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Genotypic Characterisation of Bambara Groundnut (*Vigna Subterranea* L. Verdc) Germplasm

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Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an underexploited crop with the opportunity to address some critical food requirements as it is a high-nutritional alternative food. This study aimed at characterising genetic diversity using Diversity Array Technologies Sequence Low Density (DArTseqLD) Single Nucleotide Polymorphism (SNP) markers in order to identify genomic variation that can be used for crop improvement by plant breeders. One hundred eighty-eight samples were selected for genotyping using DArTseqLD SNP markers. The study generated 1048 DArTseqLD SNP markers. Analysis of molecular variance (AMOVA) revealed 84 % and 13 % variation among and within the Bambara groundnut germplasm respectively, 3 % variation was observed among the populations. Cluster analysis based on genotypic data grouped the 188 samples into 9 clusters. Based genotypic data, it can be concluded that there is a significant degree of genetic diversity in the germplasm genotyped that can be used by plant breeders in crop improvement program.

Key words: bambara groundnut; germplasm; genetic diversity; genotyping

Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an underexploited crop with the opportunity to address some critical food requirements as it is a high-nutritional alternative food [1]. Existing literature on African orphan crops highlighted the importance of more molecular research to unlock the hidden potentials (10). Molecular research sheds new light on the population structure of native Bambara groundnut germplasm which will aid in crop improvement through modern breeding (Uba et al., 2021). This research aimed at identifying the genetic diversity that exists within Bambara groundnut germplasm using DArTseqLD makers. The information will aid plant breeders who wants to embark on crop improvement program and develop quality and high yielding Bambara groundnut varieties. Currently there is low production of Bambara groundnut in Malawi (Figure 1) and some of the major reasons for its low production and productivity is the lack of introduction of high yielding genotypes in areas of cultivation, inadequate knowledge of taxonomy, lack of genetic improvement and adaptation to specific agro-ecological zones (Pungulani et al., 2012; Uba et al., 2021). Variation within individual gene loci/among alleles of a gene, or gene combinations, between individual plants or plant populations are referred to as genetic diversity (Sood et al., 2016). Genetic variability is critical component of any crop improvement program. When there are more diverse parents, more heterosis in the progeny is achieved and a higher chance of getting transgressive segregation. Breeders must identify diverse parents with high genetic variability in order to combine desirable characteristics for development of improved crop varieties over existing cultivated varieties [4].

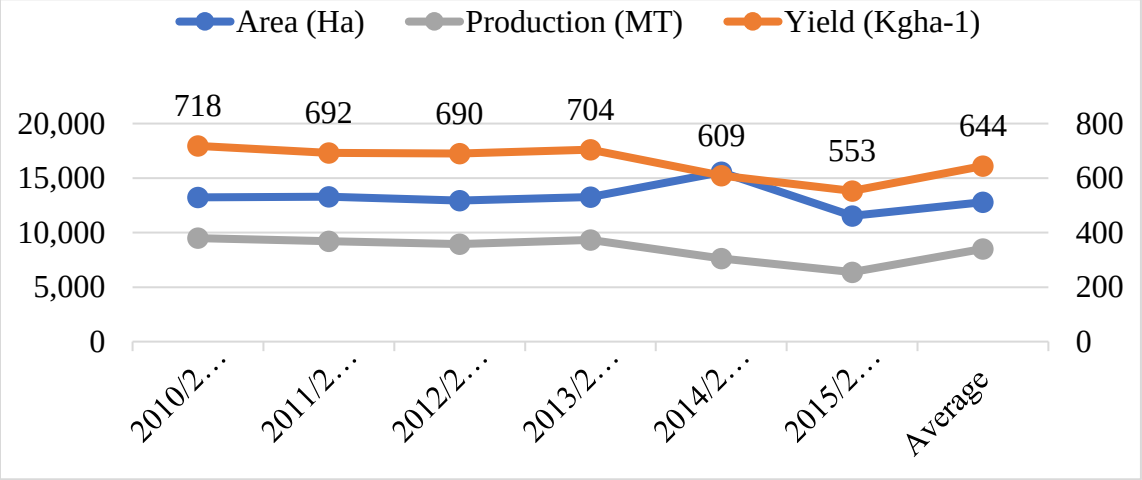

Figure 1: *Estimated Bambara groundnut production in Malawi*

Materials and Methods

Materials

A total of one hundred eighty-eight germplasm were genotyped. These were identified through a seed fair where farmer saved seeds with complete description of their attributes was showcased. Samples of some germplasms was provided by the National Plant Genetic Resources Centre (NPGRC).

