TISSUE CULTURE MEDIA

AGENCY FOR AGRICULTURE EXTENSION
AND HUMAN RESOURCES DEVELOPMENT
AGRICULTURE MINISTRY

CULTURE MEDIA is one of the critical success factors for plant propagation using tissue culture techniques.

Various compositions of culture media have been formulated to optimize the growth and development of cultured plants.

Example :

- Knudson C (1946)
- Heller (1953)
- Gamborg, B5 (1976)
- Murashige dan Skoog, MS (1962)
- Woody Plant Medium, WPM (Loyd and McCown, 1980)

CULTURE MEDIA …

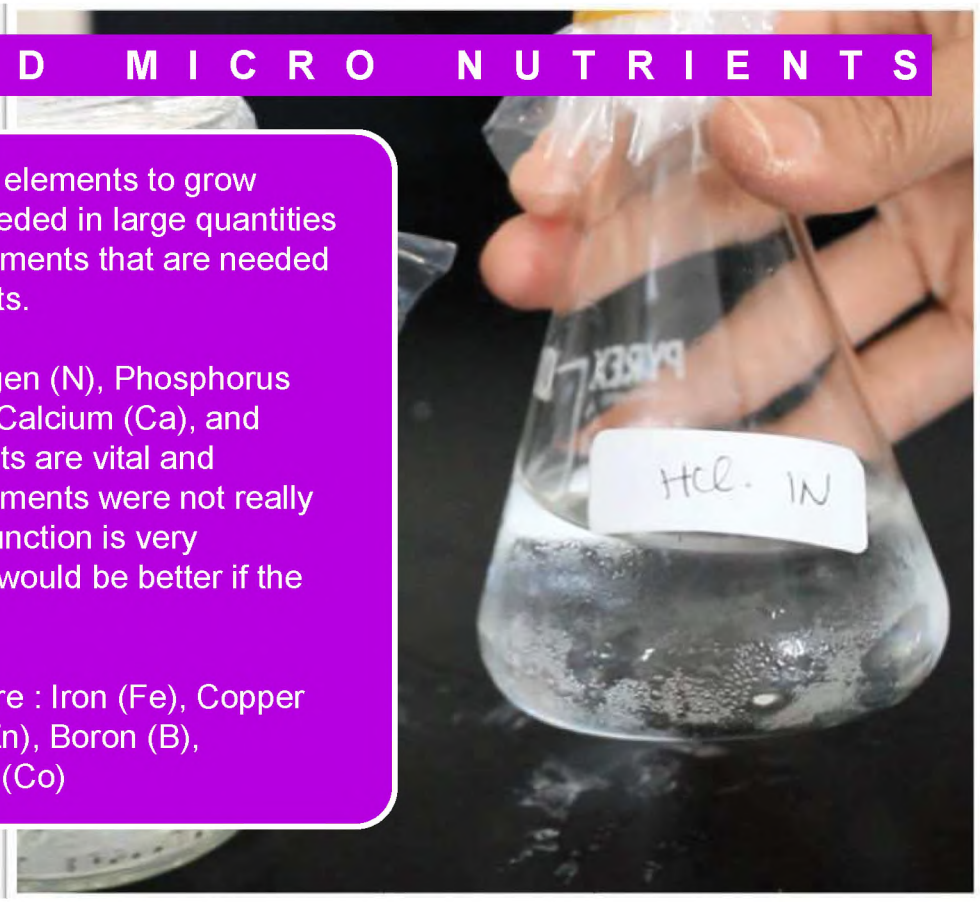
- Is the media needed so that isolated plant cells or tissues can grow and develop.
- Has a nutrient composition that can support the growth of the explants as desired.
- Nutritional requirements for optimal growth vary widely between types of plants, even between different plant parts.

