UNLOCKING THE GENETIC POTENTIAL OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* L.) TO INCREASE LEGUME CROP PRODUCTION IN NIGERIA

www.akwapolyjournal.org
P-ISSN: 2536-6733 | E-ISSN: 2616-0773

Supported by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/DESS / POLY / IKOT-OSURUA/ ARJ /1-2)

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Abstract

This research aims to improve the growth of a valuable African crop called Bambara groundnut by studying its genetic makeup. The goal is to create a detailed map of its genetic structure and to learn more about how it absorbs crucial nutrients from the soil. Bambara groundnut (BGN) is an African native legume, rich in protein, able to fix nitrogen, highly drought tolerant and with reasonably good disease resistance that bears a rich food, nutritional and cultural history for the poor resource-base farmers in sub-Saharan Africa. By doing this, we hope to find ways to help Bambara groundnuts grow better, even in environments where the soil is not very nutrient-rich. A dense genetic map is constructed in an F2 population derived from two highly divergent parents (S19-3 and Ankpa4) based on SNP and DArT markers. The linkage map consists of 1238 marker loci (859 SNPs and 379 DArTs), with good coverage (1185

cM spanning 11 linkage groups; one marker per 1 cM, on average). This genetic map is an invaluable resource for QTL analysis and represents qualitative advances in the genetic improvement of Bambara groundnut. ICP-MS analysis of a subset of the individuals (n=48) shows that Bambara groundnut is rich in phosphorus (P) and other mineral elements and that the S19-3 X Ankpa-4 F2 population developed in this project is segregating for these mineral elements. Therefore, a QTL analysis based on mineral element composition is possible if an ICP-MS analysis can be completed on the entire population (n=270). The results from the cotyledon removal experiment suggest that the cotyledon contributes to the early seedling establishment of Bambara groundnut genotypes in nutrient-poor soils. These findings have implications for the genetic improvement of Bambara groundnut to unlock the genetic potential of this essential African legume.

Keywords: *Bambara groundnut, cotyledon, genetic map, ICP-MS, QTL analysis*

Introduction

Orphan crops, such as Bambara groundnut (*Vigna subterranean*; Bambara), are an essential source of nutrition. They are highly tolerant to periods of prolonged drought and/or low soil fertility and can contribute significantly to smallholder farmers' food and nutritional security in sub-Saharan Africa (Yusuf et al., 2008; Mabhaudhi & Modi, 2013). Expanding the use of orphan leguminous crops, which are currently underutilized, can be a practical approach to diversify farming systems. These crops can be intercropped with main staples in cereal-based systems or grown separately, providing various options to introduce temporal and spatial heterogeneity into farming practices. The resulting diverse farming systems can improve resilience to biotic and abiotic stress factors, ultimately enhancing global Food, Nutritional and Environmental Security (Kendabie et al., 2020).

Although genetic variation is abundant in Bambara groundnut, crop production is still constrained by a host of biotic and abiotic factors, which can result in yield decline. Farmers have continued cultivating landraces derived by selection and domestication from wild relatives (Massawe et al.,2005). Seed yields can be low and/or unstable due to the need for improvement by controlled cross-breeding. The need for improved seed and farmers' continued use of local landraces make the situation even worse. Consistently, there is a growing interest of scientists and breeders in developing African indigenous crops (such as Bambara groundnut) for Food and Nutritional Security (Kendabie *et al*., 2015; 2016; 2020; Mayes *et al*., 2019; Waikuan *et al*.,2017). New, improved cultivars with good agronomic and consumer preference traits are needed to help secure food security and the livelihood of the farming communities in the tropics.

