UNIVERSITI PUTRA MALAYSIA

IN VITRO PROPAGATION AND MUTATION INDUCTION OF TORCH GINGER (Etlingera elatior J.)

MUHAMAD FAHMI YUNUS

FP 2013 73
IN VITRO PROPAGATION AND MUTATION INDUCTION OF TORCH GINGER
(Etlingera elatior J.)

MUHAMAD FAHMI YUNUS

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2013
IN VITRO PROPAGATION AND MUTATION INDUCTION OF TORCH GINGER (Etlingera elatior J.)

By

MUHAMAD FAHMI YUNUS

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fullfilment of the Requirements for the Degree of Master of Science

October 2013
All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
Dedicated to:

My dearest parents

Yunus bin Jamaludin
Zawiah binti Basnun

and

My Siblings

Muhamad Aqqat bin Yunus
Muhammad Ehsan Sabri bin Yunus
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**IN VITRO PROPAGATION AND MUTATION INDUCTION OF TORCH GINGER** (*Etlingera elatior* J.)

By

MUHAMAD FAHMI YUNUS

October 2013

Chairman : Associate Professor Maheran Abd Aziz, PhD

Faculty : Faculty of Agriculture

The aim of this study was to develop a protocol for *in vitro* propagation and mutation induction of *Etlingera elatior* by using gamma ray irradiation. The study included establishment an efficient *in vitro* plant propagation system in *E. elatior*, investigation of the optimum dose for radio sensitivity test, to determine the effects of various doses of gamma irradiation on multiple bud induction and also to determine the variation in genomic DNA of regenerated shoots by using random amplification of polymorphic DNA (RAPD) technique.

In this study, an efficient and systematic protocol for complete plant regeneration from suckers of *Etlingera elatior* (J.) has been developed. The addition of N6-benzyl amino-purine (BAP) (0, 3, 5, 7 and 10 mg L\(^{-1}\)) to the culture medium comprising of Murashige and Skoog (MS) basal salts, 3% sucrose, 0.4% gelrite did not show any significant effects on percentage of shoot induction and mean number of shoots produced. However, BAP at 3 mg L\(^{-1}\) was chosen as the best medium for shoot induction due to economic feasibility and it gave the highest result in all four
parameters recorded. Various concentrations of BAP, 6-furfurylaninopurine (kinetin) and N6-(2-isopentenyl) adenine (2-iP) alone at 0, 3, 5, 7 and 10 mg L$^{-1}$ were tested for shoot multiplication. BAP at all levels were found suitable for the multiplication of shoot. However, the low level of 3 mg L$^{-1}$ BAP was chosen as the best concentration of BAP due to economic feasibility. The best root proliferation was observed on MS medium without plant growth regulator (PGR). Assessment of various potting media for acclimatization showed medium containing soil: sand: peat moss (1:1:1) produced high survival of plantlets, number of leaves produced per plant and the plant height.

Mutation breeding techniques in combination with tissue culture and molecular marker methods provide a powerful tool for improvement of vegetatively propagated plants. The results of irradiation on \textit{in vitro} buds of \textit{E. elatior} showed that LD$_{50}$ to be 10 Gy with the survival of explants being sharply reduced after this dosage. The gamma irradiated shoots were subcultured for three cycles (M$_1$V$_1$ to M$_1$V$_3$) to obtain potential mutant lines. This study showed that RAPD marker was efficient in differentiating the induced mutants from the untreated control of \textit{E. elatior}. All eight selected gamma irradiated regenerants were differentiated from the untreated control based on the banding patterns obtained using 9 primers which generated 59 reproducible bands, whereby 35 (55.31\%) were found to be polymorphic. The Jaccard’s coefficient of similarity values ranging from 0.537 to 0.860 were indicative of the level of genetic variation among the mutants studied. For comparison between the potential lines (PL) and the control, a maximum similarity value (0.814) was observed in PL1 mutant while the minimum value (0.537) was observed in PL7. The
presence of polymorphic bands in 8 potential lines suggested that genetic variation occurred in all the treatments as compared to the control.

