Hygiene Problems in Plant Tissue Culture Propagation©

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INTRODUCTION

Plant tissue cultures have been used for various purposes such as mass propagation, production of disease-indexed plants, and genetic engineering. Plant tissue culture is defined as culture of plant cell, tissue, or an organ under sterile conditions in an artificial medium under aseptic conditions. This statement clearly indicates that for a successful tissue culture operation there should not be any contamination by microorganisms during the entire culture period. Contamination by microorganisms will not allow the plant cultures to grow, and the contaminants will eventually destroy plant cultures. Therefore, it is important to be aware of the major contaminants, the sources of such contaminants, and the methods available for sterilizing plant material before the introduction into tissue culture medium and to know the methods for an overall hygiene in a tissue culture operation.

Contamination caused by some common microorganisms can result in large losses during micropropagation, and their control is the most serious problem encountered in many commercial laboratories. In plant tissue culture practices, contaminants can also interfere with plant performance and final results. Therefore, it is imperative to detect and eliminate all contaminants before introducing plant material in vitro and also to be aware of the methods for avoiding contamination during routine sterile operations.

CONTAMINANTS

Fungi. Fungi are common contaminants in tissue culture at the establishment operations. Fungi could enter the system with improperly sterilized plant material or they could creep in during handling of plant material or preparation of culture medium. Infection by fungi can become apparent within a few days of culture.

Bacteria. Bacteria are the most troublesome kind of contaminants both at the establishment stage and during routine operations. Numerous genera of bacteria are found in plants grown in vivo, and these can cause problems in tissue cultures (George, 1996). Even harmless bacteria found in plants grown in vivo can interfere with the growth of in vitro cultures and cause death of tissue. Bacteria can be introduced along with the plant material or during culturing and transferring operations. In addition, some bacteria are reported to be there from the endogenous system of the plant material. This kind of infection could be plant-species specific. If this problem crops up, systemic sterilants have to be used to eradicate the infection.

Yeast and Other Microorganisms. Some common yeasts and mycoplasmas can also cause problems. However, these are not commonly found in tissue culture systems**.** Plants grown in outside environments may be systemically infected with fungal, bacterial, and virus diseases. These plants certainly carry more surface con-

taminants. Plant material can also be cultured without noticing the presence of microorganisms. Such latent or hidden contaminants can become a problem later on.

Pests. Pests such as mites and aphids can cause problems by themselves or by introducing fungal and bacterial spores into the culture vessels.

EFFECTS OF CONTAMINANTS

Once the contaminants enter the culture vessels where the plant cultures are grown it will most likely lead to a total loss of cultures. The culture medium is a highly suitable medium for most of the contaminants to establish and flourish rapidly within a short time. Once the cultures are contaminated, re-sterilization of the cultures will most likely not give successful results. Therefore, it is very important that the initial cultures are free of any microorganisms and subsequent transfers of the cultures are done with extreme care. Saprophytic fungi could grow very fast and destroy the cultures making retrieval of infected cultures extremely difficult or impossible.

Some contaminants stay hidden and cause several defects such as growth retardation, reduced root formation, and altered morphology (Long et al., 1988; de Fossard, 1977; Hanus and Rohr, 1987). In such cases, it is advisable to re-initiate cultures from the beginning. Although there have been some reports of beneficial microorganisms, such organisms are not common (Hamill et al., 2005). Clean and disease-free cultures are more vigorous and grow faster than infected cultures.

MAIN SOURCE OF CONTAMINANTS

Plant material is the main source of contaminants. The culture medium has to be properly sterilized and stored away under favourable conditions such as a clean area at low temperature for safe and long-term storage. The instruments used for cutting and transferring the cultures have to be properly sterilized. The overall sterile techniques have to be perfect to avoid any contamination.

STERILIZATION OF PLANT MATERIAL

Several sterilization techniques are used for surface sterilizing the initial plant material before introducing them into the culture medium**.** The most common sterilant is sodium hypochlorite (NaHOCl). The 1% to 2% of active chlorine present in this compound usually eradicates most contaminants on the surface when treated over 15–20 min. It is advisable to use a wetting agent as well during the sterilization process. There are other sterilants, such as calcium hypchlorite and mercuric chlorite, that can be used for such purposes. Additional steps such as sterilization under vacuum can be used for more recalcitrant contaminants. It is important to prepare the mother plants free of diseases, and the plants must be in good healthy condition. Greenhouse-grown plants are better sources than plants grown outside because the plants from outside will usually have more contaminants.

