GENETIC VARIABILITY AND ASSOCIATION MAPPING FOR YIELD AND YIELD-RELATED TRAITS IN AFRICAN YAM BEAN [*Sphenostylis stenocarpa* (Hochst ex. A. Rich) Harms]

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CERTIFICATION

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DEDICATION

To my parents Deacon and Deaconess M.A. Olomitutu and my siblings Francis and Victor Olomitutu.

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ABSTRACT

African Yam Bean (AYB) is an underutilised legume producing tubers and seeds rich in dietary proteins and minerals, but its Seed Yield (SY) is low. Landraces of AYB are repositories for potential beneficial alleles for the development of varieties with enhanced yield and qualities. However, limited information is available on the extent of genetic variation within available AYB landraces, the genetic basis of the variations and relative importance of SY-related traits, which are required for the development of varieties with improved SY and agronomic characteristics. Hence, genetic variability among some AYB accessions for SY and association of genomic regions with the yield-related traits were assessed.

One hundred and ninety-six AYB accessions were evaluated for two years at Ibadan, Kano and Ubiaja following standard practices. The experimental design was 14×14 lattice with three replicates. Data were collected on Days to Pod Maturity (DPM), Pod Weight (PDW), Pod Length (PL), Seed Length (SL), Shelling Percentage (SP), 100-Seed Weight (HSW), Seeds Per Pod (SPP) and Seed Thickness (ST), while SY was estimated. Data were subjected to principal component analysis, cluster analysis, correlation analysis, path coefficient analysis, descriptive statistics and ANOVA at $\alpha_{0.05}$. Estimates of variance components, Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV) and broad-sense heritability were computed for the traits. Yield stability index was used to identify superior and stable accessions. The 196 accessions were genotyped using 5,416 DArTseq-based Single Nucleotide Polymorphism (SNP) markers, from which 2,491 markers and 195 accessions were retained after quality filtering. Marker-trait associations were determined using the mixed linear model.

Accessions, environments and accession×environment interaction effects were significant for all the traits. The DPM ranged from 118.5±14.3 (TSs-8, Ubiaja) to 220.0±6.0 (TSs-59, Kano), PW ranged from 4.2±0.3 g/plant (138A, Kano) to122.7±17.6 (TSs-421, Ibadan), while PL was shortest in accession TSs-22B (12.0±1.3 cm, Kano) and longest in TSs-51 (27.3±0.6 cm, Ibadan). The SY ranged from 1.3±0.1 (TSs-326, Kano) to 77.6±10.4 g/plant (TSs-421, Ibadan). Variances due to environment and accession×environment interaction were higher than the genotypic variance for all the traits. Also, estimates of PCV were higher than GCV for all traits. Broad-sense heritability ranged from 17.1±3.5% (DPM) to 66.4±0.2% (SL). The first three principal components accounted for 59.7% of the total variation among the accessions. Five major clusters were delineated based on phenotypic characteristics. Shelling percentage (r_g=0.76), 100-SW (r_g=0.29), DPM (r_g=0.45), PW (r_g=0.89), SPP (r_g=0.20) and ST (r_g=0.41) had significant genetic correlations with SY, and exhibited positive direct effects on SY. Accessions TSs-119, TSs-101, 138A, TSs-4, TSs-157A and TSs-61 were identified as superior and stable. Across locations, 24 SNP markers were significantly associated with the traits at a threshold of –log (p) =4, and explained 7.1 to 12.8% of the phenotypic variation among the accessions.

A wide genetic variation exists among the African yam bean accessions. Selection criteria for improved seed yield in African yam bean should include shelling percentage, 100-seed weight, days to pod maturity, pod weight, seeds per pod and seed thickness.

Keywords: African yam bean, Marker-trait association, Genotypic variance, Yield stability index

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMMI	Additive Main Effect and Multiplicative Interaction
ASV	AMMI Stability Value
AYB	African Yam Bean
D50F	Days 50% to Flowering
DArT	Diversity Array Technology
DNA	Deoxyribonucleic Acid
DPM	Days to Pod Maturity
G × E	Genotype × Environnent Interaction
gDNA	genomic DNA
GFP	Grain Filling Period
GLM	General Linear Model
GRC-IITA	Genetic Resource Center, International Institute of Tropical Agriculture
GVC	Genotypic Coefficient of Variation
GWAS	Genome-Wide Association Mapping
HWS	100-Seeds Weight
LD	Linkage Disequilibrium
MLM	Mixed Linear Model
NLPPD	Number of Locules Per Pod
NPPPL	Number of Pods Per Plant
NTPL	Number of Tuber Per Plant
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDL	Pod Length
PVC	Phenotypic Coefficient of Variation
PW	Pod Weight
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RNA	Ribonucleic Acid
SL	Seed Length
SNP	Single Nucleotide Polymorphism
SP	Shelling Percentage
SPP	Seeds Per Pod
SSR	Simple Sequence Repeat
ST	Seed Thickness
SW	Seed Width
SY	Seed Yield
TWPL	Tuber weight Per Plant
YSI	Yield Stability Index

CHAPTER 1
INTRODUCTION

African yam bean (AYB) [*Sphenostylis stenocarpa* (Hochst ex. A. Rich.) Harms] is an underutilized tropical African legume. It is a member of family Fabaceae, subfamily Papilionoideae, tribe Phaseoleae, sub-tribe Phaseolinae and genus *Sphenostylis* (Allen and Allen, 1981). The genus *Sphenostylis* is very small and has growth habit which can be erect or climbing. Of the seven species within this genus, AYB is the most economically important species (Potter, 1992) and one of the most important tuberous legumes in African food cultures and peasant agriculture.

African yam bean produces two major food substances (tubers and seeds), whose protein value is higher than what is found in most tuberous and leguminous crops (Okigbo, 1973). The tuber protein content is more than twice the protein in tuber crops (sweet and Irish potato or yam) and ten times that of cassava storage root (NRC 2006; Norman and Cunningham, 2006). The crop (tuber) also has medicinal properties (Potter, 1992), socio-cultural importance (Ojuederie *et al*., 2015; Nnamani *et al.*, 2017), wide adaptive nature to different climatic and soil conditions (Aremu *et al*., 2020b), high nitrogen-fixing ability (Oganale, 2009) and less susceptible to most field and storage leguminous pests' due to inherent lectin in the seed (Omitogun *et al.*, 1999). Despite these benefits and other numerous potentials of AYB, the crop is in danger of extinction (Klu *et al.*, 2001).

Though genetic erosion and extinction of AYB remains a concern, the Genetic Resource Centre of the International Institute of Tropical Agriculture (GRC-IITA), Ibadan, had done tremendous work in the collection and conservation of a little over 450 landraces from different locations in countries of Africa (Abberton *et al*., 2022). Variation within these accessions could be explored for genetic improvement of the crop to the benefit of humankind. Previous studies (Akande, 2009; Popoola *et al.*, 2011; Adewale *et al.*, 2012a; Aremu and Ibirinde, 2012; Aremu *et al*., 2019; Ibirinde *et al.*, 2019; Aina *et al*., 2020) had reported characterisation of some AYB accessions using morphological traits in few environments. These studies have reported specific phenotypic traits that are very strong in distinguishing AYB germplasm and further suggest that investigation of

molecular diversity of AYB germplasm will help to lay a solid foundation for genetic improvement.

Molecular tools have been notably used to unravel intra-specific diversity in AYB germplasm: Moyib *et al.* (2008) used Random Amplified Polymorphic DNA (RAPD) technique, Ojuederie *et al.* (2014) and Adewale *et al.* (2015) used Amplified Fragment Length Polymorphism (AFLP) technique and Shitta *et al.* (2016) used Simple Sequence Repeat (SSR) markers. These technologies suffer some drawback: RAPD is PCR reaction dependent and it is a dominant marker, AFLP requires purified high molecular weight DNA and the bands are not always independent, and SSR depends on prior sequence information for the species to be studied. Shitta *et al.* (2016) used cowpea derived SSR markers since there is no prior sequence for AYB genome. They have all reported high diversity among the accessions studied. Next-generation molecular markers (such as Diversity Array Technology) are currently being used to explore the diversity existing among GRC-IITA AYB germplasm (Paliwal *et al*., 2020). Adewale *et al.* (2015), however, suggested a shift in focus to trait-maker association studies in AYB germplasm.

Crop yield has become the most important agronomic character in crop breeding due to the problem of feeding the increasing world population under uncertain or unpredictable climatic condition (Xu *et al*., 2018). Yield is a complex trait with low heritability and is the product of multiple interacting component traits (Zhao *et al.*, 2016). Its improvements in crop breeding programmes involves optimization and selection of heritable yield components. Selecting any heritable component trait or traits involves a complex pathway that leads to the formation of the complex trait. The use of correlation coefficients alone is not always adequate, as it provides only one-dimensional information without taking into account the inter-relationships among all yield component traits (Nwofia *et al*., 2014*;* Kang, 2015). Path analysis can be employed to partition correlation coefficients between yield components and yield into direct and indirect effects. This is useful in partitioning the traits into order of importance for selection and improvement purposes (Cramer and Wehner, 2000, Nwofia *et al*., 2014*;* Kang, 2015). Previous studies (Nwofia *et al*., 2014; Aremu *et al*., 2019) had reported number of seeds per pod, time of pod filling, pod length, one hundred seeds weight and number of pods per plant as traits with positive direct association to seed yield in AYB. Combining both correlation and path analysis provides a better appreciation of the causal relationship between pairs of characters (Kumar *et al*., 2015). Though correlation and path coefficient analysis are very useful in yield improvement, differences in the performance of genotypes across many

environments for specific phenotypic trait makes prediction of its performance in wide environment impossible (Perkins and Jinks, 1968). More so, stable genotypes do not necessarily give the best yield. Therefore, there is a need for approaches that incorporate both mean yield and stability in a single index, hence the Yield Stability Index (YSI) (Bose *et al*., 2014).

Recent advances in high-throughput genomic platforms has created the opportunity for genome-wide level understanding of the genetic basis of variation in complex traits. The utilization of such genetic information in AYB could facilitate the development of improved genotypes. Association Mapping (AM), originally developed for mapping human disease genes (Corder *et al*., 1994), is now a popular method of mapping Quantitative Trait Loci (QTL) in plants. Association mapping detects linkage disequilibrium (LD) (i.e., the non-random association of alleles) between genetic markers and genes controlling the trait of interest by exploiting the ancestral recombination events in a natural population (Ruggieri *et al*., 2014). It evaluates whether certain alleles and/or genes within a population are found more frequently with specific phenotypes than expected (Flint-Garcia *et al*., 2005). Based on the size and objectives of study, AM have two broad categories, (1) candidate-gene association mapping and (2) genome-wide association mapping (GWAS) or genome scan (Risch and Merikangas, 1996). Researchers interested in a specific trait often exploit candidate-gene association mapping; however, others conduct comprehensive genome scan for numerous traits by testing thousands of molecular markers across the genome for association using GWAS (Zhu *et al.,* 2008). Association mapping has several advantages over traditional linkage mapping. These include an increased resolution, a reduced research time (use existing populations rather than generating population via biparental crosses) and a higher allele number detection per locus as opposed to only two (Yu and Buckler, 2006; Semagn *et al*., 2010; Zhao *et al*., 2011). Association mapping also suffers some shortcomings, such as detection of false positives in population structure which is a result of linkage between causal and non-causal sites, more than one causal site, and epistasis. Advancement in statistical methods has helped to reduce the rate of false positives (Larsson *et al*., 2013).

Previous studies of genetic variability within AYB germplasms are limited by the number of accession and environments used. Evaluating a larger population in greater number of environments will give more information and help underpin improvement programs. Likewise, the only available report of association mapping in AYB is the preliminary assessment for nutritional qualities by Oluwole et al. (2020), there is also a

need to understand the genetic basis of yield-related traits to facilitate rapid improvement. The objectives of this study therefore were to:

1. Evaluate agronomic traits (yield and yield component traits) of 196 AYB accessions in three agro-ecologies of Nigeria.
2. Investigate inter-trait relationships and the direct and indirect effects of some yield-related traits on seed yield in AYB.
3. Investigation of quantitative trait loci (QTL) linked to seed yield and yield-related traits of AYB using genome-wide association studies.

CHAPTER 2
LITERATURE REVIEW

2.1 Agronomy and Botany of *Sphenostylis stenocarpa*

Sphenostylis stenocarpa is cultivated as an annual crop. Planting usually starts when rain has stabilised, in Nigeria, between May and July (Okpara and Omaliko, 1995). The crop is grown as a minor crop in mixed cropping system, especially with yam and cassava in different part of the country (Saka *et al*., 2007). Staking is an important cultural practice for optimum seed yield (Okpara and Omaliko, 1995). Seeds are sown at varied spacings at the base of the heaps of other major crops (Saka *et al*., 2007; Adewale and Odoh, 2013). Hypogeal germination occurs between four to seven days after planting (Adewale, 2011). The yield and other traits of AYB can be improved through fertilizer applications (Togun and Olatunde, 1998).

Sphenostylis stenocarpa has a vigorously climbing viny stem whose height can be up to 3 metres or more depending on the length of the stakes and cultivar. The crop branches profusely from the axils of the leaves. The branches twine strongly on available stakes. The main vine/stem may be reddish pigmented. The vegetative growth stage is noted with profound production of pinnately trifoliate leaves. (Okigbo, 1973; Adewale and Dumet, 2009; Adewale and Odoh, 2013).

The flowers are borne in racemes on long peduncles of 2 to 20 cm in length. Each peduncle can produce up to 20 flowers, most of which are usually aborted leaving only about 4 to 10 per raceme (Okigbo, 1973; Adewale, 2011). The flowers exhibit self-pollination. A peduncle can hold up to three or more long unicarpel pods that turn brown when matured. Most pods do dehisce when dried (Adewale and Odoh, 2013). Pods usually house about 20 seeds which may vary in size, shape (oval, oblong, rounded or truncated) and basal colour pattern (cream, black, grey, light or dark brown, purple and white) (Adewale *et al*., 2012a). African yam bean also produces small underground tubers very similar to sweet potatoes (Adewale and Dumet, 2010).

2.2 Eco-geographical distribution of *Sphenostylis stenocarpa*

Sphenostylis stenocarpa is known to tolerate wide climatic, edaphic and geographical ecologies (Aremu *et al*., 2020b). African yam bean is known to have originated from Africa (Potter and Doyle, 1992; Potter and Doyle, 1994). Its centre of diversity as indicated by Germplasm Resources Information Network, GRIN (2009), include the following countries in tropical Africa region: Angola, Burundi, Central African Republic, Chad, Cote d'Ivoire, Ethiopia, Ghana, Guinea, Kenya, Mali, Malawi, Niger, Nigeria, Togo, Tanzania, Uganda, Zaire, Zambia and Zimbabwe. In Nigeria however, AYB is extensive cultivation in the eastern (Abbey and Berezi, 1988), western, and southern (Saka *et al.,* 2004) parts of the country.

2.3 Economic potentials and uses of *Sphenostylis stenocarpa*

African yam bean is economically the most important species in the genus *Sphenostylis* and the most important tuberous legume of tropical Africa (Potter and Doyle, 1992; Adewale *et al.,* 2010). It produces two major food substances (tubers and seeds), whose protein value is higher than what could be obtained in most tuberous and leguminous crops (Okigbo, 1973). The protein content of AYB tuber is more than twice the protein in sweet potato (*Ipomea batatas*) or Irish potato (*Solanum tuberosum*) or yam (*Dioscorea spp*.) and ten times that of cassava (*Manihot esculenta*) storage root (NRC 2006; Norman and Cunningham, 2006). The tuber contains on average, 15.5% crude protein, 1.3% crude fat, and 68.3% carbohydrate (Ojuederie *et al*., 2020). The seed contains 22.46% protein, 53.68% carbohydrate, and 3.59% crude fat content (Baiyeri et al., 2018).

According to Nnamani *et al.* (2017), the utilisation of AYB as food is a function of cultural diversity in Nigeria. The tuber has medicinal importance (Potter, 1992). Paste made from the seeds of AYB is used in the treatment of acute drunkenness and as a cure for stomach aches (Asuzu, 1986). African yam bean also has a very high nitrogen-fixing ability (Assefa and Kleiner, 1997; Oganale, 2009). Due to the inherent lectin in the seed, AYB is less susceptibility to most field and storage leguminous pests (Omitogun *et al.,* 1999). AYB also have wide adaptive nature to different climatic and soil conditions (Aremu *et al*., 2020b).

2.4 Limitations of *Sphenostylis stenocarpa*

Despite its numerous benefits, AYB is faced with several constraints that affect productivity and acceptability of the crop. The presence of high anti-nutritional factors

and long cooking time (Fasoyiro *et al.*, 2006), low seed yield (Saka *et al.*, 2004), agronomic demand for stakes, photoperiodic sensitivity and long maturation period (Okpara and Omaliko, 1995) have negatively influenced the crop.

2.5 Genetic diversity within species

Genetic diversity is any measure that quantifies the magnitude of genetic variability within a population. It is a major source of biodiversity (Hughes *et al*., 2008). It is the foundation for sustainability because it provides raw material for adaptation, evolution, and survival of species under changed environmental conditions over centuries (Hammer, 2004). Diversity assessment is an integral part of plant breeding that leads to selection and development of superior varieties with human-preferred traits (Mondini *et al*., 2009; Govindaraj *et al*., 2015). It plays important role in identifying groups with similar genotypes for conservation and future utilisations (Geleta *et al*., 2006). Genetic diversity assessment between and within plant populations can be performed using different data such as; i) morphological; ii) biochemical and; iii) DNA (or molecular) (Mondini *et al*., 2009; Govindaraj *et al*., 2015).

2.5.1 Morphological characterisation of *Sphenostylis stenocarpa*

Variation occurs in plants in many ways, either within the lifespan of an individual or throughout evolutionary time. Variability is a lifestyle in plants, where they modify response physiologically and morphologically to all forms of environmental change. It can range from subtle to dramatic within (intraspecific) and among (interspecific) plant populations. Humans have applied the knowledge of intraspecific variability to the domestication and genetic improvement of several plant species through direct selection on multiple aspects of plant development. Furthermore, varieties of many cultivated plant species of interest as ornament and food with improved yield and nutrient content have been released (Alonso-Blanco *et al*., 2004; Kalisz and Kramer, 2008).

Morphological characterisation is an account of plant character based on visually accessible trait variations, either throughout its life span or at a particular growth stage. It involves documentation of highly heritable characters which are easily seen with the naked eyes as expressed in an environment using phenotypic descriptors (Adewale, 2011). Morphological characterisation is the first step in germplasm identification and classification. It is easy to record and inexpensive, and very reliable in estimating variability and heritability. Morphological characterisation is preferred in developing

countries where labour is available at reduced cost, the reverse may be the case in developed country (Govindaraj *et al.*, 2015; Mengistu *et al.*, 2015; Nelimor *et al.*, 2020).

In morphological characterisation studies of germplasm, large number of variables are often measured, some of which are insufficient in discriminating the germplasm evaluated. As such, Principal Component Analysis (PCA) is used to reveal pattern of variation and eliminate redundancy in data (Das *et al*., 2017). While PCA explains the extent of variability in a population, cluster analysis plays a complementary role of classifying the variability into separate groups based on their similarity for one or more morphological traits (Alake and Porbeni 2020). As such, heterosis can be exploited through the hybridization of genotypes belonging to different groups, and desired segregants can also be obtained. Heritability and variance component estimates are key parameters used in unravelling the gene action governing a desired trait or variable for breeding. Heritability is important in determining the response to selection (Tiwari *et al*., 2019) while genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) reveals relative amounts of genotypic and phenotypic variation in different traits.

Seeds of AYB alone is known to harbour vast genetic variability for Colour, shapes and size (Kay, 1987, Oshodi *et al*., 1995; Adewale *et al*., 2010; Adewale *et al*., 2012a). Adewale *et al*. (2010) suggest six seed characters (seed length, width and thickness and their ratios) as unique parameters for differentiating AYB accessions. Traits like peduncle length, grain filling period, peduncles per plant, pods per peduncle, pods per plant, pod length, 100-seed weight, days to germination, days to flowering, days to pod maturity, seeds per plant, seeds per pod, seed yield per plant, seed set percentage, shelling percentage, seed yield ($Kgha^{-1}$), protein content, oil content, dry matter per plot, nodule yield and tuber yield per plant have been reported to vary significantly among AYB accession (Aremu, and Ibirinde, 2012; Adewale *et al*., 2012b, Ibirinde and Aremu, 2013; Nwofia *et al*., 2014; Osuagwu *et al*., 2014; Adesoye, and Ukwueze, 2015; Ojuederie *et al*., 2015; Adewale and Kehinde, 2016; Ibirinde *et al*., 2019; Aremu *et al*., 2019, Aremu *et al*., 2020a).

