

ETHNOBOTANY AND GENETIC INTRA-SPECIFIC VARIABILITY OF *Moringa oleifera* LAM. (DRUMSTICK) IN NIGERIA

POPOOLA JACOB OLAGBENRO

MATRIC NO: CUGP100228

B. TECH (LAUTECH), M. Sc (FUNAAB)

**A THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES,
SCHOOL OF NATURAL AND APPLIED SCIENCES, COLLEGE OF SCIENCE
AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN
BIOLOGY (PLANT MOLECULAR SYSTEMATICS)**

April, 2015.

CERTIFICATION

This is to certify that Jacob Olagbenro POPOOLA (Matric. No: CUGP100228) carried out this research work in partial fulfillment of the requirements for the award of a Doctor of Philosophy (Ph.D) degree in Biology (Plant Molecular Systematics) of Covenant University, Ota, under our supervision.

Prof. O.O. Obembe

Department of Biological Sciences Signature Date

(Supervisor)

Dr. A.C. Omonhinmin

Department of Biological Sciences Signature Date

(Co-Supervisor)

Dr. E. E. J. Iweala

Department of Biological Sciences Signature Date

(Ag. Head of Department)

DECLARATION

It is hereby declared that this research work titled “Ethnobotany and Genetic Intraspecific Variability in *Moringa oleifera* LAM. (Drumstick) in Nigeria was undertaken by Jacob Olagbenro POPOOLA.

Prof. O.O. Obembe

Supervisor Signature and Date

Dr. A.C. Omonhinmin

Co-Supervisor Signature and Date

Dr. E. E. J. Iweala

Ag. Head, Department of Biological Sciences Signature and Date

Covenant University, Ota, Ogun State, Nigeria.

Prof. Shalom Chinedu

Dean, College of Science and Technology, Signature and Date

Covenant University, Ota, Ogun State, Nigeria.

Prof. Charles Ogbulogo

Dean, School of Postgraduate Studies, Signature and Date

Covenant University, Ota, Ogun State, Nigeria.

DEDICATION

This Ph.D thesis is dedicated to God Almighty for His Exceeding Grace. To Him alone be all glory and honour.

ACKNOWLEDGEMENTS

I express my sincere gratitude to my supervisors, Prof. O.O. Obembe and Dr. A.C. Omonhinmin for their guidance, constructive advice, and assistance during the course of my studies. The roles played by Dr. A.C. Omonhinmin during survey trips cannot be forgotten. I wholeheartedly appreciate the opportunity given to me to study at Covenant University and the enabling environment provided. I thank the Acting Head, Department of Biological Sciences, Dr. E. J. Iweala for the positive roles played during the course of this study. I express my gratitude to Professor A. E. Adegbite for his guidance, mentoring and advice. I highly appreciate the support I got from him. I also appreciate the administrative support and encouragement provided by Dr. I. S. Afolabi. I also acknowledge Dr. Solomon Oranusi for assistance provided during the course of this study.

I am highly indebted to Deacon Richard Adewoyin, Mr. Francis Okusodo, Tosin Adelani and the entire staff members of Covenant University farm for all the field assistance and encouragement provided during field experiments and data collection. My appreciation also goes to the entire members of staff of the Department of Biological Sciences for their encouragement, support and prayers.

I will be ungrateful if I fail to acknowledge the efforts of Tope Owoye and Victoria Nwagbara of BioScience, International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria for the roles they played during molecular characterization of the samples. I sincerely appreciate Hon. Oladapo Popoola, Mrs. Taiwo Oladoyinbo and Rev. Ben Atoyebi for their support, prayers and encouragement throughout the period of the study. To my wife Mabel Aderonke Popoola and children David and Daniel Popoola, I say thank you for your understanding, care, love, support, encouragement and prayers.

