UNIVERSITI PUTRA MALAYSIA

IMPROVEMENT IN ORGANOGENESIS AND THE DEVELOPMENT OF A TRANSFORMATION PROCEDURE FOR CUCUMBER AND MUSKMELON

A. K. M. MOHI UDDIN

FSMB 1998 20

IMPROVEMENT IN ORGANOGENESIS AND THE DEVELOPMENT OF A TRANSFORMATION PROCEDURE FOR CUCUMBER AND MUSKMELON

By

A. K. M. MOHI UDDIN

Dissertation Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Food Science and Biotechnology, Universiti Putra Malaysia.

December 1998

Specially Dedicated
To My

Father (Alhajj Md. Lutful Hoque)
Mother (Tahera Hoque)
Father-in-law (Md. Salimullah)
Mother-in-law (Masuma Salim)
and
Wife (Nazneen Salim)

ACKNOWLEDGEMENTS

First and foremost, my deepest thanks to **ALLAH** for He has guided me through and provided me wisdom, strength and comfort to complete the degree successfully.

I would like to extend my sincere gratitude to my supervisors, Dr. Suhaimi Napis, Dr. K. Harikrishna, Dr. Zaliha Christine Abdullah, Dr. M. Kamal Uddin Chowdhury and Dr. Tan Siang Hee for all their guidance, help, tutelage and invaluable advice during my Ph. D. project and the preparation as well as completion of this thesis. Their criticisms and suggestions have been most constructive and are highly appreciated. Their patience, trust and enthusiasm have left depth of feeling that could not be expressed in words. I express my sincere thanks to Professor Marziah Mahmood for her advice too.

I am profoundly indebted to my parents, father and mother-in-law, brothers, sisters and wife for their sacrifices and encouragement to do higher study in the field of Plant Biotechnology and would like to express my sincere thanks and deepest gratitude to them for their invaluable love and prayers throughout the years to complete my study. I dedicated this work to my parents, father and mother-in-law, with all my love. To my wife, whose love, help, understanding, and encouragement has been the biggest motivation in completing this degree, I dedicated this work to her, with all my love, too. My daughter, **Ramisa** also deserves appreciation for her patience and sacrifice. A special thanks to Professor Md. Sharif, Ex. Chairman and Head of Department of Botany, Jagannath University College, Bangladesh, Assistant Professor Dr. Md. Imdadul Hoque, Department of Botany, University of Dhaka, Bangladesh and Mrs. Nurjahan Begum, Lecturer, Department of Botany, Jagannath University College, Bangladesh for their valuable advice and help.

Accordingly, I would like to say thank to the Ministry of Science, Technology, and the Environment, Malaysia for financial support (Graduate Assistantship) (IRPA Grants, 50304 and 51267) which gave me the opportunity to pursue my Ph. D. degree in Malaysia. I also express my sincere thanks to the Government of The People's Republic of Bangladesh for providing me one way plane fare. Indeed, I wish to thank the Rubber Research Institute of Malaysia (RRIM) for providing *Agrobacterium* strains.

I would also like to thank my friends, entire staff of Faculty of Food Science and Biotechnology and Graduate School, Universiti Putra Malaysia and staffs of Tissue Culture and Genetic laboratory, Biotechnology Department for their friendship, invaluable help, and encouragement throughout my endeavour here.

I hope any who are not mentioned by name will recognise my gratitude for their kindness, advice, and moral support on completion of my degree at Universiti Putra Malaysia. To all others who have contributed as well as assisted me in providing different inputs in one way or another to the successful completion of my study throughout my student life, they are conferred my appreciation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATION

ANOVA	analysis of variance
BAP	6-benzylaminopurine
Bp	base pair
CaMV	cauliflower mosaic virus
CAT	chloramphenicol acetyl transferase
Cf	Cefotaxime
CPA	p-chlorophenoxyacetic acid
cv	cultivar
cvs	cultivars
d	day
DMRT	duncan multiple range test
2,4-D	2,4-dichlorophenoxyacetic acid
g	gram
GUS	β-glucuronidase
h	hour
IAA	indole acetic acid
IBA	indole-3-butyric acid
2iP	6-(γ-γ-dimethylallylamino) purine
Kg	Kilogram
Km	kanamycin
L	liter
LB	left border
LB medium	Luria-Bertani medium
M	molar
Min	minute
ml	millilitre