In summary, the combination of techniques of in vitro propagation, multiplication, gamma irradiation, and RAPD analysis for early screening of mutants can facilitate breeding programme of E. elatior.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk Ijazah Master Sains

PROPAGASI IN VITRO DAN ARUHAN MUTASI POKOK KANTAN
(Etlingera elatior J.)

Oleh
MUHAMAD FAHMI YUNUS
Oktober 2013

Pengerusi : Professor Madya Mahera Abd Aziz, PhD
Fakulti : Fakulti Pertanian

Tujuan penyelidikan yang dijalankan ialah untuk membangunkan teknik propagasi in
vitro dan aruhan mutasi Etlingera elatior dengan menggunakan penyinaran sinar
gamma. Kajian ini merangkumi penghasilan satu sistem propagasi tumbuhan secara
in vitro bagi E. elatior, kajian mengenai dos optimum bagi ujian sensitiviti radio,
penentuan kesan-kesan pelbagai dos sinaran gamma dan juga penentuan variasi pada
genom DNA dari pucuk yang dihasilkan dengan menggunakan teknik penanda
molekul amplifikasi rawak DNA polimorfik (RAPD).

Di dalam penyelidikan ini, protokol yang effisien dan sistematik untuk regenerasi
tumbuhan dari sulur E. elatior (J.) telah dibangunkan. Penambahbaian N6-benzil amino-
purin (BAP) pada kepekatan 0, 3, 5, 7 dan 10 mg L^{-1} pada kultur medium yang
mengandungi nutrien asas Murashige dan Skoog (MS), 3% sukrosa, 0.4% Gelrite
didak menunjukkan sebarang kesan yang signifikan terhadap peratusan
penginduksian pucuk dan juga jumlah min penghasilan bilangan pucuk.
Walaubagaimanapun, BAP pada kepekatan 3 mg L^{-1} telah dipilih sebagai paras
kepekatan terbaik disebabkan faktor ekonomi dan ia memberikan keputusan terbaik
di dalam semua empat parameter yang direkodkan. Pelbagai kepekatan tunggal pengawalatur pertumbuhan BAP, 6-furfurilaminopurin (Kinetin) dan juga N6-(2-isopentenil) adenin (2-iP) pada kepekatan 0, 3, 5, 7 dan 10 mg L\(^{-1}\) telah diuji untuk penggandaan pucuk. BAP pada setiap kepekatan telah diuji berkesan untuk penggandaan pucuk. Sungguhpun demikian, BAP pada kepekatan 3 mg L\(^{-1}\) telah dipilih sebagai paras kepekatan terbaik berdasarkan sifat ekonomi yang dimiliki.

Medium penggandaan akar yang terbaik ialah medium MS tanpa sebarang penambahan pengawalatur pertumbuhan. Penilaian pelbagai media berpasu bagi tujuan aklimitasi menunjukkan bahawa medium yang mengandungi tanah: pasir: tanah gambut berlumut (1:1:1) memberikan kadar kemandirian yang tinggi kepada anak pokok, penghasilan daun per anak pokok dan juga tinggi anak pokok.

Teknik pembiakbaka mutasi dengan kombinasi teknik kultur tisu dan penanda molekul boleh menjadi teknik yang berkesan untuk penambahbaikan tumbuhan yang dibiakkan melalui kaedah tampang. Keputusan irradiasi tunas in vitro E. elatior menunjukkan bahawa LD\(_{50}\) ialah pada 10 Gy dengan kadar hidup berkurangan dengan drastik selepas dos ini. Pucuk yang telah disinari dengan sinar gamma telah disubkultur untuk tiga kitaran (\(M_0\)V\(_1\) ke \(M_1\)V\(_3\)) untuk memperolehi titisan mutan yang berpotensi. Kajian ini menunjukkan RAPD adalah berkesan untuk membezakan antara mutan yang diaruh daripada kawalan E. elatior yang tidak diaruh. Kesemua lapan regenerasi yang diaruh dengan gamma telah dibezakan daripada kawalan yang tidak diaruh berdasarkan corak jalur yang diperolehi dengan menggunakan 9 primer yang menghasilkan 59 jalur reproduksi, di mana 35 (55.31%) adalah polimorfik. Nilai pekali pesamaan Jaccard berada di antara julat 0.537 hingga 0.860 menunjukkan paras kepelbagaian genetik antara mutan yang dikaji. Sebagai
perbandingan, antara titisan berpotensi (PL) dan juga kawalan, nilai kesamaan maksimum (0.814) telah diperolehi pada mutan PL1 manakala nilai minimum (0.537) diperolehi pada PL7. Penghasilan jalur polimorfik pada 8 titisan berpotensi menyarankan bahawa variasi genetik berlaku pada semua rawatan berbanding dengan kawalan.