Genetic linkage maps are essential for marker-assisted selection, map-based cloning, comparative genomics, targeted genome sequencing and QTL studies (Lucas *et al*., 2011; Young & Bharti, 2012). The availability of molecular markers, mapping populations, genetic maps, and sequence

information provided by marker-trait associations would enhance the breeding process by applying marker-assisted selection (MAS) of favourable alleles in Bambara groundnuts. Genetic linkage mapping has rapidly moved to under-researched legumes (Varshney *et al*., 2010; Bohra *et al*., 2014). For example, linkage mapping in cowpeas, an essential member of the legume family and perhaps the closest relative to Bambara groundnut, has advanced with marker technology to yield informative and increasingly dense genetic maps (Quedraego *et al*., 2002; Muchero *et al*., 2009; Lucas *et al*., 2011). However, in comparison to other legume crops of equal or less economic importance to farmers in the developing world, platforms (viz., high-resolution genetic maps, mapping populations, and the whole genome sequence) for map-based cloning in Bambara groundnut are lagging in development (Ahmad *et al*., 2013; Ho *et al*., 2017). A major challenge before the Bambara groundnut community has been the development of saturated genetic maps from large mapping populations that will facilitate the identification of important QTLs. High throughput genotyping of DArTs and SNPs using NGS technologies has allowed us to generate many sequence-derived markers before on-model species. The availability of these markers should facilitate genetic mapping and QTL analysis in Bambara groundnut.

Bambara is nutritionally dense despite a lack of a dedicated breeding programme. However, studies have yet to be conducted on the genetics of mineral element composition and, hence, the potential to breed for more nutrient-dense cultivars. Quantifying these mineral elements and the preferences and traits favoured by smallholder farmers will lay the foundations for future breeding efforts in this orphan crop. The aim of this project was genetic analysis to unlock the genetic potential of Bambara groundnut for increased legume crop production in Nigeria. Consequently, the proposed research will develop resources and new knowledge for an orphan crop and support its development and use in mitigating food insecurity and resilient cropping systems for changing climates. The key objectives of this TETFund IBR grant awarded to the PI at the Federal Polytechnic Ekowe were achieved, and this report

presents results on the construction of a high-density genetic linkage map, mineral element composition of some lines in the cross S19-3 X Ankpa-4 and a cotyledon removal experiment to facilitate Bambara groundnut crop improvement.

Materials and Methods

For the linkage mapping project, an F2 segregating mapping population (SN=263) derived from Bambara groundnut genotypic landraces (S19-3 and Ankpa-4) was used (Figure 1). These parental lines have been well characterised previously in day length experiments, and are divergent in their response to extreme day length conditions (>12 h). From July to December 2022, 6 plants of each parent and 121 F2 lines of the cross S19-3 × Ankpa 4 were grown in as Creen house at the Niger Delta University. Plant materials were grown in soil bed sand planting distance of 25 cm x 25 cm between and within rows was maintained. Irrigation was supplied manually, once in two days in the morning or evening throughout the experiment period.

Figure 1: Segregation for testa color in the cross S19-3 (Black) x Ankpa4 (Brown) at F2generation, SN18, SN44 and SN50 are three different populations derived from this cross.

Genomic DNA was isolated from fresh young leaves of both parental genotypes. Each individual of the F2 population was grown using the Qiagen Plant genomic DNA kit (Qiagen), following the manufacturer's instructions with slight modifications. Approximately 100 mg of leaf tissue was ground in a mortar with liquid nitrogen for better yield of DNA. All DNA concentrations were estimated by electrophoresis of samples in a 1 % agarose gel alongside standard lambda DNA. Microsatellite markers previously reported (Ahmad *et al.*, 2013) were tested on parents, and polymorphic markers were used to analyze 94 individuals of the F2segregating population. As previously reported, a polymerase chain reaction (PCR) was done (Ahmad*etal*.,2013). After confirming the segregation in the F2 population, this cross was later sent off to DArT Pty Ltd (Canberra, Australia) for genotyping by sequencing. This also generated 64bp sequence tags associated with each marker (DArT and SNPs) used for linkage mapping. The Join Map4 software (van Ooijen, 2006) was used to construct the linkage map, comprising sequence-derived DArT and SNP marker data.