Genetic variability and trait relationship that exists among yield and associated traits in AYB was studied by Nwofia *et al*. (2014) in the 2009 and 2010 cropping seasons. Compared to phenotypic variance, the genetic variance was slightly lower for all measured traits, suggesting higher environmental effect in the expression of these traits. The compares is also in agreement with what is observed in other studies (Adewale *et al*.,

2012b; Ibirinde *et al*., 2019; Alake and Porbeni, 2020). In both cropping seasons, low GCV and PCV were recorded for days to flowering, days to pod maturity, grain filling period, pod length and number of seeds per pod. Low PCV and GCV were reported for days to flowering by Ibirinde *et al*. (2019). Alake and Porbeni, (2020), also reported low GCV and PCV in days to flowering, days to maturity, number of pods per plant, 100-seeds weight and seed yield per plant. Ibirinde and Aremu, (2013) and Aremu *et al*. (2019) reported high GCV and PCV in maturity date, seed number per plant, seed yield per plant, number of seeds per pod, filled pods per plant, number of pods per plant, and pod filling duration. Seed length, width and thickness and their ratios exhibited high and substantial genetic variance but low PCV and GCV (Adewale *et al*., 2010).

High heritability estimate was reported for different characters in different studies: pod length, number of pods per plant, seed length, seed width, seed thickness, pod length, number of filled pod, seed yield, 100-seed weight, seed weight per pod, days to flowering, days to seedling emergence, peduncles per plants, pod length, seed per pod, days to maturity, peduncle length and number of peduncle per plant (Adewale *et al*., 2010; Ibirinde and Aremu, 2013; Nwofia *et al*., 2014; Adewale and Kehinde., 2016; Aremu *et al*., 2019; Ibirinde *et al*., 2019; Alake and Porbeni, 2020). However, moderate heritability estimate had been reported in 100-seeds weight (Ibirinde and Aremu, 2013), number of seeds per pod and 100-seeds weight (Nwofia *et al*., 2014; Alake and Porbeni, 2020) as well as days to flowering, pod length and number of pods per plant (Adesoye and Ukwueze, 2015). Moderate to high heritability estimate suggests less environmental effect in the expression of these traits. Low heritability estimate was recorded in number of seeds per pod while the time of pod filling and seed yield per plant had moderate heritability in the study of Aremu *et al*. (2019). Alake and Porbeni (2020), suggested the use of genetic correlation coefficient in selecting progenies with desirable seed attribute with low heritability estimate to increase selection efficiency in crop improvement programmes.

Akande (2009) and Popoola *et al.* (2011) assessed genetic diversity within 32 AYB collections from Southwest Nigeria based on 16 morphological characters and in 25 IITA AYB accessions for intraspecific variability using 36 morphological traits, respectively. Wide genetic variability, considered useful in selection and improvement of AYB was found in both cases. Leaf size (mid-leaflet surface area, length and width), Pod per plant and seed yields per plant were observed to have contributed larger proportion of the observed variation (Akande, 2009). The 32 AYB accessions were resolved into

five distinct clusters. Popoola *et al.* (2011) reported that number seeds per pod, pod length and seed weight contributed mostly to variations in studied populations. The 25 AYB accessions were grouped into six distinct clusters.

Morphological diversity analysis by Adewale *et al.* (2012a) on 79 IITA AYB accessions based on 24 morphological traits, identified accessions with desirable agronomic characters, such as earliness to flowering, longer pod, higher seeds per pod, non-shattering, and higher seed set percentage. In Aremu and Ibirinde (2012) bio-diversity studies on 50 accessions of AYB, branching pattern, pod length, pod and peduncle number, seed number and seed yield contributed significantly to observed variation. The same observation was made by Ibirinde *et al.* (2019) for seed yield variation.

2.5.2 Molecular characterisation of *Sphenostylis stenocarpa*

Molecular markers are noticeable DNA sequences found at distinct locations in the genome and are transferred from parents to off spring. They are naturally occurring polymorphism which includes proteins and nucleic acids that are detectably different. Molecular markers are landmarks in the genome that do not affect the phenotype of the traits of interest. They work by either measuring directly or indirectly, a specific DNA sequence difference between various genotypes (Semagn *et al*, 2006; Govindaraj *et al.,* 2015).

Compared to morphological markers, molecular markers offer numerous advantages: they are stable and detectable in all tissues regardless of growth, not influenced by environmental factors, pleiotropic, and epistatic effects (Milee *et al*, 2008; Govindaraj *et al.,* 2015). Different types of molecular markers are used in plant research, most of which are limited in their applications because of their availability and the high cost of conducting analyses on a large scale. The characteristics that a good marker has to fulfil are dependent on the number of genes segregating in a population and the size and composition of the plant population (Collard and Mackill, 2008).

According to Garrido-Cardenas *et al*. (2017), molecular marker techniques can be classified into three categories based on the method of analysis: 1) non-Polymerase Chain Reaction (PCR) based or hybridization-based, i.e., Restriction Fragment Length Polymorphisms (RFLPs); 2) PCR-based techniques, i.e., RAPD, Sequence Characterized Amplified Region (SCAR), Cleaved Amplified Polymorphic Sequences (CAPS), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), and

Direct Amplification of Length Polymorphisms (DALP) and; 3) sequence-based marker techniques, i.e., SNPs, which are the most abundant in a genome (Zhu *et al*., 2008).

Molecular tools have been notably used to unravel intra- specific diversity in AYB. Moyib *et al.* (2008) assessed for genetic diversity within twenty-four accessions of African yam bean at the Agronomy Department, University of Ibadan using nine polymorphic RAPD primers. Eight distinct DNA cluster groups were identified at 0.80 similarity indexes. Principal component analysis result showed no clear-cut zonal demarcation among the 24 accessions, suggesting low environmental mutation among the studied accessions. However, reproducibility in RAPDs is low and it is a dominant marker (Dubcovsky *et al*., 2001; Govindaraj *et al.,* 2015).

Adewale (2011) revealed high similarities across some of the genomic loci of 80 accessions of AYB with 5 AFLP primer combinations. Two AFLP primer combinations (E-ACT/M-CAG and E-ACG/M-CTG) were used by Adewale *et al.* (2015) to access diversity within 77 accessions, E-ACT/M-CAG was found to be the most efficient primer combination for polymorphic detection (85.5%). Four distinct cluster groups were identified. Higher levels of diversity among the accession were also reported, a result that concur with that of Ojuederie *et al.* (2014) on diversity study in 40 accessions of AYB using AFLP makers. The dominant nature of AFLP marker and requirement for purified high molecular weight DNA is however a major drawback.

Shitta *et al.* (2016) undertook diversity studies in 67 AYB accessions using 13 transferability cowpea derived SSR markers. Eight of the SSRs amplified above 60% of studied accessions and generated 55 polymorphic fragments with an average of 6.9 per primer. High diversity was found within accession like those above. The dependence of SSRs marker on prior sequence information is a problem, as there is no prior sequence for AYB genome. The information's from these studies are very useful, Adewale *et al.* (2015), however, suggest a shift in focus to trait-maker associations in this AYB population.

Next-generation molecular markers such as Diversity Array Technology (DArT) are currently being used to explore the diversity existing among GRC-IITA AYB germplasms (Paliwal *et al*., 2020). Diversity Array Technology was developed only in early 2000 to overcome the limitations of available marker technologies such as AFLP, RAPD, SSR at the point in time. It was developed to enable whole-genome profiling of crops without the need for sequence information (Jaccoud *et al.,* 2001). This makes it suitable for genetic mapping and diversity studies (Wenzl *et al.,* 2004). Diversity Array

Technology uses a microarray platform to score hundreds to thousands of SNPs and insertion/deletion (InDel) polymorphisms across the genome in a single assay (Jaccoud *et al.,* 2001; Wenzl *et al.,* 2004; Wittenberg, 2007) to offer high throughput genotyping procedure.

Compared to existing molecular marker technologies, DArT has several advantages: 1) it requires no prior DNA sequence information, thus making it a marker of interest to crop species with limited or no sequence are available (i.e. orphan crops); 2) Willingness to share improvements (open source platform); 3) flexibility of application; and 4) parallel rather than serial analysis of marker data used in gel electrophoresis dependent technologies (Wittenberg, 2007). The detail procedure of DArT technology platform as described by Wittenberg (2007) and Egea *et al.* (2017) are summarised as follows: i) complexity reduction, where the genomic DNA (gDNA) will be digested using a combination of restriction enzymes. The number of DArT markers that can be obtained is dependent on the germplasm diversity and the complexity reduction method used; (ii) polymorphic fragments cloning into *Escherichia coli* bacteria for genomic library construction; (iii) library amplification by polymerase chain-reaction (PCR); (iv) amplicons cleaning and evaluation through capillary electrophoresis sizing; (v) fragments sequencing; (vi) creation of FASTQ file with generated sequence reads which including sequences between 30 and 60 base pairs (bp) of the polymorphic fragments; (vii) internal alignment using other reads from the library (in case of incomplete or absence of reference genome, like AYB); (viii) markers (SNP and SilicoDArT) search and filtering using algorithms; and (ix) the SNP and the SilicoDArT markers presentation in matrices.

2.6 Inter-relationship among *Sphenostylis stenocarpa* traits

Yield is a complex trait with relatively low heritability and is the product of multiple interacting component traits (Zhao *et al.,* 2016). Its biggest improvements in crop breeding are associated with optimization and selection of heritable yield components. Selecting any heritable component trait or traits involves a complex pathway that leads to the formation of the complex trait (Nwofia *et al*., 2014*;* Kang, 2015). Correlation coefficients help to measure the level of relationship existing between a pair of character. It shows the interaction between a dependent variable and mutually associated components and where there is any change in one component disturb the whole network of cause and effect system. Correlation is very effective in determining yield

contributing characters and in indirect selection (Kumar *et al*., 2015; Sesay *et al*., 2017). The use of correlation coefficients alone is not always adequate, as it provides only one-dimensional information without taking into account the interrelationships among all yield component traits (Nwofia *et al*., 2014*;* Kang, 2015). Path coefficient analysis is a standardized partial regression statistical technique that untangles correlation coefficients into direct and indirect effects, in such a way that the contribution of each causal character to yield is known. It estimates the direct effect of a component trait on yield and its indirect effects through another predictor component trait and assists in partitioning the traits into order of importance for selection and improvement purpose (Dewey and Lu, 1959; Cramer and Wehner, 2000, Nwofia *et al*., 2014*;* Kang, 2015; Kumar *et al*., 2015; Sesay *et al*., 2017).

Seed yield per plant or seed weight per plant correlates positively with pod weight and number of seeds per pod using Pearson's correlation (Ojuederie *et al*., 2015). Seed yield/ha had a significant positive correlation with number of pods per plant, 100-seeds weight, pod weight per plant, seed weight per pod and seed weight per plant (Nwofia *et al*., 2014; Ojuederie *et al*., 2015). Significant positive genotypic correlation between seed yield and number of pods per plant, number of peduncles, number of filled pods per plant, and pod length and a negative genotypic correlation with 100-seeds weight was reported by Ibirinde and Aremu (2013). Alake and Porbeni, (2020) reported significant positive genotypic correlation between seed yield per plant and 100-seed weight. Aremu *et al*. (2019), reported significant positive phenotypic and genotypic association between seed yield per plant and time of pod filling, number of peduncle per plant and number of seeds per pod and negative phenotypic and genotypic association with days to maturity. The aforementioned studies have also reported significant positive relationships between these yield-related components, suggesting a possibility for simultaneous improvement of the traits and seed yield. Aremu *et al*. (2019) identified number of seeds per pod, pods per plant, time of pod filling, and number of days to maturity as the first order predictor variables of seed yield. One hundred seeds weight, pod length and number of pods per plant had positive direct effects on seed yield (Nwofia *et al*., 2014).

2.7 Concept of Yield Stability

For any quantitative traits such as seed yield, a significant genotype × environment interaction results in inconsistency of genotypes rank from one environment to the next. This has grossly affected the formulation of crop breeding programmes (Kang

et al., 1987), such that assessment of phenotypic performance of genotypes in different environments has become a necessary component of breeding programmes. If there were no interaction G × E, the best genotype in an environment would also be the best genotype across environments. Should this be the case, crop variety trials would be conducted in one location without replication to provide universal results (Gauch and Zobel, 1996). Hence, stability analysis will not be required.

The term stability of a genotype in a breeding programme according to Purchase (1997) and Purchase *et al*. (2000) is often used in different senses and based on different statistical analyses. Three concepts of stability were identified by Lin *et al*. (1986) as: i) a genotype is stable if its variance over a range of environments is small; ii) a genotype is stable if its response to environments is parallel to that of the mean response of all the genotypes in the trial; iii) a genotype is stable if the residual mean squares from the regression model on the environment index are small (Eberhart and Russel, 1966). Becker and Leon (1988) also proposed two different concepts of stability: i) static stability, where a stable genotype posse an unchanged performance regardless of any variation in environment i.e. zero variance over environment; ii) dynamic stability where a genotype have a predictable response to environments. Several statistical determination methods have been used to access G×E interaction and stability in crop performance. These include: the coefficient of determination (Pinthus, 1973), coefficient of variability (Francis and Kannenberg,1978), genotypic variances across environments (Roemer, 1917), regression coefficient (Finlay and Wilkinson, 1963), Shukla's stability variance (Shukla, 1972), regression coefficient and deviation from regression (Eberhart and Russell, 1966; Perkins and Jinks, 1968), and the two most widely used, the additive main effect and multiplicative interaction (AMMI) model (Gauch, 1992) and GGE biplot (Yan *et al*., 2000).

The AMMI method is a unified approach that combines analysis of variance for genotype and environment main effects with the principal component analysis of the G×E interaction into one (Zobel *et al*., 1988). Based on this, AMMI stability value (ASV) can be estimated using interaction principal component axes (IPCA) 1 and 2. Genotypes with the least ASV are considered stable or adapted genotypes (Purchase, 1997). Also, a near-zero IPCA score indicates more stable genotypes and large values suggest less stable genotypes (Adjebeng-Danquah *et al*., 2017).

Adewale and Kehinde (2016), in thier study of inheritance and stability of some agronomic traits in 30 accessions AYB using the joint regression analysis identified TSs-

24 as the most stable genotypes for days to seedling emergence, TSs-82 for seed weight per pod, TSs-61 for days to 50% flowering and TSs-84 for 100-seeds weight. Accessions TSs-18, TSs-12, TSs-148, TSs-61, and TSs-69 which have smaller IPCA scores were identified as the most stable genotypes across environments by Aremu *et al*. (2020b) in assessing yield stability of 23 accessions of AYB. However, the idea that the most stable genotypes do not necessarily give the best yield has created the need for approaches that incorporate both mean yield and stability in a single index, hence the Yield Stability Index (YSI) (Bose *et al*., 2014). Accession TSs-61 was ranked most desirable based on YSI by Aremu *et al*. (2020b).

2.8 Quantitative Traits and Marker-Trait Association

Traits that are affected by numerous genes of large and small effect, or a combination of both are called quantitative traits or complex traits or multi-factorial or polygenic. Though these traits can be measured and their phenotypic expressions are affected by the cumulative action of many genes and the interaction of these genes with the environment. This interaction varies among individuals over a given range to produce a continuous distribution of phenotypes (Falconer and Mackay, 1996; Sham *et al*., 2002). Many agriculturally important traits such as disease resistance, nutritional quality and yield are examples of quantitative traits (Sham *et al*., 2002).

The numerous genes which are assumed to be controlling genetic variation of a quantitative trait are known as Quantitative Trait Loci (QTLs) and identification of QTLs based only on conventional phenotypic evaluation is not possible (Lynch and Walsh, 1998). Quantitative Trait Loci mapping (marker-trait association) is a strategy that detects associations between a quantitatively inherited phenotype and genetic markers (Beyene and Erena, 2016). The two commonly used methods for QTL mapping are experimental populations for linkage-based QTL mapping and natural populations for linkage disequilibrium-based association mapping (Frary *et al*., 2000; Ranc *et al*., 2012; Ruggieri *et al*., 2014; Beyene and Erena, 2016).

2.8.1 Linkage mapping

In linkage-based QTL mapping, a segregating plant population is required. The parents selected for the mapping population will be divergent for the trait of interest. These populations include: backcross (BC), Double Haploids (DH), F2 derived families and Recombinant Inbred Lines (RILs) (Beyene and Erena, 2016). Each population type possesses its advantages and disadvantages. F2 and BC populations are the simplest types

of linkage-based mapping populations because they require only a short time to produce. They suffer some drawbacks; the populations require only few meiosis such that markers that are far from the QTLs they are strongly associated with it and this hamper the precise location of the QTLs. Also, the populations used for linkage mapping are highly heterozygous and cannot be propagated through seeds indefinitely or evaluated multiple times in different environmental conditions. Lastly, study of epistatic interactions could impossible both populations (Beyene and Erena, 2016). The RILs and DH populations produce homozygous true-breeding lines that are eternal resources for QTL mapping, this allows for multi-locational and replicated trials. The major disadvantage of RILs is the length of time needed to produce the population while DH populations is only possible in crops amenable to tissue culture (Paterson, 1996; Beyene and Erena, 2016).

While linkage map approach has proven useful, it is limited by the resolution of the mapping population, because only two extremely divergent parents are used to generating the segregating population, therefore limited recombination events are studied (Flint-Garcia et al., 2005). More so, the discovery of new genes associated phenotypic traits variation is limited to those having large effects (Buckler *et al*., 2002).

2.8.2 Association mapping

Association mapping is a method that can address the shortcomings of linkage mapping. It is based on linkage disequilibrium (LD) and was originally developed for mapping human disease genes (Flint-Garcia *et al.,* 2005). It has gain favourability in genetic research since its introduction to plants. This is due to the advancement in high-throughput genomic platforms, interests in identifying novel alleles and improve statistical methods (Zhu *et al.,* 2008). Association mapping allow researchers to use different genomic platforms to exploit natural genetic diversity. Genetic diversity is very important to plant breeders and geneticists, however, its utilization is only on a small scale pre-genomics era (Zhu *et al.,* 2008). This method establishes marker-phenotype associations by exploiting the ancestral/historical recombination events in a natural population (Ruggieri *et al*., 2014). It evaluates whether certain alleles and/or genes within a population are found more frequently with specific phenotypes than expected (Flint-Garcia *et al*., 2005).

Depending on the size and objectives of study, association mapping have two broad categories: 1) candidate-gene association mapping, that relates polymorphisms in particular genes which have putative roles in regulating the phenotypic variation of

specific traits and; 2) genome-wide association mapping, that seek to find markers associated with various complex traits by surveying genetic variation in the entire genome (Risch and Merikangas, 1996). Researchers interested in a specific trait often exploit candidate-gene association mapping; however, others conduct comprehensive genome scan for numerous traits by testing thousands of markers across the genome for association using GWAS (Zhu *et al.*, 2008).

The success of association analysis is dependent on the choice of germplasm (Flint-Garcia *et al*., 2003; Yu *et al*., 2006). Phenotypic variation within the germplasm is important. Phenotypic data should be filtered to remove outliers that can shift phenotypic data from normal distribution without having any meaningful effect on the data. Phenotypic data which lack normal distribution will constitute major limitation to association mapping. Heritability explains how much the phenotypic variation is linked to the genotype, therefore only traits with moderate to high heritability estimates should be included in the association mapping. Low broad-sense heritability limits the power of association. Due to strong genotype × environment interaction, genotype accessed in multi-environments will have reduced heritability. Therefore, best linear unbiased predictor (BLUP) and best linear unbiased estimator (BLUE) can be used to adjust the phenotypic data across environments for better estimates of the phenotypic values (Alqudah *et al*., 2020). Population size is also very important in improving the power of associations. According to Poland *et al*. (2012) a population of between 100 to 500 individuals is suitable for Association mapping.

