

**GENETIC DIVERSITY IN EGGPLANT  
*SOLANUM* L. AND RELATED SPECIES  
FROM SOUTHERN NIGERIA**

**BY**

**SIFAU, MUTIU OYEKUNLE**

**MATRIC NO: 940802071**

**B.Sc (HONS.) 1998, M.Sc. (2003) LAGOS**

**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE  
STUDIES, UNIVERSITY OF LAGOS, AKOKA, LAGOS, NIGERIA, FOR  
THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D.) DEGREE IN  
CELL BIOLOGY AND GENETICS.**

**AUGUST 2013**

**SCHOOL OF POSTGRADUATE STUDIES  
UNIVERSITY OF LAGOS**

**CERTIFICATION**

THIS IS TO CERTIFY THAT THE THESIS:

**GENETIC DIVERSITY IN EGGPLANT *SOLANUM* AND  
RELATED SPECIES FROM SOUTHERN NIGERIA**

SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, UNIVERSITY OF LAGOS FOR  
THE AWARD OF THE DEGREE OF

**DOCTOR OF PHILOSOPHY (Ph.D.)**

**IN**

**CELL BIOLOGY AND GENETICS.**

IS A RECORD OF ORIGINAL RESEARCH CARRIED OUT BY

**SIFAU, MUTIU OYEKUNLE**

IN THE DEPARTMENT OF CELL BIOLOGY AND GENETICS.

.....    .....    .....

AUTHOR'S NAME	SIGNATURE	DATE
---------------	-----------	------

.....    .....    .....

1 <sup>ST</sup> SUPERVISOR'S NAME	SIGNATURE	DATE
-----------------------------------	-----------	------

.....    .....    .....

2 <sup>ND</sup> SUPERVISOR'S NAME	SIGNATURE	DATE
-----------------------------------	-----------	------

# DEDICATION

This work is dedicated to

- **Almighty Allah** (SWT), the Lord of the Universe and the Fountain of Knowledge.
- My late father, Pa **Majeobaje Akanmu Sifau** for leaving a legacy of steadfastness, obedience and service to Allah and Humanity for the children.
- **All teachers** who render services that can neither be quantified nor paid for: monetarily or materially to humanity.

## ACKNOWLEDGMENTS

I am very grateful to Almighty Allah for sparing my life to completion of this work. I also thank Him for His protection and mercies on me all the time. “O Allah, give unto me in the world that which is good and in the Hereafter that which is good, and guard me from the doom of fire” (Q2:201).

My profound gratitude goes to my Supervisors Prof. Bola Oboh and Prof. O. T. Ogundipe for their wonderful supervision, encouragement, parental guidance and support (both financially and morally) to me. I pray that God in His Infinite Mercies reward, bless them abundantly and protect them and their families from all evil. I wish to express my heartfelt gratitude to Prof. Gabriel Olufemi Williams for giving me the inspiration to further my studies up to this level right from my undergraduate days and for his constructive comments and contributions all the time. May Allah in His Infinite Mercies reward you, bless you and your entire family with comfort in this world and the hereafter. Special appreciation goes to Drs. K. O. Adekoya and L. A. Ogunkanmi for their constructive criticism and scrutiny of the work and words of encouragement when they matter most. I say to them Jazakumullahu khairan. Dr. K. O. Adekoya who is also the departmental PG Coordinator, specifically made all my correspondence with the School of Postgraduate Studies faster and easier. I pray Allah to continue strengthening him. My sincere appreciation goes to the following for their financial support without which this study may not have been possible: UNILAG Central Research Committee (CRC) for providing Grant (N0: CRC 2005/03, UNILAG) for the work; UNILAG Muslim Alumni (UMA) for award of Scholarship for two consecutive sessions; and School of Postgraduate Studies for award of Graduate Fellowship.