TABLE OF CONTENTS

SUBJECT	PAGE
Title.....	i
Certification.....	ii
Declaration.....	iii
Dedication.....	iv
Acknowledgement.....	v
Table of Contents.....	vi
List of Tables.....	x
List of Figures.....	xi
List of Plates.....	xii
Abstract.....	xiii
CHAPTER ONE	INTRODUCTION
1.1 Background.....	1
1.2 Ethnobotany and Cultural diversity.....	2
1.3 Constraints to <i>Moringa oleifera</i> Cultivation.....	3
1.4 Statement for Research Problem	4
1.5 Justification of the Study.....	5
1.6 Limitations of the Study.....	6
1.7 Aim of Study.....	6
1.8 Objectives	7
CHAPTER TWO	LITERATURE REVIEW
2.1 Taxonomy of the genus <i>Moringa</i>	8
2.2 Synonyms and some indigenous lingual of <i>Moringa oleifera</i>	9
2.3 Ecology of <i>Moringa oleifera</i>	10
2.4 Geographical distribution.....	11
2.5 Morphology and life cycle of <i>Moringa oleifera</i>	12
2.6 Reproduction and dispersal of <i>Moringa oleifera</i>	13
2.7 Propagation and Planting of <i>Moringa oleifera</i>	13
2.7.1 Seed Propagation.....	14
2.7.2 Vegetative Propagation.....	14
2.8 Cytogenetic and Reproductive Biology of <i>M. oleifera</i>	14

2.9	Biological Diversity / Genetic Resources Diversity.....	15
2.10	Evaluation of Genetic Diversity.....	18
2.10.1	Morphological Markers and their limitations.....	18
2.10.2	Biochemical Markers.....	19
2.10.3	Molecular Markers.....	20
2.10.3.1	Restriction Fragment Length Polymorphism (RFLP).....	21
2.10.3.2	Random Amplified Polymorphic DNA (RAPD).....	22
2.10.3.3	Amplified Fragment Length Polymorphism (AFLP).....	22
2.10.3.4	Limitations of Molecular Markers.....	23
2.11	Limitations to Tree breeding Improvement.....	24
CHAPTER THREE MATERIALS AND METHODS		
3.1	Ethnobotany.....	25
3.1.1	Study area and Socio-demographic characteristics.....	25
3.1.2	Informed Consent.....	25
3.1.3	Data Collection.....	27
3.1.4	Use Categories.....	27
3.2	Geographical Distribution	27
3.3	Data Analysis.....	32
3.4	Genetic Diversity Studies.....	33
3.4.1	Morphological Study.....	33
3.4.2	Germplasm / Seed Collection.....	33
3.4.3	Sources of Materials.....	33
3.4.4	Nursery and Field Preparation.....	33
3.5	Development of descriptor list for characterization of <i>M. oleifera</i>	35
3.6	Morphometric characterization.....	37
3.6.1	Qualitative traits.....	37
3.6.2	Quantitative vegetative, reproductive, pod and seed traits and germination %.....	37
3.7	Data Analysis.....	38
3.7.1	Pearson Correlation Coefficient Analysis (r).....	38
3.7.2	Principal Component (PC), Cluster Analysis (CA) and Discriminant Analysis (DA)	38
3.7.2.1	Principal Component Analysis (PCA).....	38
3.7.2.2	Cluster Analysis and Discriminant Analysis.....	38
3.8	Molecular Characterization.....	39

3.8.1	Sample Preparation.....	39
3.8.2	DNA Extraction.....	39
3.8.3	Preparation of 1 L 20 % SDS.....	40
3.8.4	Electrophoresis gel /Checking the DNA Quality.....	40
3.8.4.1	Gel Electrophoresis Preparation.....	40
3.8.4.2	DNA Treatment with Poly Vinyl Pyrolidone.....	41
3.8.5	Primer combination and AFLP Adaptors.....	41
3.8.6	Amplified Fragment Length Polymorphism – AFLP Analysis.....	43
3.8.7	Gel Electrophoretic Separation	43
3.8.8	Gel Analysis.....	43
3.8.9	Data Analysis.....	44

