, carbon dioxide.

OVENS AND FURNACES:

An equipment that uses heat energy to heat the substances and are also store in it. These are used in chemical plants.

STOCK SOLUTION:

A concentrated solution that is used to provide appropriate nutrients in limited quantities to the media. Stock solutions are made from mixing 4 groups of compounds that are macro, micro nutrients and iron sources also amino acids and vitamins.

REQUIREMENTS OF STOCK SOL.:

Synthetic media is defined as a group of defined organic, inorganic compounds. eg; MS media, Gamborg or BS media nitish media.

MS media is categorised into 4:

- **Macronutrients**
- **Micronutrients**
- **Iron sources**
- **Vitamin and amino acid**

Along with stock solutions, we require definite amount of hormones for growth, therefore with stock solution we need hormones too.

M.S media: Take about 200ml of distilled water, then mix all required amount of stock and hormones. Dissolve required amount of sucrose (7.5mg). Making final volume - 250 ml. Adjust the pH 5.8. then melt, boil the agar.

HORMONES: These are important chemicals secreted in the animal body and plant body. Here we are going to discuss the plant hormones like auxin etc.

These are chemicals that control the physiological and growth activities of plants. These are an important factor that is provided to the media to ensure proper growth of plant.

We have 5 types of hormones that are secreted by plants.

- **auxin**
- **Cytokinnins**
- **Gibberellins**
- **Abscisic acid**
- **Ethylene**

AUXIN:

- **These are produced by apices of stems, root, shoot from they are**

migrated to other regions.

- induce parthenocarphy.
- controls xylem differentiate and helps in cell division.
- used as herbicides.

CYTOKININS:

- Have special effect on cytokinesis.
- overcome the apical dorminance.
- delay the leaf senecence.
- produces new leaves and chloroplasts.

GIBBERLLINS:

- increase in length of axis
- elongates and increases sizes and shapes
- another type of PGR
- they are acidic

ABSCISIC ACID (STRES HORMONES):

- acts as antagonists
- inhibits seed germination
- important role in seed development and dormancy
- increases maturation.

ETHYLENE:

- simple gaseous PGR
- apical hook formation
- promotes root growth and root hair formation
- breaks seed and bud dormancy, intiates germination

DESIGNING OF CULTURE:

A plant tissue culture lab can be used for research or for commercial purpose or for generation of new hybrids.

Following factors to be followed before designing the labs:

- There should be proper arrangement of washing of glassware,plasticware.

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- Proper arrangement or storage of media nutrient.
 - data collection and photographic facility.
 - maintaining of cultures, temperature, light, humidity and ambience.
 - assimilation of invitro developed plants. The overall design must focus on maintaining aseptic condition.

A culture is designed using culture medium or growth medium. A culture medium is a solid, liquid or semisolid composition that helps in the growth of microorganism or cells.

There are two major types of growth media, that cell culture and microbiological culture.

CELL CULTURE: In this specific types of cells derived from plants or animals.

MICROBIOLOGICAL CULTURE: This is used for growing microorganisms, such as bacteria or fungi.

The most used growth media for microorganism are nutrient broths and agar plates.

ENRICHED MEDIA: It contains nutrients required to support growth of a wide variety of organisms. It is used to harvest different types of microbes.

BLOOD AGAR: This is agar enriched with whole blood supplements the basic nutrients.

CHOCOLATE AGAR: This is heat treated blood that turns to brown and gives the medium a chocolatey color.

AGAR PLATE: It is a petri dish that contains a solid growth media. In this sometime selective nutrients are added to enhance the growth, like antibiotics.

The most commonly used agar or culture in microbiological lab is Nutrient agar, is rich in nutrients and is used to generate microbes. It is popular as it can grow a many types of bacteria and fungi.

COMPOSITION OF NUTRIENT AGAR:

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- **0.5% OF PEPTONE**
 - **digest the protein ,and principal source organic nitrogen for growth of bacteria.**
 - **0.3% OF BEEF EXTRACTS/YEAST EXTRACTS**
 - **It is water soluble substance ,repare the growth .**
 - **1.5% AGAR**
 - **Solidifying agent.**
 - **0.5% of NACL**
 - **Maintains a salt concentration in the medium.**

DISTILLED WATER

- **Essential for growth and reproduction also in transporting of materials.**
- **pH**
- **Neutral (7.4) at 25 degree celsius.**

USES:

- **Used for isolation and purification**
- **Means of producing the bacterial lawns needed for antibiotic sensitivity tests.**

RESULT AND DISCUSSION

The above report tells us about the tissue culture techniques and there applications used in todays world .Basically we in report discussed about the method and types of instruments and glassware the plasticware we have used in this. During the practical of the topic we tried to growth fully flaged plants from small explants of some plants like tulsi,pomogranate,and roses.Also in some we observed the contamination of some culture tubes of black color therefore we need to discard them and the remaining healthy tubes we incubated in the incubators.At the end we observed some plant and then potted them in the soil to provide appropriate environment to them so that they can grow.To perform these techniques one should have skill and experience.As the technology in today 's world is upgrading day day therefore we are able to culture these plants more easily and faster.The population of the world is increasing therefore we need more advanced techniques and method to grow them.