My sincere appreciation also goes to the other distinguished and wonderful lecturers in my department for their easily understood teachings and demonstrations: Profs. Joy Okpuzor (The

HOD), Sesan Omidiji, P. G. C. Odeigah, M. O. Akinola, Drs. I. A. Taiwo, K. L. Njoku, O. A. Adeyemo, L. A. Ogunyebi, A. Oloyede, Messers C. G. Alimba and O. A. Adebessin. To the other non-Teaching staff of the department, I say thank you for your useful contributions to the study. To my course mates and fellow Graduate Assistants : N. M. C. Nwakanma, A. Oloyede, T. Yahaya, Micheal Ukaegbu, Mairiga, J. P., Ayorinde Adenola, etc; I say thanks for your comradeship.

Let me also extend my appreciations to my religious constituency, President General and all members of Nakou-deen Society of Nigeria, executives and members of University of Lagos Muslim Alumni (UMA), for their prayers and encouragements. To my spiritual father, Sheik-I-Islam Alhaji (Prof.) Zakariyau Hassan Akorede, I say Jazakallahu khaera for your ceaseless prayer and spiritual upbringing. May Allah increase you in knowledge, forgive all your shortcomings and strengthen you the more. My other Ustas: Ustas Sikirulahi Oloyede, Alfa Sabiu Musa, and Sheu Abdul-Quadri Muhammad-I Awwal and others, I thank you for your admonition and spiritual upbringing. I pray Almighty Allah to grant you all that which is good in this world and make you among the inmates of Aljanah in the hereafter. My appreciation is also extended to all Muslims that follow the Sunnah of the Noble Prophet Muhammad (SAW) till the end of time.

I equally appreciate specially my best friend, Ashade Akinyemi for his support both morally and financially all the time. May the good Lord continue to shower more and more of His blessings on you and all yours all the time. To all others: Oseghale Cornelius, Ayandele Musbau, Alhajis Abideen Solagbade, Wasiu Tijani, Idowu Akeem, Mutoleeb Solagbade, Wasiu Tiamiyu, Ibrahim Ayoub, Mr and Mrs Afolabi, etc, thank you for your true friendship and companionship. May the friendship never gone sour. I also appreciate the entire work force at National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, starting from the Director/ CEO, Mr. Wasiu

Odofin, the AD (R&D), Dr. S. E. Aladele, up to the last in the cadre; thanks for the support and teamwork opportunity given to me.

This acknowledgment will not be completed without special thanks to members of my family both immediate and extended: My wife, Hajia Khadijat Iyabo Sifau, who made sure that the home front is intact and unshaken as a result of my absence from home most of the time, due to demands of the research. She provided the much-needed love, warmth and succour whenever I return. My lovely children; Mustapha, Barakah and Fareedah for their understanding, affection and smiles that gave the impetus to forge ahead with humility and zeal needed for the research.

I am most thankful to my mother, Madam Sabitiyu A. Sifau for her love, support and words of encouragement especially during those trying periods. May Almighty Allah spare and strengthen you to eat from the fruits of your labour. I must also not forget to remember my stepmother, Madam Habibah A. Sifau of blessed memory, for instilling discipline and moral in me especially during childhood. May her soul be favoured by Allah and be granted Aljannah firdaus (Amen). I fondly remember my elder brothers and their families; Mr and Mrs Fatai Alani Sifau, Mr and Mrs Musbau Sifau, and Mr and Mrs Mojeed Sifau. My other siblings and family; Mr and Mrs Tirimisiyu Sifau, Mr and Mrs Babatunde Sifau, Mr and Mrs Saheed Sifau, Mr and Mrs Adamo Sifau, Mrs Suliyat Mustapha, Moshood, Idowu and Sharafadeen. May Allah grant all your heart desire in this life and hereafter. I am also grateful to all other members of my extended family who have played one role or another in my life. My prayer to them is that God Almighty should bless them abundantly.

Finally, I thank Allah for His goodness, favour and mercies on me all the time. I pray Allah for more of these here in this world and in the Hereafter.

