



UNIVERSITY OF MALAYA

R

***IN VITRO* MUTATION INDUCTION BY GAMMA
IRRADIATION FOR THE IMPROVEMENT OF A
TRIPLOID BANANA PISANG BERANGAN (AAA)**

By

SHADIA ABDELGADIR MOHAMED RAYIS

**INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA**

**DISSERTATION PRESENTED FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY
UNIVERSITY OF MALAYA
KUALA LUMPUR
(2002)**

Perpustakaan Universiti Malaya



A510576282

DEDICATED TO:

MY PARENTS
&
MY HUSBAND

ACKNOWLEDGEMENTS

I would like to express my deepest indebtedness and gratitude to my supervisors, Professor Mak Chai and Assoc. Prof. Dr. Rofina Yasmin Othman for suggesting the subject of this study and for their supervision and keen interest, helpful guidance, criticism and encouragement during this research.

Part of the field work was carried out in the United Plantations Bhd., Teluk Intan, Perak. The author is thankful to Dr. Gurmit Singh and Mrs Y. W. Ho for all the facilities and assistance throughout the duration of experiment. Thanks are due to members of this Institute of Biological Science especially Mr. Ang Whi Leng, my friends and colleagues for their continuous assistance during this work. Last, but not least many thanks and good wishes to my sister Dr. Hjh. Sarminah Samad at the Dean Office, Faculty of Science for her helpful and valuable assistance.

I am also grateful to the Ministry of Higher Education, Sudan Government, for providing me with the opportunity and sponsorship to undertake this post-graduate study. My sincere thanks are extended to Sudan Embassy especially to Mrs Afaf Mohamedani for their care and encouragement.

Finally, I wish to express my gratitude to my understanding and supportive husband, Ahmad Al Tigani Ali Gowai, for his help, patience and filling the void during my absence. I would also like to record my utmost appreciation to my family for their encouragement and support.

The close of my thanks (Al-Hamdu Lil-Hahi Rabiul Alameen) Praise be to Allah, Lord of the World.

ABSTRACT

Tissue culture and induced mutation techniques were used to generate variation for the improvement of Pisang Berangan cv. 'Intan' (AAA). Best field performing banana *in vitro*-plants were multiplied and evaluated for better field performance, which included improved agronomic traits such as earliness in fruiting, short plant stature, high yielding capacity with desirable fruit quality and tolerance to *Fusarium* wilt.

A suitable micropropagation medium for Pisang Berangan was established through modification of MS basic medium, which resulted in significant increase in shoot multiplication. Shoot-tip meristems of Pisang Berangan were then irradiated at 20, 30, 40 and 60 Gy to induce mutations. The cultivar exhibited differences in dose responses and post-irradiation recovery based on the number of shoots produced per explant. The irradiated explants were multiplied *in vitro* to produce M_1V_4 plants before being field planted for evaluation.

Gamma irradiation caused many mutagenic changes in both qualitative and quantitative traits. Variants of morphological traits include pseudostem: size, length and pigment; leaf: deformation, colour and size and deformation of bunch/ finger etc. attributed to the bulk of the variation accounting for 25 – 30% while agronomic traits (plant height, bunch weight and fruit characters) accounting for only 3 –4%. There were also somaclonal variations observed in tissue cultured plants (without irradiation). However, frequency of somaclonal variation was much lower than mutagenic changes.

Nuclear DNA content of mutated plants was determined by using flow cytometric techniques. The results showed differences in DNA content between variants indicating the effect of gamma-irradiation on the genotype of these plants. Variants of short plant stature or stunted growth showed great differences in DNA content compared to control (non-irradiated). Generally, nuclear DNA contents decreased with an increase of gamma-dose from 20 Gy to 60 Gy.

Random amplified polymorphic DNA (RAPD) analysis was used to examine changes in the mutants. Four out of 10 primers tested showed polymorphism, which could differentiate between normal and dwarf variants. Among 450 mutant tested, only 50 samples showed clearly reproducible bands suitable for further analysis. Primer OPA-03 and OPA-05 showed major bands of 1.5 Kbp and 0.7, 0.5 and 0.4 Kbp respectively, which were absent in 40 Gy samples when compared with control and plants treated at 20 and 30 Gy, while the major bands of 1.5 Kbp and 1.0 Kbp were absent in 60 Gy treated plants. Primers OPA-03 and OPA-09 detected a higher range of variability at 40 and 60 Gy doses which produced variants that were either very short or with very low bunch weight or no bunching.

Detection of dwarf off-types caused by somaclonal variation and mutation induction of Pisang Berangan was examined by using Gibberellic Acid (GA_3) at 29 $\mu\text{mol/L}$ and

59 $\mu\text{mol/L}$ concentrations at *in vitro* culture stage and at 289 $\mu\text{mol/L}$ for plants at deflasking stage. Differences in response between dwarf variants and control plants were not constant for treatment at the *in vitro* stage. However, at the deflasking stage elongation of the sheath of the first emerged leaf after GA_3 treatment was about 2-fold greater in control plants compared with dwarf mutants, which is useful in discriminating between normal and dwarfs. The method is simple and cheap, and could prevent the waste of resources on non-productive dwarf plants.