According to Yu and Buckler, (2006) and Yu *et al.*, (2006), plant populations suitable for association studies can be classifiable into one of the five groups; i) those with precise familial relatedness and population structure, ii) multi-family sample, iii) those with population structure, iv) those with both familial relatedness and population structure, and v) those with severe familial relationship and population structure. Breseghello and Sorrells, (2006) further stated that classification of population for mapping is dependent on breeding history, local adaptation and selection. The difference in the relatedness of individuals in the population used for association mapping at the genetic level leads to the formation of population structure that can cause spurious associations between genotype and the trait of interest (Alqudah *et al*., 2020). The principal component analysis (PCA) approach developed by Price *et al*. (2006) and STRUCTURE program by Pritchard *et al*. (2000) which takes into account pairwise relatedness matrix or kinship matrix are the two commonest methods of estimating the

proportion of subpopulations in genotype data and also control structure. In the STRUCTURE method individuals with high genetic similarities are clustered in a group. While in the PCA approach, if the genotypes form no clear groups i.e. they are randomly distributed in the PC plots, then absence of population structure is can be inferred and vice versa (Alqudah *et al*., 2020).

Allele frequency also affects the power of association mapping. Rare allele leads to a lack of resolution power (Soto-Cerda and Cloutier, 2012). Detection of functional allele at low frequency is difficult unless they are having high impact on the phenotype. Therefore, most studies focus on common variants and use a major allele frequency of ≥ 5%. Unfortunately, low-frequency alleles or rare alleles can also explain natural variation in complex traits in specific populations (Youssef *et al*., 2017)

Association mapping has several advantages over traditional linkage mapping in bi-parental populations: i) increases mapping resolution; ii) natural populations are used rather than generating a population through biparental crosses, thereby reducing research time; iii) large number of alleles per locus can be surveyed simultaneously (Yu and Buckler, 2006 Semagn *et al*., 2010; Zhao *et al*., 2011). Association mapping also suffers some shortcomings, such as detection of false positives in population structure which is a result of linkage between causal and non-causal sites, more than one causal site, and epistasis. However, advancement in statistical methods has helped to reduce the rate of false positives (Larsson *et al*., 2013).

2.9 Gene and protein relationship

Genes are basic units of inheritance that are passed from generations to generations in a predictable manner. They are deoxyribonucleic acid (DNA) segments that code for protein production to determine distinct traits of individuals. The DNA contains the genetic code which is responsible for all cellular functions such as cell reproduction, DNA replication, protein synthesis, molecule transportation, etc. Each gene is located on a chromosome and can exist in different forms called alleles. These alleles are transmitted from parents to offspring through sexual reproduction (Knight and Andrade, 2018; Chen, 2020).

Deoxyribonucleic acids are long double helix structure with the resemblance of spiral staircase. It has two antiparallel strands with 5′ and 3′ ends that are reverse and complementary to each other. Nucleotides are the building blocks of these spiral staircase like structure. Each nucleotide consists of: 1) five-carbon sugar (deoxyribose), 2)

phosphate groups both forming the rails and 3) nitrogenous bases (adenine, cytosine, guanine and thymine) forming the rungs structures. The nitrogenous bases pair up in a predictable manner: Adenine with Thymine (A-T) and Cytosine with Guanine (C-G) (VanPutte *et al*., 2017). The bases on a single strand are arranged in triplet coding information (such as GCT and GTT) to produce an amino acid. The process by which the DNA is used to synthesize functional products such as functional RNAs and proteins is called gene expression.

Several steps involved in the gene expression processes can be categorized as: 1) transcription and post-transcription modification (i.e., Ribonucleic acid (RNA) splicing, 3′poly A adding, 5-capping), and 2) translation and post-translational modification (i.e., protein splicing, folding, and processing). During transcription a RNA polymerase bind to the promoter (a small sequence of DNA located at the beginning of the 5′ ends) to produce a complementary RNA primary transcript called messenger RNA. In the RNA, the base uracil (U) replaces the T. The addition of a 5′ cap and 3′ poly-A tail together with RNA splicing occur during the post-transcription. The 5′ cap and 3′ poly-A tail protects the transcripts from degradation and also facilitate the transportation to ribosomes. In the ribosome (part of the cell that makes proteins), the instruction on the RNA read to synthesize protein in a process called translation. The transfer RNA brings amino acids into the ribosome on the instruction of the messenger RNA (the codon on its strand) to produce a long chain of protein that late folded into a complex three-dimensional structure. The protein then goes off to perform its functions. Protein function as a major component of cells and perform wide variety of tasks ranging from cellular differentiation, morphogenesis, adaptability, and diversity. The presence or absence of proteins creates phenotypic characters/traits (Chen, 2020).

CHAPTER 3
MATERIALS AND METHODS

3.1 Research Sites

Field experiments were carried out during the 2018 and 2019 cropping season in three agro-ecologies of Nigeria namely: derived savanna (Ibadan), humid forest (Ubiaja), and sudan savannah (Kano). Prior to planting at each site, soils samples were randomly collected to 30 cm depth using soil auger for physical and chemical analyses (Table 3.1). Data were also collected on selected weather indices at each location during the cropping seasons (Table 3.2).

3.2 Germplasm used

A total of 196 accessions of AYB, comprising 91 from Nigeria, two from Bangladesh, one from Ghana and 102 of unknown origin, were collected from GRC-IITA and used for this study (see Appendix 1).

3.3 Experimental Design and Field Plot Management

The experimental design was a 14×14 partially balanced lattice design replicated three times. Planting dates were 25th August, 2018 and 5th June, 2019 in Ubiaja; 2nd August, 2018 and 6th August, 2019 in Ibadan; 20th July, 2018 and 17th July, 2019 in Kano. Plots consisted of single 4 m long ridges spaced 0.75 m apart. Seeds were dusted with Mancozeb 80% WP and planted 0.5 m apart on ridges. One seed was planted per hill to give a population density of 26,666 plants/ha. Pre-emergence herbicide (S-Metalachlor EC 960 g/L – 1.5 L/ha and Glufosinate-Ammonium – 3 L/ha) was applied one day after planting. Three weeks after planting, plants were staked and Triple superphosphate fertilizer was applied at the rate of 50 kg P/ha. Fortnightly, Cypermethrin 30 g/L+Dimethoate 250 g/L EC and Mancozeb 80% WP were applied at 1 L/ha and 2 Kg/ha, respectively from the inception of flowering to harvest to control floral and pod pests and fungi disease, respectively. Manual weeding was done regularly to reduce weed interference following pre-emergence herbicide application.

Table 3.1. Pre-field soil physical and chemical properties and GPS coordinates of the experimental site

	IBADAN		KANO		UBIAJA	
Properties	2018	2019	2018	2019	2018	2019
pH (1:1)	5.9	6.8	5.8	5.4	4.8	5.2
Bray P (mg/kg)	9	5	12	10	2	3
OC (g/kg)	4.3	4.2	3.8	8.7	5.1	4.8
N (g/kg)	0.6	1.3	0.2	0.6	1.1	2.8
Exchangeable bases (cmol/kg)						
Ca	1.55	1.92	1.31	0.97	0.67	2.26
Mg	0.8	0.13	0.77	0.04	0.04	2.26
K	0.12	0.21	0.12	0.16	0.04	0.1
Na	0.09	0.08	0.1	0.07	0.08	0.07
ECEC	2.55	2.34	2.29	1.24	0.8	4.68
Micronutrient (mg/kg)						
Zn	2.53	1.14	2.38	1.38	1.643	0.84
Cu	4.15	1.04	1.78	1.22	1.39	0.78
Mn	260.79	300.04	25.07	137.74	133.39	110.15
Fe	24.89	146.67	11.78	133.33	168.67	13.83
Particle size (g/kg)						
Sand	750	650	850	770	810	850
Silt	60	80	60	140	60	90
Clay	190	270	90	90	130	60
Textural class	SL	SCL	LS	SL	SL	LS
coordinate	N 7°29'12.89", E 3°54'07.38", 237.07 m altitude	N 7°29'07.95", E 3°54'03.79", 211.6 m altitude	N 12°08'21.97", E 8°40'05.55", 427.8 m altitude	N 12°08'23.59", E 8°40'11.03", 425.5 m altitude	N 6°40'09.40", E 6°20'28.08", 334.4 m altitude	N 6°40'09.40", E 6°20'28.08", 334.4 m altitude

SL: Sandy loam; LS: Loamy sand; SCL: Sandy clay loam

Table 3.2. Weather condition in each cropping season in Ibadan, Kano and Ubiaja

Month	Year	Rainfall/ day (mm)	Solar Radiation (MJ/m²/day)	Tempera ture Min (°C)	Tempera ture Max(°C)	Relative Humidity (%)	Month	Year	Rainfall/ day (mm)	Solar Radiation (MJ/m²/day)	Tempera ture Min (°C)	Tempera ture Max(°C)	Relative Humidity (%)
Ibadan 2018							**Ibadan 2019**						
August (4-31)	2018	6.54	13.2	22.23	28.33	84.91	August (6-31)	2019	13.93	14.916	22.27	28.95	82.35
September	2018	11.98	14.81	22.44	29.39	82.66	September	2019	16.96	15.73	22.29	28.96	81.14
October	2018	15.57	18.15	21.92	30.9	78.08	October	2019	13.63	16.92	22.05	29.23	79.06
November	2018	8.45	18.94	23.39	32.04	69.28	November	2019	10.8	18.18	23.3	32.3	70.05
December	2018	0	18.77	20.54	33.83	51.48	December	2019	9	18.6	21.51	33.84	60.39
January	2019	7.1	14.28	22.1	35.02	57.86	January	2020	0	18.93	19.81	34.71	46.69
February	2019	14.3	13.16	23.54	35.09	63.42	February	2020	0	19.27	21.72	36.19	53.8
Average		10.66	15.90	22.31	32.09	69.67	Average		12.86	17.51	21.85	32.03	67.64
Kano 2018							**Kano 2019**						
July (20-31)	2018	5.31	19.18	22.04	31.59	88.33	July(17-31)	2019	7.614	20.44	22.19	31.15	71.34
August	2018	8.06	17.85	21.45	30.28	92.26	August	2019	7.88	18.99	21.16	29.21	79.22
September	2018	2.852	19.98	21.72	31.94	89.09	September	2019	2.43	20.38	21.96	31.79	71.63
October	2019	2.07	20.34	21.53	31.8	67.83	October	2019	2.07	20.34	21.53	31.8	67.83
November	2018	0	19.96	16.17	34.54	54.88	November	2019	4.3	20.78	18.03	34.08	41.21
December	2018	0	16.99	14.12	30.19	42.2	December	2019	0	20.63	11.87	30.42	26.88
January	2019	0	20.21	14.17	32.05	18.71	January	2020	0	20.09	12.22	29.4	25.67
February (1-19)	2019	0.17	20.79	14.52	31.5	16.1	February (1-16)	2020	0	20.23	13.23	30.47	19.59
Average		3.69	19.41	18.22	31.74	58.68	Average		4.86	20.24	17.77	31.04	50.42
Ubiaja 2018							**Ubiaja 2019**						
August (25-31)	2018	13.07	17.47	21.911	28.33	88.9	June	2019	10.53	16.55	22.9	29.07	88.36
September	2018	10.85	16.43	22.37	28.01	90.35	July	2019	7.79	16.38	22.32	28.21	89.2
October	2018	4.52	18.45	22.57	28.58	89.53	August	2019	12.46	15.65	21.99	27.77	90.09
November	2018	2.95	19.58	22.54	30.14	83.15	September	2019	13.05	16.51	22.46	27.81	91.14
December	2018	0.01	19.03	19.27	29.72	66.92	October	2019	13.46	16.63	22.19	27.94	90.08
January	2019	0.93	17.78	20.52	30.4	72.76	N0venber	2019	2.09	19.03	22.75	30.05	84.45
February	2019	0.96	17.98	21.7	30.34	73.56	December	2019	0.9	19.28	19.19	30.2	66.29
March (1-24)	2019	2.43	18.01	23.58	30.57	84.58	Average		8.61	17.15	21.97	28.72	85.66
Average		4.47	18.10	21.81	29.51	81.22							

3.4 Data Collection

Data were recorded on a plot basis using AYB descriptors (Adewale and Dumet, 2011) as described below:

1. Days to flowering: Number of days from sowing to when 50% of the plants begin to anthesize.
2. Days to pod maturity: Number of days from sowing to when 90% of the pods in a plot are matured.
3. Grain filling period: Number of days from anthesis to pod maturity/ripening.
4. Number of pods per plant: Mean number of pods estimated using harvested pods from five representative plants.
5. Pod weight (g/plant): Mean weight of pods harvested from five representative plants.
6. Pod Length (cm): Mean length of ten randomly selected pods harvested from five representative plants.
7. Number of locules per pod: Mean number of seed cavities in ten randomly selected pods.
8. Number of seeds per pod: Mean number of well-formed seeds in ten randomly selected pods.
9. Shelling percentage (%): The seed weight per plant ratio to pod weight per plant expressed as a percentage.
10. 100-seed weight (g): Weight of 100 randomly selected seeds taken from total seed yield/plot.
11. Seed yield (g/plant): Mean weight of seeds produced per plant from five representative plants.
12. Seed length (mm): Mean distance between the two ends parallel to the hilum of ten representative seeds.
13. Seed width (mm): Mean distance from hilum to the keel of ten representative seeds.
14. Seed thickness (mm): Mean distance perpendicular to the seed length of ten representative seeds.
15. Number of tubers per plant: Mean number of tubers produced per plant; from five sample plants.
16. Tuber yield (g/plant): Mean weight of tubers produced/plant; from five sample plants.

Due to the inconsistency in tuber production in different location, only the mean of tuber traits (number of tubers per plant and tuber yield) in each environment were documented. The remaining fourteen agronomic and seed yield traits were used for further analysis.

3.5 Phenotypic Data Analysis

3.5.1 Analysis of Variance and Estimation of Genetic Parameters

Analysis of variance (ANOVA) was computed for each location and combined locations using plot means. For each location and combined locations ANOVA, location–year combinations were regarded as environment. Environments were considered fixed effects, whereas accessions, replications within environments, and blocks within replication and environments were regarded as random effects. Count data were log-transformed while square root transformation was used for data in percentages before subjecting them to ANOVA to reduce the heterogeneity of variances.

Mixed-model analysis with the restricted maximum likelihood procedure (SAS Institute Inc., 2017) was used to estimate variance components. Phenotypic variance and broad-sense heritability were then computed as:

$$\text{Phenotypic variance } (\sigma_P^2) = \sigma_g^2 + \frac{\sigma_{ge}^2}{\text{nlocs}} + \frac{\sigma_e^2}{(\text{nlocs} \times \text{nreps})}$$

$$\text{Broad-sense heritability } (H^2) = \frac{\sigma_g^2}{\sigma_P^2}$$

where σ_g^2, σ_{ge}^2, σ_e^2, nreps and nlocs are the genotypic variance, genotype × environment variance, error variance, number of replicates and number of locations, respectively. The heritability estimates were categorized as low (0 - 30%), medium (30 - 60%) and high (>60%) (Robinson *et al*., 1949).

Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) were computed for all the traits according to Singh and Chaudhary (1977) using the equations:

$$\text{Phenotypic coefficient of variation} = \frac{\sqrt{\text{phenotypic variance}}}{\text{Grand mean}} \times 100$$

$$\text{Genotypic coefficient of variation} = \frac{\sqrt{\text{genotypic variance}}}{\text{Grand mean}} \times 100$$

The PCV and GCV values were classified as described by Sivasubramanian and Menon (1973) as low (0 to 10%), moderate (10 to 20%) and high (>20%).

3.5.2 Principal Component Analysis (PCA) and Cluster Analysis

To obtain information on traits most effective in discriminating among the accessions, PCA was carried out. In the analysis, principal components (PCs) with eigenvalues ≥ 1.0 were retained. A PCA biplot analysis of the first two PCs was run to further explain the relationships between the two PCs and the variables. Cluster analysis (with constellation plot and pairwise Mahalanobis genetic distances between clusters was performed on standardized means using the Ward minimum variance method (Ward, 1963). The JMP Pro 14.1.0 (SAS Institute Inc 2017) was used for both the PCA and cluster analyses.

3.5.3 Inter-trait relationship

To determine the inherent relationship between paired traits, phenotypic (r_p) and genotypic (r_g) correlation coefficients were estimated using META-R version 6.04 (Alvarado *et al*., 2015). For further information on the interrelationships among the traits studied, path coefficient analysis was done to determine the direct and indirect effects of each trait to seed yield according to the procedure described by Kang (2015). Genotypic correlation coefficients were used in the path analysis as suggested by Kang *et al*. (1991) to avoid spurious association in the phenotype as a result of artificially created relationships among traits in a pathway.

3.5.4 Yield Stability Analysis

3.5.4.1 Additive Main Effects and Multiplicative Interaction (AMMI) Analysis

Plot means of seed yield per plant in each environment were subjected to AMMI analysis in GEA-R (Genotype × Environment Analysis with R for Windows) Version 4.11 (Pacheco *et al*., 2015). The AMMI model is given as:

$$Y_{ij} = \mu + Gi + \text{Ej} + \sum_{k=1}^{n} \lambda_k \alpha_{ik} \gamma_{jk+} e_{ij}$$

Where;

Yij = mean of yield of *i*th accessions in the *j*th environment;

μ = grand mean;

Gi = the ith accession mean deviation;

Ej = the jth environment mean deviation;

λ_k = square root of the eigenvalue of the PCA axis k;

α_{ik} and γjk = the ith accession and jth environment PCA scores;

e_{ij} = residual.

3.5.4.2 AMMI Stability Value (ASV)

The ASV suggested by Purchase (1997), was proposed to rank genotypes according to their yield stability value because AMMI model does not provide a specific stability measure. The ASV is the distance from zero in a two-dimensional scatterplot of interaction principal component analysis (IPCA) 1 scores against IPCA2 scores. The distance from zero is then determined using the Pythagorean Theorem. The measure is proposed as:

$$ASV = \sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}}\ (IPCA1_{score})\right]^2 + (IPCA2_{score})^2}$$

Where;

SSIPCA1 = Sum of squares of IPCA1;

SSIPCA2 = Sum of squares of IPCA2;

The smaller the ASV value (irrespective of dimension), the more stable the accession across environments (Purchase, 1997).

3.5.4.3 Yield Stability Index (YSI)

The YSI was calculated based on the rank of the mean of seed yield of accessions across the six (3 location and 2 years) environments and the rank of ASV as described by (Bose *et al*., 2014).

$$YSI = RASV + RSY$$

Where;

RASV = rank of the accessions based on the AMMI stability value;

RSY= rank of the accessions based on seed yield across environments.

Accessions with the least YSI i.e. high mean yield and low ASV, are considered superior for combining high performance and stability (Tumuhimbise *et al*., 2014).

3.6 Genome-Wide Association Studies

3.6.1 DNA Extraction, Genotyping and Quality Control

Leaf samples were collected from three weeks old seedlings of each of the 196 AYB accession and kept in -80°C freezer. Genomic DNA (gDNA) was extracted using the DArT protocol (www.diversityarrays.com/files/DArT_DNA_isolation.pdf). The gDNA was run on a 2% agarose gel to check the quality. Nanodrop 2000 spectrophotometer was used to measure the purity and concentration. Genotyping of the AYB accession was performed using a whole-genome profiling service of DArTseq at the DArT Pty Ltd., Canberra, Australia. The detailed methodology was described by Egea *et al.* (2017).

A raw dataset of 5,416 Diversity Array Technology sequence (DArTseq) generated Single Nucleotide Polymorphisms (SNPs) markers were generated. The 5,416 DArTseq SNPs generated were filtered using average reproducibility (≥ 95%), call rate (≥ 80%), minor allele frequency (MAF) (≥ 0.01) and missing SNP (30%) to remove bad SNPs. After quality control, 2,491 SNPs and 195 accessions remained for further analysis.

3.6.2 Phenotypic Data Analysis

The plot means of the remaining 195 accessions (in each and combined locations) were used to calculate the best linear unbiased estimates (BLUE) using META-R. Pearson correlation between traits was calculated using the estimated BLUEs across location using the Proc corr procedure of SAS. A frequency distribution plot was also generated for all traits using the estimated BLUEs in R-studio Version 3.5.

