



Preliminary Assessment of the Association between DArT-SEQ SNP and some Nutritional Traits in African Yam Bean

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ABSTRACT

African yam bean (AYB) is an underutilized tropical legume rich in protein and starch content. This study's aim was to use Diversity Arrays Technology (DArT) sequencing to study the genome-wide association studies (GWAS) of nutritional traits. The study was carried out on 137 accessions in IITA, Ibadan. Seeds were harvested at maturity and analysed for protein, oil, and starch using Kjeltex, Soxtec, and Phenol-sulfuric acid assay method. Mixed Linear Model (MLM) was used for GWAS analysis by employing the TASSEL software. AYB protein content ranged from 17.13-27.15%, while the oil and starch content ranged from 0.74-2.27% and 60.58-69.84%, respectively. A total of 3.6K SNP was generated using a high-throughput DArTseq genotype-by-sequencing SNP approach in the AYB population. These generated SNPs were filtered for quality control, and the remaining useful 2.48K SNPs were used for the preliminary GWAS. We identified a promising source of genetic material for important grain nutritional traits that could be used in AYB breeding research. Identification of QTL/gene of protein, oil, and starch will contribute to our understanding of the genetic basis of natural variation in AYB grain nutritional traits.

Keywords: African yam bean, Single Nucleotide Polymorphism, Underutilized legume, Nutrition.

Introduction

The void between the amount of food supply and the growing human population is now a global threat; and this gap can be bridged by exploiting underutilized food sources.^{1,2} Food insecurity and malnutrition are major challenges in Africa which need to be addressed via a multi-sectoral approach.^{3,4} In Africa, malnutrition resulting from habitual consumption of diets low in essential nutrients is a significant challenge. Malnutrition in children and adults is characterized by impaired cognitive mental development, belly protrusions, physical stunted growth, poor pregnancy outcomes, reduced productivity, and increased mortality rates.^{5,6} Several methods of resolving malnutrition are being employed in Africa. Among these are food import, genetic biofortification, supplement distribution, and dietary diversification.⁷ Despite these, there are calls from different quarters for crop improvement strategy to improve the nutritional content of commonly eaten food crops to reduce nutrient deficiencies in sub-Saharan Africa.^{8,9} In achieving this, so much emphasis is placed on the major staple crops, neglecting indigenous underutilized low-cost energy-protein legume and vegetable crops, including African yam bean, *Cucurbita pepo*, *Solanecio*

biafrae, and *Telfairia occidentalis*.¹⁰⁻¹³ African yam bean (AYB) is an underutilized tuberous legume, indigenous to tropical Africa. It can alleviate malnutrition due to its rich nutritional content. The neglect of AYB is considered a disadvantage as it limits access to cheap protein sources by category of Africans under high poverty and malnourished index margin.¹⁴ The seed of AYB is high nutritional content: protein range between 20% - 29%; 50% carbohydrate; 2-5% crude fat content.^{15,16,17} Significant variations have been reported for these nutritional contents,^{18,19,14} suggesting possibilities for improvement. However, quantitatively inherited traits are controlled by several genes, environment, and interaction between these genes and the environment,²⁰ rendering them subject to continual change and making conventional selection difficult. Hence, there is a need for a genome-wide level understanding of the genetic basis of variation for these traits for the development of improved genotype. Due to the availability of high-throughput genomic platforms, genome-wide association study (GWAS) has become a standard tool for identifying genes and loci responsible for the quantitative variation of complex traits.²¹ Once genes and loci are identified and validated, they could be fixed to provide AYB germplasm with durable nutritional content. There is no study on the identification of Quantitative Trait Locus (QTL) for any trait in AYB. This work was proposed as a preliminary study for the identification of putative QTLs controlling protein, starch, and oil contents in AYB.

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Materials and Methods

Genotyping of african yam bean germplasms

A total of 137 accessions of AYB were obtained from the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture

ture (IITA), Ibadan, Nigeria. Young leaves of each accession were collected, and genomic DNA was extracted using the CTAB method.²² The quality of the DNA was measured with nanodrop spectrophotometer and agarose gel electrophoresis and its concentration was adjusted to 50-100 ng/mL in volume of 50 mL before sending them for DNA sequencing. All the 137 DNA extracts were sent for the SNP genotyping using genotype-by-sequencing (GBS) method at Diversity Array Technology (DArT) Pty Ltd, Canberra, Australia. Genotyping characterization was done using DArTseq high-depth GBS technology (<https://www.diversityarrays.com/technology-and-resources/dartseq/>) and further raw sequenced FASTQ data of each samples were processed using proprietary DArT analytical pipeline (Diversity Array Technology, Australia) and generated total over 3600 genome-wide SNPs in 137 African yam bean accessions.

Field trials and assessment of some AYB nutritional contents

All the 137 AYB accessions were planted in IITA, Ibadan. Seeds were bulked at harvest and analysed for the protein, oil, and starch content. Protein, oil, and starch analyses were performed using Kjeltac, Soxtec, and Phenol-sulfuric assay method.^{23,24,25} Correlation analysis was performed using SAS v9.4.

Association mapping

Mixed Linear Model (MLM) was used for the preliminary GWAS analysis using TASSEL software. A $-\log(p\text{-value})$ of 2.03 was used for the significant threshold.