The Components Of Tissue Culture Media

1. Distilled Water
2. Macro and Micro Nutrients
3. Sugar
4. Vitamins
5. Amino Acids
6. Plant Growth Regulator
7. Compactor
8. Activated Charcoal

- Distilled water is used as a solvent.
- Making stock solutions must use distilled water because it no longer contains minerals and organic compounds.
- Well water or tap water cannot be used for media production because it contains too much organic, inorganic or microorganism contamination.
- Distilled water is produced from a water distillation device by converting the water into water vapor, then condensing the water vapor into distilled water which no longer contains minerals and organic compounds.

1.DISTILLED WATER



2. MACRO AND MICRO NUTRIENTS

- Each plant requires at least 16 elements to grow normally. Elements that are needed in large quantities are called macro elements, elements that are needed a little are called micro elements.

- The macro elements are Nitrogen (N), Phosphorus (P), Potassium (K), Sulfur (S), Calcium (Ca), and Magnesium (Mg). NPK elements are vital and necessary, while S, Ca, Mg elements were not really necessary, but because their function is very supportive for tissue growth, it would be better if the elements are available.

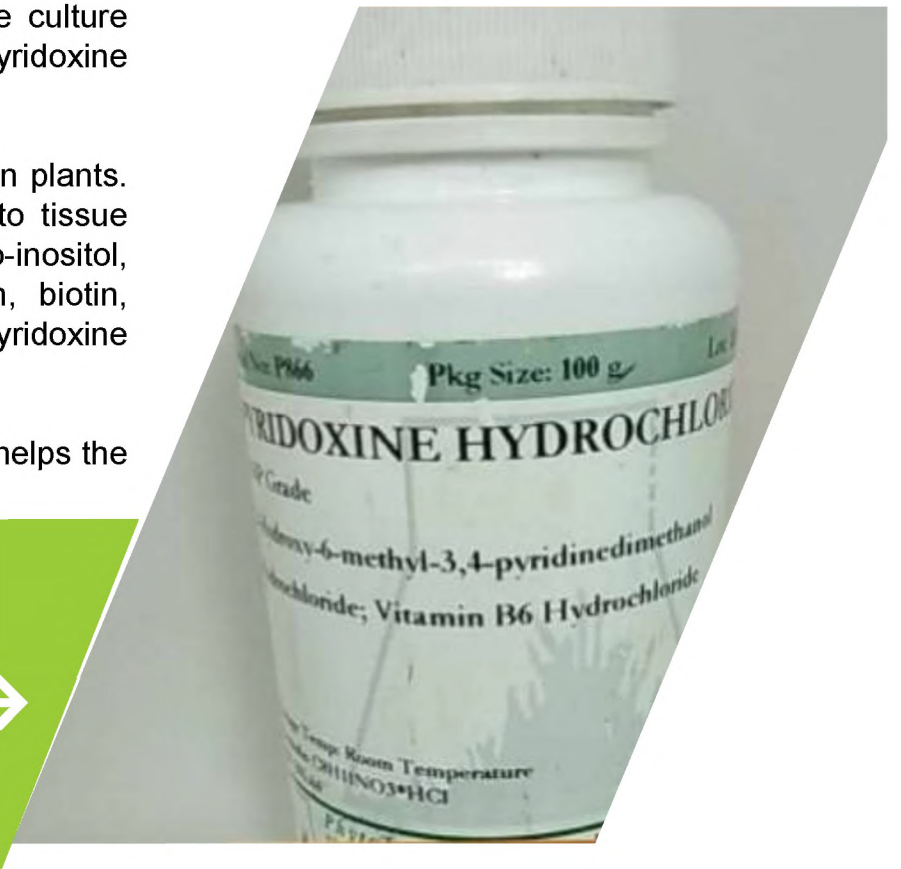
- The micro nutrient elements, are : Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn), Boron (B), Molybdenum (Mo), and Cobalt (Co)