CHAPTER FOUR

RESULTS

4.1	Ethnobotany.....	45
4.1.1	Use Categories and Ethno-ecological Knowledge of <i>M. oleifera</i>	45
4.1.2	Use Pattern of <i>M. oleifera</i>	48
4.1.3	Use Knowledge Variation of <i>M. oleifera</i>	55
4.1.4	Geographical Distribution and History of <i>M. oleifera</i> in Nigeria.....	57
4.2	Genetic Diversity Studies.....	58
4.2.1	Morphometric variability involving qualitative traits	58
4.2.2	Morphometric quantitative variation.....	62
4.2.3	Correlation Matrix of all the quantitative characters of <i>M. oleifera</i> accessions.....	65
4.2.4	Principal Component Analysis of the 40 accessions of <i>M. oleifera</i> studied.....	68
4.2.5	Cluster Analysis of the 40 accessions of <i>M. oleifera</i> studied.....	71
4.2.6	Discriminant Analysis	73
4.3	Molecular Studies.....	75
4.3.1	DNA Extraction.....	75
4.3.2	Restriction digestion of DNA.....	75
4.3.3	Polymorphism as detected by amplified fragment length polymorphisms (AFLPs)...	75
4.3.4	Cluster analysis of <i>M. oleifera</i> accessions data.....	81
4.3.4.1	Cluster membership.....	81
4.3.5	Principal Coordinate Analysis of <i>M. oleifera</i> accessions data.....	83
4.3.6	Comparative Study of Morphometric and AFLP Analyses.....	85

CHAPTER FIVE	DISCUSSION	
5.0	Ethnobotany.....	87
5.2	Knowledge variation on use and geographical distribution of <i>M. oleifera</i> in Nigeria	90
5.3	Genetic Diversity Studies.....	91
5.3.1	Morphometric Studies of Intra-specific variability in <i>M. oleifera</i>	91
5.3.2	AFLP Analysis of Intra-specific variability in <i>M. oleifera</i>	93
5.3.2.1	Genetic variation and Geographic Distribution of <i>M. oleifera</i> in Nigeria	94
5.3.2.2	Center of Endemism and Spread Pattern in Nigeria.....	96
5.3.3	Comparative Analysis: Ethno-Uses and Genetic diversity.....	96
5.4	Summary and Conclusion.....	97
5.5	Contribution to Knowledge.....	98
5.6	Impact of the Findings and Areas of Further Research.....	99
5.7	Recommendations from this study.....	100
	REFERENCES.....	101
	Appendix I Questionnaires used for Ethnobotany	118
	Appendix II Quantitative and qualitative traits studied and their brief descriptions.....	119
	Appendix III Molecular weight Ladder used for DNA quantification.....	121
	Appendix IV AFLP Reactions.....	122

LIST OF TABLES

Table NO.	Title	Page
3.1	Accession number, area of collection, location, age and origin of the accession...	29
3.2	Summary of Passport data of the 40 accessions used for genetic diversity studies	34
3.3	Descriptor state of qualitative and quantitative traits used for characterization of <i>M. oleifera</i>	36
3.4	AFLP Adapters and Primer Sequences for <i>M. oleifera</i> amplification.....	42
4.1	Socio-demography of the Sampled Populations in the Study Areas.....	46
4.2	Ethnopharmacological Use Pattern of <i>Moringa oleifera</i> among the Sampled Ethnic Groups in Nigeria	49
4.3	Preparation and utilization of parts of <i>M. oleifera</i> among the Sampled respondents	51
4.4.	Fidelity level of Use Among the Ethnic, Gender and Age groups Sampled.....	54
4.5	Overall use value of <i>M. oleifera</i> According to Ethnic, Gender and Age groups Sampled.....	56
4.6	Qualitative characters of the 40 accessions of <i>M. oleifera</i> studied.....	59
4.7	Descriptive statistics and variance of characters from the 40 accessions of <i>M. oleifera</i> accessions studied.....	63
4.8	Correlation Coefficient (r) of all the quantitative characters of <i>M. oleifera</i> accessions studied.....	67
4.9	Eigen values, proportion of variation and contribution of the 24 quantitative traits to the total variation in the first six PC axes of the 40 accessions.....	69
4.10	Standardized Canonical Discriminant Function (DF) Coefficients of <i>M. oleifera</i> traits with predictive values.....	74
4.11	Number of percentage polymorphisms, gene diversity and polymorphic information content	78
4.12	Comparative study of morphometric characterization and molecular analysis using AFLP markers.....	86