# TABLE OF CONTENTS

Caption	Page
TITLE PAGE	i
CERTIFICATION	ii
DEDICATION	iii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	vii
LIST OF FIGURES	xii
LIST OF TABLES	xiii
LIST OF PLATES	xiv
ABSTRACT	xvi

## Chapter one: INTRODUCTION

1.0	Introduction and Background of Study	1
1.0.1	The Genus <i>Solanum</i> L.	1
1.0.2	Diversity within vegetable <i>Solanum</i>	4
1.2	Statement of Problem	5
1.3	Aim and Objectives	6
1.3.1	Aim	6
1.3.2	Objectives	6
1.3.2.1	General Objective	6
1.3.2.2	Specific Objectives	7
1.4	Significance of Study	7

## TABLE OF CONTENTS (contd.)

1.5	Research Questions	8
1.6	Operational Definition of Terms and Abbreviations	9
1.6.1	Definition of Terms	9
1.6.2	Abbreviations and Acronyms	10

### Chapter two: LITERATURE REVIEW

2.0	Literature Review	12
2.1	Crop Histories, Origin and Domestication	12
2.2	Distribution and Habitat	16
2.3	Taxonomy of Eggplant	18
2.4	Taxonomic Classifications of <i>Solanum</i> .	22
2.5	Phylogeny of Genus <i>Solanum</i>	24
2.6	Characterization of Eggplant	27
2.7	Molecular tools in Eggplant <i>Solanum</i> Characterization	28
2.8	Botanical Description	31
2.9	Economic Importance	34

### Chapter three: MATERIALS AND METHODS

3.1	Sample Collections and Areas of Collection	36
3.1.1	Methods of evaluation	37
3.2	Morphological Study	37
3.3	Identification of the Plant Samples Collected	38



## TABLE OF CONTENTS (contd.)

3.4	Molecular Studies	39
3.4.1	Total Genomic DNA Extraction	39
3.4.2	Purification of extracted DNA	40
3.4.3	Verification of DNA Quality by Electrophoresis	41
3.4.4	Spectrophotometric Analysis	41
3.4.5	Polymerase Chain Reactions (PCR) and Amplification	42
3.4.5.1	Amplification of the internal transcribed spacer (ITS) region.	43
3.4.5.2	Amplification of trnL-trnF spacer plus trnI intron	43
3.4.5.3	Purification of amplified DNA and Gel Electrophoresis	44
3.4.6	Cycle Sequencing	44
3.4.6.1	DNA Sequencing, Editing and Alignment	45
3.4.6.2	Sequencing of ITS region	45
3.4.6.3	Sequencing of trnL-trnF spacer plus trnI intron	45
3.4.6.4	Sequence Editing and alignment	46
3.4.6.5	Basic Local Alignment Search Tool (BLAST)	46
3.5	Data Analysis	47
3.5.1	Morphological Data	47
3.5.2	Molecular Data	48
3.5.2.1	Phylogenetic Estimation using Maximum Likelihood (PhyML)	48

## TABLE OF CONTENTS (contd.)

### Chapter Four: RESULTS

4.1	Morphological Characterization.	49
4.1.1	Sample Collections	49
4.1.2	Authenticated Voucher Specimens	51
4.2	Description of Morphological Characters of Collected Samples	54
4.2.1	<i>Solanum nigrum</i>	56
4.2.2	<i>S. scabrum</i>	58
4.2.3	<i>S. macrocarpon</i>	60
4.2.4	<i>S. melongena</i>	63
4.2.5	<i>S. gilo</i> Raddi	71
4.2.6	<i>S. aethiopicum</i>	74
4.2.7	<i>S. dasyphyllum</i> Schum. & Thonn	76
4.2.8	<i>S. torvum</i> Sw.	78
4.2.9	<i>S. erianthum</i>	80
4.2.10	<i>S. macranthum</i> A. Rich.	82
4.2.11	<i>S. incanum</i>	84
4.2.12	<i>S. indicum</i> subsp. <i>distichum</i> var. <i>distichum</i>	86
4.3	Taxonomic Key	88
4.4	Analyses of Morphological Data.	89
4.4.1	Plant's Stem/Branches Characteristics	95
4.4.2	Leaf Characteristics	97
4.4.3	Inflorescence/ Flower and Fruit Characteristics	100
4.5	Pair-wise Analysis of Morphological Data	104