Sebagai kesimpulan, kombinasi antara teknik propagasi in vitro, penggandaan, penyinaran gamma, dan juga analisis RAPD untuk penyaringan awal mutan boleh membantu program pembiakbakaan E.elatior.
ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful. Foremost, I would like to express my deep and sincere gratitude to my supervisor, Associate Professor Dr. Maheran Abd Aziz for the continuous support of my MSc study and research. Her wide knowledge, understanding, encouraging and personal guidance have provided a good basis for the present thesis and have been of great value for me. Besides, I would like to thank the rest of my thesis committee: Associate Prof. Dr. Mihdzar Abdul Kadir and Associate Prof. Dr. Siti Khalijah Daud; Mr. Azmi Abdul Rashid, a lecturer in the Faculty of Agriculture for their encouragement, insightful comments and suggestions.

The financial support from the Ministry of Science, Technology and Innovation Malaysia for me to undertake this study is gratefully acknowledged. I would like to thank Nor Asiah binti Ismail, Research Officer from MARDI Jerangau, Terengganu for providing the seed materials. I am also grateful to the staff at Gamma Ray Laboratory, School of Applied Physics, Faculty of Science, Universiti Kebangsaan Malaysia, Selangor for providing the facilities to carry out the gamma irradiation of *E. elatior*.

I thank my fellow labmates in Agrobiotechnology Laboratory, Department of Agriculture Technology, Faculty of Agriculture for the stimulating discussions, and for all the fun we have had in the last two years. Last but not the least, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Maheran Abd Aziz, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Mihdzar Abdul Kadir, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Siti Khalijah Daud, PhD**  
Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

________________________________

(BUJANG BIN KIM HUAT, PhD)  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

Declaration by Graduate Student

I hereby confirm that:

• this thesis is my original work;
• quotations, illustrations and citations have been duly referenced;
• this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
• intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
• written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
• there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _______________________ Date: 4 October 2013

Name and Matric No.: Muhamad Fahmi Yunus and GS26314
Declaration by Members of Supervisory Committee
This is to confirm that:

• the research conducted and the writing of this thesis was under our supervision;
  Guide to Thesis Preparation

• supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate
  Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _______________Signature: _______________
Name of Chairman of Name of Member of
Supervisory Supervisory
Committee: ____________ Committee: ____________
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>xiv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>x</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xviii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xx</td>
</tr>
<tr>
<td>CHAPTER 1 INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>1.1 Background</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Problem Statement</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Significance of the Study</td>
<td>3</td>
</tr>
<tr>
<td>1.4 Objective</td>
<td>5</td>
</tr>
<tr>
<td>2 LITERATURE REVIEW</td>
<td></td>
</tr>
<tr>
<td>2.1 Taxonomy</td>
<td>6</td>
</tr>
<tr>
<td>2.2 Botany of Torch Ginger</td>
<td>8</td>
</tr>
<tr>
<td>2.3 Comercial Potential of Torch Ginger</td>
<td>11</td>
</tr>
<tr>
<td>2.4 Plant tissue culture</td>
<td>12</td>
</tr>
<tr>
<td>2.4.1 Direct Regeneration</td>
<td>12</td>
</tr>
<tr>
<td>2.4.2 Indirect Regeneration</td>
<td>14</td>
</tr>
<tr>
<td>2.4.3 Plant Growth Regulator</td>
<td>14</td>
</tr>
<tr>
<td>2.4.4 Interaction between Auxin and Cytokinin</td>
<td>17</td>
</tr>
<tr>
<td>2.5 Conventional Breeding of Torch Ginger</td>
<td>19</td>
</tr>
<tr>
<td>2.6 Mutation Breeding</td>
<td>19</td>
</tr>
<tr>
<td>2.6.1 Effect of Mutagen on Plants</td>
<td>20</td>
</tr>
<tr>
<td>2.6.2 Conventional Mutagenesis</td>
<td>21</td>
</tr>
<tr>
<td>2.6.3 <em>In vitro</em> Mutagenesis</td>
<td>21</td>
</tr>
<tr>
<td>2.6.4 Advantages of <em>In Vitro</em> Mutagenesis</td>
<td>22</td>
</tr>
<tr>
<td>2.7 Radiation Process</td>
<td>24</td>
</tr>
<tr>
<td>2.8 Gamma Rays</td>
<td>25</td>
</tr>
<tr>
<td>2.9 Radiosensitivity Test</td>
<td>25</td>
</tr>
<tr>
<td>2.10 Lethal Dose 50</td>
<td>26</td>
</tr>
<tr>
<td>2.11 Chimera</td>
<td>27</td>
</tr>
<tr>
<td>2.12 Molecular Marker</td>
<td>29</td>
</tr>
<tr>
<td>2.12.1 Random Amplification of Polymorphic DNA</td>
<td>29</td>
</tr>
</tbody>
</table>
3 MATERIALS AND METHODS
Part I: In vitro propagation
3.1 Plant Materials 31
3.2 Surface Sterilization Techniques 32
3.3 Effects of Different Concentration of BAP on Shoot Induction 33
3.4 Effects of Different Types and Concentrations of Cytokinin on Shoot Multiplication 34
3.5 Effects of Different Types and Concentrations of Auxin on Root Induction 35
3.6 Effects of Different Types of Growth Media on Acclimatization 35
3.7 Effects of 2,4-D and BAP Concentration in ½ MS Medium on Percentage and Intensity of Callus Formation 36
3.8 Callus Proliferation 38
3.9 Experimental Design and Statistical Analysis 38

Part II: In vitro Mutagenesis and RAPD Analysis
3.10 Plant Materials 39
3.11 Effect of Gamma Irradiation on Regeneration of Shoots from In Vitro Buds 39
3.12 Post Mutagenesis Handling 40
3.13 Experimental Design and Statistical Analysis 42
3.14 Plant Materials and Genomic DNA Extraction 42
3.15 DNA Quantification 43
3.16 Primer Selection 44
3.17 Polymerase Chain Reaction and Primer Screening 45
3.18 Statistical Analysis 45

4 RESULTS AND DISCUSSION
Part 1: In vitro Propagation
4.1 Effects of Different Concentration of BAP on Shoot Induction 47
4.2 Effects of Different Types and Concentration of Cytokinin on Shoot Multiplication 53
4.3 Effects of Different Types and Concentration of Auxin on Root Induction 60
4.4 Effects of Different Types of Growth Media on Acclimatization 64
4.5 Effects of 2,4-D and BAP Concentration in ½ MS Medium on Percentage of Callus Formation and Intensity of Callus Formation 68
Part II: *In vitro* Mutagenesis and Molecular Marker

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6 Effect Of Gamma Irradiation On Regeneration Of Shoots From <em>In Vitro</em> Buds</td>
<td>73</td>
</tr>
<tr>
<td>4.7 Management of Chimera and Biological Effect Of Gamma Rays</td>
<td>79</td>
</tr>
<tr>
<td>4.8 Post Mutagenesis Handling and Modification of the Culture Conditions</td>
<td>81</td>
</tr>
<tr>
<td>4.9 Evaluation of Genetic Variation of Gamma Rays Treated Regenerants with RAPD Markers</td>
<td>82</td>
</tr>
</tbody>
</table>

