MEDIA CREATION IN TISSUE CULTURE

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Abstract

Plant tissue culture is part of an unconventional vegetative propagation technique. The difference in this technique compared to conventional vegetative propagation techniques is usually located in different situations and locations. Tissue culture media is one of the factors that can determine the success rate of in vitro plant propagation. Components of culture media are environmental factors that provide elements of plant growth such as macro nutrient elements, micro nutrient elements, carbohydrates, vitamins and growth regulators, organic salts, natural complex compounds, activated charcoal and compacting materials (Various formulations or compositions of growing media It was found to optimize the growth and development of cultured plants, there are several types of media, but the culture media commonly used are media with the Murashige and Skoog (MS) formulation, MS media are basic media that have a very complete formulation.In general, tissue culture media are divided into basic media and treatment media. Recipe for basic media is a recipe for a combination of substances containing essential nutrients (macro and micro), sources of energy and vitamins.

INTRODUCTION

Plant tissue culture is part of a non-conventional vegetative propagation technique. The difference between this technique and conventional vegetative propagation techniques usually lies in different situations and locations. The application of plant tissue culture techniques requires indoor conditions (laboratory) and aseptic nature (sterile from pathogens). Leading to aseptic conditions, it is necessary to explain that all activities related to tissue must be in aseptic conditions.

In addition to tissue culture equipment, media is one of the main factors in the success of culture. Tissue culture media is one of the factors that can determine the level of success of in vitro plant propagation, in this case tissue culture. Various formulations or compositions of planting media have been found to optimize the growth and development of cultured plants. The role of culture media is related to the provision of nutrients and energy as well as other substances needed for the growth and development of explant materials in culture bottles so that it greatly influences the success of tissue culture. Seeing the important role of culture media, through this practicum, the creation of culture media is carried out properly and correctly in accordance with existing procedures.

Media that is too dense can make it difficult for roots to grow, because the roots have difficulty penetrating the media. While media that is too soft will cause failure in the work. Failure can be in the form of sinking of the planted explants, especially heavy explants such as wartel explants, melinjo, garlic explants, soybean explants, and so on. The use of liquid media is more emphasized on cell suspension, namely to grow plb (protocorm like bodies or also called protocormus). From this protocarmus, it can later grow into planlets if transferred into a suitable solid media (Hendaryono and Wijayani, 2007).

In vitro media commonly used are usually solid media because they have several advantages, including the use of the smallest explants will be easier to see, the explants are above the surface of the media so that they do not require aids for aeration, shoots and roots will grow more easily in still media. However, liquid media also has several advantages that are not available in solid media, including not requiring additional solidifying agents, suitable for protoplasm and cell culture processes, exudates released by explants do not accumulate around the explants, and greater contact between explants and media (George and Sherington, 1984).

In the process, the success of tissue culture is not only due to controlled environmental conditions but also determined by the culture media. Culture media is one of the determining factors of success. Culture media is a component of environmental factors that provide plant growth elements such as macro nutrients, micro nutrients, carbohydrates, vitamins and growth regulators, organic salts, natural complex compounds, activated charcoal and solidifying agents (George and Sherington, 1984).

The culture media commonly used is media with Murashige and Skoog (MS) formulation. MS media is a basic media that has a very complete formulation. The composition of MS media can generally be used on almost all types of plants (Wattimena, 1992).

In general, tissue culture media are divided into basic media and treatment media. Basic media recipes are recipes for combinations of substances containing essential nutrients (macro and micro), energy sources and vitamins. In tissue culture techniques, dozens of types of basic media are known. The naming of basic media recipes is generally taken from the name of the inventor or researcher who first used it in a special culture and obtained an important

result. Some basic media that are widely used include:
(1). Murhasige and Skoog (1962) basic media which can be used for almost all types of culture, especially in herbaceous plants, (2). Knop media can also be used to grow carrot callus, (3). B5 basic media for soybean, alfalfa and other legume cell cultures, (4). White's basic media (1934) which is very suitable for tomato plant root cultures, (5). Vacin and Went basic media which is usually used for orchid tissue culture, (6). Nitsch and Nitsch basic media which is usually used in pollen culture and cell culture, (7). Schenk and Hildebrandt basic media (1972) or SH media which is suitable for monocotyledonous plant tissue cultures, (8). Special medium for woody plants or Woody Plant Medium (WPM), (9). N6 media for cereals, especially rice.