## **TABLE OF CONTENTS (contd.)**

4.6	Principal Component Analysis of Morphological Data	110
4.7	Molecular Characterization	116
4.7.1	Spectrophotometry	118
4.7.2	Polymerase Chain Reaction (PCR)	122
4.7.3	DNA Sequencing of both ITS and trnL-trnF spacer Plus trnI intron Regions.	128
4.7.4	Basic Local Alignment Search Tool (BLAST) Results.	130
4.7.5	Phylogenetic estimation using Maximum Likelihood (PhyML)	133

### **Chapter Five: DISCUSSION OF RESULTS**

5.0	Discussion of Results	136
-----	-----------------------	-----

### **Chapter six: SUMMARY OF FINDINGS/CONCLUSIONS**

6.0	Summary of Findings/Conclusions	144
6.1	Contributions to Knowledge	146
	<b>References</b>	147
	<b>Appendices</b>	170

## List of Figures

Figures	Title	Page Number
<b>1</b>	The dispersion of <i>Solanum</i> members around the world	17
<b>2</b>	Pollination in <i>Solanum</i>	33
<b>3</b>	Map of Southern Nigeria showing some Points of Sample Collection.	50
<b>4a</b>	A UPGMA dendrogram of Pair-wise (Similarity) analysis showing relationships among the samples using Data for Qualitative characters only.	105
<b>4b</b>	Dendrogram of the Combined Morphological Data (Both Qualitative and Quantitative) at a Similarity Coefficient of 43.4%.	107
<b>4c</b>	Dendrogram of the Combined Morphological Data at a Similarity Coefficient of 51 %.	109
<b>5a</b>	Relationship among 49 <i>Solanum</i> Accessions Studied Based on Principal Components Analysis using Data for Eleven Quantitative Morphological Traits	111
<b>5b:</b>	Projection of the 49 <i>Solanum</i> Accessions Morphologically Characterized on a Three Dimensional Basis.	113
<b>6</b>	Relative absorbance of DNA samples of accessions of eggplant <i>Solanum</i> at 260nm	119
<b>7</b>	Relative absorbance of DNA samples of accessions of eggplant <i>Solanum</i> at 280nm	120
<b>8</b>	Relative absorbance Ratio ( $A_{260/280}$ ) of DNA samples of accessions of eggplant <i>Solanum</i> .	121
<b>9</b>	Phylogenetic Relationships within eggplant <i>Solanum</i> using <i>C. anuum</i> as the outgroup.	134

## List of Tables

<b>Tables</b>	<b>Title</b>	<b>Page Number</b>
<b>1</b>	Modified informal group classification of <i>Solanum incanum</i> and <i>S. melongena</i>	21
<b>2</b>	Taxonomic classifications of <i>Solanum</i> based on different authors.	23
<b>3</b>	Collected samples and their Identification names	52
<b>4</b>	List of both Qualitative and Quantitative Characters evaluated in this study	90
<b>5</b>	Range of Variation in Quantitative Descriptors and Coefficient of Variations given by the Ratio of the Standard Deviation to the Mean	91
<b>6</b>	Mean values and standard deviation of Quantitative descriptors	92
<b>7</b>	Plant's Stem/Branches Characteristics observed for the <i>Solanum</i> samples Characterized	96
<b>8</b>	Leaf characteristics recorded for all the samples	98
<b>9</b>	Wide diversity in fruit Characters observed in eggplant <i>Solanum</i> accessions	101
<b>10</b>	Serial number and respective sample I.D with name used for Principal Component Analysis.	114
<b>11</b>	Different Primers used for PCR and their Sequences.	123
<b>12</b>	List of Primers used for Sequencing	129
<b>13</b>	BLAST Results and Percentage Maximum Identity	131

