RBCL GENETIC RELATEDNESS AND ANTIMICROBIAL PROPERTIES OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa*) SEEDS

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(18PCO02061)

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE IN APPLIED BIOLOGY AND BIOTECHNOLOGY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY.

ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfilment of the requirements for the award of the degree of Master of Science in Applied Biology and Biotechnology in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria

Mr. John A. Phillip

(Secretary, School of Postgraduate Studies) Signature and Date

Prof. Akan B. Williams

(Dean, School of Postgraduate Studies) Signature and Date

DECLARATION

I, **ERUEMULOR, DAVELYNE IFECHUKWUDE (18PCO02061)** declare that this research was carried out by me under the supervision of Dr. J.O Popoola of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the research 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 research are duly acknowledged.

ERUEMULOR, DAVELYNE IFECHUKWUDE **Signature and Date**

CERTIFICATION

We certify that this research titled "**RBCL GENETIC RELATEDNESS AND ANTIMICROBIAL PROPERTIES OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa*) SEEDS"** is an original research work carried out by **ERUEMULOR, DAVELYNE IFECHUKWUDE (18PCO02061)** in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Dr. J.O. Popoola. We have examined and found this work acceptable as part of the requirements for the award of the Master of Science in Applied Biology and Biotechnology.

Dr. Jacob O. Popoola

(Supervisor) 07/10/2021 **Signature and Date**

Dr Isaac O. Ayanda

(Departmental PG Coordinator) **Signature and Date**

Prof. Solomon Oranusi

(Head of Department) **Signature and Date**

Prof. Akan B. Williams

(Dean, School of Postgraduate Studies) **Signature and Date**

DEDICATION

I dedicate this research work to God Almighty, for confirming his word with manifestations that has led to the completion of this research. He alone is worthy of my praise.

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ABSTRACT

African yam bean (AYB) (*Sphenostylis stenocarpa*) is an underexploited leguminous crop belonging to the Fabaceae family. AYB has the potential to significantly boost food security due to its considerable nutritional and medicinal qualities. To efficiently utilize AYB genetic resources for its improvement, it is necessary to understand the crop's sequence information and other ways it can be utilized not just for food but also for therapeutic purposes. A total of 24 accessions of AYB seeds were collected from the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA) via the Standard Material Transfer Agreement (SMTA) of the International Treaty on Plant Genetic Resources. Genomic DNA was extracted from two-weeks old leaf samples of the accessions and subjected to PCR amplification and sequencing using chloroplast Ribulose-1,5-bisphosphate carboxylase large subunit (rbcL). The generated sequences were cleaned, assembled, and aligned in BioEdit and Geneious Prime. The sequences were also submitted to the Genbank and accession numbers were assigned to the submitted nucleotide sequences (GenBank OK254173- OK254196). The genetic relatedness among the accessions was determined using nucleotide and amino-acid composition, pairwise distances between the accessions, and hierarchical clustering method of unweighted pair group method with arithmetic mean (UPGMA). The antimicrobial assay was determined using average zones of inhibition with standard error values. The aligned sequence of 534 bp revealed high genetic similarities in their nucleotide and amino acid compositions. The pairwise genetic distance between the 24 accessions ranged from 0.00 (TSs 4) to 0.026 (TSs 303) indicating proximity except for TSs 303. The cluster analysis segregated the 24 accessions into three major clusters of high genetic similarities (65 % to 95 %). Two accessions (TSs 333 and TSs 357) are the most closely related in their nucleotide sequences while only one accession (TSs 303) stands alone as a cluster. There were substitutions in nucleotide bases of some accessions. At nucleotide position 132, three accessions (TSs 311, TSs 303, and TSs 331) had T (thiamine) instead of G (guanine) common to all other accessions. However, only accession TSs 303 showed consistency in the base substitutions at different nucleotide positions. For instance, at 34, 66, 123, 132, 183, 210, 216, 309, 372, 373, 510 and 528 postions, TSs 303 had different bases from other accessions. These substitutions might have accounted for the separation of TSs 303 into a unique cluster. The average zones of inhibition among the tested microorganisms; *Aspergillus niger* (19.06 ± 4.50), *Bacillus subtilis* (19.26 ± 5.84), *Candida sp* (21.17 ± 1.25), *Escherichia coli* (22.67 ± 4.52) amongst others showed

considerable antimicrobial properties. The results of the antimicrobial assay indicate that AYB seeds can be used as antifungal and antibacterial agents in the food and health sectors.

Keywords: African yam bean, antimicrobial activity, RbCl, genetic relatedness, substitution