Somaclonal variants and induced mutants at 20, 30, 40 and 60 Gy were evaluated for tolerance to *Fusarium* wilt disease by using 'double-tray' technique as well as testing in heavily infested field (Hot-spot). In double-tray screening, the results showed that all inoculated plantlets succumbed to disease infection gradually, ranging from 10 days to one month. In field evaluation, all suckers tested did not survive more than 6 months, though some mutants survived longer period than others.

ABSTRAK

Teknik kultur tisu dan aruhan mutasi digunakan untuk menghasilkan variasi dalam meningkatkan prestasi Pisang Berangan kultivar Intan (AAA). Pokok-pokok pisang *in vitro* yang mempunyai prestasi ladang terbaik digandakan dan dinilai untuk menghasilkan prestasi ladang yang lebih baik termasuk ciri-ciri agronomi seperti pembuahan awal, pokok rendah, hasil kualiti buah yang tinggi serta ketahanan terhadap penyakit layu *Fusarium*.

Suatu media mikropropagasi yang sesuai untuk Pisang Berangan menggunakan medium asas MS yang diubahsuai memberikan peningkatan ketara dalam penggandaan pucuk. Meristem pucuk Pisang Berangan diiradiasikan pada dos 20, 30, 40 dan 60 Gy untuk mengaruh mutasi. Kultivar berkenaan memberikan perbezaan di dalam tindakbalas terhadap dos radiasi dan pemulihan selepas radiasi berdasarkan bilangan pucuk yang terhasil setiap eksplan. Eksplan teraruh digandakan secara *in vitro* bagi menghasilkan pokok-pokok M₁V₄ sebelum ditanam di ladang untuk penilaian.

Radiasi gamma mengakibatkan banyak perubahan mutagenik bagi ciri-ciri kualitatif dan kuantitatif. Variasi ciri morfologi termasuk pseudostem: saiz, panjang dan pigmen, daun: keabnormalan, warna dan saiz serta buah: saiz, keabnormalan tandan/sikat dan sebagainya merupakan variasi tertinggi iaitu 25 – 30% sementara ciri-ciri agronomi (tinggi pokok, berat tandan dan ciri buah) hanya kira-kira 3 – 4% sahaja. Variasi somaklon juga didapati pada pokok-pokok kultur tisu normal (tanpa radiasi). Bagaimanapun frekuensi variasi somaklon berbeza dan ianya sangat rendah berbanding perubahan mutagenik.

Kandungan DNA nuklear mutan ditentukan menggunakan teknik aliran sitometri. Perbezaan kandungan DNA antara mutan menunjukkan kesan radiasi gamma ke atas genotip pokok berkenaan. Variasi bagi ketinggian pokok dan tumbesaran bantut menunjukkan perbezaan kandungan DNA yang besar berbanding kawalan (tidak teraruh). Secara amnya, kandungan DNA nuklear menurun dengan peningkatan dos gamma dari 20 ke 60 Gy.

Analisis amplifikasi rawak DNA polimorfik (RAPD) digunakan untuk mengkaji perubahan-perubahan DNA pada mutan. Empat dari 10 primer menunjukkan polimorfisme yang boleh membezakan di antara pokok normal dan bantut. Daripada 450 mutan yang diuji, hanya 50 sampel menunjukkan jalur-jalur yang jelas dan sesuai untuk analisis. Primer-primer OPA-03 dan OPA-05 menunjukkan jalur-jalur utama 1.5 dan 0.7, 0.5 dan 0.4 kbp masing-masing dalam pokok yang dirawat pada dos 40 Gy berbanding kawalan dan pokok-pokok yang dirawat pada dos 20 dan 30 Gy. Jalur-jalur utama 1.5 kbp dan 1.0 kbp pula wujud dalam pokok-pokok yang dirawat pada dos 60 Gy. Primer-primer OPA-03 dan OPA-09 dikesan menghasilkan variabiliti yang tinggi pada dos 40 dan 60 Gy yang menghasilkan variasi pokok renek, berat tandan yang rendah serta tiada tandan.

Pengenalpastian pokok renek hasil variasi somaklon dan aruhan mutasi Pisang Berangan dilakukan menggunakan Asid Gibberalik (GA₃) pada kepekatan 29 µmol/L dan 59 µmol/L pada peringkat *in vitro* dan 289 µmol/L pada peringkat deflask. Perbezaan tindakbalas antara variasi renek dan kawalan tidak mantap untuk rawatan pada peringkat *in vitro*.

Bagaimanapun, pada peringkat deflask, pemanjangan berkas daun pertama muncul selepas rawatan GA₃ kira-kira dua kali ganda lebih dari kawalan berbanding mutan renek di mana ia berjaya membezakan diantara normal dengan yang renek. Kaedah ini adalah mudah dan murah disamping berkesan mengelakkan pokok renek yang tidak produktif.