## List of Plates

Plates	Title	Page Number
1	Photographs of some vegetable <i>Solanum</i> species	55
2	<i>Solanum nigrum</i>	57
3	<i>S. scabrum</i>	59
4	Flower morphology in <i>S. macrocarpon</i>	61
5	<i>S. macrocarpon</i>	62
6	Clusters of <i>S. melongena</i> plant	64
7	<i>S. melongena</i> showing different flower colours and shapes	66
8	<i>S. melongena</i> showing prickles or spines	67
9	Different colours of <i>S. melongena</i> fruits	69
10	<i>S. melongena</i> showing different predominant fruit shapes	70
11	<i>S. gilo</i>	72
12	<i>S. gilo</i> plants showing different morphology	73
13	<i>S. aethiopicum</i> .	75
14	<i>S. dasyphyllum</i>	77
15	<i>S. torvum</i> plant showing leaves, flowers and fruits.	79
16	<i>S. erianthum</i> showing simple leaves, white flowers and fruits.	81
17	<i>S. macranthum</i> plant	83
18	<i>S. incanum</i>	85
19	<i>S. indicum</i>	87
20	Electrophorogram of extracted DNA sample	117
21	Electrophorogram of ITS2 – ITS5 amplification	124
22	Electrophorogram of ITS3 – ITS4 amplification	124

## **List of Plates (Contd.)**

<b>23</b>	Electrophorogram of re-cleaned ITS3 – ITS4 amplification of Samples 11 and 12	125
<b>24</b>	Electrophorogram of trnl C-trnl D amplification	127
<b>25</b>	Electrophorogram of trnl E-trnl F amplification	127

## **ABSTRACT**

*Solanum* L., with an estimated number of over 2000 species, and the largest genus in the Solanaceae, includes economically important species such as the tomato, potato, and eggplant. The common name “Eggplant” is given to vegetable *Solanum* which has been used as nomenclature to refer to several *Solanum* species important for human diet and health. Many vegetable *Solanum* species that occur in Nigeria are sources of food and of medicine. Despite the importance of this genus, its taxonomy and phylogenetic relationships among these taxa are currently unclear. The advent of molecular biology has revolutionized the field of plant systematics and is being applied for elucidating the phylogenetic relationships at all taxonomic levels of *Solanum* species. The objective of this study was to assess and measure the genetic diversity in eggplant and related *Solanum* species’ genetic resources in Southern Nigeria using both morphological and molecular methods. This is in order to establish the phylogenetic relationship among the taxa studied and identify agronomic marker traits useful for classification with a view to promoting their conservation, effective management and sustainable use. A total of forty nine (49) eggplant *Solanum* samples and related species were collected during exploration. These represent 12 different species of which two additional *Solanum* species were discovered for Nigeria. Taxonomic key was generated for all the species collected for ease of identification after they have been morphologically described in detail. The dendrogram constructed using morphological data from collected samples of different species of vegetable *Solanum* (eggplants) and related species through UPGMA clustering method showed the phenetic relationship among samples studied. Principal Component Analysis (PCA) revealed that fruit characters were important agronomic marker traits with a coefficient of variation (>50%) that most effectively discriminated among eggplant accessions and hence useful in establishing a simple but effective eggplant classification



system in Nigeria. DNA sequence data from one nuclear region (ITS) and two chloroplast regions (trnI C- trnI D and trnI E – trnI F) resulted in a well resolved phylogenetic hypothesis, with results strongly suggesting that most of the *Solanum* accessions in this study are monophyletic. Of the three gene regions, trnL-trnF spacer was especially useful for phylogenetic inference, with both a high percentage of parsimony-informative sites as well as a low level of homoplasy. The highest bootstrap value of 100 was observed between *S. macrocarpon* L. and *S. torvum* Sw., and between *S. macranthum* A.Rich. and *S. indicum* L. This was closely followed by a value of 99 between *S. aethiopicum* L. and *S. dasyphyllum* Schum. & Thonn. All these indicate a close relationship between these species and a possibility of a common ancestor is strongly proposed. There were situations whereby results from molecular study contradicted those of morphological analysis. There were also cases whereby observations noticed in morphological analysis were equally noticed in molecular analysis. Therefore, molecular analysis was able to reveal more about relationships among the accessions studied by either confirming or negating the result of morphological analysis. The combination of molecular markers and morphological evaluations as used in this study has greatly helped in cultivar identification and clarification of the phylogenetic affinities of the large and complex genus *Solanum*.