ETHNOBOTANY AND GENETIC DIVERSITY ASSESSMENT OF *Telfairia occidentalis* HOOK F. (FLUTED PUMPKIN) IN SOUTHERN NIGERIA

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A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D) IN BIOLOGY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA, OGUN STATE, NIGERIA

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ACCEPTANCE

This is to attest that this dissertation has been accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Biology in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria.

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DECLARATION

I, **AWORUNSE, OLUWADUROTIMI SAMUEL (17PCO01624)** declare that this research was carried out by me under the supervision of Prof. Olawole O. Obembe and Dr. Jacob O. Popoola of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that this thesis has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this thesis are duly acknowledged.

AWORUNSE, OLUWADUROTIMI SAMUEL

Signature and Date

CERTIFICATION

We certify that this thesis titled **"ETHNOBOTANY AND GENETIC DIVERSITY ASSESSMENT OF *Telfairia occidentalis* HOOK F. (FLUTED PUMPKIN) IN SOUTHERN NIGERIA"** is an original research work carried out by **AWORUNSE, OLUWADUROTIMI SAMUEL (17PCO01624)**, in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Prof. Olawole O. Obembe and Dr. Jacob O. Popoola. We have examined and found this work acceptable as part of the requirements for the award of Doctor of Philosophy (Ph.D) degree in Biology.

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DEDICATION

This work is dedicated to my late parents, Chief Japheth Moyo Aworunse and Mrs. Rhodes Abike Aworunse of blessed memory.

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LIST OF ABBREVIATIONS AND SYMBOLS

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
AP-PCR	Arbitrarily primed polymerase chain reaction
cDNA	Complementary DNA
CTAB	Cetyl trimethyl ammonium bromide
CV	Coefficient of variation
DAF	DNA amplification fingerprinting
DAPC	Discriminant analysis of principal components
DArTseq	Diversity array technology sequencing
DBI	Days to female flower bud initiation
DFFL	Days to 50% female flowering
DFB	Days to 50% female flower bud initiation
DFF	Days to first female flowering
DFPSF	Defatted fluted pumpkin seed flour
DFR	Days to first fruiting
DGM	Days to germination
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DFFR	Days to 50% fruiting
DRP	Days to 95% ripe pod
ESTs	Expressed sequence tags
FAO	Food and Agricultural Organisation
FL	Fidelity
FLC	Flower colour
FLW	Fresh leaf weight
Fst	Fixation index
GBS	Genotyping by sequencing
gSSRs	Genomic simple sequence repeats
H	Nei’s gene diversity
I	Shannon-Weaver’s diversity index

IBS	Identity-by-state
INL	Internode length
IPGR1	International Plant Genetic Resources Institute
ISSR	Intersimple sequence repeat
LAPS	Leaf apex shape
LFA	Leaf area
MAF	Major allele frequency
Maf	Minor allele frequency
Max.	Maximum
MCMC	Markov Chain Monte Carlo
Min.	Minimum
MLY	Marketable leaf yield
Na	Number of different alleles
NBP	Mean number of branches per plant
Ne	Effective number of alleles
NGS	Next generation sequencing
NLP	Number of leaves per plant
Nm	Gene flow
NMB	Number of monomorphic bands
NPB	Number of polymorphic bands
NPP	Number of pods per plant
NSP	Number of seeds per pod
NVP	Number of vines per plant
OUV	Overall use value
PAST	Paleontological statistics software package for education and data analysis
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDC	Pod circumference
PDL	Pod length
PDW	Pod weight
PFP	Pod formation period
PGR	Plant genetic resources
PIC	Polymorphic information content
PPB	Percentage of polymorphic bands