Leaf sampling for DNA extraction

A total of one hundred eighty-eight Bambara groundnut germplasm samples from farmers and NPGRC was used as the source of samples for DNA extraction. These were planted on 23rd December, 2021 in the Screen house of the Lilongwe University of Agriculture and Natural Resources (LUANAR). The plants were allowed to grow for three weeks to allow full establishment. Tender leaves from 188 plants were sampled for DNA extraction. Leaf samples were sampled using a procedure as described by Intertek (Parmar et al., 2021). Briefly, the two leaf discs were collected using a leaf puncher from a fresh clean leaf and placed in the 96 well Abgene plates until the 94 wells were filled while two wells were left blank as control. Then the samples were oven dried at 48 ºC for 24 hours. After 24 hours the plates were sealed with silicone mats and sent to Intertek laboratory in Sweden for DNA extraction process.

DNA extraction and genotyping

High Molecular Weight (HMW) DNA was extracted from the tissue samples and its quality was checked in Intertek Sweden and then sent for the high-throughput DArTseqLD Single Nucleotide Polymorphism (SNP) genotyping at the Diversity Arrays Technology Pty Ltd. Canberra, Australia. This entailed subjecting the DNA samples to complexity reduction by digestion/ligation reactions as described by Kilian et al. (2012) but replacing the single PstI-compatible adaptor with two adaptors corresponding to PstI and MseI restriction enzymes' overhangs. The PstI-compatible adapter were designed to include Illumina flowcell attachment sequence, barcode region, while the reverse adapter contained flowcell attachment region and MseI-compatible overhang sequence. Only mixed fragments (PstI-MseI) were effectively amplified in PCR. Subsequently, equimolar amounts of PCR product from each sample were bulked and sequenced by the Hiseq2500/ Novaseq6000 (Illumina® Inc., San Diego, CA, USA). Thereafter, the generated sequences were processed using proprietary DArT analytical pipelines. Approximately 250,000 sequences per barcode/sample were used in marker call. Identical sequences were collapsed into FASTQCOL files followed by SNPs calling using the software package DArTsoft14.

Genotypic data analysis

Germplasm samples data was filtered for quality using a minor allele frequency (MAF) threshold of 0.05 and call rate cut-off of 0.7 with the rest of the missing calls imputed by mean. Basic diversity metrics for the germplasm were inferred using the snpReady package (Granato & Fritsche-Neto, 2018). These included the polymorphism information content (PIC), MAF, Nei's genetic diversity (GD), and observed heterozygosity (Ho). Grouping of the genotypes was evaluated using principle component analysis (PCA) as computed using the factoextra package (Kassambara & Mundt, 2016). A euclidian distance matrix was used for dendrogram plotting using the adegenet package (Jombart et al., 2010). The analyses was done in the R software environment (R Core Team, 2021). Analysis of Molecular Variance (AMOVA) was done using GeneAlEx v 6.5 (Peakall and Smouse 2012).

Results and discussion

Analysis of Molecular Variance (AMOVA) for the Bambara groundnut germplasm samples

Percent variation exists within and between the Bambara groundnut germplasm (Figure 2). Genetic analysis revealed that there is a 13 % variation within the genotyped Bambara groundnut germplasm and 84 % variation among the genotyped Bambara groundnut germplasm while 3 % variations were observed among the populations. Results are in support with the study conducted by Uba et al., (2021) in which the AMOVA reported that 89% of genetic variation occurred among the populations, 8% between regions, and 3% between populations. The greater the genetic diversity of the germplasm, the greater the likelihood of success in breeding desirable traits. The high percentage value of genetic diversity within-population obtained from AMOVA could be due to natural adaptation or extensive seed exchange among farmers between environment, and it could be due to the population's common origin, which could have resulted in Bambara groundnut growers using the same seed continuously, without new introductions. Seed sources for planting Bambara groundnut in Malawi include farmer-saved seeds, exchange and market purchase (Pungulani et al., 2012). Due to the crop's autogamous breeding system, this is likely to result in a heterogeneous population of landraces and thus higher intra-landrace diversity, as opposed to the homogeneous population that would be expected.

