AVOID OF BACTERIAL CONTAMINANTS IN TISSUE CULTURES

By

AMANY ELIWAY ALI SAID
B. Sc. Agric. Sci. (Agric. Biochemistry), Fac. Agric., Cairo Univ., 2011

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APPROVAL SHEET

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Title of Thesis: Avoid of Bacterial Contaminants in Tissue Cultures
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ABSTRACT

This research was designed to study the biological effects of actinomycetes and essential oils on microbial contaminants in banana plants tissue culture *in vitro*. Microbial contaminations are considered to be of the most serious constraints facing banana plant in tissue culture proliferation *in vitro.* Four antibiotics, namely rifampicin, gentamicin, chloramphenicol and vancomycin each at 50, 100 and 200 mg /litre and three antifungal agents, namely ketoconazole, fluconazole and nystatin each at 50, 100 and 200 mg/litre were used in the culture susceptibility tests of the identified bacteria and fungi, respectively.

To control banana plants contaminated by bacteria *in vitro*, actinomycetes (*Streptomyces bobilii* or *S. grisiobrunneus)* were tested. Actinomycetes as antimicrobial agent were tested in two ways, first in Petri dishes to test the antagonism between antimicrobial agents from actinomycetes and bacteria as well as fungi contamination, second in culture medium from the beginning at concentration of (0, 5, 10 and 15%) in different stages of banana indirect stages (shooting and rooting). Data obtained showed that, after twelve weeks, *S. grisiobrunneus* was more effective than *S. bobilii* at any concentration tested and at different stages. Addition of *S. grisiobrunneus* at the concentration of 10 % to the culture media during all studied stages was the prefer treatment which caused the best number of explants free contamination and the best number of survived explants.

Moreover, seven commercial pure essential oils were tested as antimicrobial activities *i.e.* Peppermint Oil, Coriander Oil, Ginger Oil, Marjoram Oil, Nigella Sativa Oil, Onion Oil, and Garlic Oil as 1.0 cm oil per 50 ml *Murashige* and *Skoog* (MS) medium before solidified medium. Media supplemented with essential oils, were compared to control medium, autoclaved at 121°C for 20 min. From our results we found that, Coriander Oil was the best in controlling the contamination and also more healthy on plant. The ratio of contamination after addition of coriander oil on *Murashige* and *Skoog* (MS) medium was reached to 33.3%. While, the other best oil used was *Nigella sativa* which gave a contamination reached to 41.6% and also healthier plant.

Key words: Antibiotic treatment, Antifungal treatment, Actinomycetes, Bacteria, Fungi, Essential oil, Banana, Contaminants and MS medium

DEDICATION

*In the Name of **Allah**, the most gracious, the Most Merciful, all praise be to **Allah** and prayers and peace be upon **Prophet Mohammed** His servant and messenger. I dedicate this work to my dear and beloved **father** and **mother**, my dear **brothers**, for all the support they lovely offered during my post-graduate studies and all my life.*

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First of all, Alhamdulillah, all praise be to ***Allah****, the Lord of the Worlds. HE the Almighty gave me the strength to complete this work and made me lucky to be supervised by so kind and helpful supervisors I wish to express my sincere thanks, deepest gratitude and appreciation to* ***Prof. Dr. Mohammed Zakaria Sedik****, Professor of Agricultural Microbiology, Faculty of Agriculture, Cairo University and* ***Prof. Dr. Ali Talaat Sabbour*** *professor of plant department, faculty of Agriculture, Cairo University and* ***Dr. Abd El Rahman Metwally*** *for suggesting the problem, supervision, continued assistance, and guidance through the course of my study and for their revision of the manuscript of this thesis. Grateful appreciation is also extended to all staff members of the Agricultural Microbiology Department, Faculty of Agriculture, Cairo University.*

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CONTENTS

INTRODUCTION

Banana is an important fruit crops in Egypt, due its great economic importance as well as nutritional value and high availability throughout the year. Recently, the use of tissue culture increased remarkably, as it is considered one of the modern breeding methodologies for many crops, vegetables and fruits. Banana is the most important crop is bred in a manner tissue culture, which is characterized by a great deal in increasing the quantity produced of the crop, in addition to product quality and excellence through a great deal compared to the traditional method, which is highly exposed to nematode infections, especially in the roots. The fedden production ranges (14-25 tons) of a tissue culture manner compared to the traditional way, which has a yield per fedden (8-14 tons), in order to select a high-yield plants before breeding by tissue culture. The most important banana varieties deployed in Egypt, which are bred in the laboratory, are Jrndnan, Williams and Willaims Zeaf class.

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations to tipotency and the roles of hormones in cytodifferentiation and organogenesis. Currently, tissue-cultured plants that have been genetically engineered provide insight into plant molecular biology and gene regulation. Plant tissue culture techniques are also central to innovative areas of applied plant science, including plant Biotechnology. For example, select plants can be cloned and cultured as suspended cells from which plant products can be

harvested. In addition, the management of genetically engineered cells to form transgenic whole plants requires tissue culture procedures; tissue culture methods are also required in the formation of somatic haploid embryos from which homozygous plants can be generated. Thus, tissue culture techniques have been, and still are, prominent in academic and applied plant science.