3. Sugar (Energy Source)

- The standard sources of carbon are sucrose and glucose. The most widely used for tissue culture is sucrose, but if it is difficult to find it can use white sugar that is used daily. This white sugar also qualifies for culture growth.
- The concentration of sucrose used for culture also varies depending on the needs. According to Livy Widya Gunawan (1987) for callus and shoot culture the concentration is 2 - 3%, for embryo culture it can be up to 12%. The use of sucrose above 3% causes cell wall thickening.
- If there are additional organic materials such as coconut water, the percentage is 2%, because coconut already contains sugar

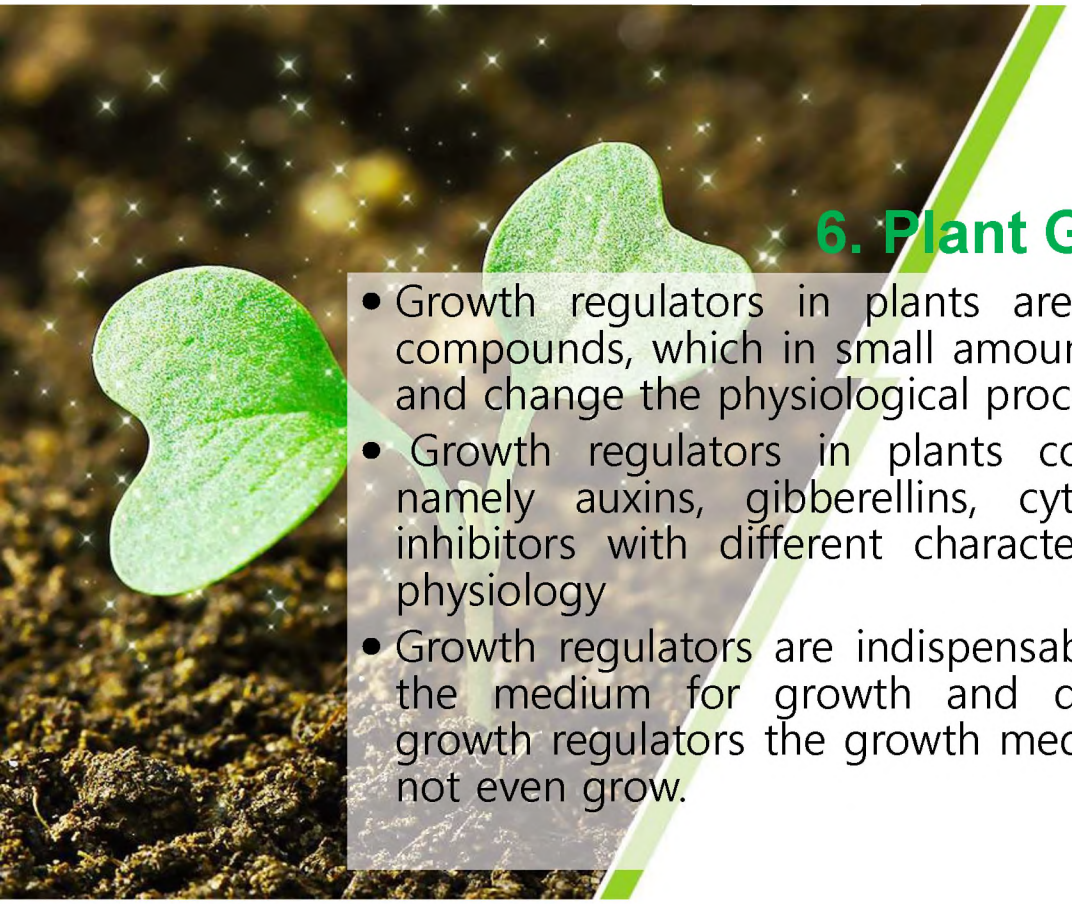
4. VITAMIN →

- Vitamins that are often used in tissue culture media include thiamine (vitamin B1), pyridoxine (Vit B6) and nicotinic acid.
- These vitamins are commonly found in plants. Other vitamins that are often added to tissue culture media are: niacin, glycine, myo-inositol, folic acid, cyanocabalamin, riboflavin, biotin, choline chloride, calcium pantetone, pyridoxine phosphate.
- Myo inositol is often added because it helps the cell division process.