Variasi somaklon dan aruhan mutasi pada dos-dos 20, 30, 40 dan 60 Gy disaring terhadap ketahanan terhadap penyakit layu *Fusarium* menggunakan teknik 'double-tray' dan ujian ladang yang terinfeksi 'Hot Spot'. Keputusan daripada kaedah 'double tray', menunjukkan semua pokok yang diinokulasi mati akibat infeksi penyakit antara 10 hari hingga sebulan. Daripada penilaian ladang pula, semua pokok yang disaring tidak dapat hidup melebihi 6 bulan. Bagaimanapun terdapat beberapa mutan yang hidup lebih lama berbanding yang lain.

LIST OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
ABSTRAK	vi
LIST OF CONTENTS	x
LIST OF TABLES	xv
LIST OF FIGURES	xix
LIST OF PLATES	xxi
ABBREVIATIONS AND ACRONYMS	xxv

CHAPTER ONE: INTRODUCTION

1.1 Importance of Bananas and Plantains	1
1.2 Genetic Improvement of Banana	5
1.3 The Objectives of the Study	6

CHAPTER TWO: LITERATURE REVIEW

2.1 General Introduction to Plant Breeding	8
2.2 Classification of Banana Germplasm	9
2.3 Banana Breeding Systems and Genetics	12
2.4 Propagation of banana germplasm	13
2.5 Strategies for Banana Improvement	15
2.6 Approaches to Banana Improvement	18
2.6.1 Conventional breeding of cultivated bananas	18
2.6.2 Non-conventional Breeding	20
2.6.2.1 Somaclonal variations	21
2.6.2.2 Mutation Breeding	24
2.6.2.2.1 Sources of Mutation	27
(A) Natural Mutations (Spontaneous)	27
(B) Induced Mutations and Mutagenesis	28
(C) Mutation Breeding of Vegetatively propagated Crops	30
Including <i>Musa</i> spp.	
2.6.3 Success in Mutation Breeding	33
2.6.4 Constraints in Mutation Breeding	34
2.6.5 Ploidy Effects in Bananas Phenotypic Appearance	35
2.7 Flow Cytometry for Analysis of <i>Musa</i> genome	37

2.8 Molecular Markers in <i>Musa</i> Breeding Programs	39
2.8.1 DNA Markers	40
2.8.2 Polymerase Chain Reaction: PCR-Based Fingerprinting of Bananas	41
2.9 The role and potential of Gibberellic acid	43
2.9.1 Detection of Dwarf off-types in Micropropagated Bananas	44
2.9.1.1 The use of Gibberellic acid for detection of dwarf off-types	45
2.9.1.2 The use of Molecular Marker for detection of dwarf off-types	47
2.10 Constraints in Banana Production	48
2.10.1 <i>Fusarium</i> Wilt, distribution and spread	49
2.10.1.1 Pathogen Variability	50
2.10.2 Breeding for Disease Resistance/Tolerance	51

CHAPTER THREE: MATERIALS AND METHODS

3.1 Culture Initiation	56
3.2 Mutation Induction	58
3.2.1 Evaluation of gamma irradiated plants	59
3.2.2 Morphological variation evaluated in the Greenhouse and the field	60
3.3 Somaclonal Variation in Pisang Berangan (AAA)	62
3.3.1 Characterisation of variants and evaluation for Important agronomic traits	63
3.4 Analysis of Nuclear DNA Content	64
3.4.1 Plant Materials and Sample Preparation	65
3.4.2 Instrument setting and alignment	65
3.4.3 Sample analysis	66
3.5 Molecular Characterization of Mutants	66
3.5.1 Plant Material	66
3.5.2 DNA extraction	68
3.5.3 Quantification of DNA	69
3.5.4 RAPD Primers	70
3.5.5 Random Amplified DNA marker for the analysis of mutants	71
3.6 Detection of Dwarfism by using Gibberellic Acid	73
3.6.1 The growth Cabinet experiment	73
(i) During <i>in vitro</i> culture	73
(ii) At deflasking stage	74
3.6.2 The nursery experiment	75
3.6.3 The green house experiment	75
3.7 Screening for Tolerance to <i>Fusarium</i> wilt Disease by using 'double- tray' system and field evaluation	76

3.7.1 Plant materials	76
3.7.2 Isolates and inoculum preparation	77
3.7.3 Inoculation Procedure	78
3.7.4 Assessment of Disease symptoms	79
3.7.5 Field Screening for Tolerance to FOC Disease in the <i>Fusarium</i> 'Hot-spot'	81
3.8 Statistical Analysis	82