Since tissue culture is a viable alternative for the production of agricultural commodities, high yield and quality. Therefore, the efficiency of targeted research study is applying the method of tissue culture bananas in a manner comparable with traditional agriculture and methods of raising the efficiency of production in a manner tissue culture, with recognition of the most important problems facing the production of this style and empowered to find solutions to those problems.

The practice of plant tissue culture has contributed towards the propagation of large number of plant from small pieces of stock plant in relatively short period of time (Daniel, 1998). Basically, the technique consists of taking a piece of a plant (such as a stem tip, node, meristem, embryo, or even a seed) and placing it in a sterile, (usually gel-based) nutrient medium where it multiplies. In most of the cases the original plant is not destroyed in the process a factor of considerable importance to the owner of a rare or unusual plant. The micropropagation has also been used extensively in the improvement of selections of plant with enhanced stress or pest resistance, production of pathogen free plants and somatic hybridizations (Daniel, 1998). The formulation of the growth medium depends upon whether it is intended

to produce undifferentiated callus tissue, multiply the number of Plantlets, grow roots, or multiply embryos for "artificial seed. The nutrient media in which the plant tissue is cultivated is a good source of nutrient for microbial growth. These microbes compete adversely with plant tissue culture for nutrient. The presence of these microbes in these plant cultures usually results in increased culture mortality, the presence of latent infections can also result in variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting (Kane, 2003).

For *in vitro* micropropagation of banana, microbial contamination is a great problem. Although initially surface sterilization works, latter on microbial contamination at the base of the explants was observed within 3 to 5 days after inoculation. Bacterial growth was also observed around the explants in the culture media. Huge number of explants was destroyed in the culture due to endogenous bacteria. The principal microbial contaminants frequently reported in plant *in vitro* cultures are bacteria and fungi. The bacterial contaminants of banana *in vitro* cultures were *Proteus* sp., *Erwinia* sp .and *Klebsiella* sp. while, the fungal contaminants were *Aspergillus* sp., *Fusarium* sp, *Penicillium* sp. and *Candida* sp (Meghwal *et al*., 2000 and Maina *et al*., 2010).

Despite following these aseptic procedures, microbial contamination still remains a major problem affecting banana *in vitro* propagation in laboratory. The objectives of this study were (I) to identify bacterial and fungal contaminants of banana *in vitro* cultures (ii) to evaluate the efficacy *S. bobilii* and *S. grisiobrunneus* on the suppression of the identified microbial contaminants (iii) to know

effect *S. bobilii* and *S. grisiobrunneus* on plants and (iv) to study the effect of different essential oils (peppermint oil, coriander oil, ginger oil, marjoram oil, Nigella Sativa oil, onion oil, and garlic oil) as microorganisms inhibitors as well as sterilization agents on MS medium or eradicating microorganisms causing agents of contaminants.

REVIEW OF LITERTURE

1. Origin of banana

Banana belongs to Planate kingdom, Musaceae family and Musa genus. Musa has two major species namely *Musa acuminate* and *Musa balbisiana* (IITA, 1998). Edible *Musa* spp. was originated in South - Eastern Asia from Eastern India and South to Northern Australia. Banana is likely to have been first domesticated in Papua New Guinea. Early Filipinos probably spread the banana eastward to the Pacific Islands and Hawaii.

Banana and plantains are mostly sterile triploid hybrids between the species *Musa acuminate* (A genome) and *Musa balbisiana* (B genome). All cultivated commercial bananas are triploid, 2n=3x=33 with genome constitution of AAA (mainly the sweet dessert banana), AAB, ABB (mainly but not exclusively starchy plantains eaten after cooking). There are also seedless cultivated AA and AB diploids, Tetraploid (2n=4x=44) with genome constitution of AAAA, AAAB, AABB, ABBB and sterile, except a few parthenocarpic AA and AB diploids (ISAAA, 1999) and most banana cultivars are hybrids of these species. Banana cultivars vary greatly in plant and fruit size, plant morphology, fruit quality, and disease as well as insect resistance. Most bananas have a sweet flavour when ripe; exceptions to this are cooking bananas and plantains. Plantains are hybrid bananas, in which the male flowering axis is either degenerated, or possess only relicts of male flowers. Plantains are always cooked before consumption and are higher in starch than bananas. The two groups of plantains, French and

Horn, produce fewer fruit per plant than sweet bananas. The groups differ in whether the male parts of the inflorescence are persistent or absent.

Banana is the second largest food-fruit crop of the world produced in the tropical and subtropical regions of mostly the developing countries (Singh *et al* ., 2011).The crop is a staple food for 400 million people in the tropics, a major staple food and a source of income for over 20 Million people in Eastern Africa . Total world production is estimated at around 97 million tonnes, of which approximately one third is produced in subsistence farms in Africa (FAOSTAT, 2011). Also (FAO, 2009 and FAOSTAT, 2011) reported that, banana is the largest cultivated fruit throughout the world.

2. Banana tissue culture

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of to impotency and the roles of hormones in cyto differentiation and organogenesis. Currently, tissue-cultured plants that have been genetically engineered provide insight into plant molecular biology and gene regulation. Plant tissue culture techniques are also central to innovative areas of applied plant science, including plant biotechnology and agriculture. For example, select plants can be cloned and cultured as suspended cells from which plant products can be harvested. In addition, the management of genetically engineered cells to form transgenic whole plants requires tissue culture procedures; tissue culture methods are also required in the formation of somatic