Total generated 3.6K SNPs were further filtered using call rate ($\geq 80\%$), average reproducibility ($\geq 95\%$), minor allele frequency (MAF) (≥ 0.01) and SNP missing data 30% and maintained total 2485 quality SNPs for GWAS analysis of the seed oil, protein, and starch content. The association between SNP markers and quality traits was analysed using Mixed Linear Model (MLM) that provide advantage in association analysis over general linear model (GLM) and avoid spurious association by using population structure (Q) and relatedness kinship (K) matrix as covariates in GWAS analysis. TASSEL software²⁶ was used for the preliminary GWAS analysis of quality traits of African yam bean and a $-\log(P\text{ value})$ of > 2.03 was used for the significant threshold to confirm association between SNP and traits.

Results and Discussion

AYB protein content ranged from 17.13-27.15%, while the oil and starch content ranged from 0.74-2.27% and 60.58-69.84%, respectively. The correlation between seed protein and oil was observed to be significantly negative with -0.04953 (Table 1).

A total of 3.6K SNP was generated using a high-throughput DArTseq genotype-by-sequencing SNP approach in the AYB population. These generated SNPs were filtered for quality control, and the remaining quality 2,485 SNPs were used for the preliminary GWAS. Out of the 2,485 SNPs used for this study, 405 SNPs aligned with a common bean, the reference genome, while 2,065 SNPs did not align with the common bean reference genome. A total of 50 putative QTLs were observed to be associated with AYB seed protein, oil, and starch content using a significant threshold of $-\log(p\text{-value})$ of 2.03 and P-value (< 0.009). Out of the 50 putative QTLs, 31 QTLs were associated with protein trait, while oil and starch content showed 8 and 11 QTLs, respectively (Figure 1).

The efforts in underutilized crops for genetic linkage mapping and QTL/gene identification of value traits of biotic, abiotic, yield related, agronomical and nutritional traits are key factor for initiating crop improvement program using genomic assisted breeding tools.²⁷ The seed protein, oil, and starch are quantitative traits that are controlled by multiple genes and their interaction with the environment.²¹ We need to identify the genes responsible for each trait to understand how each of these traits is segregated,²⁸ hence the GWAS of AYB. The GWAS for AYB seed protein and oil revealed that some QTLs were found on chromosome 1 that aligned on common bean reference genome, while others where unknown because the reference genome of AYB is not available yet.

Table 1: Pearson Correlation Coefficients

| Nutritional Traits | Oil | Protein | Starch |
|--------------------|----------|---------|--------|
| Oil | 1 | | |
| Protein | -0.04953 | 1 | |
| Starch | 0.02633 | 0.17495 | 1 |
| | 0.6643 | 0.0037 | |

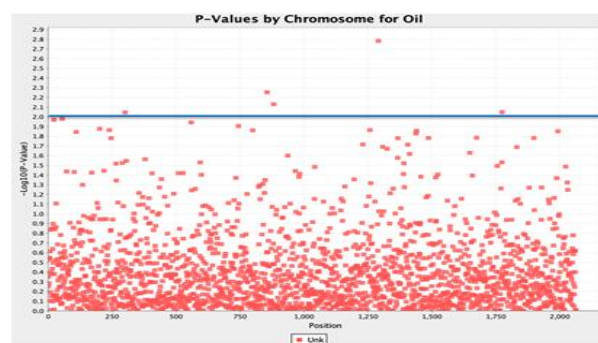
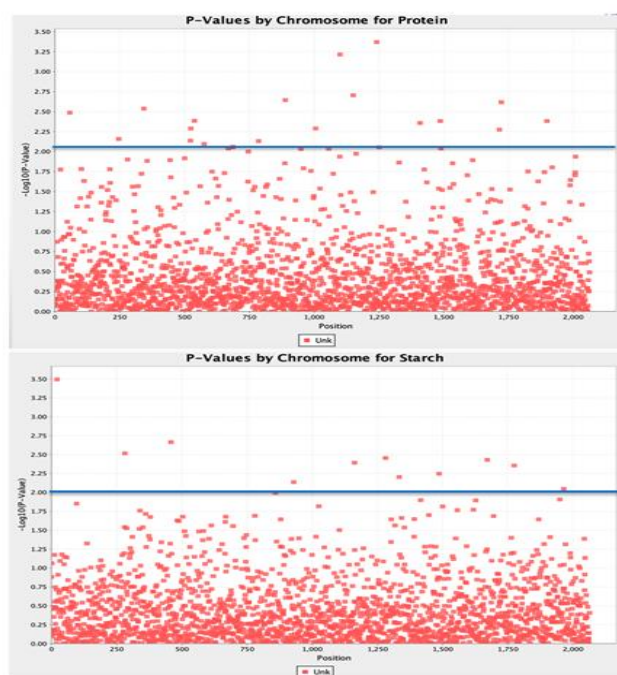


Figure 1: Genome-wide SNP markers associated with nutritional traits in a set of 137 AYB accessions.

Manhattan plots show GWAS results for protein, oil, and starch. The SNPs are shown on the X-axis and the genome-wide scan $-\log_{10}(P\text{-values})$ are shown on the Y-axis. The horizontal blue lines indicates significant association with traits based on threshold at $-\log_{10}(P\text{ value}) \geq 2.03$.

Conclusion

The identified regions may be of particular interest to geneticists and breeders for the alteration of the seed protein, oil, and starch levels. So far, this is the first and preliminary report on the GWAS on AYB nutritional traits. Research is still on-going to validate the QTLs and determine if the QTLs exhibit genetic stability.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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