5. AMINO ACID

- Amino acids are a source of organic N which is taken up more quickly than inorganic N in the same medium. Amino acids play an important role in callus growth and differentiation.
- The need for amino acids for each plant is different.
- Casein hydrolysate is a source of added amino acids to improve growth and morphogenesis, especially media that does not contain ammonium ions.



6. Plant Growth Regulator

- Growth regulators in plants are non-nutrient organic compounds, which in small amounts can support, inhibit and change the physiological processes of plants.
- Growth regulators in plants consist of five groups, namely auxins, gibberellins, cytokines, ethylene and inhibitors with different characteristics and effects on physiology
- Growth regulators are indispensable as a component of the medium for growth and differentiation. Without growth regulators the growth medium is stunted or may not even grow.

Auxin

Auxins inside the plant body have a very complex effect and can control growth.

The physiological roles of auxins in plants such as:
- Enlargement of cells and organs,
- Root formation,
- Maintains apical dominance,
- Stimulate vascular tissue differentiation,
- Triggers ethylene synthesis

Types of auxins used in tissue culture techniques:
- Natural auxins, for example **IAA (Indol Acetic Acid)**
- Synthetic auxins, for example **IBA (Indolebutyric acid), NAA (Naphthalene acetic acid) and 2,4-D (2,4-dichlorophenoxy acetic acid)**

Auxin

The roles of auxins in tissue culture techniques are:
1. Inducing roots in this case auxin can accelerate root growth, increase the number of roots and improve the root system of the explants.
2. Induce callus, for the purpose of producing secondary metabolites.
3. Induce somatic embryogenesis.
4. Increase ethylene synthesis.

Auxins are synthesized at the tips of roots or shoots.

Cytokinins

Cytokinins are the name of a group of growth regulators that are very important as a stimulant for cell division and morphogenesis in tissue culture.

More than 30 types of natural cytokinins are found, but two are widely used in tissue culture, such as:
- Zeatin (4-hydroxy-3-memethyl-trans-2-butenylaminopurine)
- 2-iP (N6- (2-isopentyl) adenine)

Synthetic cytokinins that are commonly used in tissue culture activities are:
- Kinetin (6-furfurylaminopurine)
- BAP (6-benzylaminopurine)
- Thidiazuron (N-phenyl-N'-1,2,3-thidiazol-5-phenylurea)

Cytokinins

In tissue culture activities, cytokinins have been shown to stimulate the occurrence of :
- Cell division,
- Callus proliferation,
- Formation of shoots,
- Flowering,
- Chloroplast formation and,
- Inhibits root formation.

GIBBERELLIN

In whole plants, gibberellins:

- Can affect the elongation of the stem or stem segment,
- Encourage flowering,
- Fruit induction, and
- Breaker of bud dormancy.

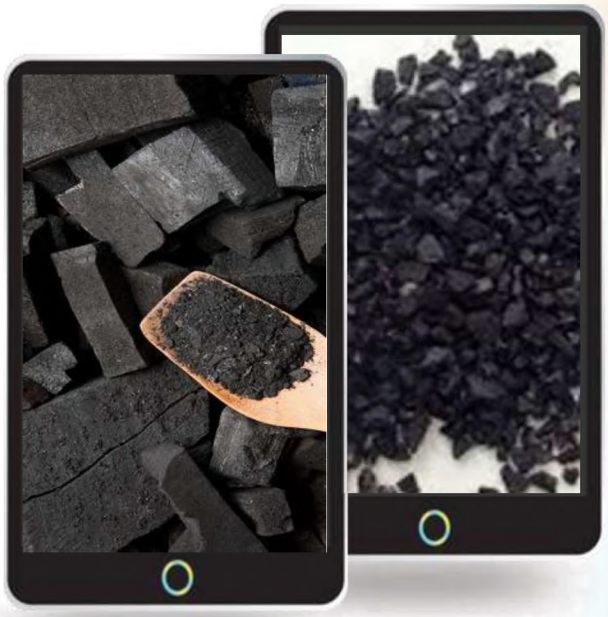
The growth regulator of the gibberellin group that is often used in tissue culture is Gibberillic acid (GA3).