Phenotypic characterization of seed mineral contents was evaluated in two parents and forty-eight (48) F2 lines of the Bambara groundnut mapping population derived from the cross (S19-3xAnkpa-4). These lines segregate for seed size, and 120 lines were planted for evaluation in the greenhouse (good nutrient conditions). They have already been genotyped with thousands of 64bp staged GBS (DArTseq and SNPs) and polymorphic markers. The ICP-MS analysis for two parents and 48 F2 segregating lines was performed in collaboration with research partners (University of Nottingham). ICP-MS is a quantitative methodology for the simultaneous determination of 29 elements (including phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), copper (Cu), calcium (Ca), sulphur (S), iron (Fe), manganese (Mn) and zinc(Zn) in tissues using state-of-the-art inductively coupled plasma-mass spectrometry (ICP-MS). We also characterized the effect of cotyledon excision on seedling growth (root and shoot) in a Bambara groundnut genotype (DodR) in pots experiment in 3 replicates (4 plants per replicate) in the greenhouse. For the cotyledon excision experiment, seeds were sown in 96 cell trays and 8-day-old seedlings (15-17 days after sowing) were transferred to 4-litre pots containing rivers and only (control group with cotyledon and treatment without cotyledon).

Results and Discussion

The DArT-based GBS of the F2 mapping population (S19-3 x Ankpa4) and a diversity panel comprising the parents of the F2 cross and 111 genotypic landraces returned 8,872 (3092 SNP sand 5,780 Silico DArTs) potential markers. After filtering this data set, 3852 markers (1,312 SNPs and 2540 Silico DArTs) were identified as high-quality data polymorphic between the two parents used to generate the mapping population. This accounts for 43.4% of sequence-derived markers that could be used for linkage analysis and map construction. However, to produce a linkage map with minimal missing data, 1,238 markers (859SNPs and 379 Silico DArTs) were used to construct a genetic map for 263 individuals. The segregation data assembled for polymorphic markers tested for goodness of fit and the proportion of segregation distortion was automatically detected by Join Map at ($p < 0.05$ for significance). The locus genotype frequency suggested 1117 (772 SNPs and 345 Silico DArTs, 90.2%) markers showed goodness of fit of 1:2:1 and 3:1 for both SNP and Silico DArT marker types, respectively, while the remaining 121 (87 SNP and 34 Silico DArTs, 9.2%) showed significant deviation from Mendelian ratios in the map for all 263F2lines. Segregation distortion is a common phenomenon observed in genetic mapping projects utilizing bi-parental crosses, and chromosomal regions responsible for distorted segregation ratios have been mapped (Vogl & Xu, 2000).

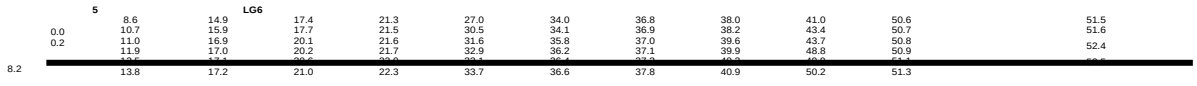
Markers linked to segregation distorted locus show distorted segregation, thereby contributing to a deviation in locus genotype frequency from the expected Mendelian ratios (Vogl & Xu, 2000). In the present study, GBS (SNPs and Silico DArTs) markers showed distorted segregation of 9.2% for the map involving all 263F2 lines. Markers that showed noticeable distortion were excluded from the linkage analysis. In contrast to earlier studies that reported a high proportion of distorted marker segregation for SSRs, DArTs and AFLPs in two mapping populations (32 and 27%, respectively) used for map construction and QTL analysis in Bambara groundnut (Ahmad,2013), the influence of segregation distortion on QTL analysis will be negligible. Segregation distortion may result from several factors, such as residual heterozygosity, gametic or zygotic selections, embryo lethal genes, genotyping errors, non-homologous

recombination, transposable elements and environmental agents (Xian-Liang*etal*.,2006). A genetic map was produced for all 263 F2 individuals, with total map lengths of 1185 cm (Figures 2 and 3).

In this map, mapped markers were precisely assigned to 11linkage groups(LGs) using a LOD threshold of 6.0, which is in agreement with the haploid chromosome number(n=x=11) in Bambara groundnut (Uguru *et al*., 2006). On average, the distances between adjacent markers were 1.2 cM (Table 1). Linkage groups were arranged in order of magnitude, with LG1 being the most significant and LG11 being the shortest, in agreement with the chromosome lengths of Bambara groundnut genotypes (Uguru *et al*., 2006). The length of LGs ranged from 78 (LG11) to 132 cM(LG1)in the genetic map.