CHAPTER FOUR: RESULTS

4.1 Culture Initiation and Production of <i>in vitro</i> Explants	83
4.1.1 Mutation Induction, Post-irradiation recovery and Radiosensitivity	88
4.1.2 Variability Induced by Gamma Irradiation	97
4.1.2.1 Variation at culture stage	97
4.1.2.2 Variation at Nursery stage	100
4.1.2.3 Variation at Field stage (UP and UM fields)	108
4.1.3 Frequency of mutagenic changes	117
4.1.4 Mutagenic changes in quantitative traits	120
4.1.4.1 Frequency distribution of some characters in gamma Irradiated plants	122
(1) Plant height at flowering stage	122
(2) Girth circumference	125
(3) Days to flowering	126
4.1.4.2 Mutagenic Changes in Bunch characters	129
4.2 Somaclonal Variation in Pisang Berangan	136
4.2.1 Quantitative Aspects of Somaclonal Variation	137
4.2.1.1 Variation in Stature	139
4.2.1.2 Variation of Vegetative characters	142
4.2.1.3 Inflorescence and Fruit Variations	146
4.3 DNA content of variants induced by Gamma Irradiation	150
4.3.1 Flow cytometric analysis for ploidy and DNA content	151
4.3.2 DNA Content of different types of variants induced By gamma irradiation (mutants)	154
4.4 Molecular Analysis of variants induced by gamma-irradiation	161
4.4.1 Optimization and reproducibility of DNA amplification	162
4.4.2 Preliminary screening for polymorphism	164
4.4.3 Analysis of polymorphism	169
4.4.4 Association of RAPD markers with Dwarf variants	181
4.4.5 Association of radiation doses and variability	182
4.4.6 Data analysis	183
4.5 Detection of Dwarf off-types by using Gibberellic Acid (GA ₃)	185
4.5.1 Verification experiment	185
4.5.2 Growth Cabinet experiment	193
4.5.2.1 Response to GA ₃ at <i>In vitro</i> culture and Deflasking stages	193

(1) Leaf sheath	194
(2) Leaf Petiole	201
(3) Pseudostem length	203
4.5.3 Nursery experiment	207
4.5.3.1 Response to GA ₃ at <i>In vitro</i> culture and deflasking stages	207
4.5.3.2 Deflasking stage	213
4.5.4 Greenhouse Experiment at deflasking stage	222
4.5.5 Useful results obtained from using GA ₃ in Detection of dwarfism	226
4.6 Screening for tolerance to <i>Fusarium</i> wilt disease of gamma-irradiated Plants	227
4.6.1 Screening for <i>Fusarium</i> wilt in the nursery plants using the double-tray technique	227
4.6.1.1 External symptoms used for evaluation	228
4.6.1.2 Disease Tolerance Evaluation	233
4.6.2 Screening for tolerance to FOC in the field	241

CHAPTER FIVE: DISCUSSION

5.1 General Introduction	245
5.2 <i>In vitro</i> Propagation of Pisang Berangan	245
5.3 Exploitation of <i>In vitro</i> mutation for banana improvement by using gamma-ray.	247
5.3.1 Post-irradiation recovery and radio-sensitivity	251
5.3.2 Mutant evaluation	254
5.4 Detection of Dwarfism in Mutant plants	262
5.5 Screening for Disease tolerance to <i>Fusarium</i> wilt disease In Pisang Berangan	267
5.6 Other Characteristics of the mutants	270
5.6.1 Flow Cytometry analysis	271
5.6.2 Random Amplified Polymorphic DNA (RAPD) by Polymerase Chain Reaction (PCR) of mutated plants	274
5.7 Somaclonal Variation studies	277

CHAPTER SIX: SUMMARY	279
----------------------	-----

REFERENCES	284
------------	-----

APPENDIX

(A) Preparation of Modified MS Medium	307
(B) Preparation of Potato Dextrose Agar	308
(C) Dendrogram generated from RAPD fingerprinting profile in mutated Pisang Berangan (Fig. 4.6-F).	309
(D) Proximity Matrix for dissimilarity distance values (Table 1).	310
(E) Statistical Analysis	311
(F) Optimization experiment of DNA concentration	315

LIST OF TABLES

Table No.	Page
1.1 : Fruit Hectareage in Peninsular Malaysia in 1997	2
2.1 : Methods using Mutations in Plant Breeding	26
3.1 : Composition of culture Medium used for banana culture	57
3.2 : Number of meristem tips treated by Gamma-rays	58
3.3 : The number of generated banana plants (irradiated and control) for field evaluation	60
3.4 : Types and sample size of mutants (variants) used for RAPD analysis	67
3.5 : Random oligonucleotide Sequences (Primers) for Preliminary evaluation	70
3.6 : PCR reaction Mixture (reagent) used in RAPD method	72
3.7 : The number of plants used for different GA ₃ treatment in Growth Cabinet experiment	73
3.8 : The number of plants used for different GA ₃ treatments in nursery environment	75
3.9 : The number of plantlets produced for the screening of FOC resistance by 'Double-tray' experiment	76
3.10 : The age and number of plantlets inoculated with conidial suspension of FOC	78
3.11 : Types and disease symptoms examined at double tray experiment	79
3.12 : Scale of Leaf Symptom Index (LSI) and Rhizome Discolouration Index (RDI).	80
4.1 : The Multiplication rates, Contamination % and number of shoot/bud per explant of different batches for 6 subcultures	85
4.2 : Effects of gamma treatments on shoot multiplication	91
4.3 : Variability induced by gamma irradiation observed at <i>in vitro</i> , urserly and field stages for 40 and 60 Gy, for all batches and Control.	98