3.6.3 Population Structure and Genome-Wide Association Analysis

Population structure analysis was carried out using the PCA approach. A kinship matrix was also calculated to infer familial relatedness. Based on the estimated BLUEs for phenotypic traits and filtered SNPs, marker-traits associations were determined using the Mixed Linear Model (MLM), taking the population structure and familial relatedness into account. The MLM approach was preferred in this study to detect marker-trait associations due to its ability to reduce the false-positive associations by controlling both types I and II errors in comparison with other models for such as the general linear model (GLM). The statistical formula for the MLM approach proposed by Yu et al. (2006) is given as:

$$Y = X\beta + W\alpha + Qv + Zu + \varepsilon$$

Where;

Y = observed vector of means;

β = fixed effect vector (p × 1) other than molecular markers effects and population structure;

α = fixed effect vector of the molecular markers;

ν = fixed effect vector from the population structure;

u = random effect vector from the polygenic background effect;

X, W, Q, and Z = incidence matrixes from the associated β, α, ν, and u parameters;

ε = residual effect vector.

Based on the distribution of p-values for all the traits, marker-trait associations were declared significant at P-values of –log (p) = 4 (Mogga *et al*., 2018; Adewale *et al*., 2020). Manhattan and quantile-quantile (Q-Q) plots were constructed accordingly. All analyses were performed in Tassel software.

Molecular markers are known as reference landmarks for genes in the genome. To further validate the significant marker-trait association found in this study using related legume genome. A blast search was performed for trimmed nucleotide sequence of significant AYB markers in the combined location analysis on Common bean (*Phaseolus vulgaris*) genome (*Phaseolus vulgaris* G19833 genome v2.0) database in Legume information system (Dash, 2016). Syntenys of related legumes (*Glycine max* 2.0, *Vigna angularis* 3.0, and *Cajanus cajan* 1.0) were also included in the search. The scroll was zoomed to 1 Mb (500 Kbp up and downstream) to check for the surrounding genes and their encoding protein products and know if they regulate the traits of interest. These was done because the lack of AYB reference genome limits candidate gene mapping.

CHAPTER 4
RESULTS

4.1 Evaluation of Agronomic Traits of 196 AYB Accessions

4.1.1 Mean Square, Means and Coefficient of Variation of Agronomic Traits of 196 AYB Accessions

The mean square for seed yield and its components for the 196 accessions of AYB in each location and across environments is presented in Table 4.1. In the combined analysis, there were significant differences ($p < 0.05$) in the accessions for all traits measured. The effects of environments and accession × environment interaction were significant for the traits. Significant differences ($p < 0.05$) were also observed among the accessions for different traits in each location (Table 4.1.). The distinguishing traits among the accessions in each locations are: days to 50% flowering, days to pod maturity, pod length, seed yield per plant, 100-seed weight, seed length, seed width and seed thickness significantly distinguish the accessions in Ibadan, pod weight per plant, seed yield per plant, number of pods per plant, number of locules per pod, shelling percentage and seed thickness in Kano, and pod weight per plant, pod length, seed yield per plant, number of pods per plant, 100-seed weight, seed length, seed width, and seed thickness in Ubiaja. Significant environment and accession × environment interaction effects were also observed for different measured traits in each location.

Mean days to flowering ranged from 83.9 (TSs-90) to 101.4 (TSs-153), days to pod maturity from 139.9 (89A) to 163.0 (TSs-19) and grain filling period from 52.6 (TSs-13) to 76.5 days (TSs-61). The mean seed yield was 15.2 g/plant and ranged from 7.3 (TSs-309) to 31.6 (TSs-421). Pod weight ranged from 56.9 (TSs-195) to 17.7 g/plant (TSs-309), while one hundred seed weight ranged from 16.2 (TSs-368) to 25.1 g (TSs-151A). The lowest number of pods per plant was produced by TSs-217 (4.1) while TSs-162 (16.3) had the highest. Number of seeds per pod and locules per pod were lowest in TSs-6 (9.3, 10.1) and highest in TSs-96 (18.0, 18.7, respectively). Accessions TSs-1 (18.0 cm) and TSs-297 (23.4 cm) had the shortest and the longest pods, respectively. Shelling percentage ranged from 32.4% (TSs-31) to 58.7% (TSs-46). Experimental CVs for all the

Table 4.1. Mean squares for 14 agronomic traits of 196 accessions of AYB evaluated during the 2018 and 2019 cropping season in three agro-ecologies of Nigeria

SOV	DF	D50F	DPM	GFP	NPPPL	PW	SP	PDL
IBADAN								
Accession	195	30.03***	40.89***	57.16***	0.06	1274.51	1.41	12.1***
Environment	1	9365.57*	20.07	10308*	0.01	90635	51.77	1482*
Rep(environment)	4	1110.41***	1634.48***	794.21***	2.6***	58592***	10.2***	119.64***
Block(rep*environment)	78	23.12**	27.09***	41.65***	0.08***	1647.51***	0.94	4.57
Accession*environment	195	18.49*	2.62	22.3***	0.06***	1099.43***	1.53***	6.99***
Error	702	14.77	12.25	23.228375	0.04	616.23	0.8	4.9
KANO								
Accession	195	91.18**	614.4**	658.62**	0.2***	620.05**	2.73***	21.75*
Environment	1	0.81	305331***	308038***	5.63***	2804.71*	2.29	2074.69***
Rep(environment)	4	145.17	1356.03**	1343.61**	0.1	248.46	1.02	10.73
Block(rep*environment)	78	52.14**	231.86**	281.37**	0.06	195.96	1.04*	8.21
Accession*environment	195	62.29***	403.97***	453.2***	0.12***	424.9***	1.37***	16.02***
ERROR	702	34.45	158.89	176.53	0.05	191.42	0.77	6.44
UBIAJA								
Accession	195	120.82	108.85	124.08	0.06*	310.22	1.32*	13.53***
Environment	1	327734***	279427***	631.14	46.95***	173659***	2.29	3088.81**
Rep(environment)	4	135.88	1276.38***	1385.82***	0.51***	741.81	13.56***	58.14***
Block(rep*environment)	78	113.63***	144.04***	152.45**	0.06***	452.48***	0.78	7.11*
Accession*environment	195	108.39***	112.95***	138.33**	0.05***	258.82*	0.97***	7.68***
Error	702	57.99	60.45	98.92	0.03	200.28	0.63	5.33
COMBINE ANALYSIS								
Accession	195	111.12***	248.33*	300.56**	0.11**	774.96*	1.8*	19.61***
Environment	5	105468***	410740***	252643***	18.17***	178126***	156.3***	3725.93***
Rep(environment)	12	463.82***	1422.3***	1174.56***	1.07***	19861***	8.26***	62.84***
Block(rep*environment)	234	62.96***	134.33***	158.49***	0.07***	765.32***	0.92**	6.63*
Accession*environment	975	64.13***	209.3***	236.84***	0.09***	644.24***	1.52***	11.74***
Error	2157	35.84	75.46	97.98	0.04	336.72	0.73	5.54

*, **, ***, significant at 0.05, 0.01 and 0.001 levels of probability, respectively. Days to 50% flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PL).

Table 4.1. continued

SOV	DF	SY	NLPPD	NSPPD	HSW	SL	SW	ST
IBADAN								
Accession	195	455.73*	0.01	0.01	39.22***	0.69***	0.89***	0.58***
Environment	1	45558	2.94**	2.55**	467.76	2.24	22.52**	9.07**
Rep(environment)	4	20545***	0.09***	0.1***	96.42***	0.4	0.54**	0.49
Block(rep*environment)	78	543.87***	0.01	0.01	14.27	0.23	0.12	0.23
Accession*environment	195	347.89***	0.01**	0.01**	19.96***	0.25	0.18***	0.34***
Error	702	202.49	0.01	0.01	13.74	0.2	0.11	0.21
KANO								
Accession	195	99.97**	0.04***	0.04**	44.74*	0.96***	0.63**	0.79***
Environment	1	283.6	2.23***	1.23**	388.19*	6.89***	0.25	1.25
Rep(environment)	4	51.83	0.01	0.02	54.25*	0.16	0.16	0.25
Block(rep*environment)	78	30.56	0.01	0.02*	19.2	0.22	0.17	0.2
Accession*environment	195	69.45***	0.02***	0.03***	33.16***	0.55***	0.41***	0.41***
Error	702	30.43	0.01	0.01	15.94	0.22	0.19	0.25
UBIAJA								
Accession	195	83.21	0.01***	0.02***	28.09***	0.63***	0.29***	0.45***
Environment	1	37282***	5.12***	3.73***	292.66	55.06**	9.8**	8.91*
Rep(environment)	4	306.35	0.06***	0.04***	49.34	1.07**	0.41*	0.82**
Block(rep*environment)	78	123.12***	0.01	0.01	17.95*	0.22	0.12	0.19
Accession*environment	195	72.66**	0.01***	0.01***	16.57**	0.31**	0.14	0.24*
Error	702	54.47	0.01	0.01	12.75	0.24	0.12	0.19
COMBINE ANALYSIS								
Accession	195	225.74*	0.02***	0.02**	52.18***	1.26***	0.55***	0.88***
Environment	5	56656**	8.71***	9.56***	2582.22***	59.79***	57.48***	63.52***
Rep(environment)	12	6967.85***	0.05***	0.05***	66.67***	0.54**	0.37**	0.52**
Block(rep*environment)	234	232.52***	0.01	0.01*	17.14*	0.22	0.14	0.2
Accession*environment	975	181.26***	0.02***	0.02***	25.69***	0.42***	0.28***	0.38***
Error	2157	96.36	0.01	0.01	14.1	0.22	0.14	0.21

*, **, ***, significant at 0.05, 0.01 and 0.001 levels of probability, respectively. Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

traits were generally low (< 21%), except pod weight and seed yield with CV of 56.4% and 64.6%, respectively (Table 4.2).

Based on a better performance than the average mean, 45% of the accession yielded more seeds than the average mean, 56% flowered earlier, 48% matured earlier, 48 % had a shorter grain filling period, 44% produced more pods, 49% and 39% had higher pod weight and hundred seed weight, respectively; 50% produced longer pods, 52% had higher shelling percentage and 50% had more seed per pods. (See Appendix 2).

Tuber production was inconsistent across environments (Table 4.3). In 2018, 25.5% produced tuber in Ibadan, 66.3% in Kano, and 14.8% in Ubiaja, while in 2019, 76.5% produced tuber in Ibadan, 54.6% in Kano, and 24.5% in Ubiaja. Only TSs-121 produced tuber in all six environments. Seventeen accessions (119A, TSs-113, TSs-143, TSs-192, TSs-249, TSs-255, TSs-276, TSs-309, TSs-367, TSs-421, TSs-424, TSs-437, TSs-44C, TSs-56A, TSs-61, TSs-63, TSs-69) produce tuber in five environments and 27 produced tubers in at least four environments. Four accessions (138A, TSs-22, TSs-314, TSs-87B) produced no tuber in any of the six environments. Highest number of tubers per plant was recorded in TSs-166 (12), followed by TSs-216 (11), TSs-156A (10) and TSs-249 (9), all in Kano during the 2019 season. The first 20 accessions with the highest tuber weight were from the two planting seasons in Kano, with TSS-133 having the highest weight of 1104.4 g/plant (Table 4.3).

4.1.2 Estimation of Genetic Parameters

For all traits, the estimates of genotypic coefficients of variation were lesser than those of phenotypic coefficients of variation (Table 4.4). Environmental variance and the variance to due accession × environment interaction were higher than the genotypic variances. The GCV ranged from 1.0 to 11.3%, while the PCV ranged from 2.5 to 24.2% for the traits studied. The highest GCV and PCV were observed for seed yield (11.3%, 24.3%) followed by the number of pods per plant (10.1%, 21.7%), while the lowest was recorded in days to maturity (1.0%, 2.5%). Low to high broad-sense heritability estimates were obtained for the studied traits. Only seed length (66.4%) showed high heritability. Moderate heritability estimates were obtained for seed thickness (57.8%), 100-seeds weight (51.6%), seed width (50.0%), days to flowering (45.0%), pod length (42.0%) and number of seeds per pod (30.8%). Shelling percentage recorded the lowest heritability estimate of 16.3%. Seed yield per plant had 21.6% heritability estimate (Table 4.4).

Table 4.2. Descriptive statistics of 14 agronomic traits of selected accessions of African yam bean evaluated during the 2018 and 2019 cropping season in three agro-ecologies of Nigeria

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
89A	86.00	139.93	57.21	6.69	20.08	45.69	19.93	12.85	12.14	20.14	10.81	8.08	6.10	5.65
TSs-1	84.22	153.39	69.17	10.79	30.69	42.33	17.95	11.41	10.66	17.42	14.76	7.55	6.10	5.99
TSs-13	92.47	143.07	52.64	7.59	28.14	48.84	21.22	13.31	12.05	21.29	14.19	8.23	6.14	5.71
TSs-151A	89.89	155.67	65.78	9.03	33.01	39.75	22.77	11.62	10.11	25.06	13.16	8.87	6.71	6.73
TSs-153	101.39	162.61	61.22	8.94	36.56	45.15	22.43	14.55	13.05	19.23	20.32	7.93	6.25	6.15
TSs-162	90.89	159.06	68.17	16.28	49.40	43.50	19.94	12.91	11.81	21.54	21.02	8.28	6.48	5.93
TSs-19	94.56	163.00	68.13	6.38	27.06	35.27	20.54	11.19	10.58	19.52	13.41	8.00	6.27	6.28
TSs-195	91.00	151.39	60.39	14.33	56.90	46.42	19.96	12.85	11.66	21.50	27.54	7.81	6.11	5.96
TSs-217	92.72	151.83	59.11	4.09	18.25	46.72	21.43	14.17	12.58	17.54	8.45	7.96	6.05	5.48
TSs-297	91.61	160.39	67.11	9.96	34.69	44.68	23.40	12.35	11.23	20.39	16.41	7.95	6.32	6.01
TSs-309	87.61	149.22	61.56	6.28	17.71	43.91	20.01	10.46	9.91	19.05	7.31	8.15	6.07	5.87
TSs-31	85.72	153.33	67.56	6.05	22.95	32.35	21.95	12.42	12.23	18.78	8.93	7.82	6.05	5.93
TSs-368	89.17	154.78	65.56	7.55	28.67	39.69	22.78	11.64	10.37	16.20	12.46	7.70	5.86	5.50
TSs-421	91.06	160.00	68.94	9.51	53.07	44.43	20.50	11.9	11.11	22.74	31.63	8.39	6.42	6.17
TSs-46	90.83	161.00	70.17	7.99	31.62	58.67	18.98	11.23	10.22	21.75	17.22	7.67	6.21	6.43
TSs-6	86.61	153.78	67.17	7.35	24.24	40.02	18.26	10.12	9.32	20.68	10.63	8.05	6.27	6.09
TSs-61	87.33	158.28	76.50	10.71	41.69	46.55	20.53	12.42	11.6	22.31	18.52	8.22	6.29	6.15
TSs-90	83.89	153.39	69.50	8.04	20.76	50.55	18.95	10.85	10.63	19.63	9.52	7.75	6.13	5.63
TSs-96	85.72	154.11	68.39	12.48	39.29	46.31	20.25	15.49	14.46	18.59	17.70	7.95	6.43	6.26
MIN.	83.89	139.93	52.64	4.09	17.71	32.35	17.95	10.12	9.32	16.20	7.31	7.26	5.58	5.47
MAX.	101.39	163.00	76.50	16.28	56.90	58.67	23.40	15.49	14.46	25.06	31.63	8.87	6.71	6.73
MEAM	89.11	153.03	64.05	9.23	32.52	43.8	20.69	12.35	11.42	20.58	15.19	7.98	6.19	5.97
SEM	0.24	0.47	0.40	0.12	0.50	0.24	0.07	0.07	0.07	0.08	0.27	0.01	0.01	0.01
CV(%)	6.72	5.68	15.45	21.56	56.43	13.12	11.37	7.98	8.56	18.24	64.62	5.89	6.06	7.76

Days to 50% flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight per (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Table 4.3. Mean of tuber traits of selected accessions of African yam bean evaluated during the 2018 and 2019 cropping season in three agro-ecologies of Nigeria

ACCESSION	UBIAJA 2018		UBIAJA 2019		KANO 2018		KANO 2019		IBADAN 2018		IBADAN 2019	
	NTPL	TW	NTPL	TW	NTPL	TW	NTPL	TW	NTPL	TW	NTPL	TW
119A	0	0	1	28.06	4	343.85	4	85	5	63.1	2	35.45
30A	0	0	0	0	2.5	58.35	3.5	124.5	1	10.2	5	56.75
60B	3	10	0	0	2	157.65	5	370	3	17.4	0	0
TSs-101	1.5	25.75	0	0	2	94.4	4.67	314.67	0	0	3.5	79.15
TSs-104	0	0	0	0	4.25	199.77	4.08	237	4.33	230.5	3.5	60.1
TSs-113	0	0	2.5	51.95	4.5	385.8	3	192	1.75	37.93	2.33	37.73
TSs-119	0	0	0	0	2	47.3	1.5	76.5	2	8	3	24.3
TSs-121	2	27	1	22.29	2.5	245.7	1.5	32	6	368.63	4.22	237.99
TSs-133	0	0	0	0	5	1104.4	4	176	0	0	3.67	48.6
TSs-14	1	40.1	3	63.41	0	0	0	0	2	10.3	2.33	60.03
TSs-143	0	0	2	68.5	2	137.3	4	125	3	28.7	3	37.43
TSs-150	0	0	0	0	2	42.6	6.33	194.67	2	24.9	3.14	51.02
TSs-153	0	0	1	9.29	3.5	186.1	7	329.5	0	0	4	37.3
TSs-156A	0	0	1	19.27	1	35.9	10	182	0	0	3.17	53.1
TSs-161	0	0	3	77.81	3	373.43	5	205	0	0	4	53
TSs-166	0	0	2.33	29.75	3	52.75	12	753	0	0	3.67	31.2
TSs-192	2	7.4	0	0	2	30.7	2	126	4	105.1	3	53.41
TSs-2015-06	1	9.1	0	0	1	15.4	2	69.75	0	0	2	97.52
TSs-224	0	0	3	16.1	1	107.2	2	19	0	0	3	68.1
TSs-249	1	14.6	0	0	3.5	199.85	9	609	4	93.93	2.78	59.82
TSs-255	2	8.6	3	44.25	2.5	185.95	5	322	0	0	3.83	64.73
TSs-273	0	0	1	5.42	3.25	73.82	5.75	370.67	0	0	2	29.2
TSs-276	0	0	1	8	3	196.5	6	159	3	75.15	3.33	64.15
TSs-294	1	16.2	0	0	6	525.7	5.5	194.5	0	0	1	7.6

NTPL = Number of tuber per plant; TW = Tuber weight.

Table 4.3. continued

ACCESSION	UBIAJA 2018		UBIAJA 2019		KANO 2018		KANO 2019		IBADAN 2018		IBADAN 2019	
	NTPL	TW	NTPL	TW	NTPL	TW	NTPL	TW	NTPL	TW	NTPL	TW
TSs-307	0	0	0	0	1.5	48.8	3	73	2	52.1	3.25	40.98
TSs-309	0	0	1	14.26	4	64.9	2	33	2	30.5	2.5	17.45
TSs-320	0	0	0	0	3	160.6	6.5	156	2	8.3	4	37.9
TSs-367	0	0	2	14.96	2.5	173.35	4	88	5	49.1	2.5	92.35
TSs-369	0	0	0	0	5	396.4	4	634	2.5	95.5	3.33	56.18
TSs-38	0	0	0	0	1	131.8	2	159	2.5	24.13	2.13	17.04
TSs-421	2	12.4	1.5	35.27	3	156.3	0	0	4.5	116.45	4.52	98.93
TSs-424	0	0	1	6.54	2	96.2	3.5	281.5	2	18	2	16.3
TSs-437	0	0	1.5	61.05	4	191.85	2	295	1.75	36.15	4.73	120.91
TSs-441	0	0	1	16.05	2.88	193.09	5.83	154.58	0	0	4	46.95
TSs-443	0	0	2	32.94	1	41.4	2	40	0	0	1	8.1
TSs-44C	2	19.35	0	0	5	406.55	1	36	3	36.3	2.7	50.9
TSs-48	0	0	3	53.31	2.5	107.4	0	0	3	8.2	3	45.2
TSs-56	0	0	1	10.33	1	12.6	5	287	0	0	3	23.3
TSs-56A	2	13.3	1	36.9	3	287.5	5.56	192.61	0	0	3.5	83.35
TSs-61	0	0	1	32.78	3	328.55	4.5	264.67	4	25	4	36.1
TSs-62B	0	0	1	37.69	4	322.9	0	0	5	160.1	2	52.1
TSs-63	1	18.8	2	11.2	3	119.2	5	505	0	0	4.75	14.2
TSs-69	1	9.6	0	0	4.5	369.15	2	24	2	34.8	2	15.5
TSs-84	0	0	0	0	1.5	98.05	6	117	2	14.9	4	75.5
TSs-92	2	16.5	2	37.34	0	0	0	0	3	19.1	2	17.3
TSs-96	1	15.3	0	0	3	144.6	0	0	2	105.7	6.5	43.95

NTPL = Number of tuber per plant; TW = Tuber weight.