Table1: Marker distribution across11linkage groups (263F2lines): linkage groups and map lengths, number of mapped markers, and marker intervals

Linkage groups	Map length, cM	Number of loci	Average inter-marker distance, cM
LG1	132	150	0.88
LG2	128.67	113	1.14
LG3	125.49	57	2.2
LG4	123.97	73	1.7
LG5	114.8	102	1.13
LG6	113.51	91	1.25
LG7	109.54	59	1.86
LG8	89.58	112	0.8
LG9	87.17	67	1.3
LG10	80.63	73	1.1
LG11	78.31	112	0.7
Total	1184	1009	1.2



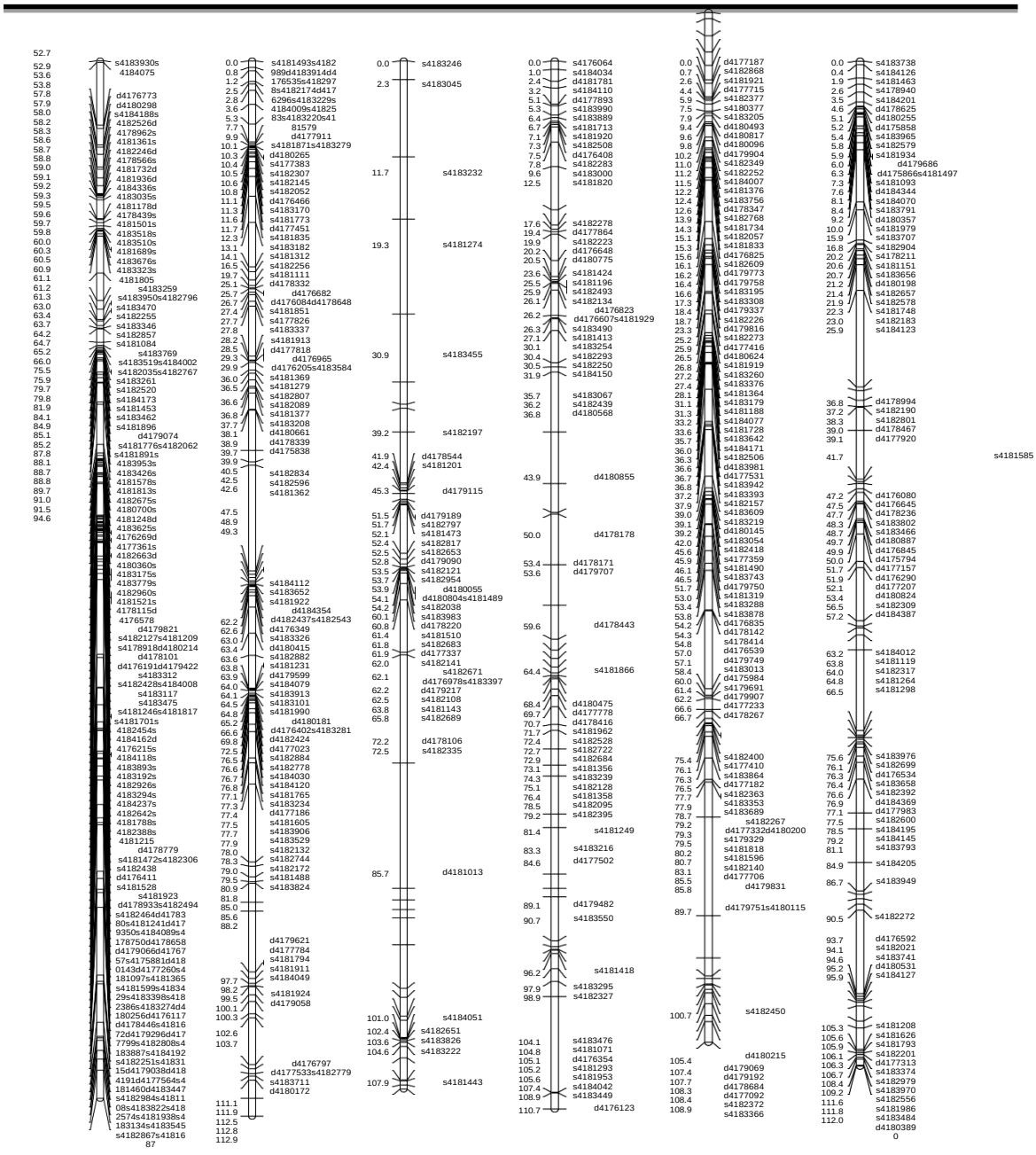


Figure2: Genetic linkage map of 11linkage groups (LGs1,2, 3, 4, 5 and 6). This map was constructed using 263 F2 lines derived from a S19-3 × Ankpa4 cross. Positions are given in centimorgan (Kosambi units) to the left of the linkage groups and the name of the marker to the right. A total coverage of 1184cMw as obtained with 1009 markers (683SNPs, 326 DArTs).