4.4	: Variability induced by gamma irradiation observed on field grown plants for 20 and 30 Gy	99
4.5	: Types and frequency of variants of gamma-irradiated Pisang Berangan at UM and UP fields	119
4.6	: Performance of Gamma irradiated plants (Pisang Berangan)	123
4.7	: Bunch characteristics of gamma-irradiated plants	130
4.8	: Materials used at different batches and planting dates	136
4.9	: Frequency of Somaclonal variants at different growth stages of Pisang Berangan cv. Intan (AAA), among tissue culture derived plants	138
4.10	: The early fruiting tissue cultured-plants (TC) as compared to those derived by suckers	138
4.11	: Differences in characteristics of Dwarf variants and normal plants of Pisang Berangan (AAA) at field stage	140
4.12	: Somaclonal variation of bunch characters amongst tissue cultured and conventionally propagated plants (from suckers)	147
4.13	: Samples of different Variants induced by gamma-irradiation selected for ploidy analysis and DNA content	151
4.14-A	: Flow cytometric estimation of nuclear DNA content in short stature variant of gamma-irradiated Berangan (AAA) with comparison to non-irradiated (control)	156
4.14-B	: Flow cytometric estimation of nuclear DNA content in bunch abnormalities variants of gamma-irradiated Berangan (AAA) with comparison to non-irradiated (control)	157
4.14-C	: Flow cytometric estimation of nuclear DNA content in late to flowering variant of gamma-irradiated Berangan (AAA) with comparison to non-irradiated (control)	158
4.15	: Summary and statistical analysis of results obtained by Flow cytometric estimation of nuclear DNA content in <i>Musa</i> (Pisang Berangan, AAA) for different types of selected variants	159
4.16	: Summary and statistical analysis of results obtained by Flow Cytometric estimation of nuclear DNA content in <i>Musa</i> (Pisang Berangan, AAA) at different gamma doses	160

4.17	: Types and number of mutagenic variants selected for RAPD Analysis	161
4.18	: The nucleotide sequences (5' to 3') of the primers from OPERON Tech., USA, used for initial screening (RAPD) by PCR	162
4.19	: Summary of results of optimization experiment	163
4.20-A):	The polymorphisms of primers used for the different gamma-irradiated variants and non-irradiated plants (N)	165
4.20-B):	The polymorphisms of primers used for the different gamma-irradiated variants and non-irradiated plants (N)	166
4.21	: The number of bands produced by each Four primers and percentage of polymorphism	167
4.22	: 50 Random samples representing one group of mutated (20, 30,40 and 60 Gy) and control plants collected from the field, used in RAPD technique	168
4.23	: DNA patterns of mutated Berangan biotypes obtained by using primer	170
4.24	: The number of variants at different gamma-doses that showed polymorphism with the four primers analyzed	182
4.25	: The leaf sheath length (cm) of Pisang Berangan and Pisang Serendah after application of GA3 (0, 29 and 59 $\mu\text{mol/L}$ at <i>In vitro</i> and deflasking stages (289 $\mu\text{mol/L}$) and their control	186
4.26	: The length of leaf petiole (cm) of Pisang Berangan and Pisang Serendah after application of GA3 (0, 29 and 59 $\mu\text{mol/L}$ at <i>In vitro</i> and deflasking stages (289 $\mu\text{mol/L}$) and their control	187
4.27	: Pseudostem length (cm) of Pisang Berangan and Pisang Serendah after application of GA3 (0, 29 and 59 $\mu\text{mol/L}$ at <i>In vitro</i> and deflasking stages (289 $\mu\text{mol/L}$) and their control	188
4.28-A :	Analysis of variance for leaf sheath at <i>in vitro</i> stage	190
4.28-B :	Analysis of variance for leaf sheath at deflasking stage	190
4.29-A :	Analysis of variance for leaf petiole at <i>in vitro</i> stage	191
4.29-B :	Analysis of variance for leaf petiole at deflasking stage	191
4.30-A :	Analysis of variance for pseudostem length at <i>in vitro</i> stage	191
4.30-B :	Analysis of variance for pseudostem length at deflasking stage	191