7. COMPACTOR

- The compactor that is often used is agar, the advantages of using agar are in order to freeze at 45 ^{o}C and melt at 100 ^{o}C, so that in the culture temperature range the agar will be in a stable frozen state, not digested by plant, enzymes, and does not react with compounds media.
- Agar is a polysaccharide mixture made from several species of algae, containing a small amount of Ca, Mg, K and Na elements.
- The hardness of the media increases with increasing agar concentration, and is also influenced by the type of agar used, the pH of the media, and the addition of activated charcoal. Activated charcoal content of 0.8 to 1% inhibits agar freezing.
- The high agar concentration causes the nutrient uptake process to decrease.

8. Activated Charcoal

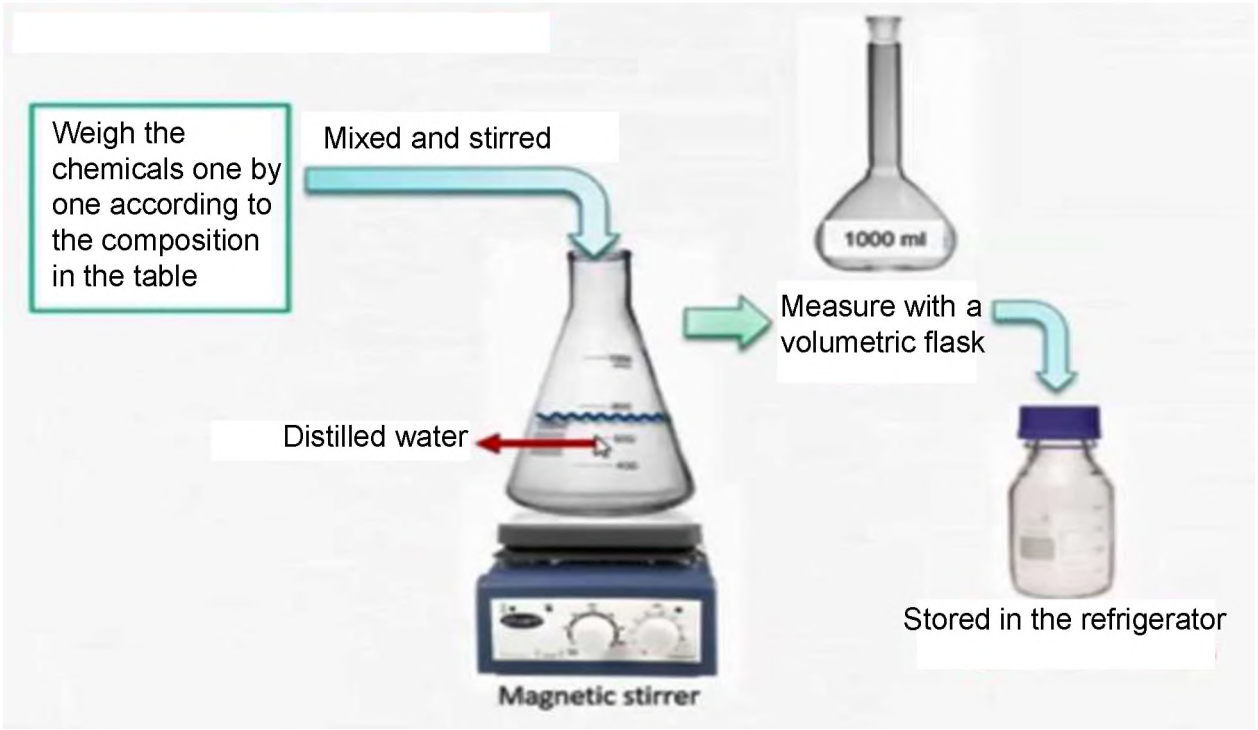
- Activated charcoal is charcoal that has been heated for several hours using steam or hot air. This material has a very strong adsorption power.
- Effect of adding activated charcoal to the media
  - Can adsorb toxic compounds which can inhibits the growth of culture, especially the phenolic compounds tissue injured at initiation
  - Stimulates roots by reducing light reach the explants parts in the media
- The added activated charcoal is evenly distributed in the media, by shaking it after sterilization in an autoclave before freezing.