5 CONCLUSION 89

REFERENCES 91
APPENDICES 100
BIODATA OF STUDENT 112
LIST OF PUBLICATIONS 113
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
</tr>
<tr>
<td>8</td>
<td>54</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
</tr>
<tr>
<td>10</td>
<td>65</td>
</tr>
<tr>
<td>11</td>
<td>70</td>
</tr>
<tr>
<td>12</td>
<td>77</td>
</tr>
<tr>
<td>13</td>
<td>83</td>
</tr>
<tr>
<td>14</td>
<td>85</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Suckers (arrow) of <em>E. elatior</em> used in this study.</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>Explants used in shoot induction study. The outer scales and dead surface of the sterilized suckers were trimmed off before placing on the culture medium.</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>Effects of different concentrations of BAP on shoot induction from <em>E. elatior</em> suckers after 12 weeks of culture on (A) BAP 3 mg L(^{-1}) (B) BAP 5 mg L(^{-1}) (C) BAP 7 mg L(^{-1}) and (D) BAP 10 mg L(^{-1})</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>Development of shoots from sucker’s of <em>E. elatior</em> on MS medium containing 3 mg L(^{-1}) BAP after (A) 5 weeks (B) 6 weeks (C) 8 weeks (D) 9 weeks (E) 11 weeks and (F) 12 weeks of culture</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>Effects of cytokinin type and concentration on shoot multiplication of <em>E. elatior</em> after 12 weeks of culture on (A) MSO</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>Effects of cytokinin type and concentration on shoot multiplication of <em>E. elatior</em> after 12 weeks of culture on (B) BAP 3 mg L(^{-1}) (C) BAP 5 mg L(^{-1}) (D) BAP 7 mg L(^{-1}) and (E) BAP 10 mg L(^{-1})</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>Effects of cytokinin type and concentration on shoot multiplication of <em>E. elatior</em> after 12 weeks of culture on (F) KIN 3 mg L(^{-1}) (G) KIN 5 mg L(^{-1}) (H) KIN 7 mg L(^{-1}) and (I) KIN 10 mg L(^{-1})</td>
<td>57</td>
</tr>
<tr>
<td>5</td>
<td>Effects of cytokinin type and concentration on shoot multiplication of <em>E. elatior</em> after 12 weeks of culture on (J) 2-iP 3 mg L(^{-1}) (K) 2-iP 5 mg L(^{-1}) (L) 2-iP 7 mg L(^{-1}) and (M) 2-iP 10 mg L(^{-1})</td>
<td>58</td>
</tr>
<tr>
<td>6</td>
<td>Rooting of <em>E. elatior</em> shoots on MS media with different types and concentrations of auxin after 8 weeks of culture on (A) MSO</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td>Rooting of <em>E. elatior</em> shoots on MS media with different types and concentrations of auxin after 8 weeks of culture on (B) IBA 1 mg L(^{-1}) (C) IBA 2 mg L(^{-1}) (D) IBA 3 mg L(^{-1}) (E) NAA 1 mg L(^{-1}) (F) NAA 2 mg L(^{-1}) and (G) NAA 3 mg L(^{-1})</td>
<td>63</td>
</tr>
</tbody>
</table>
7 Effect of potting media on plantlet performance of *E. elatior* after six weeks of acclimatization on (A) Sand (B) Soil (C) Peat moss and (D) Sand + soil + peatmoss (1:1:1).