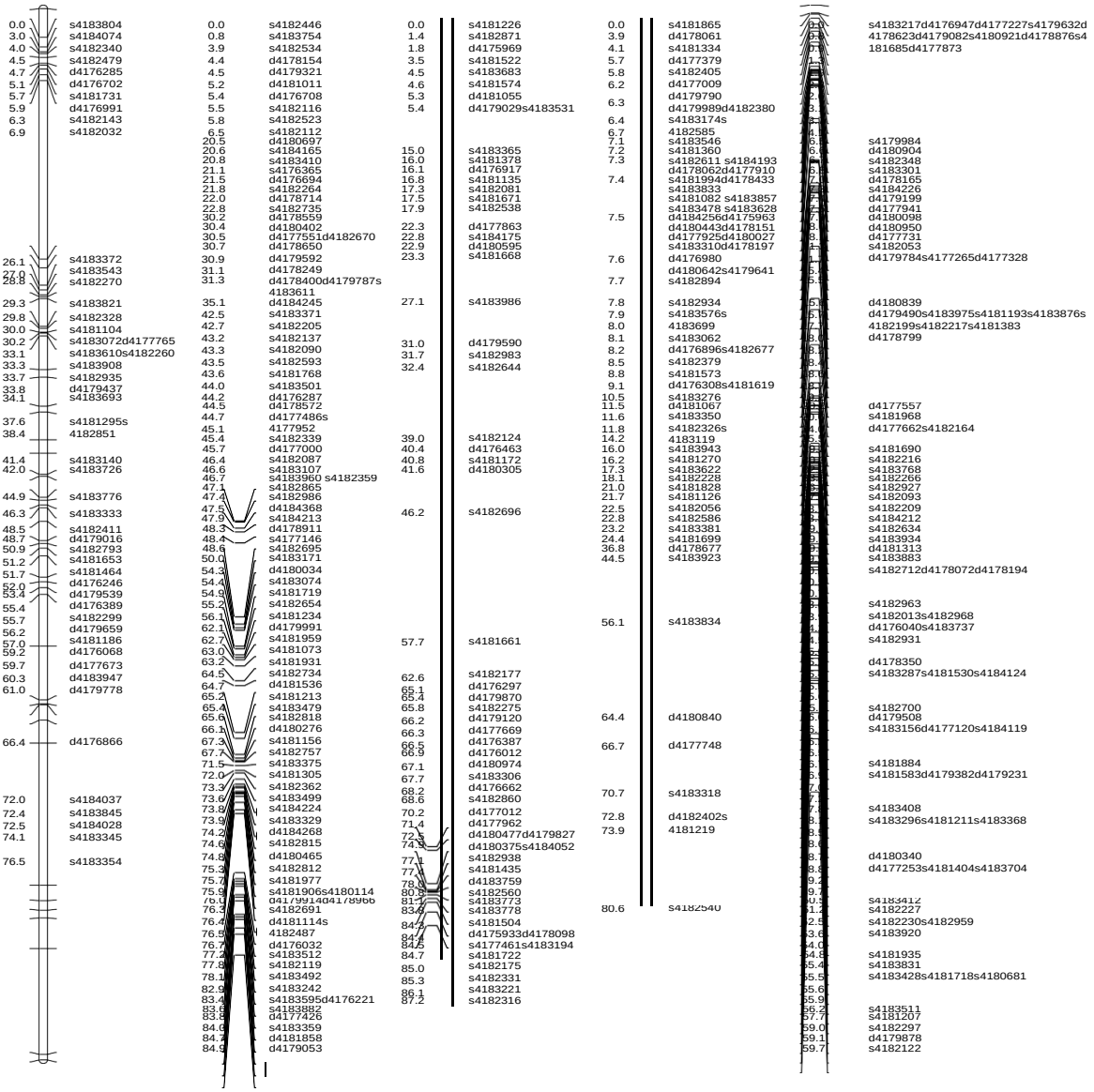


Figure3: Genetic linkage map of 11 linkage groups (LGs7, 8, 9,10 and 11). This map was constructed using 263 F2 lines derived from a S19-3 × Ankpa4 cross. Positions are given in centimorgan (Kosambi units) to the left of the linkage groups and the name of the marker the right. A total coverage of 1184cM was obtained with 1009 markers (683SNPs,326 DArTs).

This high-density map provides complete coverage of the Bambara groundnut nuclear genome, and the number of linkage groups corresponds to the 11 chromosome numbers and lengths reported for the Bambara groundnut genome (Uguru *et al*., 2006). The current Bambara groundnut linkage map is considerably improved over the previous Bambara groundnut genetic linkage maps built on SSR, AFLP and DArT markers (Ahmad, 2013). The broad genome coverage achieved in this study was due to the capacity of GBS markers to detect a high level of genetic polymorphism between the genetic cross S19-3 (from Namibia) x Ankpa4 (Nigeria, West Africa), large F2 population size and maximum recombination events between the two parents of the same species. Such a high-density intra-specific molecular linkage map based on genic-SNP and genic-SSR markers is also available for pigeon peas, covering a genome map length of 1520.22 cm (Kumawat *et al*., 2012). While the number of markers assigned to each linkage group could reflect the relative amount of genetic variation present among the linkage groups, their map distances, on the other hand, reveal the similarity in chromosome lengths (Osuji *et al*., 2005; Uguru *et al*., 2006). Furthermore, this map was constructed using 64-bp sequence-derived markers of the Bambara groundnut genome; therefore, it will be beneficial for comparative genome mapping and synteny studies with other legume genomes with reference genetic maps and a draft sequenced genome.