4.31	: The mean values of leaf sheath (cm) after Gibberellic Acid treatment on gamma-irradiated and dwarf Serendah at <i>in vitro</i> and deflasking stages	196
4.32	: The mean values of leaf petiole (cm) after Gibberellic Acid treatment on gamma-irradiated and dwarf Serendah at <i>in vitro</i> and deflasking stages	202
4.33	: The mean values of pseudostem length (cm) after Gibberellic Acid treatment on gamma-irradiated and dwarf Serendah at <i>in vitro</i> and deflasking stages	204
4.34	: The mean values of the effect of Gibberellic acid on leaf sheath (cm) of gamma-irradiated and dwarf Serendah at <i>in vitro</i> and deflasking stages	209
4.35	: The mean values of leaf petiole (cm) after treatment of Gibberellic acid on gamma-irradiated non-irradiated and dwarf Serendah at <i>in vitro</i> and deflasking stages	212
4.36	: The mean values of pseudostem length (cm) after treatment of Gibberellic acid on gamma-irradiated non-irradiated and dwarf Serendah at <i>in vitro</i> and deflasking stages	212
4.37	: The mean values of leaf sheath and plant height (leaf 1) for mutated and non-irradiated plants treated with GA_3 (289 μ mol/L)	222
4.38	: The mean values of leaf sheath and plant height (leaf 2) for mutated and non-irradiated plants treated with GA_3 (289 μ mol/L)	223
4.39	: The number of gamma-irradiated and control plants used in "Double-tray" technique for screening <i>Fusarium</i> wilt tolerance	227
4.40	: Results of leaf and Rhizome Scales for <i>Fusarium</i> wilt screening	234
4.41	: Effect of inoculation of FOC on banana plants mutated and Control (Berangan and Novaria)	236
4.42	: Survival of suckers obtained from gamma-irradiated plants in the <i>Fusarium</i> 'hot-spot'.	242

LIST OF FIGURES

Figure No.	Page
4.1 : The mean of multiplication rates of four batches of Pisang Berangan at different for 6 subcultures	86
4.2 : Number of buds/shoots produced by different subcultures for different Gamma-doses in 4 batches of mutation induction	93
4.3-A : Frequency Distribution of plant height at flowering in Pisang Berangan (AAA) gamma-irradiated at 0, 20, 30, 40 and 60 Gy's.	124
4.3-B : Frequency Distribution of Girth circumference in Pisang Berangan (AAA) gamma-irradiated at 0, 20, 30, 40 and 60 Gy	127
4.3-C : Frequency Distribution of Days to flowering in Pisang Berangan gamma-irradiated (AAA) at 0, 20, 30, 40 and 60 Gy	128
4.4-A : Frequency Distribution of Bunch weight in Pisang Berangan (AAA) gamma-irradiated at 0, 20, 30, 40 and 60 Gy	131
4.4-B : Frequency Distribution of Comb weight in Pisang Berangan (AAA) gamma-irradiated at 0, 20, 30, 40 and 60 Gy	133
4.4-C : Frequency Distribution of number of Comb/bunch in Pisang Berangan (AAA) gamma-irradiated at 0, 20, 30, 40 and 60 Gy	134
4.5 : Histogram of relative nuclear DNA content obtained after analysis of nuclei isolated from young leaf tissues of Soya bean (<i>Glycine max</i> cv. palmetto) and Pisang Berangan (mutants)	153
4.6-(A,B, C and D) : RAPD profiles of mutated and normal (control) plants generated by Primers: (A) OPA-03 (B) OPA-05 (C) OPA-07 and (D) OPA-09	172
4.6-A ₁ : RAPD profile generated by Primer OPA-03	173
4.6-A ₂ : RAPD profile generated by Primer OPA-03	174
4.6-B ₁ : RAPD profile generated by Primer OPA-05	175
4.6-B ₂ : RAPD profile generated by Primer OPA-05	176
4.6-C ₁ : RAPD profile generated by Primer OPA-07	177
4.6-C ₂ : RAPD profile generated by Primer OPA-07	178
4.6-D ₁ : RAPD profile generated by Primer OPA-09	179
4.6-D ₂ : RAPD profile generated by Primer OPA-09	180

4.6-E	: Dendrogram- RAPD of 50 samples of mutated and control plants based on 4 random primers and Ward's method (1963)	184
4.7	: Difference between gamma irradiated Berangan B(M), non-irradiated Berangan B(N) and Serendah (S) in leaf sheath and Pseudostem height at <i>in vitro</i> stage	197
4.7-1	: Response of gamma irradiated Berangan and dwarf Serendah at <i>In vitro</i> stage, treated at 0, 29 and 59 $\mu\text{mol/L}$ GA ₃	198
4.7-2	: Leaf sheath length (cm) control (non-irradiated), treated (gamma-irradiated) of Pisang Berangan and dwarf Serendah	200
4.7-3	: Pseudostem height (cm) in control (non-irradiated), gamma-irradiated of Pisang Berangan and dwarf Serendah	200
4.7-4	: Response of mutated Berangan B(M) and dwarf Serendah (S) treated with 289 $\mu\text{mol/L}$ GA ₃ at leaf I stage, for leaf sheath, leaf petiole and pseudostem height	205
4.7-5	: Response of mutated Berangan B(M) and dwarf Serendah (S) treated with 289 $\mu\text{mol/L}$ of GA ₃ at leaf II stage for leaf sheath, leaf petiole and pseudostem height	206
4.7-6	: Leaf sheath, leaf petiole and pseudostem height at leaf I stage of gamma irradiated Berangan B(M), non-irradiated B(N) and Serendah (S) treated with 289 $\mu\text{mol/L}$ of GA ₃ (leaf I)	206
4.7-7	: Leaf sheath, leaf petiole and pseudostem height at leaf II stage of gamma irradiated Berangan B(M), non-irradiated B(N) and Serendah (S) treated with 289 $\mu\text{mol/L}$ of GA ₃ (leaf II)	208
4.7-8	: Leaf sheath length and pseudostem height of gamma irradiated Berangan B(M), non-irradiated B(N) as compared to control Plants in leaf I stage	224
4.7-9	: Leaf sheath length and pseudostem height of gamma irradiated Berangan B(M), non-irradiated B(N) as compared to control Plants in leaf II stage	225
5.1	: The Development of Radiation Damage in Plant Cells	249
5.2	: Earliest time for the identification of Somaclonal variation	278