The nutrients in the culture media consist of several components, as follows:

1. Macro nutrients used in all culture media formulations.
2. Micronutrients are always used. There are some media compositions that use only iron or iron-chelate.
3. Vitamins and amino acids and organic N, are generally added in varying amounts. Vitamins, amino acids and other organic materials such as myo inositol are media components that have a good effect on culture growth. The group of vitamins that are often used are from the vitamin B group, namely Thiamin-HCL (B1), Pyrodoxin-HCL (B6), ASAN Nicotinate and Riboflavin (B2) (Nugroho, 1997).
4. Energy and carbon sources in the form of sugar are a must, except for very specific purposes. The optimum concentration of sucrose depends on the type of tissue being cultured. In callus and shoot cultures, the sucrose concentration used is between 2-4% which is the optimum concentration. However, in embryo culture, the sugar concentration can reach 12%. According to Szweykowske, 1974 as quoted by George & Sherrington (1984), the division of Ceratodon purpureus protonema cells is influenced by the interaction between glucose and 2iP. Sugar has a dual function in the media, namely as an energy source and as a balancer of the media's osmotic pressure. According to George & Sherrington (1984), 4/5 of the osmotic potential in White media is caused by sugar, while in MS media only half of the osmotic potential is due to sugar.
5. Natural complex organic compounds such as: coconut water, yeast extract, green banana juice, bean sprouts, pineapple, potatoes and so on.
6. Plant Growth Regulators (PGR): there are several types, including: auxin, cytokinin, gibberellin, abscisic acid, ethylene and so on. PGR is an important component in tissue culture media. The type and concentration of PGR used is very dependent on the type of garden and the purpose of the culture (Nugroho, 1997).
7. Buffer (chelating agent). The addition of amino acids often also acts as an organic buffer. The addition of KH2PO4 itself is not effective as a buffer. Many previous researchers such as Tausson and Kordan (George & Sherringtone, 1984) suggested adding FeSO4 and Na-EDTA to the media to act as a buffer.
8. Solidifying agent. This material is used to make solid media, commonly used is agar. The advantages of using agar are:

   Agar freezes at a temperature of ≤ 45o C and thaws at a temperature of 100o C, so that within the culture temperature range, the agar will be in a stable frozen state, is not digested by enzymes produced by plant tissue, does not react with the compounds that make up the media.

   In commercial propagation and experiments not intended to study cell metabolism, the use of pure agar is not a necessity considering the price of pure agar is very high. Unwanted materials from agar can be removed by soaking in distilled water for 24 hours. The agar is then rinsed with ethanol and dried in an oven at 60o C for 24 hours. The concentration of agar given ranges from 0.6-1.0%. (Deberg, (1982 in Gunawan 1988).
9. Another important factor is the pH which must be adjusted in such a way that it does not interfere with the function of the cell membrane and the pH of the cytoplasm. In addition to considering the physiological interests of the cells, pH adjustment must also consider the following factors: Solubility of the salts that make up the media, Uptake of growth regulators and other salts and, Efficiency of agar freezing.

   Plant cells require a slightly acidic pH ranging from 5.5-5.8 (Gamborg and Shyluk, 1981). Ericaceae plants such as Rhododendron pH adjustment is usually done using NaOH (or sometimes KOH) or HCl when all components have been mixed, some time before being sterilized by autoclaving. Even though the media has been determined, often after sterilization the pH changes. In general there is a decrease in pH after being sterilized in an autoclave. To achieve a pH of around 5.7-5.9, Nann et al. (in George and Sherrington, 1984) made a pH of 7.0 in unsterilized media. To avoid significant changes in pH. Murashige and Skoog suggest that heating is carried out to dissolve the agar and heating the media in the autoclave for several minutes, then the determination of the media is carried out sterilized in the autoclave. In a large container, the media is sterilized and then titrated with sterile NaOH/HCl to the desired pH. After that, the media is poured into a sterile culture container that has been prepared in a laminar air flow cabinet.
10. Activated charcoal, functions to absorb toxic compounds produced by explants as an antioxidant and is also often used to stimulate root growth.

