CHAPTER 3

Sterilization Techniques in Plant Tissue Culture

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INTRODUCTION

Plant tissue culture techniques have been used routinely in the study of totipotency and the roles of hormones in cytodifferentiation and organogenesis. Tissue-cultured plants that have been genetically engineered provide insight into plant molecular biology and gene regulation which are key to plant and agricultural biotechnology. Clonal selection of elite plants and production of secondary metabolites using cultured suspended cells. Tissue culture methods are often used in the formation of somatic haploid embryos from which homozygous plants can be generated.

Tissue culture involves the use of small pieces of plant tissue called explant which are cultured in a nutrient medium under sterile conditions. Using the appropriate growing conditions for each explant type, plants can be induced to rapidly produce callus (a group of undifferentiated cells), somatic embryos (embryos developing from vegetative cells), suspension cultures, new shoots, and, with the addition of suitable hormones new roots. The new plants are then hardened in the control conditions, placed in soil and grown in the field condition as a normal plant grows in nature.

Plant tissue or cell culture requires various combinations of nutrients, minerals, plant growth substances, vitamins and sugars (as carbon source). Unfortunately, these culture media are also suitable for the rapid growth of bacteria and fungi. Once these microbes invade the plant tissue or cell culture they usually grow quickly, depleting the medium of nutrients and producing toxins that can affect the growth of, and ultimately kill, the cultured plant tissue.

Thus, it is imperative to use sterile techniques for all *in vitro* plant culture manipulations. The preparation and maintenance of tissue culture system requires the sterilization of the culture medium, the culture container, surface-sterilization of the seed or plant tissue to be cultured, and sterilization of any instruments used to handle or manipulate the plant tissue. In addition, any subsequent manipulation of the plant tissue must typically be carried out in a sterile room or cabinet and filtered-air environment like laminar air-flow cabinet. Contamination by bacteria and fungi is a frequent problem that continually threatens plant tissue cultures. Even a fungal spore or bacterial cell that comes into contact with the growth

media will rapidly contaminate the plant tissue culture materials and instruments as well. Killing or removing all forms of microbial life (including endospores) in a material or an object is defined as the process of sterilization. This review deals with the techniques and methods of sterilization to maintain the laboratory, equipments and the plant materials used in tissue culture.

ASEPTIC STERILIZATION TECHNIQUE

The essence of sterilization or aseptic technique is the exclusion of invading microorganisms during experimental procedures. If sterile tissues are available, then the exclusion of microorganisms is accomplished by using sterile instruments and culture media concurrently with standard bacteriological transfer procedures to avoid extraneous contamination. Media and apparatus are rendered sterile by autoclaving at 15 lbs/inch^2 (121°C) for 15 minutes. The uses of disposable sterile plastic ware are convenient. Filter sterilization is employed for heat-labile substances like cytokinins. Aseptic transfers can be made on the laboratory bench top by using standard bacteriological techniques using a laminar flow hood. Plant tissue collected from field or which have a probability of microbial contamination needs surface sterilization with ethyl alcohol and/or chlorox and added surfactant. Concentration of thesurface-sterilant and exposure time are determined empirically depending on the type of explant used.

STERILIZING CULTURE ROOMS AND TRANSFER HOODS

Tissue culture lab is compartmentalized into work elements in separately specified areas such as, media preparation, glassware washing, sterilization, microscopy, and aseptic transfers, in order to facilitate all operations and enhances cleanliness. Tissue culture media and nutrient agar, and Laminar flow hoods are available from several suppliers.

Large transfer rooms are best sterilized by exposure to ultraviolet (UV) light. Sterilization time varies according to the size of the room and should be done when no experiment is in progress. Ultraviolet radiation is harmful to the eyes. Transfer rooms can also be sterilized by washing them 1-2 times a month with a commercial brand of antifungal liquids. Smaller transfer rooms and hoods also can be sterilized with UV lights or by treatment with bactericides and/or fungicides. Laminar flow hoods are easily sterilized by turning on the hood and wiping down all surfaces with 95% ethyl alcohol 15 min before initiating any operation under the hood.

Culture rooms should be initially cleaned with detergent-brand soap and then carefully wiped down with a 2% sodium hypochlorite solution or 95% ethyl alcohol. All floors and walls should be washed gently on a weekly basis with a similar solution; extreme care must be used to avoid stirring up any contamination that has settled. Commercial disinfectants diluted at manufacturer's recommended rates can be used to disinfect work surfaces and culture rooms. Formaldehyde gas is an excellent disinfectant for larger culture area and rooms. Commonly used as formalin, a 37% aqueous solution inactivates viruses and bacteria by inactivating proteins by forming covalent cross-links with several functional groups.