Construction of a detailed genetic map and QTL analysis relies on identifying sufficient markers revealing polymorphism among parents used in a genetic cross and the availability of relevant mapping populations. In the present study, the mapping population was based on a pair of genetically diverse genotypic landraces (IITA-S19-3 and Ankpa 4), for which a high percentage of polymorphic markers (43.4% of SNPs and SilicoDArTs) with comprehensive genome coverage were identified. The significant genetic distance between the parental lines of the mapping population in the present study provided a high degree of polymorphism for markers across most of the linkage groups (Table 1). This finding is at variance to the report (Ahmad *et al.*, 2013) that

observed lower polymorphism for DArTs in a narrow cross, as evident from the reported in the identification of 236(3.1%) of polymorphic DArTs out of 7680DArTs screened between the parents of the narrow cross. However, a 36.3% and 33.1% polymorphic rate for SSRs was also reported in Bambara groundnut's narrow and wide cross populations, respectively (Ahmad *et al*., 2013).

Bambara groundnut seeds and seedlings of genotypes Ankpa-4 and S19-3, as well as the ICP-MS result for P, K, Fe and Zn are presented below:

Ankpa-4 seeds and 2-days seedling

S19-3 seeds and 2-days old seedling

Genotypes	100 seed weight (g)	P (mg/kg)	K (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
Ankpa-4	60-80	3950	14840	56.94	28.55
S19-3	48-60	3846	15606	28.65	36.14

Result from cotyledon removal in nutrient poor soil (sand)is also presented below:

Variation in seed size between the two parental genotypes (Ankpa-4 and S19-3) from this cross, suggests that the F2 population is segregating for seed size and for mineral element composition.The results from cotyledon removal experiment suggests that the cotyledon contributes to the early seedling establishment of Bambara groundnut genotypes in nutrient poor soils.

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