    Addition of activated charcoal. Activated charcoal 0.8-1 g/l inhibits agar coagulation (Horner et al (1977 in George & Sherrington, 1984). Activated charcoal is charcoal that has been heated for several hours using

steam or hot air (George & Sherrington, 1984). This material has very strong adsorption properties. Activated charcoal can be added to the media at various stages of culture development. This material can be added to the initiation media, regeneration media, or rooting media. The addition of activated charcoal can help the growth of culture development, depending on the type of culture. In general, the effects of activated charcoal are as follows: Adsorbing toxic compounds found in the media that can inhibit culture growth, such as phenolic compounds from injured tissue during initiation, and 5-hydroxymethyl furfural compounds which are thought to be formed from sugars in weak acid solutions and heated under high pressure (Nitsch et al, 1968 in Gunawan 1988), Adsorbing growth regulators so as to prevent unwanted callus growth, such as in androgenesis and shoots that are to be rooted, and also assisting embryogenesis in cultures in regeneration media without auxin, possibly by acting as a sink that draws auxin from within the cells so that embryogenesis can occur (Drew, 1979 in George & Sherringtone, 1984) and, Stimulating rooting by reducing the level of light reaching the part of the explant contained in the media.

METHODS

This practicum was conducted at the YAHDI Plant Tissue Culture Laboratory, Jalan Tanah 600 Marelan Raya, Medan, North Sumatra.The tools used are pan, stirrer, scales, stove, culture bottle, pH paper, autoclave..The materials used are MS Media, Aquades, Agar-agar, NH4NO3, KNO3, Mio inositol, Sucrose, Vitamins, Stock Solutions C, D, E, F, IAA, Coconut Water

Work procedures

How to Make MS Tissue Culture Media with 3 Media Combinations of 750ml

1. Stock A. NH4NO3 = 1.65 gr/L $= \frac{1{,}65x750mL}{1000} = 1{,}2375gr$

   B.KNO3 = 1.90 gr/L $= \frac{1{,}90x750mL}{1000} = 1{,}425gr$

   Mio Inositol = 0.1 gr/L $= \frac{0{,}1x750mL}{1000} = 0{,}075gr$

   Sucrose = 30 gr/L $= \frac{30x750mL}{1000} = 22{,}5gr$

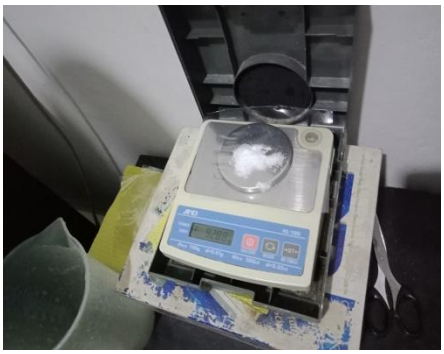
   Then all the ingredients are weighed and mixed

   So that = 7 gr/L = 1.75

2. Stock Pipettes C,D,E,F = 5 ml/L = 3.75 ml

   Vitamin = 4 ml/L = 3 mL

   BAP 1 ppm = 7.5 Ml



   ZPT IAA 1ppm = V1 . N1 = V2 . N2

   = 750 . 1 = V2. 100 ppm

   V2= 7.5 ml

3. Added Aquades = 637.5 ml (Divided by 3 in Media)

For the first = 250 ml

Second = 212.5 ml (Dissolved in 250 ml)

Third = 175 ml (Dissolved in 250 ml)

Dissolve Coconut Water as much as:

1. AK O % = 0 mL

2. AK 15% $= \frac{15x250mL}{100} = 37{,}5mL$

3. AK 20% $= \frac{30x250mL}{100} = 75mL$

4. Measure pH 4.8 – 5.8

5. Weigh the agar = 1.75 gr/ 250 ml

Into 3 parts

6. Cook the media until it boils while stirring.

7. Pour into a sterile culture bottle.

8. Autoclave at 121 0C for 15 – 20 minutes.

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RESULTS AND DISCUSSION

Results

Table . Contamination in tissue culture media

NO	Group	Initial Media Amount	Contamination	
			Contam	Sterile
1.	Group 1	**12**	**12**	**0**
2.	Group 2	**12**	**12**	**0**
3.	Group 3	**12**	**12**	**0**
Average		**12**	**12**	**0**
Standard Deviation		**0**	**0**	**0**