Table 4.4. Mean, variance components and genetic estimates for 14 agronomic traits of 196 accessions of African yam bean evaluated during 2018 and 2019 cropping seasons in three agro-ecologies of Nigeria

Traits	Mean	σ^2e	σ^2p	σ^2g	σ^2ge	GCV	PCV	H^2 (%)
D50F	89.11	36.09	6.61	2.97	9.76	1.9	2.9	45.0
DPM	153.03	75.67	14.76	2.53	48.17	1.0	2.5	17.1
GFP	64.05	98.48	17.87	4.15	49.52	3.2	6.6	23.2
NPPPL	9.23	25.46	4.02	0.87	10.42	10.1	21.7	21.6
PW	32.52	334.79	45.72	7.86	115.58	8.6	20.8	17.2
SP	43.8	112.38	16.2	2.72	43.43	3.8	9.2	16.8
PDL	20.69	5.54	1.18	0.5	2.27	3.4	5.3	42.0
NLPPD	12.35	4.96	0.79	0.22	1.74	3.8	7.2	28.1
NSPPD	11.42	4.74	0.79	0.25	1.65	4.4	7.8	30.8
HSW	20.58	14.24	3.07	1.58	4.17	6.1	8.5	51.6
SY	15.19	95.7	13.62	2.97	32.01	11.3	24.3	21.8
SL	7.98	0.22	0.08	0.05	0.08	2.8	3.5	66.4
SW	6.19	0.14	0.03	0.02	0.05	2.1	2.9	50.0
ST	5.97	0.21	0.05	0.03	0.06	3.0	3.9	57.8

Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST); Environmental variance (σ^2e); Genotypic variance (σ^2g); phenotypic variance (σ^2p); Phenotypic coefficient of variation(PCV); Genotypic coefficient of variation (gCV); broad-sense heritability (H^2).

4.1.3 Principal Component Analysis (PCA)

The eigenvalues and percentage variance of the first six principal components (PCs) with Eigenvalues higher than 1.0, and the eigenvectors of fourteen morphological variables are presented in Table 4.5. The six PCs accounted for 86.6% of the total variation among the 196 accessions. All 14 seed yield and yield-related traits significantly contribute to the first three PCs which cumulatively explains 59.7% of the total variation.

The first PC explained 26.8% of the total variation and was positively associated with number of pods per plant, pod weight, shelling percentage, number of locules per pod, number of seeds per pod, 100-seeds weight, seed yield, seed length, seed width and seed thickness (Table 4.5). The second PC which accounted for 17.8% of the total variance is positively associated with grain filling period, days to pod maturity, 100-seeds weight and the three seed metric traits (seed length, seed width and seed thickness) and negatively associated with number of seeds and locules per pod. The major positive contributors to the third PC, explaining 15.1% of the total variation, were days to flowering, pod length, number of seeds and locules per pod, 100-seeds weight, seed length and seed width, while days to pod maturity, grain filling period, number of pods per plant and pod weight contributed negatively. The fourth, fifth and sixth PCs accounted for 10.4%, 8.8% and 7.7% of the total variation, respectively.

A trait biplot of the first and second PCs which accounted for a cumulative variance of 44.6% is shown in Figure 4.1. Except for days to flowering, all other traits had positive contributions to the first PC. Grain filling period, days to pod maturity, 100-seeds weight and the three seed metric traits made positive contributions to second PC, while the contributions of days to flowering, number of locules per pod, number of seeds per pod, pod length number of pods per plant, pod weight, seed yield and shelling percentage were negative. Based on vector lengths, days to flowering and pod length had relatively low contributions to both PCs. Accessions were scattered across the four quadrants.

4.1.4 Cluster Analysis

The cluster history with 195 morphotypes or clusters is presented in Appendix 3. The dissimilarity among the accessions spanned a distance of between 0.92 and 19.29. Accessions TSs-366 and TSs-294 were the most similar (with the least distance of 0.92). The 196 accessions were grouped into five main clusters, with each cluster further divided into sub-groups (Figure 4.2)

Table 4.5. Eigen vectors of the first six principal components and the variance proportion and cumulative contributions for 14 agronomic and yield traits of 196 accessions of African yam bean evaluated during the 2018 and 2019 cropping seasons at three agro-ecologies of Nigeria

Variables	PC1	PC2	PC3	PC4	PC5	PC6
D50F	-0.01	-0.03	0.25	0.13	0.60	0.59
DPM	0.09	0.22	-0.37	0.51	0.36	0.12
GFP	0.10	0.22	-0.49	0.41	-0.02	-0.26
NPPPL	0.33	-0.19	-0.36	-0.13	-0.19	0.17
PW	0.41	-0.18	-0.24	-0.13	-0.14	0.27
SP	0.24	-0.07	0.04	-0.28	0.43	-0.26
PDL	0.12	-0.10	0.27	0.47	-0.36	0.28
NLPPD	0.23	-0.42	0.24	0.30	0.05	-0.24
NSPPD	0.23	-0.43	0.21	0.27	0.06	-0.30
HSW	0.31	0.36	0.20	-0.08	-0.04	0.01
SY	0.41	-0.18	-0.19	-0.21	0.07	0.22
SL	0.20	0.32	0.25	0.07	-0.31	0.22
SW	0.35	0.33	0.20	0.00	0.01	-0.15
ST	0.32	0.30	0.15	-0.03	0.16	-0.24
Eigenvalue	3.75	2.49	2.12	1.46	1.23	1.08
Proportion %	26.8	17.7	15.2	10.4	8.8	7.7
Cumulative %	26.8	44.5	59.7	70.1	78.9	86.6

Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST); Principal Component (PC).

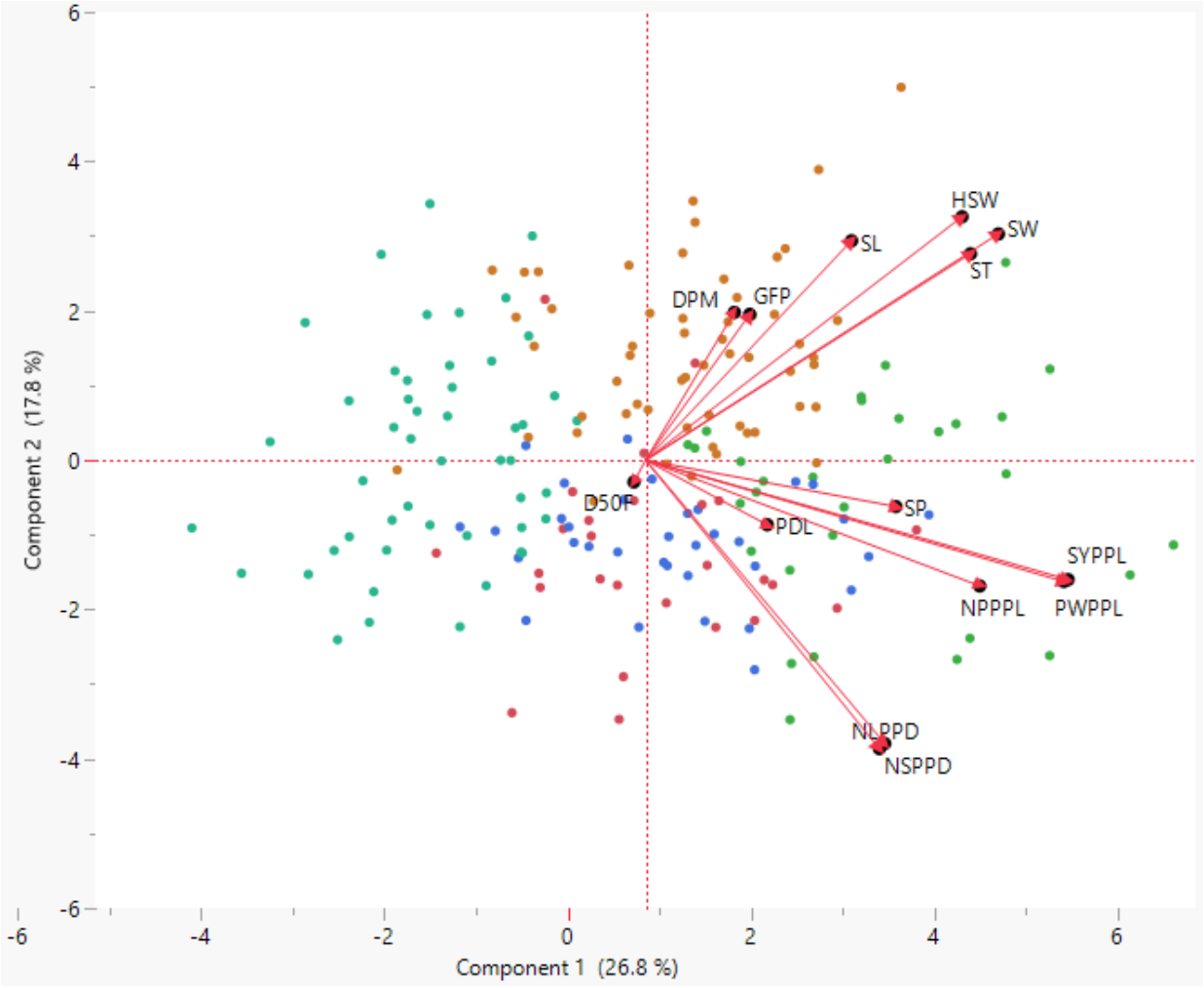

Figure 4.1. PCA biplot of 14 agronomic traits of 196 accessions of African yam bean evaluated during the 2018 and 2019 cropping seasons in three agro-ecologies of Nigeria. Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight per plant (PWPPL); Shelling percentage (SP); Pod length (PDL); Seed yield per plant (SYPPL); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST). The colored points show the distribution of accessions into 5 different clusters

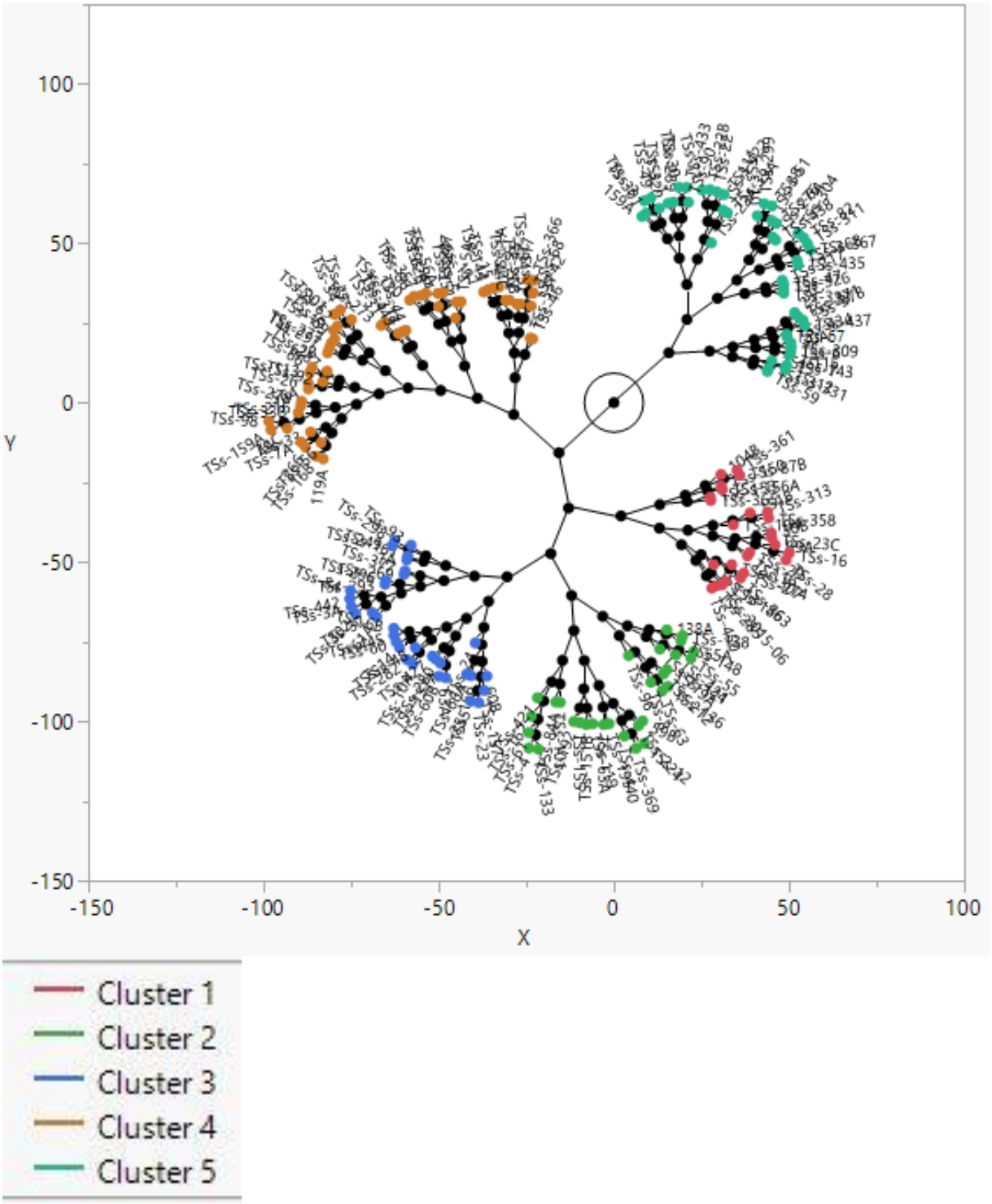

Figure 4.2. A constellation plot depicting genetic relatedness among 196 accessions of African yam bean evaluated during 2018 and 2019 cropping season in three agro-ecologies of Nigeria

The number and proportion of accessions in each cluster were 26 (13.3%) in cluster I, 31 (15.8%) in cluster II, 34 (17.4%) in cluster III, 54 (28.6%) in cluster IV and 49 (25.0%) in cluster V (Table 4.6). Cluster I comprised of accessions that were early maturing, with short grain filling period, high shelling percentage, long pods, high number of seeds and locules per pod. Cluster II consisted of accessions with early flowering, high number of pods per plant, heavy pods and seeds weight, high seed yield, width and thickness. Accessions in cluster III had the longest grain filling period. Cluster IV comprised of accessions that were late maturing with long seeds and high seed thickness, while accessions in cluster V have low number of pods per plant, pod weight, shelling percentage, locules and seeds per pod, hundred seeds, seed yield, short pods, and small seed size (Table 4.6). Members of each clusters are presented in Appendix 4.

4.1.5 Yield Selection Indices

4.1.5.1 Relationships among Traits

The extents of association among paired traits of AYB accessions across the six environments are presented in Table 4.7. For nearly all paired traits, genotypic correlation coefficients were higher than those of their corresponding phenotypic values. Except for days to 50 % flowering, grain filling period, pod length and seed length all the other traits had significant positive phenotypic correlations with seed yield. Number of pods per plant (r_p= 0.7**) and pod weight (r_p= 0.91**) had significant high phenotypic correlation coefficients with seed yield. Positive significant genotypic correlations were recorded between seed yield and all other traits except pod and seed length. Pod length (r_g=-0.44**) had significant negative relationship with seed yield (Table 4.7).

Days to 50% flowering had a significant positive phenotypic association with only days to pod maturity (r_p=0.21**). Positive genotypic associations were recorded between days to 50% flowering and days to pod maturity, shelling percentage, pod length, number of locus per pod and number of seeds per. Grain filling period (r_p=-0.78**; r_g=-0.53**) and pod weight (r_p=-0.14*; r_g=-0.32**) had positive phenotypic and genotypic correlation with days to pod maturity. Pod maturity also had positive genetic correlation with shelling percentage, pod length and number of locus per pod phenotypic correlation with number of pods per plant. Grain filling period showed significant genotypic and phenotypic positive relationship with number of pods per plant (r_p=-0.53**; r_g=-0.27**) and pod weight (r_p=-0.4**; r_g=-0.19**) (Table 4.7).

Table 4.6. Cluster means and standard deviation of the 196 accessions of African yam bean based on 14 agronomic traits

Trait	Cluster I (26)	Cluster II (31)	Cluster III (34)	Cluster IV (56)	Cluster V (49)
D50F	90.69±3.95	88.22±1.86	88.54±2.48	88.26±2.45	89.04±2.4
DPM	148.25±4.91	151.62±4.25	155.13±2.68	155.56±3.44	151.69±3.26
GFP	58.03±2.93	63.97±4.67	66.65±3.18	66.26±3.03	62.66±3.99
NPPPL	8.56±1.52	11.04±2.19	10.5±2.01	8.81±1.47	7.99±1.46
PW	30.88±5.01	41.4±6.67	35.91±6.14	31.18±4.23	26.88±4.22
SP	46.05±3.10	45.53±3.17	44.16±2.91	43.97±4.98	41.15±3.58
PDL	21.15±1.01	20.83±0.92	21.0±0.73	20.72±1.15	20.11±1.14
NLPPD	13.14±0.74	12.99±0.99	12.8±0.62	12.05±0.66	11.64±0.74
NSPPD	12.16±0.80	12.13±0.97	11.82±0.56	11.1±0.69	10.73±0.76
HSW	20.37±1.60	21.81±1.32	19.71±1.10	21.6±1.60	19.37±1.49
SY	15.04±3.13	20.23±4.11	16.54±2.59	14.47±2.75	11.95±2.15
SL	7.95±0.28	8.06±0.24	7.90±0.20	8.15±0.25	7.83±0.27
SW	6.19±0.11	6.34±015	6.08±0.12	6.31±0.14	6.04±0.15
ST	5.98±0.20	6.12±0.19	5.82±0.18	6.12±0.19	5.81±0.18

Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locus per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST). Cluster population in parenthesis.

Number of pods per plant had significant phenotypic and genotypic correlation coefficient with pod weight and shelling percentage and significant positive phenotypic correlation with number of seeds per pods and number of locus per pod. Pod length and the three seed metric traits showed significant negative correlation coefficient with number of pods per plant. There were also significant phenotypic and genotypic association between pod weight per plant and shelling percentage, number of locules per pod, 100-seeds weight, seed width and seed thickness and only significant phenotypic association with number of seeds per pod. The shelling percentage had significant negative relationship with pod and seed length. Pod length also has significant positive genotypic and phenotypic seed length. One hundred seed weight had significant positive phenotypic and genotypic relationships with the three seed metric traits. The seed metric traits had significant positive relationships with one another both genotypically and phenotypically.

4.1.5.2 Path Coefficient Analysis

Direct and indirect effects of thirteen agronomic traits on grain yield for combined location is presented in Table 4.8. Days to pod maturity (1.493), pod weight (0.839), shelling percentage (0.389), number of seeds per pod (0.155), 100-seed weight (0.012) and seed thickness (0.017) had positive direct effect on seed yield. Negative direct effects on seed yield were recorded in days to flowering (-1.452), grain filling period (-1.757) number of pod per plant (-0.29), pod length (-0.014), number of locus per pod (-0.109), seed length (-0.061) and seed width (-0.012) (Table 4.8).