LIST OF PLATES

Plate No.		Page
4.1	: <i>In vitro</i> multiplication of Berangan on MS medium supplemented with 4.5mg/l of BAP	87
4.2	: Meristem pieces of Pisang Berangan used for Gamma-irradiation.	87
4.3	: (1) The meristem pieces of control, showing vigorous growth (2) Gamma-irradiated meristem pieces at 40 Gy, showing partial survival (3) Gamma-irradiated meristem pieces at 60 Gy, showing non-survival and blackening	96
4.3.1	: Compact leaf rosettes	101
4.3.2	: Chlorotic and necrotic variants	101
4.3.3	: Small and narrow leaves with uneven lamina	102
4.3.4	: Leaf mottling (pale-green) with dark green and yellow strips running along the veins	103
4.3.5 (C and D)	: Leaf discolouration, waxy and light yellowing stripes	103
4.3.6	: Red patches (anthocyanin) on the surface of lamina	104
4.3.7	: Leaf crinkling and rough surface	104
4.3.8	: Red and wavy leaf margin	105
4.3.9	: Yellow coloration of lamina	105
4.3.10	: Flat pseudostem with yellow colour and brown spots	106
4.3.11	: Narrow pseudostem with light brown streaking	106
4.3.12	: Extensive browning of pseudostem sheath	107
4.3.13	: Stunted growth (B) in comparison to normal plant (A)	107
4.3.14	: Variegated leaf with red sectors	109
4.3.15	: Uneven lamina with twisting midrib in the upper part of the leaf	109
4.3.16	: Yellow midrib and rough leaf surface	110
4.3.17	: Deformed lamina with yellow coloration	110
4.3.18	: Bending of young leaf, crinkling of lamina, Compact and erect leaves	111
4.3.19	: Petiole bases spreading or loose with erect margins	111

4.3.20	: Red petiole margin and yellowish petiole with rough leaf surface	112
4.3.21	: Twisting of midrib and rough surface	112
4.3.22	: Red leaf margin	113
4.3.23	: Leaf deformation, rough-surface and red margins	113
4.3.24	: Light pseudostem colour without powdery surface but abnormal suckers	114
4.3.25	: Pseudostem with high proliferation of normal suckers	114
4.3.26	: Waxy yellow pseudostem, absence of powder	115
4.3.27	: Long peduncle with persistent male flowers	115
4.3.28	: Abnormal bunch with lax and few number of combs.	116
4.3.29	: Dwarf variant with stunted growth	116
4.3.30	: Variation in mutated fruit orientation (A) Normal finger distribution, (B) Loose finger orientation and (C) Fused fingers	135
4.4.1	: Short variant with compact petioles	141
4.4.2	: Stunted dwarf (right) compared to tall variant (left) (approximately 30% taller than the normal plants)	141
4.4.3	: Mosaic variant, the upper leaf surface showing a pattern of bright spot (mottling) and is covered with depression and protuberances	143
4.4.4	: Thick, rubbery, narrow and irregular lamina at hardening stage	143
4.4.5	: Deformed lamina appearing as lobed blade at petiole edge or leaf midrib	144
4.4.6	: Unfolded cigar-leaf	144
4.4.7	: Variant showing decrease in black pigmentation and appearance of a reddish color on pseudostem, leaf sheath and petiole	145
4.4.8	: Variation in bract color. (a) Purple red (b) yellowish red and (c) Deep purple red	148
4.4.9	: Bunch variant showing persistent aborted inflorescence with long peduncle	148
4.4.10	: Bunch variant with only male flowers or persistent flowers	149

