Sterilization in plant tissue culture pdf

Exposure 1 Methods of Sterilization 2 Preparation Stock Solutions 3 Media Training MCS Medium 4 Surfacesterilization explants 5 Preparation explant for callusinducation 6 Characteristics of callus 7 Sub cultivating callus on media differentiation 8 Creating a suspension culture from grown callus 9 Extracting secondary metabolites from callus 10 Micro-propaganda plant tip and nodal method of culture 11 Anter culture for haploid production 12 Protoplast insulation 13 Preparation synthetic seeds 14 H air root induction through Agrobacterium m ediated gene transmission 15 Fast DNA extraction plants 16 Develop RAPD map EXPERIMENT No 1 STERILIZATIONTECHNIQUES INTRODUCTION : Sterilization techniques are designed to kill or remove broad spectrum of microorganisms, including the simplest fungi, Virus bacteria. Autoclave, dry thermal sterilization and filtration are sufficient for most materials of cellular and organ culture. METHODS (A) AUTOCLAVING A laboratory vehicle designed to use steam under adjustable pressure to achieve sterilization is called an autoclave. Principle: Water boils at 100 degrees Celsius depending on the pressure of steam in the atmosphere. When atmospheric pressure rises, the boiling point of the water will also rise. So if the pressure of steam inside a close vessel increases to 15 pounds/inch2. Temperatures can reach up to 121.6 degrees Celsius. The autoclave is equipped with a device that can support saturated steam at the prescribed temperature and pressure for any period of time. Description: This is essentially a double jacket vertical cylinder, made of strong metal and separated by a case made up of an iron sheet. The cover is very heavy and consists of weapons metal and screwed with screws of buffer files. It turns out to be airtight with the interposition of the asbestos washer. The cylinder contains water up to a certain level. Products that need to be sterilized are placed on a perforated aperture, serving as a platform above the water level. Heating can be made either with a gas burner or with electricity. The camera is fitted with a steam crane at the top and a pressure sensor with a protective valve. The pressure used is 15 pounds/inch for 15-20 minutes under this pressure H2O boils at 121 degrees Celsius in operation is necessary so that the air in the chamber is replaced by saturated steam otherwise the required temperature will not be obtained. It's not the pressure that kills the body, but the temperature of the vapor. The time of work to achieve infertility depends on the nature of the sterilized material, the type of containers and the volume. Sterilization by autoclave kills all organisms, including spores. Cell death and spores occur due to coagulation of proteins. Use: Sterilization of most types of solid or liquid media, distilled water, normal saline solution, discarded crops, contaminated media, apron, rubber tubes, glove gloves B USE OF HOT AIR OVEN: Dry heat or hot air sterilization is recommended where it is either undesirable or it is unlikely that steam under pressure will make direct and complete contact with materials that will be sterilized. The device working for this type of sterilization can be a special electric or gas furnace or even a kitchen stove. Principle: Sterilization is carried out by dry heat at high temperature. Bacterial cells and spores die due to dehydration. Description: It consists of a camera with a triple wall. The interior two walls are made of copper leaf and the outer part of asbestos. The camera is filled with several adjustable shelves and the thermometer is interested in recording the temperature. There are adjustable holes that are kept open during temperature increases and reopen partially when sterilization completes clean glassware like petri dishes, test tube, flasks, pipettes etc are inside and the door is closed. The electric heated is put on and the temperature of the camera is only opened after the temperature is reduced to normal. Use: A useful device for sterilizing laboratory glassware. It is also used to sterilize substances that cannot be sterilized by wet. FILTER STERILIZATION growth substances such as zetine, gibberilic acid (GA3), absic acid (ABA), urea and some vitamins are sterilized by membrane filtration of the size of pores 0.22 -0.45 µ Four types of filters are commonly used, i.e. asbestos-spec glass, non-glos and membrane filters. The membrane filters are made from cellulose acetate, cellulose nitrate or a mixture of nylon and polysulfone. A common form is paper as a disk about 50 mm in diameter and 0.1 mm in thickness. The filter installation is wrapped in aluminum foil and automatically wrapped before use. The membrane filters are stored in the petridish for the automatic filter of the swinnex model available. Procedure: Filtering is carried out under low pressure, connected to vacuum decicators with the safety of attaching the flask between them. This is where the first filtration liquid is filled. They generate positive pressure inside the bottom container. Now the cotton fork at the lower end is connected to the vacuum pump. This creates negative pressure inside the container. If any liquid is still left, take an empty syringe filled with air and blow on the lid until it is filtered out. The vacuum pump is turned off only when positive and negative pressures balance each other. (D) I don't want to. STERILIZATION OF GLASSWARE: They soak in a solution of chromaic acid for 24 hours. They are washed in a high-pressure set of tap water with cleaning followed by several rinses, first with tap water and then with distilled water and finally with distilled water. Wash the glassware, then dry in the oven (3 hours at 100 degrees Celsius). Currently, the