There are some commercial sterilants such as Alcide (Alcide Co., U.S.A.) that can be used for certain hidden contaminants. Antibiotics and chemicals such as Plant Preservative Mixture can be added to the culture medium during the growth of cultures to control the contaminants. Plant Preservative Mixture is reported to be a heat-stable biocide that can control several contaminants. However, the side effects of these chemicals on the plant performance have to be checked.

STERILE TECHNIQUES AND EQUIPMENTS

It is very important that the sterilized plant material is handled carefully by practicing proper sterile techniques. For this, sterilizer units such as Bunsen flame or electric sterilizers along with appropriate instruments such as long handled forceps and scalpels have to be used. In addition, a sterile air-flow cabinet will be useful for 100% guarantee.

CONTAMINATION DETECTION

Visual inspection of the cultures will enable the detection of most of the common fungal, yeast, and bacterial contaminants. Fungal growth usually dominates if the cultures are not sterilized properly in the first instance. Some bacteria are slow to grow, and it may take a few weeks to see such bacterial growth. Sometimes, the latent bacteria can be detected only after several months.

Plant tissue culture medium contains compounds that will favour rapid growth of many common microorganisms. Occasionally, the type of culture medium can also influence the growth of the contaminants. For example, a contaminant may show up easily in liquid culture medium, while the solid medium could prevent its visibility. Also, some compounds in the medium may inhibit the growth of the contaminant, but the contamination will show up when the cultures are transferred to new medium without such compounds. It is advisable to culture explants at the beginning and during culturing in microbiological media to detect bacteria that could be concealed in plant tissue. This procedure is called indexing, and in this method a portion of the plant tissue is transferred to a bacteriological medium and incubated to check for the growth of any microorganisms. Several endogenous and disease-causing organisms can be detected by this technique. More recently, molecular techniques have also been developed for detecting and identifying contaminants (Cassells, 1991, 1997; Hamill et al., 2005; Lata et al., 2006). Because surface sterilization mainly eliminates epiphytic organisms, any infection within the tissue (systemic infection) must be detected and treated accordingly.

CONTROL MEASURES

Considering the facts given above, careful steps taken through appropriate methods is a good strategy for avoiding the contaminants in the first place. In case of difficulties in controlling common fungi at the start of culturing, fungicides can be used in the medium. It is also important to examine the cultures on a regular basis and discard all infected cultures. The initial explants can be cultured in a medium containing yeast extract and peptone to encourage the microbial growth, and this way any escapes from the initial decontamination procedures can be detected at an early stage (Leifert and Cassells, 2001).

Heat therapy and meristem cultures can be used to eliminate virus-like organisms and other microorganisms such as mollicutes. Antibiotics can also be used to control some microorganisms (Lata et al., 2006). In some cases regeneration of plants through callus phase helped to eliminate mycoplasma-like bodies (Ulrychova and Petru, 1975). Meristem is known to be free of microorganisms; therefore cultures established using meristem are free of microorganisms including all systemic contaminants. Meristem tip culture in combination with heat-therapy could help in getting virus-free cultures.

Cultures have to be checked regularly while they are in the growth rooms or cabinets for any contamination and pest infestation. Pests such as common dust mites could cause serious damage to the cultures. In a study conducted in a commercial laboratory, three species of mites have been identified as vectors of fungal contaminants (Terras et al., 1991). These mites have pouches in which fungal spores are carried and the spores could be planted on tissue culture medium. Spores germinate readily in the medium, and the excessive fungal growth will destroy the entire cultures. The mite population will grow rapidly by feeding on the mycelium, the mites will continue to infest other culture vessels, and eventually a room full of cultures could be completely destroyed in a very short time. Frequent checking, removal of old and infected cultures, cleanliness, and use of miticide spray for the growth room will help to control such losses. All contaminated cultures should be kept away from the culture area and destroyed by autoclaving.

CONCLUSION

In order to avoid hygiene problems in plant tissue culture, the best advice is to start off the practice with effectively sterilized initial cultures and exercise good contamination control procedures, which should also include routine inspection of cultures and the growth environment in which the tissue cultures are grown for any contaminants.

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