بسم الله الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها

علي هذه الأفلام قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ١٥-٢٥ مئوية ورطوبة نسبية من ٢٠-٤٠%

To be Kept away from Dust in Dry Cool place of 15-25- c and relative humidity 20-40%

بعض الوثائـــــق الاصليـــة تالفـــة

بالرسالة صفحات لم ترد بالاصل

Using of Biotechnology Techniques in Plant Improvement and Rapid Propagation

By

Nizar Ali Mualla

B.Sc.Agric.(Hort.), Fac.of Agric., Tishreen University, Syria, 1996

D. Sc.Agric.(Hort.), Fac.of Agric., Tishreen University, Syria, 1997

M.Sc. Agric.(Plant Physiology), Fac.of Agric.,Cairo University, 2001

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APPROVAL SHEET

Name: Nizar Ali Mulla

THESIS TITLE :

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This thesis for the PH.D. Degree in plant Physiology has been approved by :

Prof. Dr. Ahmed Ezzat Abou-Salha

Prof. Dr. Abdel –Rahman Moursy Ghallab

Prof. Dr. Eglal Zaki Harb

Prof. Dr. Osama Mohamed El-Shihy

Name of Candidate: Nizar Ali Mualla **Degree Ph.D.**
Title of Thesis: Using of Biotechnology Techniques in plant Improvement and Rabid propagation

Supervisors: Prof. Dr. Eglal Mohamed Zaki Harb;Prof. Dr. Osama Mohamed AL-shihy . Prof. Dr.Ahmed Hussien Hanafy Ahmed
Department: Agriculture Botany
Branch Plant Physiology **Approval: 3/10/2005**

ABSTRACT

This study was carried out in the Plant Biotechnology Lab, Bioengineering and Plant Analysis Labs, Fac. of Agric., Univ. of Cairo, and Anatomy Lab., Fac. of Science, Ain Shams Univ. Egypt, during the study period (2002-2005).Two banana cultivars (Grande Naine and Williams cvs)were used to study th effect of six levels of water deficit stress induced by PEG (MW 6000) (0,2.5,5,10,15 and 20 %) *in vitro*. *Ex vitro* acclimatized plants were subjected to five levels of PEG (0,2.5,5,10 and 15%).Putrescine at 0, 2 and 4 ppm was added into MS culture medium, and at 0 and 2 ppm was used as foliar application under greenhouse conditions. Data were calculated *in vitro* and *ex vitro*. The obtained data reveal that the increase of PEG concentrations induced negative response in all growth characters (survival percentage, shoot height, and leaves number). Root number root weight, plant dry weight were increased as PEG level increased. Anatomical parameters of leaf (leaf blade, midrib, mesophyll, and vascular bundles thickness), and root (root and vascular cylinder diameter) as well as plant pigments, N,P,K,Ca and Mg concentrations were decreased with the increasing of PEG level either *in vitro* or *ex vitro*. Positive responses of organic components (total sugars, free amino acids ,proline ,phenols)and Na concentrations, anatomical parameters (air cavities of leaf, cortex width and number of vessels of root) were observed as PEG levels increased . On the other hand, the data indicate that the putrescine treatment significantly prevented all above morphological, chemicals and anatomical parameters from the inhibition resulted by water stress conditions.Stomata frequency was significantly decreased by the increasing of PEG concentration. The activity of superoxide dismutase (SOD) and glutathione reductase (GR) enzymes were increased under the increasing of PEG. Positive response of this enzymes was detected by application of putrescine at 2 ppm. New low ,molecular weight protein . It was found that the using of gene gun is effective method for insert new genes into apical meristem of banana . also it was found that the acceleration pressure 1100 psi gave the highest transient expression followed by pAB6 plasmid. Histochemical GUS assay in transformed plants revealed GUS expression. Gene integration was assessed by subjecting plants to PCR analysis using specific primers for *bar* and *GUS* genes.

