SEED LONGEVITY EVALUATION AND GENOME-WIDE ASSOCIATION STUDIES ON SOME NUTRITIONAL TRAITS OF AFRICAN YAM BEAN

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A Ph.D THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE 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

MAY, 2023

ACCEPTANCE

This is to attest that this thesis 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, Ogun State

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DECLARATION

I, OLUWOLE, OLUBUSAYO OLAYEMI (18PCO01853), declare that I carried out this research under the supervision of Prof. Olawole O. Obembe and Prof. Michael T. Abberton of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria and International Institute of Tropical Agriculture, Ibadan, Nigeria, respectively. I attest that the 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.

OLUWOLE, OLUBUSAYO OLAYEMI

Signature and Date

CERTIFICATION

We certify that this thesis titled **"SEED LONGEVITY EVALUATION AND GENOME-WIDE ASSOCIATION STUDIES ON SOME NUTRITIONAL TRAITS OF AFRICAN YAM BEAN"** is an original research work carried out by **OLUWOLE, OLUBUSAYO OLAYEMI (18PCO01853)**, 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 Prof. Michael T. Abberton. 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 research work is dedicated to the Almighty God and my parents, Engr. Olufemi Oluwole and Mrs Florence Oluwole.

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LIST OF ABBREVIATIONS

AA: accelerated ageing

AFLP: Amplified Fragment Length Polymorphism

ANOVA: analysis of variance

AYB: African Yam Bean

Bp: Base pairs

BLUE: Best linear and unbiased estimators

BNF: Biological nitrogen fixation

CDT: controlled deterioration treatment

Chr: Chromosome

CMLM: Compressed mixed linear model

CTAB: Cetyl trimethylammonium bromide

DArT: Diversity array technology

DArTseq: Diversity array technology sequence (DArTseq)

DF: degree of freedom

DNA: Deoxyribonucleic Acid

EDTA: Ethylene Diaminetetraacetic Acid

FAO: Food and Agriculture Organization

FT-IR: Fourier-Transform Infrared Spectroscopy

gDNA: Genomic DNA

GHG: greenhouse gases

GI: glycemic index

GLM: General linear model

GRC: Genetic Resources Centre

GWAS: Genome-Wide Association Study

IITA: International Institute of Tropical Agriculture

ISSR: Inter-Simple Sequence Repeat

LEA: late embryogenesis abundant

MAF: Minor allele frequency

MAS: Marker assisted selection

Mbp: Mega base pair

MC: moisture content

MLM: mixed linear model

mRNA: Messenger ribonucleic acid

N/A: Not available

NGS: next generation sequencing

*p*50: the time (days) it takes for seed viability to reduce to 50%

PCA: Principal component analysis

PCR: Polymerase chain reaction

Q-Q: Quantile-quantile

QTLs: Quantitative Trait Loci

RAPD: Random Amplified Polymorphic DNA

Raf: Raffinose

Rep: replicate

RFO: Raffinose family oligosaccharides

RH: relative humidity

RPM: revolution per minute

SFE: Supercritical Fluid Extraction

SID: seed information database

SNPs: Single Nucleotide Polymorphisms

SSR: Simple Sequence Repeat

TASSEL: Trait Analysis by aSSociation, Evolution and Linkage

TE: Tris-Ethylene diaminetetraacetic acid

tRNA: Transfer ribonucleic acid

TSs: Tropical *Sphenostylis sternocarpa*

ABSTRACT

African yam bean (AYB) (*Sphenostylis stenocarpa* Hochst. Ex A. Rich) is a neglected legume that has potential to contribute towards alleviating food insecurity in sub-Saharan Africa. As an underutilised legume, AYB provides a great source of protein and starch, with low oil content. Seed nutritional content is a quantitative trait influenced by environmental effects, genes and an interaction of both. These seed nutritional contents (e.g. oil content) are known to influence seed longevity. Seed longevity is the period of time a seed remains viable during storage. Understanding the genetic basis of nutritional traits will contribute to adequate plant conservation as well as food and nutrition security. The aim of this research was to identify the candidate genes associated with AYB seed oil content, seed protein content and the seed starch content, and also assess the influence of these traits on seed longevity of African yam bean. A total of one hundred and thirty-four accessions of African yam bean were used for this research. The nutritional traits i.e., the seed oil, protein and starch contents were analysed using the Soxtec, Kjeltec and Phenol-sulphuric acid method, respectively. Twelve AYB accessions were selected from the one hundred and thirty-four accessions on the basis of high and low protein and oil content for the seed longevity evaluation. The seeds were planted and at harvest maturity, the seeds were harvested, divided into five seed lots and dried at two different temperatures (17 ℃ (control) and 45 ℃). In this study, variation was observed in the seed lots subjected to initial high-temperature drying (45 ℃) and their subsequent seed longevity. Ten out of twelve accessions, which were subjected to two-stage drying at 45 ℃ and 17 ℃ showed better seed longevity when compared to seeds dried at only 17 ℃ in the drying room. A significant positive association was observed between the seed longevity (*p50*) of seeds across all the treatments indicating that seed starch content has a role to play in AYB seed longevity. A genome-wide association study (GWAS) was also carried out on some nutritional traits (i.e., seed oil, seed protein and seed starch content) collected from two planting seasons (2018/2019 and 2019/2020) for one hundred and thirty-four accessions of African yam bean based on 2,485 SNP (single nucleotide polymorphism) markers. A total of five, six, and two SNP markers were found to be significantly associated with genes responsible for oil, protein and starch content, respectively. Also, one pleotropic marker 100004767|F|0-39:A>T-39:A>T was observed to be associated with both AYB seed oil and protein content. This study identified the candidate genes that may be responsible for AYB seed oil, protein and starch content and this can expedite the molecular breeding process using marker-assisted selection. Also, the two-stage temperature drying revealed the importance of adequate seed drying for optimum ex situ conservation of seeds. The effect of high temperature drying (45 ℃) on the seed longevity of AYB provided a more efficient protocol to be used in conserving genetic resources, which indirectly contribute to food security.

Keywords: African yam bean, Genome-wide Association Studies, Seed longevity, Seed oil content, Seed protein content, Seed starch content, Sphenostylis stenocarpa.