MS	Murashige and Skoog
MUG	4-methyl umbelliferyl glucuronide
NAA	naphthalene acetic acid
NOS	nopaline syntheses
nptII	neomycin phosphotransferase II
OD	optimal density
PCR	polymerase chain reaction
P^H	hydrogen ion concentration
RB	right border
Rf	rifampicin
Ri	root-inducing
SAS	statistical analysis system
s	second
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
TDZ	thidiazuron
Ti	tumour-inducing
v/v	volume/volume
w/v	weight/volume
X-gluc	5-bromo-4-chloro-3-indolyglucuronide
µ	micro

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

IMPROVEMENT IN ORGANOGENESIS AND THE DEVELOPMENT OF A TRANSFORMATION PROCEDURE FOR CUCUMBER AND MUSKMELON

By

A.K.M. MOHI UDDIN

December 1998

Chairman: Dr. Suhaimi Napis

Faculty: Food Science and Biotechnology

A comprehensive study was carried out to optimise and improve a direct shoot organogenesis system to facilitate the transfer of reporter genes (*GUSINT* and *nptII*) into cucumber (*Cucumis sativus* L.) and muskmelon (*C. melo* L.) plants. The studies carried out were: (i) a comparative study on direct and indirect shoot regeneration (ii) the improvement of a direct shoot regeneration system by using an ethylene action inhibitor, silver nitrate and an ethylene biosynthesis inhibitor, cobalt chloride (iii) identification of suitable strains of *Agrobacterium tumefaciens* and *A. rhizogenes* that are able to infect cucumber and muskmelon plants (iv) development of an *intron* containing gene transformation system for both cucurbit species through *A. tumefaciens* and (v) analysis of transformants.

For the comparative study of direct and indirect shoot regeneration, four different types of explants from five elite commercial cucumber cultivars namely Spring Swallow, Suyo Cross, Suyo Long, Tasty Glory, Tasty Green and one muskmelon cultivar called Birdie were used. Explants from both species were cultured onto Murashige and Skoog medium containing different concentrations of *6*-benzylaminopurine alone and in combination with either 2,4-dichlorophenoxyacetic acid or naphthalene acetic acid. *6*-benzylaminopurine alone at 1.0 or 2.0 mg/l significantly ($p<0.05$) induced shoot primordia

from the largest number of proximal cotyledon and hypocotyl explants of all cucumber cultivars and muskmelon explants tested. However, the addition of either 2,4-dichlorophenoxyacetic acid or naphthalene acetic acid to *6*-benzylaminopurine, resulted in a reduction of the shoot primordia induction rate.

Higher number of shoots induced from explants as well as high numbers of morphological normal shoots were obtained when explants were cultured on medium containing *6*-benzylaminopurine alone. Specific concentrations of indole-butyric acid and naphthalene acetic acid significantly ($p<0.05$) contributed to root initiation from the largest number of cucumber and muskmelon shoots, respectively. Dark treatment was sufficient to significantly induce root formation from the non-rooting cucumber and muskmelon shoots.

The addition of either silver nitrate or cobalt chloride to the seed germination medium or shoot primordia induction medium caused a significant ($p<0.05$) enhancement of shoot regeneration rate from cucumber cv. SS and TG explants compared to the control. The regeneration rate was further enhanced when these two ethylene inhibitors were added to both SGM and SPI media. Furthermore, the number of shoots induced from explants of both Spring Swallow and Tasty Green cultivars was also enhanced upon the same treatment. However, muskmelon shoot induction and regeneration were reduced when the same treatment was employed.

In the *Agrobacterium*-mediated transformation experiments inoculation of cucumber cultivar Spring Swallow and muskmelon cultivar Birdie explants with *A. tumefaciens* and *A. rhizogenes* wild type strains revealed the different degrees of virulence of both bacteria. It was found that the virulence of both *Agrobacterium* species was enhanced when acetosyringone was added to the culture of inoculum