Eglal Harb

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Supervisors:

Prof.Dr.Eglal Mohamed Zaki Harb
Professor of Plant Physiology
Faculty of Agriculture, Cairo University

Prof. Dr. Osama Mohamed Al-Shihy
Professor of Plant Physiology and Biotechnology
Faculty of Agriculture, Cairo University

Prof. Dr. Ahmed Hussien Hanafy Ahmed
Professor of Plant Physiology
Faculty of Agriculture, Cairo University

بسم الله الرحمن الرحيم

صدق الله العظيم

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CONTENTS

INTRODUCTION

Introduction

Bananas and plantains (Musa. spp.) are major staple food for millions of people in the tropical and subtropical countries

World banana production has been estimated to be 80.6 Millions ton annually (FAO, 1999). Banana plants are monocotyledons of the genus Musa, family Musaceae. Banana and plantains, which are derived from the wild species Musa acuminate (AA) and Musa balbisiana (BB).

The genus Musa is composed of four sections, Eumusa section includes almost all cultivated varieties. The cultivars Williams and GrandeNaine however are belonging to AAA genomic group (2n=3x=33) (Okole and Sch, 1996).

They are seedless, thus the plant propagated vegetative or *in vitro* .the *in vitro* propagation is preferable because it allows the production of a large number of virus free plants in a relatively shorter time and smaller space.

Banana is cultured wherever water is available with respect to the other environmental factors. Recently, it was introduced to desert regions having lower relatively available water. This makes it necessary to investigate the plant response to water stress during its micropropagation and under greenhouse conditions.

However, banana production is threatened by various abiotic stress agents (drought, salinity, freezing ...etc) mainly drought limit growth and productivity of most species (shin *et al.* 2000). Water is a major limiting factor in world agriculture. In general, most crop plants are highly sensitive to even a mild dehydration stress.

Drought is one of the most important constraints of banana production. In fact drought can cause severe damage at any stage of banana growth, and development which leads to yield loss. The study of stress resistance mechanisms in plant is important.

Biotechnology and plant tissue culture technique are effective tools for production drought tolerant plants (Jose *et al.*2000).

In vitro selected plantlet resistant to polyethyleneglycol (PEG) induced water stress was described as osmotically adapted in various species (Dix, 1993). Sala *et al.* (1990) mentioned that the PEG lowers the water potential but doesn't at enter the cell

wall imposing as water stress could be induced in the micropropagation in media by adding osmotic and /or gelling agents such as PEG and agar, respectively.

Polyamins (PAs) have been found in all living organisms studied and are required for normal development of both prokaryoutes and eukaryotes (Tabor and Tuber, 1984).

There is a growing interest in the possible involvement of polyamine in the defense reaction of plants to environmental stress (Boucherau *et al.*1999).

Because of their poly cationic nature at physiological pH, PAs combined strongly of negative charges in cellular components such as nucleic acids, proteins and phospholipids (Smith, 1985).

Several studies recorded that the application of PAs have been shown to protect plant tissue from the determintal effects of several types of stresses; such as osmotic stress (Sun *et al.*2002).It has been proposed that PAs act as protector system in plants under stress conditions .

A common aspect of most adverse environmental conditions is the increased production of reactive oxygen species (ROS) within several subcellular compartments of the plant cell (Van Breusegem *et al.* 1999) ROS can occur as by-products of regular cellular metabolism such as in photosynthesis. However, under stress, their formation is usually exacerbated. Drought stress leads to the disruption of electron transport systems and thus under water deficit conditions the main sites of ROS production in the plant cell are organelles with highly oxidizing metabolic activities or with sustained electron flows: chloroplasts, mitochondria and microbodies. Within the photosynthetic apparatus, photosystem II (PS II) is affected most by drought stress, particularly within the oxygen-evolving complex and the reaction centers (He and Yu, 1995).

In general, ROS (particularly superoxide and hydroxyl radicals) are damaging to essential cellular components such as DNA, proteins and lipids. Lipid peroxidation disrupts the membrane integrity of the plant cell. As a result, essential solutes leak out of organelles and from the cell, causing disruption in membrane function and metabolic imbalances. DNA is the blueprint for both future form and function. Any damage to its integrity could mean that proteins that would have been essential for optimal function of the plant will not be synthesized. Similarly, denaturation of important proteins essential