Days to flowering had positive indirect effects on seed yield through days to pod maturity (0.5229), shelling percentage (0.1115), number of seeds per pod (0.0473), 100-seed weight (0.0011) and seed thickness (0.0015). Grain filling period had positive indirect influence on yield through days to pod maturity (0.7956), pod weight (0.3383), shelling percentage (0.0483), 100-seeds weight (0.0006) and seed thickness (0.0009). Number of pods per plant positively contributed indirectly to seed yield through days to pod maturity (0.0216), pod weight (0.5950), shelling percentage (0.3040) and number of seeds per pod (0.0014). Pod length also had positive indirect effect on seed yield through days to pod maturity (0.3062) and 100-seeds weight (0.0013). Number of locules per pods positively influenced seed yield indirectly through days to pod maturity (0.3063), pod weight (0.1602), shelling percentage (0.1197), number of seeds per pod (0.1510), and

Table 4.7. Genotypic and phenotypic correlation coefficients among 14 traits of 196 accessions of African yam bean evaluated during 2018 and 2019 cropping season in three agro-ecologies of Nigeria

Traits		D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SL	SW	ST
DPM	rg	0.35**												
	rp	0.21**												
GFP	rg	-0.61**	0.53**											
	rp	-0.42**	0.78**											
NPPPL	rg	-0.57**	0.01	0.53**										
	rp	-0.2**	0.16*	0.27**										
PW	rg	-0.15*	0.32**	0.40**	0.71**									
	rp	-0.09	0.14*	0.19**	0.83**									
SP	rg	0.29**	0.40**	0.12	0.78**	0.70**								
	rp	0.08	0.06	0.01	0.21**	0.21**								
PDL	rg	0.35**	0.21**	-0.14	-0.33**	-0.23**	-0.83**							
	rp	0.12	-0.003	-0.08	0.02	0.12	-0.16*							
NLPPD	rg	0.48**	0.21**	-0.2	0.12	0.19**	0.31**	0.01						
	rp	0.14	0.02	-0.06	0.18**	0.26**	0.21**	0.39**						
NSPPD	rg	0.31**	0.09	-0.14	0.01	0.11	0.27**	-0.08	0.97**					
	rp	0.08	0.02	-0.02	0.18*	0.27**	0.23**	0.33**	0.94**					
HSW	rg	0.09	0.12	0.05	-0.18*	0.26**	0.28**	0.11	-0.27**	-0.28**				
	rp	0.05	0.11	0.08	0.05	0.21**	0.26**	0.12	-0.06	-0.07				
SL	rg	0.03	0.15*	0.09	-0.16*	0.07	-0.21**	0.42**	-0.25**	-0.36**	0.73**			
	rp	0.05	0.1	0.06	-0.01	0.09	0.02	0.30**	-0.07	-0.13	0.6**			
SW	rg	0.01	0.07	0.07	-0.16*	0.25**	0.43**	0.04	0.06	-0.01	0.86**	0.50**		
	rp	0.01	0.12	0.11	0.1	0.24**	0.23**	0.11	0.11	0.08	0.68**	0.54**		
ST	rg	0.09	0.09	0.05	-0.20**	0.28**	0.50**	-0.02	0.14	0.15*	0.70**	0.19**	0.82**	
	rp	0.01	0.11	0.11	0.05	0.22**	0.26**	0.02	0.08	0.09	0.61**	0.3**	0.80**	
SY	rg	0.28**	0.45**	0.15*	0.53**	0.89**	0.76**	-0.44**	0.26**	0.2**	0.29**	-0.06	0.36**	0.41**
	rp	0.03	0.15*	0.12	0.70**	0.91**	0.44**	0.02	0.27**	0.28**	0.26**	0.05	0.26**	0.28**

*, **, *** significant at 0.05, 0.01 and 0.001 levels of probability, respectively.

Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locus per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

seed thickness (0.0023). Seed length had positive indirect contribution to seed yield through days to pod maturity (0.2191), pod weight (0.0557), 100-seed weight (0.0087), and seed thickness (0.0032). Seed width had positive indirect effect on seed yield through days to pod maturity (0.0983), pod weight per plant (0.2117), shelling percentage (0.1672), 100-seed weight (0.0102), and seed thickness (0.0137). A residual value of 0.30 was recorded (Table 4.8).

4.1.5.3 Yield Stability Index

The AMMI analysis of variance of seed yield revealed highly significant ($P \leq 0.01$) variations among accessions, environments, interaction and Interaction Principal Component (IPC) 1, 2 and 3 (Table 4.9). Accession significantly explained 9.24% of the total sum of square while environment and the accessions × environment interaction contributed 53.43% and 37.34% respectively. By partitioning the interaction term through the AMMI model, the first three multiplicative terms (PC1, PC2 and PC3) of AMMI significantly explained 51.04%, 24.15% and 12.15% of the interaction sum of squares.

In Table 4.10, TSs-143 (0.018) followed by TSs-280 (0.03), 138A (0.039), TSs-84 (0.05), TSs-69 (0.053), TSs-157A (0.057), TSs-119 (0.058), 151B (0.061), TSs-361 (0.063) and TSs-22B (0.065) had the lowest ASV. Accession TSs-195 had the highest ASV of 2.139. Accessions TSs-119 (12), TSs-101 (22), 138A (29), TSs-4 (39), TSs-157A (39) and TSs-61 (49) were the top-ranking accessions based on YSI, integrating low ASV and high mean seed yield per plant. Accessions TSs-421 (93) and TSs-195 (102) had the highest mean seed yield per plant and high ASV. Accession TSs-143, though had lowest ASV, had a mean seed yield that is below the grand mean. Accessions like TSs-104, TSs-363, TSs-29, TSs-278, TSs-19, TSs-443 and TSs-11 are low-yielding accessions with high ASV (Table 4.10).

4.2 Marker-traits association

Seed yield had high significant positive correlations coefficients with number of pod per plant (0.70***), pod weight (0.91***), and shelling percentage (0.44***). Similarly, seed length (0.61***), seed width (0.68***), seed thickness (0.60***) with 100-seed weight. Days to pod maturity and grain filling period (0.81***), number of pod per plant and pod weight (0.83***), number of lucules per plant and number of seeds per plant (0.94***), seed length and seed width (0.54***), seed width and seed thickness (0.80***) exhibited remarkably significant positive correlation coefficients with each other. (Table 4.11). A widespread with near-normal distribution were recorded in all traits used in the GWAS analysis (Figure 4.3).

A total of 5,416 SNPs were generated for the AYB Accessions using DArT sequencing technology. After quality filtering, 2,491 SNPs were retained for GWAS analysis. The first

Table 4.8. Path analysis showing the direct (diagonal bold) and indirect effect of 13 agronomic traits on seed yield of 196 accessions of African yam bean evaluated during the 2018 and 2019 cropping season in three agro-ecologies of Nigeria

Traits	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SL	SW	ST
D50F	**-1.4520**	0.5229	1.0691	0.1644	-0.1230	0.1115	-0.0050	-0.0522	0.0473	0.0011	-0.0021	-0.0001	0.0015
DPM	-0.5085	**1.4934**	-0.9362	-0.0042	0.2706	0.1568	-0.0029	-0.0223	0.0137	0.0015	-0.0090	-0.0008	0.0015
GFP	0.8834	0.7956	**-1.7573**	-0.1526	0.3383	0.0483	0.0019	0.0216	-0.0211	0.0006	-0.0054	-0.0009	0.0009
NPPPL	0.8223	0.0216	-0.9237	**-0.2903**	0.5950	0.3040	0.0046	-0.0135	0.0014	-0.0022	0.0098	0.0020	-0.0034
PW	0.2128	0.4815	-0.7083	-0.2058	**0.8394**	0.2738	0.0033	-0.0207	0.0175	0.0031	-0.0041	-0.0031	0.0046
SP	-0.4160	0.6016	-0.2182	-0.2267	0.5905	**0.3892**	0.0117	-0.0334	0.0424	0.0034	0.0128	-0.0053	0.0084
PDL	-0.5142	0.3062	0.2429	0.0959	-0.1964	-0.3236	**-0.0141**	-0.0010	-0.0131	0.0013	-0.0260	-0.0005	-0.0003
NLPPD	-0.6978	0.3063	0.3490	-0.0360	0.1602	0.1197	-0.0001	**-0.1086**	0.1510	-0.0032	0.0152	-0.0007	0.0023
NSPPD	-0.4429	0.1324	0.2395	-0.0026	0.0948	0.1065	0.0012	-0.1057	**0.1550**	-0.0034	0.0222	0.0001	0.0025
HSW	-0.1366	0.1866	-0.0919	0.0531	0.2195	0.1105	-0.0016	0.0297	-0.0447	**0.0118**	-0.0451	-0.0106	0.0116
SL	-0.0499	0.2191	-0.1557	0.0464	0.0557	-0.0809	-0.0059	0.0268	-0.0561	0.0087	**-0.0614**	-0.0062	0.0032
SW	-0.0098	0.0983	-0.1294	0.0468	0.2117	0.1672	-0.0006	-0.0065	-0.0015	0.0102	-0.0306	**-0.0124**	0.0137
ST	-0.1313	0.1379	-0.0914	0.0590	0.2328	0.1954	0.0002	-0.0147	0.0232	0.0083	-0.0118	-0.0102	**0.0166**

Residual = 0.3; Coefficient of determination = 0.91; Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Number of locus per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Table 4.9. AMMI model for seed yield in six environments and the proportion of the total variance attributable to the source of variation

Source of variation	DF	SS	MS	% G*E interaction	% SS
Environments (E)	5	297613	59522.59***		53.43
Accession (G)	195	51460.5	263.9***		9.24
Interaction (G × E)	975	207984.9	213.32***		37.34
IIPCA1	199	100915.6	507.11***	51.04	
IPCA2	197	47745.97	242.37***	24.15	
IPCA3	195	24852.99	127.45**	12.57	
Residuals	2271	333636	146.91		

, * significant at P-value <0.01 and < 0.001 respectively
DF = the degree of freedom; SS, the sum of the square; MS, mean square.

Table 4.10. Mean seed yield (g/plant), AMMI Stability Value (ASV), Yield Stability Indices (YSI) and their rank for selected accessions of AYB evaluated in six environments

S/N	ACCESSION	YLD	IPCA1	IPCA2	ASV	ASVR	YLDR	YSI	YSIR
1	TSs-119	25.67	0.01405	0.04952	0.05774	7	5	12	1
2	TSs-101	24.06	0.03266	-0.04275	0.08119	14	8	22	2
3	138A	19.23	0.01680	0.01530	0.03866	3	26	29	3
4	TSs-4	19.78	-0.03480	-0.04808	0.08787	16	23	39	4
5	TSs-157A	18.59	-0.00293	0.05649	0.05683	6	33	39	5
6	TSs-61	18.52	-0.03102	-0.05082	0.08296	15	34	49	6
7	TSs-280	16.15	0.01347	-0.01080	0.03046	2	76	78	9
8	151B	15.94	-0.00952	-0.05725	0.06068	8	80	88	14
9	TSs-361	15.09	0.02313	-0.03956	0.06288	9	94	103	22
10	TSs-84	14.30	-0.02220	-0.01895	0.05060	4	108	112	26
11	TSs-69	13.85	-0.00965	0.04951	0.05355	5	117	122	32
12	TSs-22B	13.50	-0.01413	-0.05783	0.06509	10	126	136	44
13	TSs-143	11.10	-0.00377	0.01640	0.01823	1	168	169	66
14	TSs-421	31.63	-0.85188	-0.72254	1.94010	194	1	195	93
15	TSs-195	27.54	-1.00000	0.33151	2.13943	196	2	198	102
16	TSs-11	9.94	0.27514	0.24162	0.62973	156	183	339	190
17	TSs-443	7.46	0.26875	0.13130	0.58301	148	195	343	191
18	TSs-19	11.16	-0.22291	-0.60921	0.77014	179	167	346	192
19	TSs-278	11.10	0.36462	-0.05294	0.77247	180	169	349	193
20	TSs-29	9.80	0.32886	0.18443	0.71913	170	186	356	194
21	TSs-363	10.37	-0.43499	0.36008	0.98738	188	177	365	195
22	TSs-104	8.83	0.41745	0.08941	0.88684	186	191	377	196
	Mean	15.20							

YLD = mean seed yield; ASVR = AMMI stability value rank; YLDR = mean seed yield per plant rank; YSIR = yield stability index rank; IPCA = interaction principal component analysis.

Table 4.11. Pearson correlation coefficient among traits of 195 accessions of African yam bean evaluated during the 2018 and 2019 cropping season in three agro-ecologies of Nigeria

Traits	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW
DPM	0.20**												
GFP	-0.35***	0.81**											
NPPPL	-0.21**	0.16*	0.25***										
PW	-0.09	0.14*	0.18*	0.83***									
SP	0.09	0.06	0.02	0.21**	0.21**								
PDL	0.13	-0.005	-0.08	0.01	0.11	-0.16*							
NLPPD	0.14*	0.02	-0.06	0.18*	0.26***	0.21**	0.39***						
NSPPD	0.09	0.02	-0.03	0.18*	0.27***	0.23**	0.33***	0.94***					
HSW	0.03	0.11	0.12	0.05	0.22**	0.26***	0.12	-0.06	-0.07				
SY	0.03	0.15*	0.12	0.70***	0.91***	0.44***	0.024	0.27***	0.28***	0.26***			
SL	0.04	0.10	0.10	0.001	0.09	0.02	0.31***	-0.08	-0.13	0.61***	0.05		
SW	-0.002	0.13	0.12	0.12	0.24***	0.23**	0.12	0.12	0.08	0.68***	0.26***	0.54***	
ST	0.01	0.11	0.09	0.05	0.22**	0.27***	0.02	0.09	0.09	0.60***	0.28***	0.30***	0.80***

Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

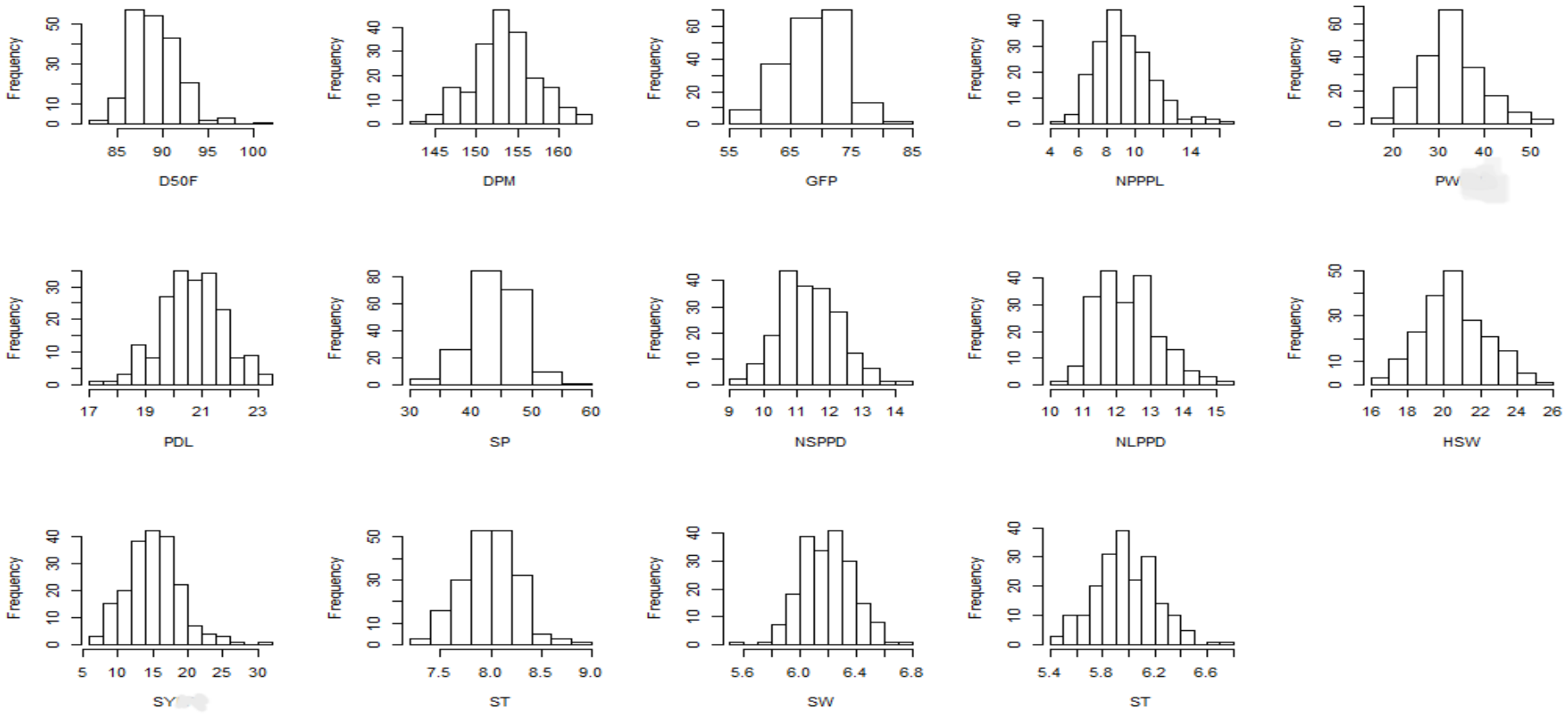

Figure 4.3. Histogram of the best linear unbiased estimates for the fourteen agronomic traits use for GWAS. Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

three PCs account for 5.9, 4.8. and 3.7 % of the variation among the AYB accessions, respectively. No clear clustering can be deduced among the accessions based on the PCA (Figure 4.4). For combined location GWAS analysis, 24 markers were found to be significantly associated with eleven different traits at a threshold of –log (p) = 4 (Table 4.12). Nine were associated with days to flowering, four with seed thickness, three each with each of number of locules per pod, number of seeds per pod and seed width, two with grain filling period and shelling percentage while one each with 100-seeds weight, number of pods per plant, pod weight and seed length. The trait variation accounted for by each significant marker R^2 varied from 7.1 in seed thickness to 12.8 % in number of locules per pod. No significant marker was associated with seed yield per plant. Six pleiotropic makers were associated with highly correlated traits. Markers 100009412|F|0-67:G>A-67:G>A, 29423119|F|0-32:A>T-32:A>T and 29422706|F|0-34:C>T-34:C>T were associated with number of lucules per pods and number of seeds per pods. Markers 29420334|F|0-52:C>G-52:C>G and 29420736|F|0-57:G>T-57:G>T were associated with seed width and seed thickness, while 29420888|F|0-53:C>T-53:C>T was associated with pod weight and number of pod per plant (Table 4.12). Manhattan and Q-Q plots of the SNP-based associations mapping for the eleven traits are presented in Figures 4.5 and 4.6. The observed p-values for all traits aligned with expected p-values as shown by the Q-Q plots. Significant markers at the set threshold are those above the blue lines in the Manhattan plots

Over the two years, 32 significant markers were associated with eight traits in Ibadan. These markers explained 5.8 to 10.9 % of the observed traits variation. Twenty-seven markers displayed significant associations with thirteen traits in Kano. Variance explained by these markers ranged from 6.7 to 14.4 %. Forty-nine makers were significantly associated with eleven traits in Ubiaja. The contribution of all the markers to the phenotypic variation ranged between 7.3 and 13.1 %. No single marker overlap for the same trait across the three locations However, 17 markers were consistently significant in one location and combine location analysis for the same traits. Twenty-six pleiotropic markers were found for different correlated traits in the three locations. Chromosome position was ascribed zero because AYB has no reference genome yet (Tables 4.13, 4.14 and 4.15).

After blast search was performed for trimmed nucleotides sequenced of the 24 significant AYB makers in the combined location analysis on *Phaseolus vulgaris* G19833 genome v2.0, fifteen markers associated with nine traits were found in common. These

trimmed nucleotides sequenced of significant AYB makers were found in chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 10 and 11 *of Phaseolus vulgaris*. Three of the markers were in chromosome 3, and two each in chromosomes in 1, 2 and 7. The blast search reveals that several genes whose encoding protein products are known to regulate traits of interest were located close to these markers at less than 500 kbp. These genes had been reported in *Phaseolus vulgaris* and *Glycine max* genome (Table 4.16). The nucleotide sequence of these markers is presented in Appendix 5.