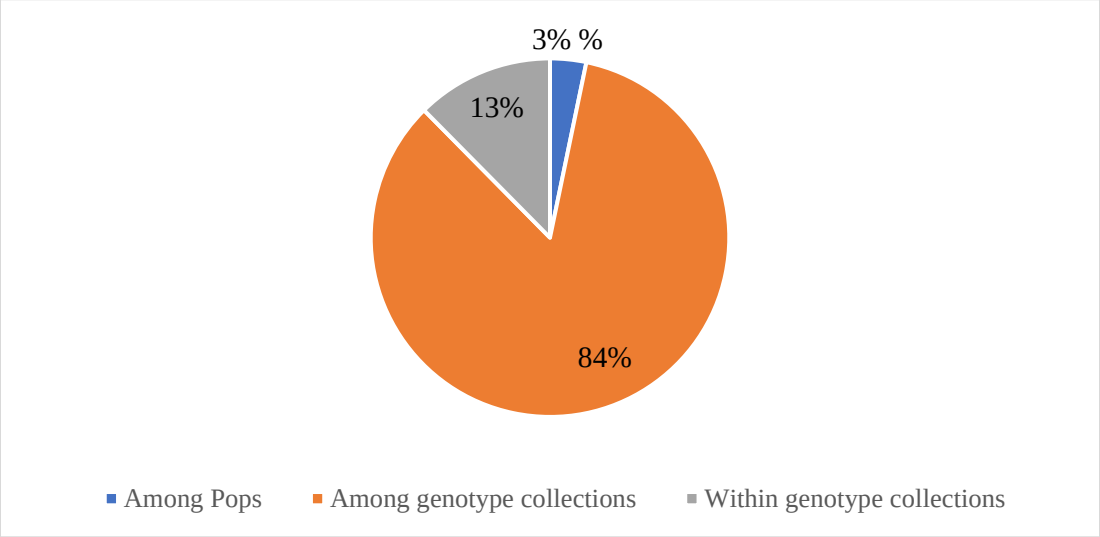

Figure 2: *Percentages of Molecular Variance for the 188 Bambara groundnut germplasm samples genotyped*

Cluster analysis of the Bambara groundnut germplasm samples

Genotypic data grouped the one hundred eighty-eight germplasm samples into 10 clusters. Clusters 4 and 6 is the largest with 34 germplasm each while the smallest cluster has 7 germplasm. Clusters 1 has a total of 17 germplasm samples with NPGRC contributing highly with 7 germplasm samples. Cluster 2 had a total of 34 germplasm samples, cluster 3, 4, 5,6,7,8 and 9 had a total of 17, 27, 15, 34, 7, 27 and 10 germplasm samples respectively with Kandeu EPA, NPGRC, Chipala and Manjawira EPA contributing highly. Similar findings has been reported by Uba et al., (2021) where the grouping of some accessions from different regions into the same cluster may indicate the degree of relatedness between accessions from different regions. This was attributed in part to the transfer and exchange of seeds between regions via gene banks and human migration. Population structure analysis improves understanding of genetic diversity and aids in association mapping studies. It also contains additional information for selecting genetically diverse germplasm for future hybridization program (Uba et al., 2021). Integrating conventional breeding with molecular breeding techniques can accelerate variety development and deployment, particularly for neglected and underutilized crops species. This could significantly shorten breeding cycles and allow varieties to be released more quickly (Majola et al., 2021).