chromium-sulfur acid method is replaced by the use of detergents to then rinse with tap water with water distilled water. Use Laminar Air Flow cabinets: they are available in a variety of sizes. The size of the 1.2m X 1.2m X 7.1m is equipped with glass on the top top half of the work bench with a sunmica top end of a small sink and door. They are equipped with U.V. hermicide for internal sterilization and cool white fluorescent tubes for uniform lighting. They gave a closed area in which air was circulating there, dust and gerb screening filters over the working surface at an even speed. They are built to keep the transmission zone under positive pressure, starting an experiment all in the journey. The surface of the hand transmission camera is sprayed with 70% ethanol for the demyssia of air pollutants the door is slightly closed and the UV is turned on for 45 minutes for internal sterilization. At the beginning of the work, the light tubes switch ON. The blower switches and the door is open. This causes positive pressure inside, so the air from can't rush in. Change into new canvas shoes. EXPERIMENT NO. 2 PREPARATION OF STOCKSOLUTIONS INTRODUCTION : Preparing a stock solution is the most important and important step in plant tissue culture technology, because to prepare any basal environment ingredients, macronutrients, trace elements, iron EDTA and vitamins are common. So, in order to prepare any basal environment it is a complex process of weighing the ingredients of trace elements, macronutrients, vitamins, iron EDTA every time, and it is a laborious process. To avoid all of the above difficulties, it is best to prepare stock solutions for standard concentration and use if necessary. RELATED: Chemical: Mineral Salt Glassware: Amber Color Bottle, Funnel, glass, glass rod, filter paper, pipette, etc. Tool: Autoclave PROCEDURE : 1. Preparing Macronutrients shares: Usually macronutrients stock solutions are prepared at a concentration of 20X. Weighing the ingredients as given in the table, how to dissolve one by one amount to 1000 ml, which is 20X concentrations. To prepare 1litre of the basal medium requires 50 ml of the above solution. 2. Preparation of micronutrient stocks: Usually macronutrient stock solutions are prepared at 100X concentrations. Weigh the elements as given in the table and dissolve in 1000 ml of distilled water, which gives 100X concentrations. To prepare 1 liter of basal environment requires 10 ml of micronutrient solution. Iron-EDTA: Iron reserve is prepared separately due to iron lysing problem. This element requires an acidic state for solubility. Usually the reserve of iron is prepared in a cheated form, like sodium salt Ferric-Ethylene Diamine Acetic Acid (Fe-EDTA). 3. Preparing vitamins: Weighing each vitamin as a In the table and dissolve in 1000 ml of distilled water, which gives 100X concentrations, 1 ml is required to prepare 1 liter of basal medium. 4. Preparation of a solution of hormonal broth: Various hormones oxin (dissolved in 70% alcohol, 1N HCl) and cytokinins (1N NaOH) are prepared at a concentration of 1 mg/ml in suitable solvents. TABLE FOR STOCK SOLUTION PREPARATION Abbildung in dieser Leseprobe nicht enthalten NOTE : 1. All stock solution should be clear and transparent, free of dust and without precipitation. 2. Stock solutions should be stored in amber-colored bottles. 3. Stock solutions are stored at 4 degrees Celsius in a dark room. These solutions are stored for a limited period of time. 4. After using the stock decision immediately keep close and do not expose for a longer time. EXPERIMENT NO. 3 PREPARATION OF MEDIAMSMEDIUM INTRODUCTION The success of the production of callus and morphogenesis of plant tissues and the application of in vitro techniques is largely governed by a better understanding of the nutritional needs of cultural cells and tissues and the composition of tissues of cultural media. Although the basic needs of plant crops are similar to those of whole plants, the need for nutrition consists of inorganic salts, carbon and energy sources, vitamins and phyto hormones, other components including organic nitrogen compounds, organic acids and complex substances. In practice, the nutritional components that contribute to optimal tissue growth in the laboratory can vary from species to species. Therefore, the composition of the media was formulated in accordance with the specific requirements of a particular system. REQUIREMENTS : Chemical: Stock solutions, distilled water, sucrose, myo-inositol, agar-agar, PVP Glassware: Cultural vessels/pipes, funnel, glass, glass rod, filter paper, pipette, measuring cylinder. Tool: Autoclave, PH meter MS - MEDIAPREPARATION Abbildung in dieser Leseprobe nicht enthalten PROCEDURE 1. Pipette of the required volume of solution, as in the given in the table. 2. Weigh the other ingredients as given in the table and add one by one effectively and dissolve. 3. Add filter sterilized growth hormone to the desired concentration. 4. Adjust pH 5.80-5.85 with 0.1N HCl or 0.1 N NaOH. 5. Finally, the agar is added to the solution and boiled until the solution is clearly formed. 6. Wednesday is allowed to cool for some time until it reaches around 45 0C to 50 0C and then dispense it into a heat culture tube sterilized around 15-20 ml/tube and coat it with a cotton plug and wrap with paper. 7. The above culture pipes with the media automatically autoclave at 15 pounds of pressure, 121 0C for 20 minutes. 8. Keep these sterilized tubes in a sloping position let them harden. Now these tubes are ready for further use. The media should be clean and milky white. NOTE 1. All stock solutions must be clear and transparent, dust-free and Precipitation. 2. Stock solutions should be stored in amber-colored bottles. 