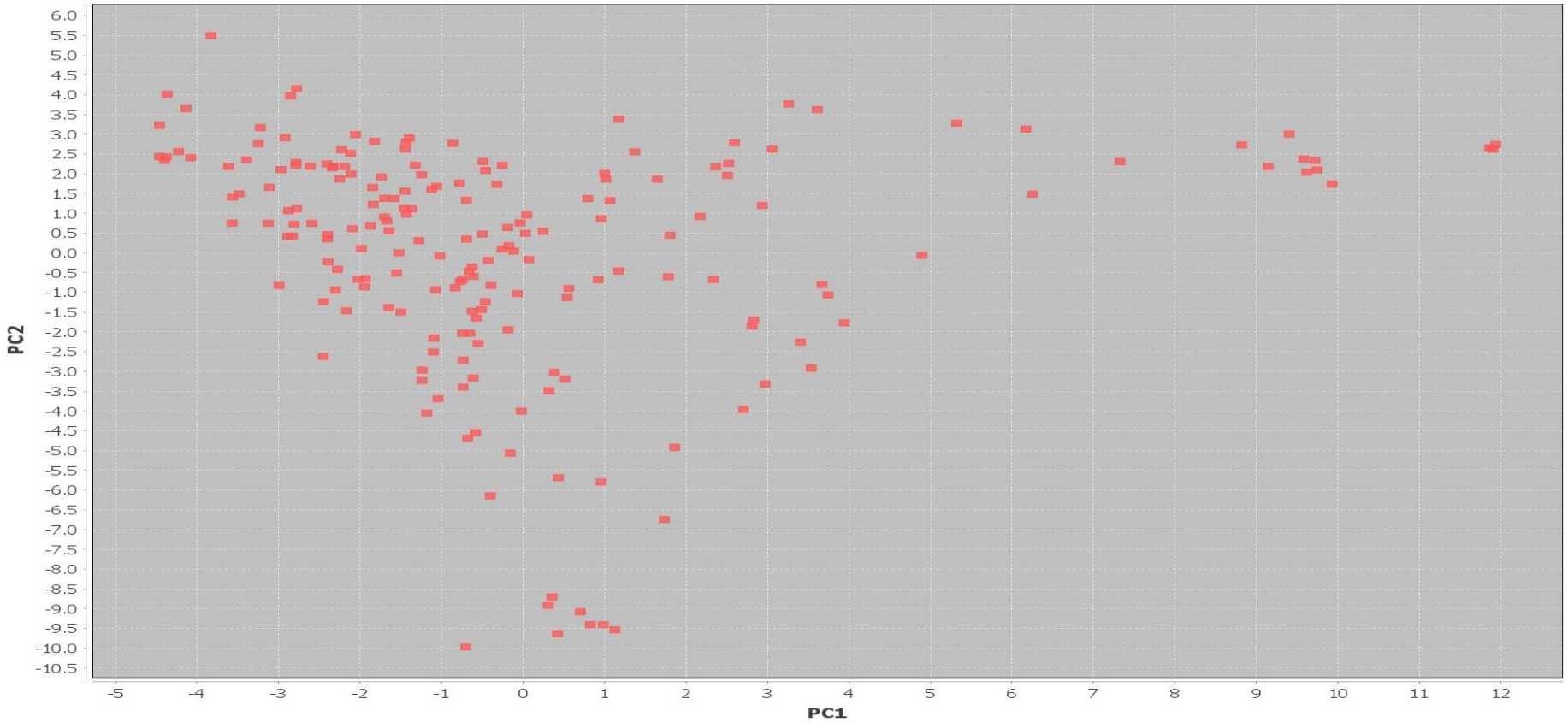

Figure 4.4. Biplot of PC1 against PCs 2 depicting population structure in 195 AYB accessions genotyped with the DarTseq SNPs marker

Table 4.12. DArTseq SNPs markers having significant association with agronomic traits of 195 accessions of African yam bean evaluated during the 2018 and 2019 cropping seasons in three agro-ecologies of Nigeria

S/N	Trait	Marker	Positions	P-value	Marker R^2
1	D50F	100005571|F|0-66:C>A-66:C>A	2088	1.05E-04	0.0956
2	D50F	29423419|F|0-65:A>T-65:A>T	1988	1.11E-04	0.1018
3	D50F	100003791|F|0-8:C>T-8:C>T	21	1.51E-04	0.0917
4	D50F	100003301|F|0-12:C>T-12:C>T	2232	1.59E-04	0.0925
5	D50F	29422735|F|0-21:G>A-21:G>A	1614	3.69E-04	0.0820
6	D50F	29419972|F|0-19:G>T-19:G>T	282	4.63E-04	0.0795
7	D50F	29420809|F|0-38:T>A-38:T>A	2408	5.46E-04	0.0778
8	D50F	29423359|F|0-48:C>T-48:C>T	1960	7.28E-04	0.0884
9	D50F	100026403|F|0-5:G>T-5:G>T	176	8.28E-04	0.0733
10	GFP	29423446|F|0-9:C>T-9:C>T	2002	1.61E-04	0.0750
11	GFP	29420466|F|0-41:C>T-41:C>T	605	5.70E-04	0.0779
12	HSW	29421549|F|0-25:A>C-25:A>C	1124	8.76E-04	0.0754
13	NLPPD	100009412|F|0-67:G>A-67:G>A	118	1.25E-05	0.1280
14	NLPPD	29423119|F|0-32:A>T-32:A>T	1836	1.61E-04	0.1162
15	NLPPD	29422706|F|0-34:C>T-34:C>T	1600	6.61E-04	0.0790
16	NPPPL	29420888|F|0-53:C>T-53:C>T	844	5.83E-05	0.1064
17	NSPPD	100009412|F|0-67:G>A-67:G>A	118	7.31E-05	0.1059
18	NSPPD	29423119|F|0-32:A>T-32:A>T	1836	3.11E-04	0.1053
19	NSPPD	29422706|F|0-34:C>T-34:C>T	1600	7.56E-04	0.0767
20	PWPPL	29420888|F|0-53:C>T-53:C>T	844	3.71E-04	0.0887
21	SL	29420365|F|0-55:C>G-55:C>G	2152	8.84E-04	0.0769
22	SP	100024379|F|0-68:C>A-68:C>A	151	1.49E-04	0.1109
23	SP	29420331|F|0-29:T>C-29:T>C	522	6.52E-04	0.0796
24	ST	100034480|F|0-31:C>A-31:C>A	216	3.42E-05	0.1036
25	ST	29420736|F|0-57:G>T-57:G>T	2364	9.84E-05	0.0925
26	ST	29420334|F|0-52:C>G-52:C>G	525	7.09E-04	0.0719
27	ST	29420680|F|0-49:T>G-49:T>G	729	7.61E-04	0.0712
28	SW	29420736|F|0-57:G>T-57:G>T	2364	3.63E-05	0.1084
29	SW	29421428|F|0-9:C>A-9:C>A	1088	2.08E-04	0.0891
30	SW	29420334|F|0-52:C>G-52:C>G	525	7.94E-04	0.0746

Days to flowering (D50F); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Number of locus per pod (NLPPD); Number of seeds per pod (NSPPD); Seed length (SL); Seed width (SW); Seed thickness (ST).

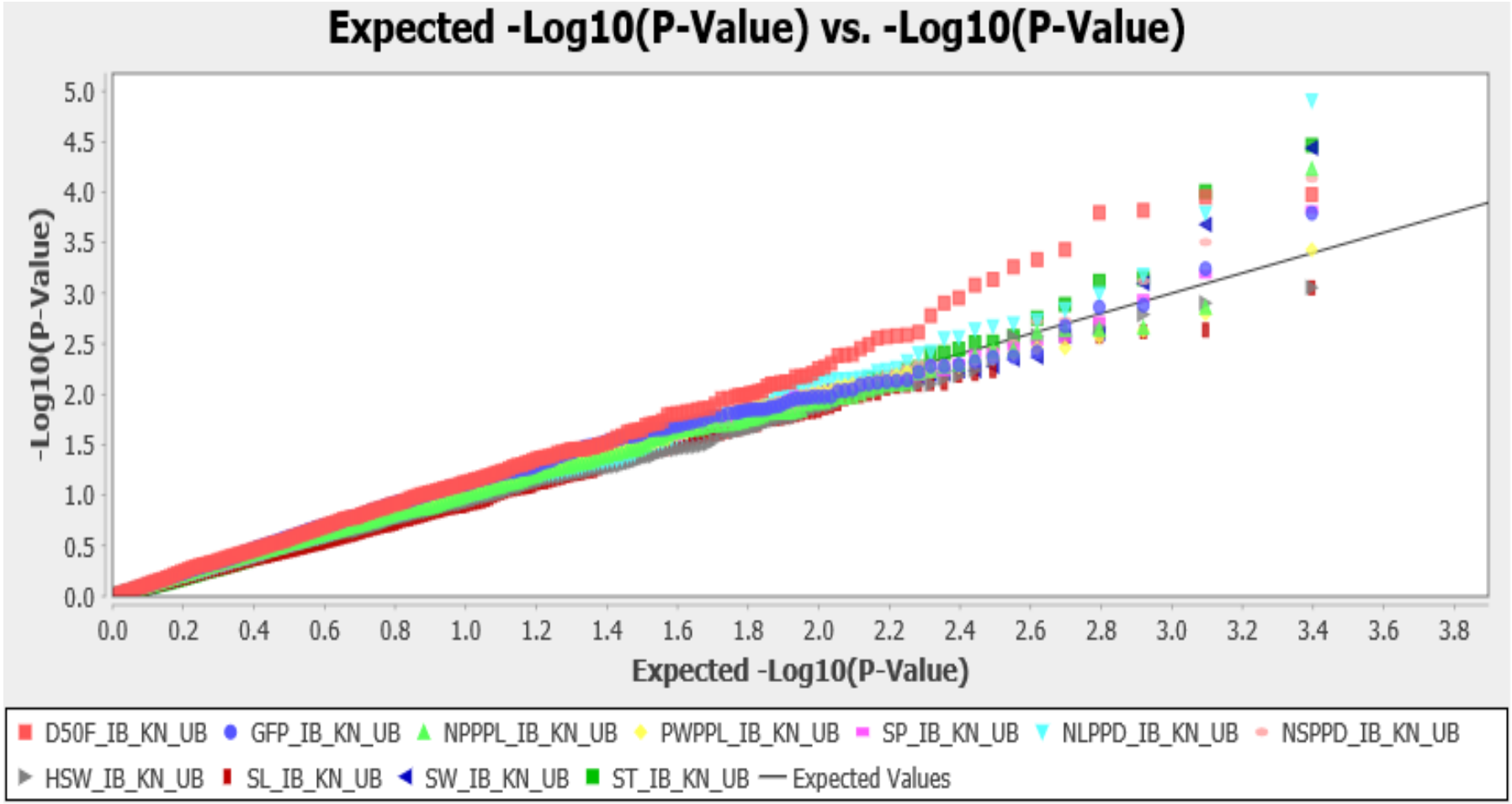

Figure 4.5. The Q-Q plot of the DArTseq SNP-based associations mapping for eleven agronomic traits. Days to flowering (D50F_IB_KN_UB); Grain filling period (GFP_IB_KN_UB); Number of pods per plant (NPPPL_IB_KN_UB); Pod weight per plant (PWPPL_IB_KN_UB); Shelling percentage (SP_IB_KN_UB); Number of locules per pod (NLPPD_IB_KN_UB); Number of seeds per pod (NSPPD_IB_KN_UB); 100-seeds weight (HSW_IB_KN_UB); Seed length (SL_IB_KN_UB); Seed width (SW_IB_KN_UB); Seed thickness (ST_IB_KN_UB)

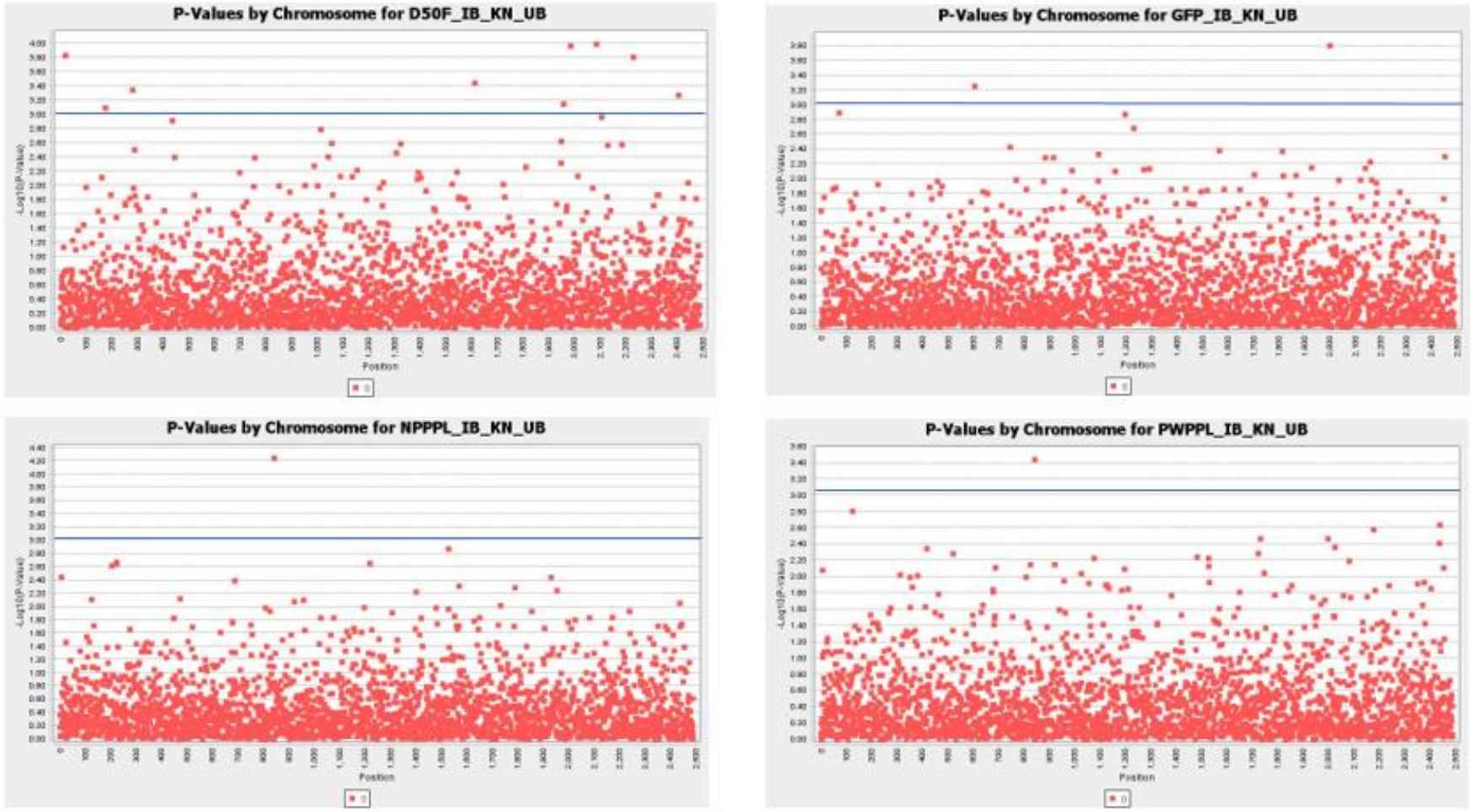

Figure 4.6. The Manhattan plot of the DArTseq SNP-based associations mapping showing significant markers at a p-value threshold of –log (p) = 4. Days to flowering (D50F_IB_KN_UB); Grain filling period (GFP_IB_KN_UB); Number of pods per plant (NPPPL_IB_KN_UB); Pod weight per plant (PWPPL_IB_KN_UB

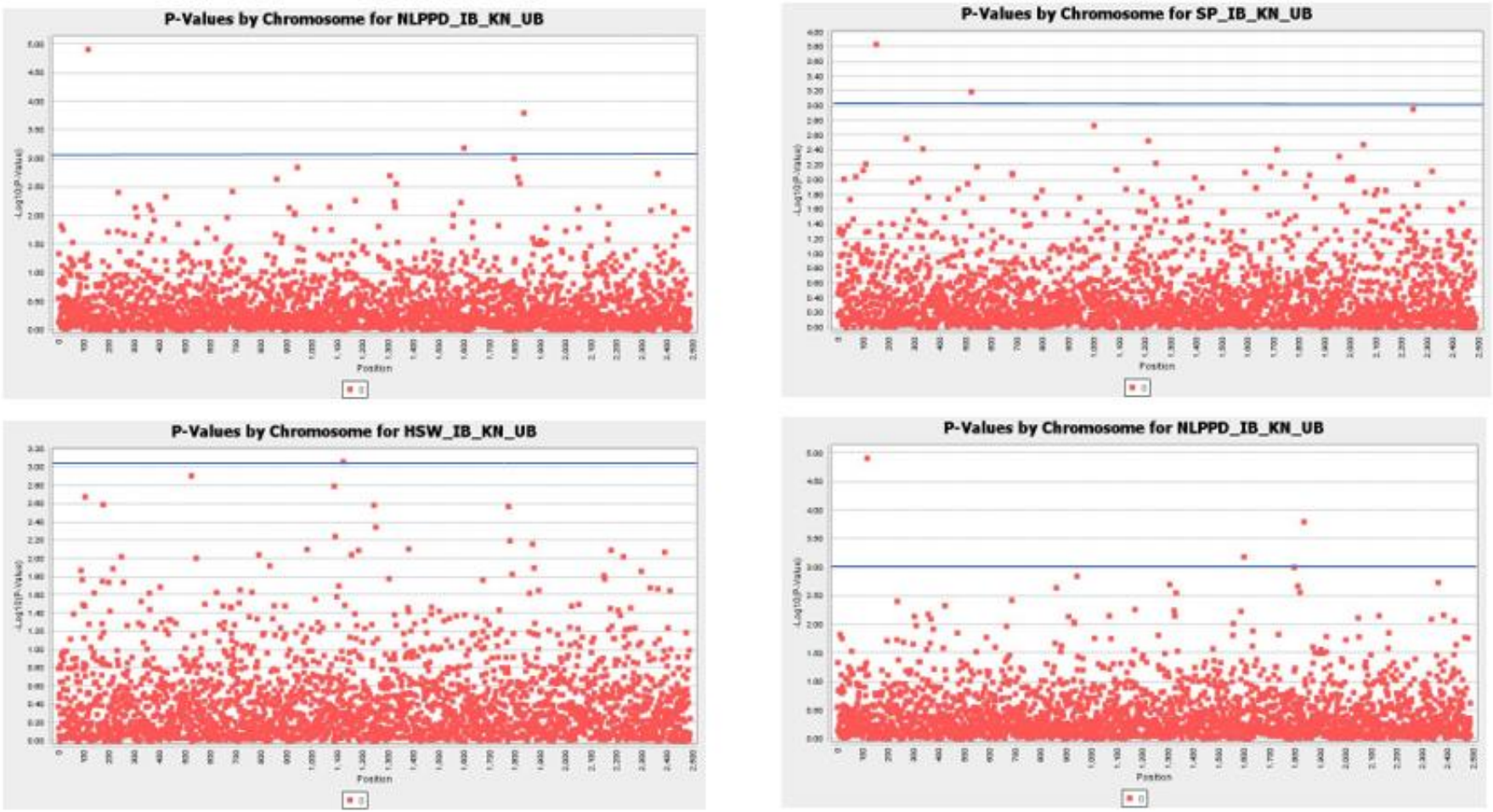

Figure 4.6. Continued. Shelling percentage (SP_IB_KN_UB); Number of locules per pod (NLPPD_IB_KN_UB); Number of seeds per pod (NSPPD_IB_KN_UB); 100-seeds weight (HSW_IB_KN_UB)