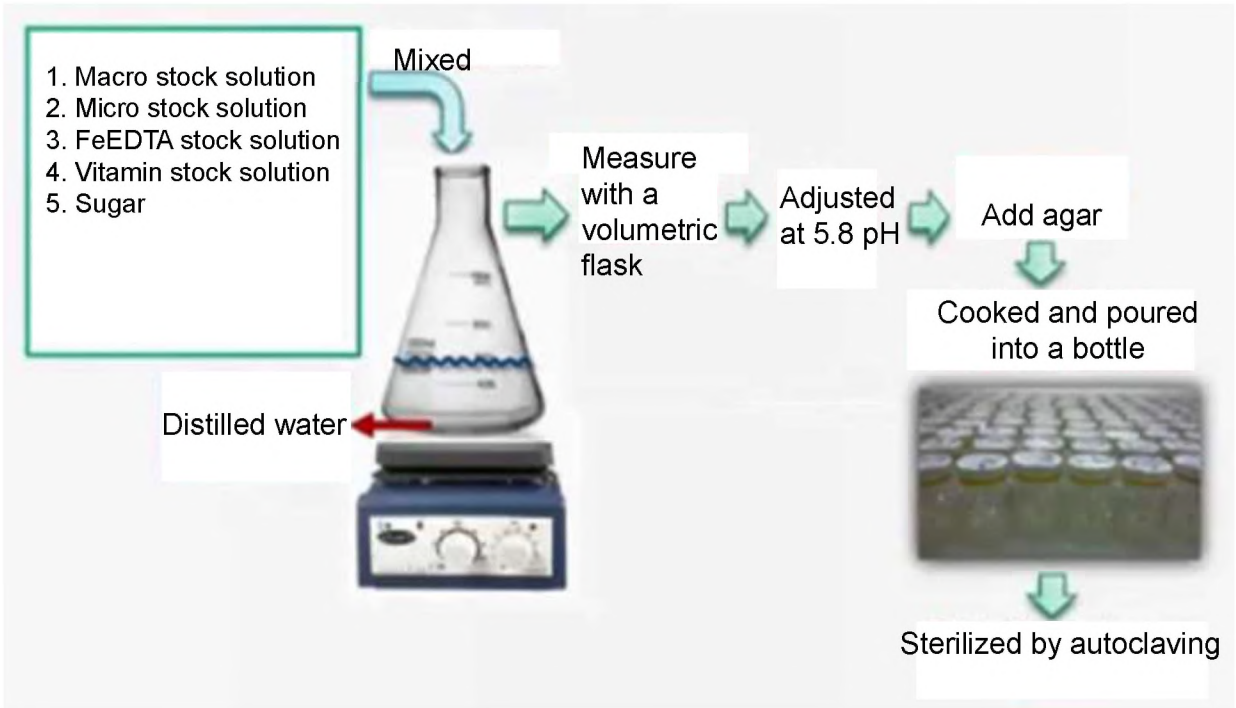
Buffer or pH Media

- The pH must be adjusted in such a way that it does not interfere with the cell membrane function and the pH of the cytoplasm. Plant cells require a slightly acidic pH ranging from 5.5 to 5.8.
- The pH adjustment is usually done by using NaOH or HCl when all the components have been mixed, sometime before being sterilized in an autoclave. The addition of amino acids is often an organic buffer

Stock Solution Procedures



MS Media Procedures




Thank You