hossam maghraby

شبكة المعلومات الجامعية

بسم اللّه الرحمن الرحيم

hossam maghraby

شبكة المعلومات الجامعية

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

hossam maghraby

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

hossam maghraby

شبكة المعلومات الجامعية

بعض الوثائق الأصلية تالفة

hossam maghraby

شبكة المعلومات الجامعية

بالرسالة صفحات
لم ترد بالأصل

Evaluation of a new transformation method in tomato

By

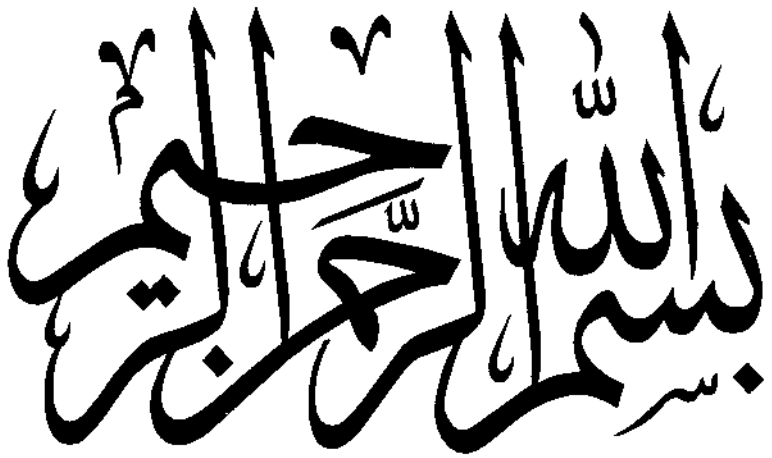
Mohamed Eid Mohamed Saad

B.Sc. Agric. Sci. Cairo University, 1994

Thesis
Submitted in partial fulfillment of the requirements for the degree
of

MASTER OF SCIENCE
IN
Vegetable Crops

Department of Vegetables
Faculty of Agriculture
Cairo University
(2001)


بسم الله الرحمن الرحيم

APPROVAL SHEET

Name: **Mohamed Eid Mohamed Saad**

Title: **Evaluation of a New Transformation Method in Tomato**

A thesis submitted for the M.Sc. degree
In
Vegetable Crops

Approved by:

Prof. Dr. Effat Badr

Prof. Dr. Ahmed A. Hamza

Prof. Dr. M. A. Badawi

Committee in charge

Date: 2 / 6 / 2001

Supervisors

Prof. Dr. Mohammed A. Badawi	Chairman of Dept. of Vegetable Crops Faculty of Agriculture, Cairo Univ.
Prof. Dr. Ebtissam H. A. Hussein	Professor of Genetics Faculty of Agriculture, Cairo Univ.
Prof. Dr. Hanaiya A. El-Itriby	Deputy Director, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center

Approved By

Prof. Dr. Effat A. Badr	Professor of Genetics Faculty of Agriculture, Alexandria Univ.
Prof. Dr. Ahmed A. Hassan	Professor of Vegetable Crops Faculty of Agriculture, Cairo Univ.
Prof. Dr. Mohammed A. Badawi	Chairman of Dept. of Vegetable Crops Faculty of Agriculture, Cairo Univ.

Name of Candidate Mohamed Eid Mohamed Saad **Degree** M.Sc.
Title of Thesis Evaluation of a New Transformation Method in Tomato

Supervisors Prof. Dr. Mohammed A. Badawi, Prof. Dr. Ebtissam H. A. Hussein and Prof. Dr. Hanaiya A. El-Itriby

Department Vegetable Crops
Branch Vegetable **Approval**

ABSTRACT

A new system for transformation of tomato was evaluated using the Helios Gene Gun. Transformation parameters were optimized using a plasmid containing the *GUS* gene. The bombarded leaflets were subjected to the *GUS* histochemical assay and the number of blue foci was counted. The highest *GUS* expression was obtained when using helium pressure of 200 psi with 0.6μm gold particle size. The microcarrier loading quantity (MLQ) of 0.25 mg/shot was found to be the most convenient for use with the Helios Gene Gun. A promoter comparison study was carried out with both the Helios Gene Gun and the Biolistic Gun. The level of expression of the promoters in driving the *GUS* expression was estimated by counting the number of blue foci expressed in the bombarded leaflets. The CsVMV promoter showed the highest expression followed by the CaMV 35S promoter then the Maize Ubi1 promoter while the Rice Act1 showed the lowest average expression. Similar results were obtained with both the Biolistic Gun and the Helios Gene Gun. Apical meristems of 12 plants from each of the 3 cultivars (Castlerock, Strain B and Peto 86) were bombarded with the construct harboring the CsVMV promoter using the Helios Gene Gun. Transformed plants were screened for putative transgenic events using PCR. No amplification products were obtained revealing no sign of stable transformation. The effects of the new system on the morphological characters of the bombarded plants were investigated. Statistical analysis of data collected on different morphological characters indicated no significant differences between the bombarded and non-bombarded plants using the Helios Gene Gun.

M. A. Badawi

To My Family

Mohamed

ACKNOWLEDGMENT

I do thank Allah for all the gifts he has given me

I would like to express my very deep gratitude to my supervisor **Prof. Dr. Mohamed Abd-El-Mageed Badawi,** Chairman of Dept. of Vegetable Crops, Faculty of Agriculture, Cairo University for his valuable guidance, for his great assistance and for the constant help, care and encouragement he provided me.

I would like to acknowledge my supervisor **Prof. Dr. Ebtissam Hussein,** Professor of Genetics, Faculty of Agriculture, Cairo University for her supervision, continuous help and valuable support during the entire work.

I would like to thank my supervisor **Prof. Dr. Hanaiya El Itriby** , Cheif Researcher and Deputy Director of the Agricultural Genetic Engineering Research Institute. (AGERI), for her supervision, valuable advice and keen interest throughout my work.

I can find no words to express my deep gratitude to **Prof. Dr. Magdy Madkour,** Director of the Agricultural Genetic Engineering Research Institute (AGERI), for his kindness and continuous support.

I would like to express my gratitude to **Dr. Gharib Abdel Raouf Gad El Karim**, Chief Researcher at AGERI, ARC, for his keen help with the statistical analyses.

Finally, I appreciate the support of my colleagues at AGERI especially Dr. **Dina El Khishin, Dr. Shireen Assem, Dr. Amina Abdel Hamid, Mohamed Abdel Sadek, Amr Ageez, Khaled Hashem, Ahmed Shokry, Nasser Abdel Razik, Dina El-Amir, Emad Hassanien, Hisham EL-Shishtawy** and our lab. assistants **Howeida El Garhey and Shafik Darwish.**

Abbreviations

neomycin phosphotransferase II	NPT II
β-glucuronidase	GUS
chloramphenicol acetyltransferase	CAT
Cauliflower Mosaic Virus	CaMV
Cassava Vein Mosaic Virus	CsVMV
Polyubiquitin	Ubi
Actin	Act
phosphinothricin acetyl transpherase	PAT
phosphinothricin	PPT
Polymerase Chain Reaction	PCR
Yeast Artificial Chromosome	YAC
green fluorescent protein	GFP
major outer membrane protein	MOMP
I-aminocyclopropane-1-carboxylate	ACC
alcoholdehydrogenase	Adh
Piperazine-N,N`-bis(2-ethanesulfonic acid; 1,4-Piperazinediethanesulfonic acid	PIPES
Sodium Dodecyl Sulfate	SDS
Polyvinylpyrrolidone	PVP
Pound per square Inch	psi
luciferase	LUC
polygalacturonase	PG

CONTENTS

List of Tables