8 Effects of 2,4-D and BAP in half MS medium on callus formation from leaf bases of *E. elatior* after 12 weeks of culture on (A) C9 (1.0 mg L\(^{-1}\) 2,4-D and 0.1 mg L\(^{-1}\) BAP) (B) C12 (6.0 mg L\(^{-1}\) 2,4-D and 0.1 mg L\(^{-1}\) BAP) (C) C18 (6.0 mg L\(^{-1}\) 2,4-D and 0.5 mg L\(^{-1}\) BAP) and (D) C24 (6.0 mg L\(^{-1}\) 2,4-D and 1.0 mg L\(^{-1}\) BAP) media

9 LD\(_{50}\) determination on survival rate of irradiated buds of *E. elatior* after 8 weeks of incubation

10 Effect of different levels of gamma irradiation on *in vitro* buds of *E. elatior*. A) Survival of explants exposed to 10 Gy after 8 weeks of culture. B) Morphological abnormality observed on irradiated explant which later died after M\(_1\)V\(_1\) stage. C) Explant irradiated with 20 Gy failed to survive after 12 weeks of culture. D) M\(_1\)V\(_1\) shoots produced from 10 Gy irradiated explant after 12 weeks of culture.

11 Amplification of genomic DNA from *in vitro* shoots (M\(_1\)V\(_3\)) treated with 10 Gy of gamma rays using various RAPD primers, A) primer OPAW11, B) primer OPU16, and C) primer OPU13. In each of the three panels, lane M corresponds to 10 kb DNA ladder; lane C corresponds to the control (non irradiated plant), lanes 2 through 10 correspond to DNA from 9 potential regenerant lines, lane NC on the third panel corresponds to the negative control. Note: solid arrows point to the polymorphic bands, while the blank arrows point to the monomorphic bands

12 Dendogram constructed from Jaccard’s similarity coefficients from RAPD data, showing the clustering of the 9 regenerants. (C: control, PL1 - PL8: Potential lines)
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>µmol m⁻² s⁻¹</td>
<td>Micromole per square meter per second</td>
</tr>
<tr>
<td>2, 4-D</td>
<td>2,4-Dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>⁶⁰Co</td>
<td>Cobalt-60</td>
</tr>
<tr>
<td>2-iP</td>
<td>N6-(2-isopentenyl) adenine</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BAP</td>
<td>N6-benzyl amino-purine</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyl trimethylammonium bromide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DMRT</td>
<td>Duncan’s Multiple Range Test</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>Gy</td>
<td>Gray</td>
</tr>
<tr>
<td>IAA</td>
<td>indole-acetic acid</td>
</tr>
<tr>
<td>IBA</td>
<td>indole-3-butyric acid</td>
</tr>
<tr>
<td>KIN</td>
<td>6-furfurylaminopurine</td>
</tr>
<tr>
<td>LD</td>
<td>Lethal Dose</td>
</tr>
<tr>
<td>MARDI</td>
<td>Malaysian Agricultural Research and Development Institute</td>
</tr>
<tr>
<td>MS</td>
<td>Murashige and Skoog</td>
</tr>
<tr>
<td>NAA</td>
<td>1-naphthaleneacetic acid</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PPM</td>
<td>Plant Preservative Mixture</td>
</tr>
<tr>
<td>RAPD</td>
<td>Random Amplification of Polymorphic DNA</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>RCBD</td>
<td>Randomized Complete Block Design</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical Analysis System</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>UKM</td>
<td>Universiti Kebangsaan Malaysia</td>
</tr>
<tr>
<td>UPM</td>
<td>Universiti Putra Malaysia</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background

Zingiberaceae is one of the largest families of the plant kingdom. It is an important family which provides many useful products for food, spices, medicines, perfume, dyes essentials oil and aesthetics to man (Jaafar et al., 2007; Poulsen, 2006). It provides plants of economic value mainly for its beautiful flowers, vegetable and also ingredient in a dish. These species are represented throughout the tropical and subtropical regions where the Indo Malayan (Indonesia, Malaysia, Brunei, Singapore, Papua New Guinea and the Southern Philippines) region is the centre of diversity for the Zingiberaceae. Of the 52 genera and 1500 species known in the world, at least 25 genera and 650 species can be found in Malaysia (Sirirugsa, 1999).