PPC	Pulp colour
PPV	Plant part value
PstI	*Providencia stuartii* strain I
PTL	Petiole length
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RCBD	Randomised complete block design
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNAse	Ribonuclease
Rpm	Revolutions per minute
SCoT	Start codon targeted marker
SDC	Seed colour
SDW	10 seeds weight
Sig.	Significance
SNP	Single nucleotide polymorphism
SNP	Single nucleotide polymorphism
*Sph*I	*Streptomyces phaeochromogenes* strain I
SPSS	Statistical package for social sciences
SSR	Simple sequence repeats
Taq	*Thermus aquaticus*
TBE	Tris Borate Ethylenediamine Tetraacetic Acid
TE	Tris EDTA (Ethylenediamine Tetraacetic Acid)
TNB	Total number of bands
UPGMA	Unweighted pair group method with arithmetic averages
UV	Ultra violet
UV	Use value
VND	Vine diameter
VNL	Vine length 6 weeks after planting
VPG	Vine pigmentation
w/v	Weight by volume
WHO	World Health Organisation
WPS	Weeks post sowing

ABSTRACT

The cultivation of *Telfairia occidentalis* constitutes a significant source of revenue for several small-holder farmers in Southern Nigeria. However, a decline in the indigenous knowledge and limited information on genetic diversity are major constraints to developing improved varieties in the species. The aim of the study was to evaluate indigenous knowledge variation, and phenotypic and genetic diversity of *T. occidentalis* in Southern Nigeria. Two hundred and ninety-five (295) respondents across four ethnic groups were interviewed. Uses cited by the respondents were grouped into categories. Quantitative ethnobotanical indices including fidelity level (FL %), use value (UV), and overall use value (OUV) of the different use categories were computed. Thirty-two (32) *T. occidentalis* landraces were evaluated for variability in 26 quantitative and 5 qualitative traits. The landraces were also assessed for molecular diversity using 8 start codon targeted (SCoT) primers and 18,469 single nucleotide polymorphism diversity array technology sequencing (DArTseq-SNP) markers. Six (6) use categories were identified, with utilisations as food and medicine exhibiting 100 % fidelity levels. The UVs differed significantly ($p < 0.05$) among the ethnic groups, gender, age group, and occupation, with the Efik/Ibibio linguistic group, females, aged respondents, and farmers possessing better ratings on local knowledge. The landraces displayed significant variability ($p < 0.05$) in all the quantitative traits evaluated except for the number of pods per plant and number of vines per plant. Principal component analysis (PCA) involved floral and vegetative traits as distinguishing characters that accounted for higher variabilities across the landraces. Cluster analysis based on the quantitative traits partitioned the landraces into five heterogeneous groups. A comparison of the cluster means revealed that ToIm002, ToIm003, ToOn002, ToIm002, ToRv003, ToRv001, ToRv002, and ToOn003 were early flowering and maturing landraces. Genetic diversity assessment using the SCoT markers amplified 66 fragments across the *T. occidentalis* genomes with an average polymorphic information content (PIC) of 0.77. A SCoT-based hierarchical clustering and principal component analysis (PCA) assembled the landraces into four clusters. Population-based genetic diversity using the SCoT markers showed a Nei's gene diversity of 0.28 ± 0.01, indicating that the landraces were of a narrow genetic base. This was further corroborated by a high genetic identity and close genetic distance between the populations. The 18,469 DArTseq-SNPs exhibited a mean PIC value of 0.17. The mean observed heterozygosity (0.13) of the populations was lower than the expected (0.18), suggesting a low genetic diversity. Discriminant analysis of principal components (DAPC), analysis of molecular variance (AMOVA) and fixation index (Fst) estimates revealed no evidence for genetic differentiation and population structure between populations of the landraces. A DArTseq-SNP cluster analysis stratified the landraces into three admixed groups without reference to the collection regions. Overall, the study showed that the indigenous knowledge and use of *T. occidentalis* are structured along gender, age group, and occupation lines. Furthermore, both SCoT and DArTseq-SNP markers revealed a narrow genetic base for the plant, despite evidence of high morphological diversity. The results of this study have significant implications in the characterisation, conservation, improvement and utilization of fluted pumpkin.

Keywords: Telfairia occidentalis, ethnobotany, genetic diversity, SCoT and DArTseq-SNP