Discussion

The success of using tissue culture method is highly dependent on the media used. Culture media is one of the determining factors of success. Culture media is a component of environmental factors that provide plant growth elements such as macro nutrients, micro nutrients, carbohydrates, vitamins and growth regulators, organic salts, natural complex compounds, activated charcoal and solidifying agents. In this parakikum, the culture media made is in solid form with Murashige and Skoog formulations.

The preparation of culture media is done by pipetting the stock solution that has been previously made and stored in the refrigerator. The stock solution is pipetted according to the search results (in Calculation) using the dilution formula and then diluted into a 1L beaker. Pipetting is done sequentially to avoid chemical reactions between solutions that can cause a decrease or degradation or salting reactions that will result in the unavailability of growth elements for explant growth. The concentration of the solution used is in accordance with the concentration in the MS media formulation. The solution that has been in the beaker glass is then diluted by adding water and 22.5 g of sucrose. Sugar has a dual function in the media, namely as an energy source and as a balancer of the osmotic pressure of the medium. This is done so that the sucrose dissolves quickly. After the sucrose has dissolved, water is added to the solution until the volume becomes 1 L, heating is continued. Then measure the pH of the solution using a pH meter. The recommended pH of the solution is between 5.8-6.0. If the pH of the solution is below 5.8, NaOH is added drop by drop until the pH rises to around 5.8. If the pH is above 6.0, KCl is added drop by drop until the pH drops to that range. Plant cells require a slightly acidic pH ranging from 5.5-5.8. Even though the media has been determined, often after sterilization the pH changes.

To avoid significant pH changes Murashige and Skoog suggested that heating be done to dissolve the agar and heating the media in an autoclave for several minutes, then the media is sterilized in the autoclave. In a large container, the media is sterilized and then titrated with sterile NaOH/HCl to the desired pH. After that, the media is poured into a sterile culture container that has been prepared in a laminar air flow cabinet.

The pH adjustment is done to ensure the availability of nutrients for the explants in the culture bottle. After the pH is balanced, then the agar is added. Because in this practicum, the media used is a solid media, a solidifying agent in the form of agar is needed. The agar given is 7 grams and is divided into 3 parts, put into the media composition solution and heated. The pH measurement is no longer carried out because if the media solution that has been added with agar is measured for its pH, it will damage the pH meter. Agar concentrations that are too high can reduce the diffusion of compounds from and to the explants so that the uptake of nutrients and growth substances is reduced, while the inhibitory substances from the explants remain collected around the explants. After reaching the boiling point which is indicated by a clear solution and there are bubbles, the solution is poured into 70 culture bottles according to the amount needed. Then the bottle is covered with plastic and wet sterilization is carried out using an autoclave for 20 minutes at a temperature of 1210C and at a pressure of 15 psi. After that, the culture bottles are placed in the culture room on the shelves that have been provided.

The results of this practicum showed that all media made were contaminated. Contamination is a fundamental problem that often occurs in in vitro culture. In media conditions containing sucrose and nutrients, as well as relatively high humidity and temperature, microorganisms and fungal spores can grow and develop rapidly. Contamination in in vitro culture can come from: Air, Explants, both externally and internally, Small organisms that enter the media, Culture bottles and tools that are not sterile, Dirty work environment and culture room. But here all contaminated media probably came from the coconut water used because the coconut water was not sterile or had been contaminated before the media was made.

CONCLUSION

Tissue culture media is one of the factors that can determine the success rate of in vitro plant propagation, in this case tissue culture. In this practicum, all media made were contaminated. Contamination is a fundamental problem that often occurs in in vitro culture. In media conditions containing sucrose and nutrients, as well as relatively high humidity and temperature, microorganisms and fungal spores can grow and develop rapidly. All contaminated media probably came from the coconut water used because the coconut water was not sterile or had been contaminated before the media was made.

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