*Etingera elatior* (Jack) R.M.Sm which belongs to the Zingiberaceae family is one of the most commonly known species of *Etingera*. This species is also known as torch ginger or wax flower due to the striking resemblance of the inflorescence to a flaming torch. Torch ginger is widely cultivated in the tropical country and possibly native to Indonesia and Malaysia. It is known as *kantan* in Malaysia and *kecombrang* in Indonesia.
1.2 Problem Statement

*E. elatior* is one of the neglected plants in the Zingiberaceae family and scientific research on their propagation technique, production, biotechnology and ecology is limited. However, this ginger species offers great scope for the development of a large range of ornamental and cut flower types (Poulsen, 2006). Ismail (2009) reported that *E. elatior* is one of the 30 popular herbs or new industrial crops that have high demand in Malaysia. It is now cultivated on a commercial scale in places like Australia, Thailand and Costa-Rica for cut flower production (Ismail, 2009; Segalen, 2010). The plant itself makes a great garden landscape, their flowers having an immense ornamental value and also has a place in an eco-garden. To sustain in the ornamental and cut flower industry, torch ginger requires continuous improvement in certain characters like flower colour, morphology, longevity, size, odour and decreased time to flower formation. Unfortunately, conventional breeding of *E. elatior* is handicapped by the cross incompatibility and poor fruit set and also low seed production (Marcisik and Hoult, 2010). Due to these factors, alternative approaches for crop improvement of *E. elatior* such as through mutation induction could be explored.

*In vitro* culture techniques provide an alternative means of propagation and a tool for crop improvement (Zheng et al., 2008). Unfortunately, this medicinal plant has not received much attention from tissue culturists and to date, there is limited information on plant regeneration of this medicinal plant. Hence, with the increasing importance of torch ginger as an ornamental species, there is a need for an efficient
protocol for plant regeneration using tissue culture techniques. The protocol developed here will be helpful for regenerating plants at much higher rates than any other conventional breeding methods and also may serve as a potential source of new variants and further genetic improvement in this species (Xu et al., 2009). In addition, development of new torch ginger cultivars with improved characters is another approach that can be explored. Possible approaches to create genetic variability for the selection of useful plants are through conventional plant breeding, mutagenesis, somaclonal variation and genetic transformation.

1.3 Significance of the Study

In a modern and industrialized horticulture, there is always a demand and necessity for new cultivars. Modern day plant breeding is based on creating variations followed by selection, evaluation and multiplication of the desired genotypes. Induced mutations have played an important role in the improvement of plants and more than 2500 mutant cultivars have been developed through mutation breeding (Patade et al., 2008). Mutation breeding is an established method for plant improvement, thus encouraging the plant breeders to use induced mutagenesis. Induction of mutation can increase the possibility a thousandfold compared with spontaneous mutation under natural condition (Broertjes and Van Harten, 1988).

In a crop improvement programme, plant breeders often combine several techniques in order to increase efficiency and reduce the time needed for the development of a new cultivar. Such combination has been exploited for the creation of new and novel plant cultivars, particularly in vegetatively propagated species (Pinet-Leblay et al.,
Successful outcome of a mutation depends on an efficient induction of mutation as well as an effective recognition and recovery of the desired mutant plants through repeated subculture (Puchooa, 2005).

The present study is divided into two parts: a) *In vitro* propagation and b) *In vitro* mutagenesis and RAPD analysis. A new technique is needed to standardize the management of chimeric tissues through multiple bud regeneration. Molecular techniques can provide an understanding of plant cell responses to mutation induction. It also facilitates a better understanding of the potential and limitations of mutation breeding, which can lead to early identification of useful variants. Major advantages of random amplification of polymorphic DNA (RAPD) are the low cost and effort required for its application (Dhakshanamoorthy, et al., 2011; Atienzar and Jha, 2006).
1.4 Objective

The objectives of this study are:

1) To establish an efficient *in vitro* plant regeneration system in torch ginger.
2) To investigate the optimum dose for radio sensitivity test and to determine the effects of various doses of gamma irradiation on multiple bud induction.
3) To determine the variation in genomic DNA of regenerated shoots by using RAPD technique.
REFERENCES