4.4.11	: Small, lax and split fingers	149
4.5.1	: The growth cabinet with all treated plants and their control	195
4.5.2	: Variation in growth responses to GA ₃ between <i>In vitro</i> plantlets and control (non-irradiated) B(N) 1 and 2, gamma-irradiated B(M) 3, and dwarf Serendah (S) 4-control	195
4.5.3	: <i>In vitro</i> culture of gamma-irradiated Berangan showed different response to GA ₃ (59, 29 $\mu\text{mol/L}$) 30 days after application	210
4.5.4	: The effect of GA ₃ (29 $\mu\text{mol/L}$) on different plant types after 30 days after application	211
4.5.5	: The effect of GA ₃ (59 $\mu\text{mol/L}$) on different plant types 30 days from application	211
4.5.6	: After 2 weeks (leaf I) stage, gamma-irradiated plants (40 Gy) treated with GA ₃ (289 $\mu\text{mol/L}$) and (GA ₃ = 0)	218
4.5.7	: Three categories in gamma irradiated plants treated with GA ₃ (289 $\mu\text{mol/L}$) after 2 weeks (leaf I)	219
4.5.8	: After two weeks (leaf I) non-irradiated Berangan treated with GA ₃ (289 $\mu\text{mol/L}$), showed only two categories	219
4.5.9	: Dwarf plants (Serendah) treated with 289 $\mu\text{mol/L}$ GA ₃ , showing length less than intermediate and longer than short	220
4.5.10	: Three categories (Tall, intermediate and short) of gamma irradiated plants treated with 289 $\mu\text{mol/L}$ GA ₃ after 4 weeks (leaf II)	221
4.5.11	: Gamma irradiated plants (40 Gy) treated with 289 $\mu\text{mol/L}$ GA ₃ and GA ₃ = 0, after 4 weeks (leaf II)	221
4.6.1	: Double-tray set up	230
4.6.2	: External leaf symptoms characterized by the yellowing of the older leaf margin progressing towards the midrib of lamina	230
4.6.3	: Gamma irradiated plants (40 Gy) at the third week after FOC race 4 inoculation	231
4.6.4	: Gamma irradiated plants showing collapse of the petiole and splitting of pseudostem with wilting of leaves at 3 to 4 weeks after inoculation	231
4.6.5	: Control Berangan plants (non-inoculated) showed vigorous growth with no Fusarium wilt symptoms	232

4.6.6	: Internal Symptoms of discolouration found to be most pronounced in the rhizome area of 40 Gy treated plantlets	238
4.6.7.(a-c)	: Total discolouration of vascular tissues (level 6) using INIBAP Scale	238
4.6.8	: Mutated plantlets (60 Gy) showed internal symptoms resembling level-6	239
4.6.9	: Non-irradiated Berangan showing dark discolouration in the vascular corm (level 6)	239
4.6.10	: Mutated Berangan at 40 Gy showing different levels of internal symptoms	240
4.6.11	: An external symptom on the leaf (yellow colour) starting from the oldest leaf to the younger ones	243
4.6.12	: Splitting of pseudostem	243
4.6.13	: Collapsed field planted Berangan plants due to damage to vascular system	244

ABBREVIATIONS AND ACRONYMS

B(M)	-	Berangan mutant
B(N)	-	Berangan normal
bp	-	base pair
BW	-	bunch weight
°C	-	degrees Celsius
CaCl ₂	-	Calcium chloride
C/ b	-	Comb per bunch
cm	-	centimeter
CW	-	Comb weight
dATP	-	deoxyadenosine triphosphate
dCTP	-	deoxycytidine triphosphate
DES	-	diethyl sulphate
dGTP	-	deoxyguanosine triphosphate
dNTPs	-	deoxyribonucleoside triphosphates
DNA	-	deoxyribonucleic acid
DMSO	-	dimethyl sulfoxide
DMRT	-	Duncan Multiple Range Test
dTTP	-	deoxythymidine triphosphate
EDTA	-	ethylene diaminetetra acetate
EMS	-	Ethylmethane sulfonate
g	-	gram
G	-	Girth
GA ₃	-	Gibberellic acid
GML	-	Ground magnesium limestone
Gy	-	Gray-unit of radiation rate
HCl	-	hydrochloric acid
IAA	-	indole-3-acetic acid
INIBAP	-	International Network for the Improvement of Banana and Plantain
kb	-	kilobases
kbp	-	kilobases pair
KCl	-	potassium chloride
L	-	litre
M	-	Molar
Mbp	-	Mega base pair
mg	-	milligram
min	-	Minute
ml	-	Mililitre
mM	-	Millimolar
mmol	-	millimoles
MNU	-	N- methyl- N- nitrosoarea
MS	-	Murashige and Skoog media
M.W	-	Molecular weight
NaCl	-	Sodium chloride
NaN ₃	-	Sodium azide

NaOH	-	Sodium hydroxide
Na ₂ SO ₄	-	disodium phosphate
ng	-	nanogram
PCR	-	Polymerase Chain Reaction
PDA	-	Potato Dextrose Agar
pg	-	picogram
PPFD	-	Photosynthetic photon flux density
RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
Rnase	-	Ribonuclease
Sec	-	second
TBE	-	Tris- borate- EDTA
TE	-	Tris – EDTA
µg	-	microgram
µl	-	microliter
µ mol / L	-	micromol per litre
UV	-	ultra violet
VCG	-	Vegetative Compatibility Group
v / v	-	volume per volume
w / v	-	weight per volume