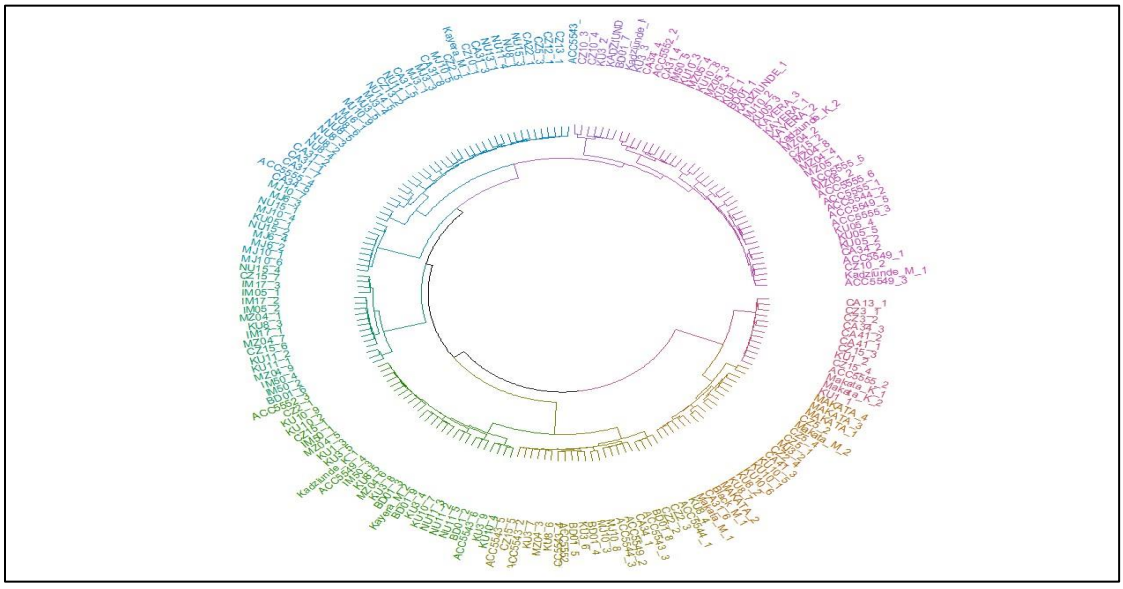

Figure 3: *Cluster analysis of Bambara groundnut germplasm samples*

Conclusion

Based on genotypic data analysis, it can be concluded that there is a significant degree genetic diversity in the Bambara groundnut germplasm evaluated in this study. Analysis of molecular variance (AMOVA) revealed 84% genetic diversity. Allelic diversity in Bambara groundnut is high among the germplasm and geographical origin. The current study's findings indicated that the DArT SNP marker is informative and selective. Therefore it could be widely used for molecular analysis of Bambara groundnut. Based on molecular characterization of Bambara groundnut germplasm from various collection sites, the DArT SNP marker revealed that variation exists among the germplasm and that the pattern of genetic diversity varied across the collection sites. There are nine major subpopulations of Bambara groundnut germplasm based on molecular characterization. As a result, the finding will make a significant contribution to the management, conservation and association mapping of Bambara groundnut for future improvement. Information on the phenotypic variation and genotypic diversity of various Bambara

groundnut germplasms is critical for strategic production and recommendations to farmers and breeders who wants to embark of seed production and breeding programs respectively.

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Statements and declarations

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Competing interest

The author declare that there is no conflict of interest exist.

Data availability

All relevant data will be available upon request and authorization by the author.

References

1. haliqi, A., Rafii, M. Y., Mazlan, N., Jusoh, M., & Oladosu, Y. (2021). Genetic analysis and selection criteria in bambara groundnut accessions based yield performance. *Agronomy*, *11*(8), 1–13
2. Mayes, S., Basu, S. M., Molosiwa, O., Redjeki et al. (2013). Molecular analysis of Bambara groundnut, an underutilised African legume crop as part of the BAMLINK project - What lessons can we learn? *Acta Horticulturae*, *979*(February 2015), 451–458.
3. Ntundu, W. H., Shillah, S. A., Marandu, W. Y. F., & Christiansen, J. L. (2006). Morphological diversity of bambara groundnut [Vigna subterranea (L.) Verdc.] landraces in Tanzania. *Genetic Resources and Crop Evolution*, *53*(2), 367–378.
4. Patil, H. E., & Modha, K. G. (2022). Genetic diversity in finger millet [Eleusine coracana (L .) Gaertn] using Genetic diversity in finger millet [Eleusine coracana (L .) Gaertn] using principal component analysis. *The Pharma Innovation Journal*, *10(9)*(July), 6.
5. Sood, S., Kumar, A., Kalyana Babu, B., Gaur, V. S., Pandey, D., Kant, L., & Pattnayak, A. (2016). Gene discovery and advances in finger millet [Eleusine coracana (L.) Gaertn.] genomics-an important nutri-cereal of future. *Frontiers in Plant Science*, *7*(November 2016), 1–17.
6. Uba, C. U., Oselebe, H. O., Tesfaye, A. A., & Abtew, W. G. (2021). Genetic diversity and population structure analysis of bambara groundnut (Vigna subterrenea L) landraces using DArT SNP markers. *PloS One*, *16*(7), e0253600.