LIST OF FIGURES

Fig. NO.	Title	Page
3.1	Geographical location of area of study.....	26
4.1	Fidelity Levels of Use Category of <i>M. oleifera</i> in Nigeria.....	47
4.2	A two dimensional plot of Principal Components Analysis of the 40 accessions of <i>M. oleifera</i> based on variance – covariance matrix.....	72
4.3	Average linkage dendrogram generated from 31 morphometric traits.....	74
4.4	Cluster Analysis of the 40 accessions of <i>M. oleifera</i> studied based on AFLP data using UPGMA method with six groups.....	84
4.5	The scatter plot of the First and Second Principal Coordinates Analysis of the 40 accessions of <i>M. oleifera</i> based on AFLP data.....	86

LIST OF PLATE

Plate NO.	Title	Page
4.1	Some parts of <i>M. oleifera</i> used for different purposes.....	52
4.2	Morphological features of <i>Moringa oleifera</i>	61
4.3	Variation in pod and seed traits among the accessions (Representative).....	64
4.4	Electrophoretic gel banding pattern of PCR products showing extracted DNA before digestion.....	78
4.5	Electrophoresis pattern of the <i>Moringa oleifera</i> accession by 2 primer combinations.....	79
4.6	Electrophoresis Pattern obtained using AFLP combination primer M-CAC/E- ACC in the 40 accessions of <i>Moringa oleifera</i> studied.....	81
4.7	Electrophoresis pattern obtained in the AFLP combination Primer M-CAG/E- ACA in the 40 accessions of <i>Moringa oleifera</i> studied.....	82

ABSTRACT

Moringa oleifera is an underutilized tree crop with enormous potential to contribute to improved food security and nutrition, medicine, incomes and environment in Nigeria. The plant has not received systematic research attention to enhance its genetic management and improvement. The local knowledge base on its utilization is weak. Genetic diversity details and knowledge about its intra-specific differences are also poorly understood. This study was aimed at collating and documenting the indigenous knowledge variation and local uses of *M. oleifera*, sampling germplasm for maintenance and use, assess geographical distribution and characterize the sampled accessions. Ethnobotanical data were collected using semi-structured questionnaires and Participatory Rural Appraisals (PRA) method. Intra-specific variabilities were evaluated using morphometric traits and Amplified Fragment Length Polymorphisms (AFLP) markers. Thirty one (31) morphometric traits involving qualitative and quantitative vegetative, floral, pod and seed traits, seed set and germination percentages were used for morphometric analysis. Two AFLP selective primer combinations (M-CAC/E-ACC and M-CAG/E-ACA) were employed for the AFLP analysis. Correlation coefficient, principal component (PCA), cluster (CA) and discriminant analysis (DA) were employed to evaluate the intraspecific relationships. Medicinal use recorded 93% fidelity level (FL), food and nutritional purposes (FL = 71.1%), fodder (FL = 60.9%), fencing (FL = 53.2%), gum (FL = 38.9%), coagulant (FL = 38.8%), and firewood (FL = 27.9%). There were significant differences ($P \leq 0.05$) among the ethnic, gender and age groups regarding the ethno-botanical use value. The use pattern of *M. oleifera* varied from one ethnic group to another. Primer M-CAG/E-ACA generated 859 bands while primer M-CAC/E-ACC gave 413 bands. Morphometric and AFLP cluster analyses using UPGMA segregated the 40 accessions into six groups. The pattern of spread of *M. oleifera* in Nigeria is southward from the North. The use of morphometric traits are less reliable, less efficient and time consuming in the assessment of genetic relationships compared to AFLP marker. AFLP proved to be more robust, fast and reliable. Accessions that are far apart based on genetic similarity coefficient (such as KnN077, ogN026, oyN003 and edN037) could be selected for breeding trials in future. Findings from this study revealed poor management and conservation of *M. oleifera* germplasm in the different agro-ecological zones of Nigeria, which calls for the development of a more effective and coordinated strategies for improved management, utilization, conservation and genetic improvement of *M. oleifera*.