3. Stock solutions are stored at 4 degrees Celsius in a dark room. These solutions are stored for a limited period of time. 4. After using the stock decision immediately keep close and do not expose for a longer time. EXPERIMENT NO. 4 SURFACE STERILIZATION OF EXPLANTS INTRODUCTION The first important condition for successful tissue culture procedures is to maintain an aseptic state. Sterilization eliminates microorganisms and thus avoids contamination by bacteria and fungi. To maintain an aseptic environment, all cultural vessels, media and tools used in tissue processing as well as explant itself must be superficially sterilized. Plant material can be sterilized by various chemicals. Some widely used sterile chemicals are as follows: 1% sodium hypochlorite (NaClO) : It is usually available with 5% active chlorine content, so that 20% can be used for normal sterilization. Calcium hypochlorite Ca (ClO)2 : This occurs in the form of powder. Usually used 100 ml Ca (ClO)2. For sterilization, the desired weight of hypochlorite is added to the water, agitated for 10 minutes, allowed for the settling and cleared filtered supernathent solution. The filter is used immediately because of the deliquescent (take water) nature. Calcium hypochlorite enters the plant tissue slowly compared to sodium hypochlorite. The standard concentration is used about 4 to 10%, and the soaking time varies from 5 to 30 minutes. B of romin water: 1 to 2% water bromine solution is used for sterilization purposes. Mercury chloride: It dissolves in water to create a solution. The concentration is used from 0.01 to 0.1% for 2-10 minutes, depending on the fabric. Mercury chloride is an extremely toxic substance for plants, so rinsing should be very thorough at least five times. Alcohol: 70% alcohol is used to sterilize plant material by immersing them for 30 sec to 2 minutes. Normally, alcohol alone is not enough to kill all microorganisms and plant material after the treatment of alcohol is considered another chemical sterile. The antibiotic antibiotic cefotaxim at a concentration of 50 mg/l in the nutrient environment is usually used to fight bacterial infection. Explants after treatment with sterile substances should be thoroughly washed with sterile distilled, as the preservation of such toxic chemicals will have a serious impact on the creation of culture. REQUIREMENTS: Tween 20 (liquid detergent), 0.1% HgCl2, 70% alcohol, sterile distilled water Glasswares glasses, sterile Petri plates, sterile blades, sterile mipps, Fabric Equipment Laminar Air Flow Hood, Autoclave PROCEDURE 1. Wash the sheet with tap water to remove the soil and dust particles laid on the surface. 2. Transfer the washed sheet to a glass glass containing tap water; Add a few drops of liquid liquid - Tween 20. 3. Cover a glass of mouth muslin cloth with gum and keep under running tap water for 1 hour to remove any wax/oil deposition on the surface of the sheet. 4. Wash his tris with distilled water. 5. Transfer the leaves explant into the laminar air flow hood for further work to avoid contamination. 6. Wash the above sheet with sterile distilled water for three times each wash should be for 3-4 minutes. 7. Treat it with a 0.1% HgCl2 solution for 60 sec. 8. After treatment with a disinfectant, wash it with sterile water for three times, each wash should be for 3-4 minutes. 9. Wash 70% alcohol for 30 seconds to remove water from the surface of the sheet. 10.Transfer t he sterile sheet on sterile petriplets. 11.Cut the sheet into small pieces about 1x1 cm with a sterile blade. 12.Now the alien is ready to be vaccinated. IT'S EXPERIMENTAL. 5 PREPARATION OF EXPLANT FOR CALLUSINDUCTION INTRODUCTION Explant is any part taken from the plant that will be used to initiate the culture in vitro. It can be a part of the shoot or leaves or even just some cage. Any part of the plant that can regenerate and cause callus can be used as an explant. Selection, preparation and vaccination explant can be done through concrete steps. The choice of explant selection explant depends on various factors like the time of the plant material, the area of the plant can be used, etc. In many types of explant different organs vary in growth and regeneration rates, while some do not grow at all. The choice of material explant also determines if plantlets developed through the culture of haploid tissue or diploid. The risk of microbial contamination with inappropriateexplants also increases. Therefore, it is very important that the appropriate choice of explant be made up to the fabric culture. Specific differences in the potential for regeneration of different organs and explants have different explanations. Significant factors include differences in cell stage in the cell cycle, the presence or ability to transport endogenous growth regulators, and the metabolic capabilities of cells. The most commonly used tissue explants are the meristematic ends of plants like the tip of the trunk, the auxiliary tip of the kidney and the tip of the root. These tissues have high cell division rates and either focus or produce the necessary substances to regulate growth, including oxins and cytokines. [...] A quote from the paper Hirenkumar Sherathiya (Author), 2012, Practical Guide to Plant Tissue Culture, Munich, GRIN Verlag, Read e-book sterilization in plant tissue culture slideshare. methods of sterilization in plant tissue culture. surface sterilization in plant tissue culture. methods of sterilization in plant tissue culture ppt. seed sterilization in plant tissue culture. importance of sterilization in plant tissue culture. define sterilization in plant tissue culture. filter sterilization in plant tissue culture

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