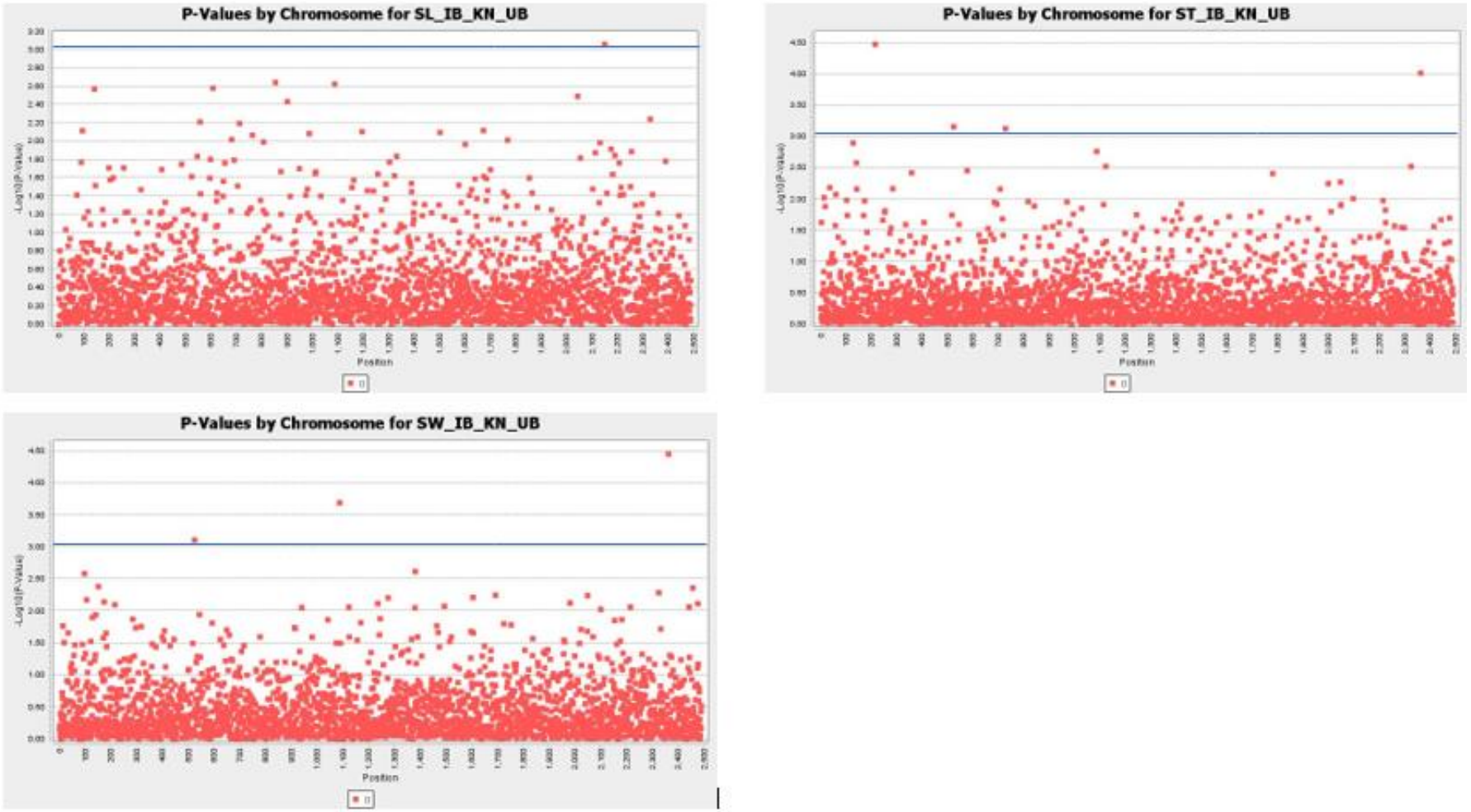

Figure 4.6. Continued. Seed length (SL_IB_KN_UB); Seed width (SW_IB_KN_UB); Seed thickness (ST_IB_KN_UB)

Table 4.13. DArTseq SNPs markers having significant association with 10 agronomic traits of 195 accessions of African yam bean evaluated during the 2018 and 2019 cropping seasons in Ibadan

SN	Traits	Markers	Positions	P-value	Marker R^2
1	DPM	29420294|F|0-18:T>G-18:T>G	500	8.92E-04	0.0583
2	GFP	29420332|F|0-44:A>G-44:A>G	523	9.10E-04	0.0771
3	GFP	100003503|F|0-19:G>A-19:G>A	13	1.40E-04	0.0783
4	GFP	29423028|F|0-16:T>G-16:T>G	1784	6.84E-04	0.0785
5	GFP	29423049|F|0-7:G>A-7:G>A	1799	7.48E-04	0.0834
6	HSW	100025280|F|0-62:G>T-62:G>T	2155	8.85E-04	0.0589
7	HSW	29423506|F|0-18:A>C-18:A>C	2041	4.18E-04	0.0894
8	HSW	29422234|F|0-7:G>C-7:G>C	2383	2.38E-04	0.0974
9	NLPPD	29422224|F|0-23:A>C-23:A>C	2095	9.85E-04	0.0754
10	NLPPD	29422698|F|0-33:C>T-33:C>T	1596	5.08E-04	0.0872
11	NLPPD	29421172|F|0-41:T>C-41:T>C	1009	5.19E-05	0.1091
12	NPPPL	29423489|F|0-22:T>A-22:T>A	2027	8.41E-04	0.0767
13	NSPPD	29421172|F|0-41:T>C-41:T>C	1009	3.21E-04	0.0873
14	PDL	29420240|F|0-61:T>A-61:T>A	464	2.63E-04	0.0896
15	PW	29423037|F|0-63:C>T-63:C>T	1790	3.81E-04	0.0684
16	PW	29421263|F|0-22:G>T-22:G>T	1057	9.80E-04	0.0752
17	PW	29422908|F|0-19:T>A-19:T>A	1713	7.94E-04	0.0775
18	PW	100018322|F|0-16:G>A-16:G>A	2379	6.10E-04	0.0805
19	PW	29420355|F|0-37:C>T-37:C>T	2180	2.95E-04	0.0888
20	SL	100033556|F|0-45:A>G-45:A>G	204	6.06E-04	0.0775
21	SL	29420902|F|0-48:C>G-48:C>G	855	2.37E-04	0.0890
22	ST	29420577|F|0-18:C>T-18:C>T	671	7.39E-04	0.0769
23	ST	29420736|F|0-57:G>T-57:G>T	2364	6.79E-04	0.0778
24	ST	100006540|F|0-20:T>G-20:T>G	70	2.52E-04	0.0889
25	ST	29421256|F|0-15:A>G-15:A>G	2225	3.50E-04	0.0897
26	SW	29420997|F|0-39:T>G-39:T>G	913	8.35E-04	0.0760
27	SW	29420035|F|0-28:C>A-28:C>A	326	8.22E-04	0.0760
28	SW	100003047|F|0-64:T>A-64:T>A	5	7.58E-04	0.0769
29	SW	100006540|F|0-20:T>G-20:T>G	70	6.84E-04	0.0781
30	SW	29420636|F|0-34:G>A-34:G>A	698	6.01E-04	0.0804
31	SW	29420560|F|0-12:A>G-12:A>G	660	3.36E-04	0.0860
32	SW	29422384|F|0-12:C>A-12:C>A	1475	2.74E-04	0.0883
33	SW	29423121|F|0-28:G>C-28:G>C	1837	2.56E-04	0.0891
34	SW	29421951|F|0-37:A>C-37:A>C	2187	8.77E-05	0.1061

Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Pod length (PDL); Number of locus per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Table 4.14. DArTseq SNPs markers having significant association with 13 agronomic traits of 195 accessions of African yam bean evaluated during the 2018 and 2019 cropping seasons in Kano

SN	Traits	Markers	Positions	P-value	Marker R^2
1	D50F	29420334|F|0-52:C>G-52:C>G	525	9.67E-04	0.0738
2	D50F	29423359|F|0-48:C>T-48:C>T	1960	1.21E-05	0.1444
3	DPM	29423446|F|0-9:C>T-9:C>T	2002	3.71E-04	0.0671
4	DPM	100008504|F|0-45:T>G-45:T>G	2380	1.82E-04	0.0902
5	GFP	29423446|F|0-9:C>T-9:C>T	2002	2.38E-04	0.0690
6	HSW	29420330|F|0-48:C>T-48:C>T	521	9.04E-04	0.0758
7	HSW	29422320|F|0-37:T>C-37:T>C	2257	4.17E-04	0.0851
8	HSW	29422467|F|0-10:G>C-10:G>C	1503	3.02E-04	0.0882
9	NLPPD	29422453|F|0-13:A>T-13:A>T	1494	8.11E-04	0.0761
10	NLPPD	29422807|F|0-31:G>A-31:G>A	2163	4.67E-04	0.0822
11	NLPPD	29423255|F|0-27:A>C-27:A>C	1905	2.57E-04	0.0889
12	NPPPL	29420438|F|0-65:T>C-65:T>C	589	1.02E-04	0.1216
13	NPPPL	100036805|F|0-42:C>T-42:C>T	225	3.46E-06	0.1396
14	PDL	29420640|F|0-24:T>C-24:T>C	702	5.67E-04	0.0867
15	PDL	29420950|F|0-66:C>A-66:C>A	882	1.56E-04	0.0969
16	PDL	29419947|F|0-52:T>C-52:T>C	267	6.60E-05	0.1064
17	PW	100036474|F|0-5:G>C-5:G>C	224	6.50E-04	0.0800
18	PW	100034020|F|0-56:T>A-56:T>A	206	5.99E-04	0.0825
19	PW	29421814|F|0-15:G>T-15:G>T	1197	3.51E-04	0.0929
20	PW	100036805|F|0-42:C>T-42:C>T	225	9.19E-05	0.1024
21	SL	29422320|F|0-37:T>C-37:T>C	2257	8.20E-04	0.0772
22	SL	29421428|F|0-9:C>A-9:C>A	1088	6.29E-04	0.0802
23	SL	29422009|F|0-31:T>A-31:T>A	1258	3.43E-04	0.0871
24	SP	29422509|F|0-26:C>T-26:C>T	1527	9.52E-04	0.0761
25	ST	29420980|F|0-33:A>G-33:A>G	902	9.15E-04	0.0748
26	ST	100043389|F|0-33:G>C-33:G>C	254	1.45E-04	0.0775
27	ST	100043388|F|0-30:T>G-30:T>G	253	1.54E-04	0.0781
28	SW	100008540|F|0-10:C>T-10:C>T	98	4.76E-04	0.0838
29	SW	100024528|F|0-45:C>T-45:C>T	152	4.67E-04	0.0840
30	SW	29421428|F|0-9:C>A-9:C>A	1088	3.43E-04	0.0875
31	SW	29420736|F|0-57:G>T-57:G>T	2364	1.50E-04	0.0970
32	SY	100034020|F|0-56:T>A-56:T>A	206	9.51E-04	0.0763

Days to flowering (D50F); Days to pod maturity (DPM); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locus per pod (NLPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Table 4.15. DArTseq SNPs markers having significant association with 13 agronomic traits of 195 accessions of African yam bean evaluated during the 2018 and 2019 cropping seasons in Ubiaja

SN	Trait	Marker	Position	P-value	Marker R^2
1	D50F	29422735|F|0-21:G>A-21:G>A	1614	6.12E-04	0.0753
2	D50F	100005571|F|0-66:C>A-66:C>A	2088	1.56E-05	0.1150
3	D50F	100003791|F|0-8:C>T-8:C>T	21	1.54E-05	0.1151
4	D50F	100003301|F|0-12:C>T-12:C>T	2232	1.62E-05	0.1161
5	D50F	29423419|F|0-65:A>T-65:A>T	1988	1.05E-05	0.1306
6	DPM	29420933|F|0-41:A>G-41:A>G	874	7.03E-04	0.0786
7	DPM	100005571|F|0-66:C>A-66:C>A	2088	6.52E-04	0.0794
8	DPM	100003301|F|0-12:C>T-12:C>T	2232	7.14E-04	0.0798
9	DPM	100003791|F|0-8:C>T-8:C>T	21	5.78E-04	0.0808
10	DPM	29423419|F|0-65:A>T-65:A>T	1988	7.15E-04	0.0919
11	NLPPD	29421805|F|0-42:G>T-42:G>T	1194	8.48E-04	0.0746
12	NLPPD	29420605|F|0-36:T>A-36:T>A	687	8.40E-04	0.0755
13	NLPPD	29423478|F|0-9:T>C-9:T>C	2021	6.88E-04	0.0769
14	NLPPD	29420257|F|0-7:C>G-7:C>G	477	3.19E-04	0.0854
15	NLPPD	29419951|F|0-35:C>T-35:C>T	271	2.74E-04	0.0871
16	NLPPD	29422706|F|0-34:C>T-34:C>T	1600	7.45E-05	0.1016
17	NLPPD	29422084|F|0-9:G>A-9:G>A	1308	2.47E-04	0.1037
18	NLPPD	29423119|F|0-32:A>T-32:A>T	1836	1.99E-04	0.1054
19	NLPPD	100009412|F|0-67:G>A-67:G>A	118	3.52E-05	0.1111
20	NLPPD	29423047|F|0-35:G>T-35:G>T	1797	5.60E-06	0.1312
21	NPPPL	29422840|F|0-21:A>T-21:A>T	1671	8.96E-04	0.0760
22	NPPPL	29422492|F|0-20:C>A-20:C>A	1515	8.37E-04	0.0768
23	NPPPL	29420610|F|0-13:G>T-13:G>T	689	7.52E-04	0.0786
24	NPPPL	29422961|F|0-30:G>A-30:G>A	1744	6.93E-04	0.0789
25	NPPPL	29422175|F|0-30:A>G-30:A>G	1356	6.46E-04	0.0797
26	NPPPL	29420738|F|0-32:G>T-32:G>T	757	5.73E-04	0.0811
27	NPPPL	29420098|F|0-25:C>G-25:C>G	370	5.04E-04	0.0825
28	NPPPL	29422296|F|0-22:G>A-22:G>A	1419	4.62E-04	0.0835
29	NPPPL	29422706|F|0-34:C>T-34:C>T	1600	4.17E-04	0.0846
30	NPPPL	29421598|F|0-23:G>A-23:G>A	1140	3.76E-04	0.0858
31	NPPPL	29423484|F|0-25:C>A-25:C>A	2023	8.50E-04	0.0869
32	NPPPL	29422378|F|0-19:G>C-19:G>C	1471	2.89E-04	0.0888
33	NPPPL	29420043|F|0-64:G>C-64:G>C	2075	2.33E-04	0.0913
34	NPPPL	29422042|F|0-40:A>G-40:A>G	1278	1.48E-04	0.0965

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Number of locus per pod (NLPPD).

Table 4.15. continued

SN	Trait	Marker	Position	P-value	Marker R^2
35	NPPPL	29421963|F|0-54:G>C-54:G>C	2456	7.99E-05	0.1035
36	NPPPL	29422682|F|0-17:A>C-17:A>C	1584	7.49E-06	0.1312
37	NSPPD	29419951|F|0-35:C>T-35:C>T	271	9.63E-04	0.0740
38	NSPPD	29420605|F|0-36:T>A-36:T>A	687	5.44E-04	0.0812
39	NSPPD	29420013|F|0-30:C>G-30:C>G	312	3.88E-04	0.0841
40	NSPPD	29423119|F|0-32:A>T-32:A>T	1836	7.01E-04	0.0886
41	NSPPD	100009412|F|0-67:G>A-67:G>A	118	1.02E-04	0.0995
42	NSPPD	29422706|F|0-34:C>T-34:C>T	1600	6.26E-05	0.1046
43	NSPPD	29422084|F|0-9:G>A-9:G>A	1308	1.69E-04	0.1115
44	NSPPD	29423047|F|0-35:G>T-35:G>T	1797	6.21E-06	0.1312
45	PDL	29420104|F|0-25:C>A-25:C>A	375	2.55E-04	0.0831
46	PW	29423092|F|0-12:C>A-12:C>A	1822	9.62E-04	0.0752
47	PW	29420043|F|0-64:G>C-64:G>C	2075	8.44E-04	0.0767
48	PW	29422490|F|0-15:G>A-15:G>A	2153	8.42E-04	0.0767
49	PW	29421598|F|0-23:G>A-23:G>A	1140	8.33E-04	0.0769
50	PW	29423047|F|0-35:G>T-35:G>T	1797	8.33E-04	0.0769
51	PW	29421992|F|0-68:A>C-68:A>C	1243	7.45E-04	0.0781
52	PW	29422706|F|0-34:C>T-34:C>T	1600	6.95E-04	0.0789
53	PW	29420156|F|0-23:C>T-23:C>T	412	6.87E-04	0.0790
54	PW	29421580|F|0-20:A>T-20:A>T	1136	6.02E-04	0.0805
55	PW	29423189|F|0-38:T>A-38:T>A	1870	4.13E-04	0.0848
56	PW	29422682|F|0-17:A>C-17:A>C	1584	3.57E-04	0.0865
57	PW	29422378|F|0-19:G>C-19:G>C	1471	3.14E-04	0.0879
58	PW	29420098|F|0-25:C>G-25:C>G	370	2.79E-04	0.0893
59	PW	29423484|F|0-25:C>A-25:C>A	2023	2.09E-04	0.1085
60	PW	29421963|F|0-54:G>C-54:G>C	2456	3.23E-05	0.1141
61	SL	29423011|F|0-50:G>A-50:G>A	1769	1.65E-04	0.0860
62	SL	29423156|F|0-27:C>T-27:C>T	1855	9.36E-05	0.0919
63	ST	100019839|F|0-33:C>G-33:C>G	142	9.34E-04	0.0729
64	ST	100034480|F|0-31:C>A-31:C>A	216	5.08E-05	0.1050
65	SW	100008911|F|0-27:T>C-27:T>C	108	5.70E-04	0.0740
66	SW	100008851|F|0-26:T>C-26:T>C	106	2.67E-04	0.0821
67	SY	29423189|F|0-38:T>A-38:T>A	1870	8.47E-04	0.0770
68	SY	29421963|F|0-54:G>C-54:G>C	2456	2.86E-04	0.0893

Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of seeds per pod (NSPPD); Seed length (SL); Seed width (SW); Seed thickness (ST).

Table 4.16 Significant markers whose nucleotide sequence were found on the *Phaseolus vulgaris* genome and the encoding protein of genes found close to them

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Roles	Reference
1	D50F	100003301|F|0-12:C>T-12:C>T	Pv11:14,478,583..14,478,651	Phvul.011G112300	*G. max*	11	Protein ULTRAPETALA 1-like	Flower development	Carles *et al*. (2004); Carles *et al*. (2005)
				Phvul.011G112500	*P. vulgaris*	11	Secretory carrier membrane protein (SCAMP) family protein	Support pollen tube growth /elongation	Wang et al. (2010)
				Phvul.011G112700	*P. vulgaris*	11	Protein kinase superfamily protein	Flower development	Lehti-Shiu and Shiu (2012); Gachomo *et al*. (2014)
				Phvul.011G109900	*P. vulgaris*	11	Myb transcription factor	Regulate flowering time	Shan *et al*. (2012); Liu *et al*. (2013)
				Phvul.011G110100	*P. vulgaris*	11	Makorin RING-zinc-finger protein	Flower development	Yang *et al*. (2014); Zang *et al*. (2016)
2	D50F	100005571|F|0-66:C>A-66:C>A	Pv03:808,484..808,549	Phvul.003G002200	*P. vulgaris*	3	Myosin 1	Regulate flowering time	Peremyslov et al. (2011); Ojangu *et al*. (2012)
				Phvul.003G001500	*P. vulgaris*	3	U2 small nuclear ribonucleoprotein A	Regulate flowering time	Wang and Brendel (2006)
				Phvul.003G008700	*P. vulgaris*	3	Pentatricopeptide repeat (PPR) superfamily protein	Leads to embryo abortion	Rahaman *et al*. (2018)
				Phvul.003G001000	*P. vulgaris*	3	Protein arginine methyltransferase 6	Regulate flowering time	Niu *et al*. (2007)
				Phvul.003G000900	*P. vulgaris*	3	Eukaryotic aspartyl protease family protein	Flower development	Simões and Faro (2004)

Days to 50% flowering (D50F)

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Roles	Reference
				Phvul.003G000700	*P. vulgaris*	3	serine/threonine protein phosphatase 2A	Regulate flowering time	Kim *et al*. (2002); Heidari et al. (2013)
				Phvul.003G006700	*P. vulgaris*	3	zinc finger (C3HC4-type RING finger) family protein	Flower development	Yang *et al*. (2014)
				Phvul.003G007700	*P. vulgaris*	3	histone-lysine N-methyltransferase	Flower development	Gu *et al*. (2016)
				Phvul.003G007900	*P. vulgaris*	3	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein	Regulate flowering time	Singh *et al*. (2020)
				Phvul.003G008400	*G. max*	3	putative Myb family transcription factor At1g14600-like isoform X2	Regulate flowering time	Shan *et al*. (2012); Liu et al. (2013)
				Phvul.003G008800	*P. vulgaris*	3	squamosa promoter binding protein-like 14;	Regulate flowering time	Preston and Hileman (2013); Xu *et al*. (2020)
3	D5OF	29420809|F|0-38:T>A-38:T>A	Pv07:40,040,933..40,041,001	Phvul.007G269300	*P. vulgaris*	7	Eukaryotic aspartyl protease family protein	Flower development	Simões and Faro (2004)
				Phvul.007G270100	*P. vulgaris*	7	ubiquitin-conjugating enzyme 20	Regulate flowering time	Zhao *et al*. (2019)
				Phvul.007G270200	*G. max*	7	serine/arginine-rich splicing factor 4-like isoform X1	Regulate flowering time	Yan *et al*. (2017)
				Phvul.007G270600	*G. max*	7	zinc finger protein CONSTANS-LIKE 4-like	Regulate flowering time	Yano *et al*. (2000); Steinbach (2019)
				Phvul.007G273000	*P. vulgaris*	7	ethylene-responsive transcription factor 1B	Flower development	Nakano *et al*. (2014)

Days to 50% flowering (D50F).

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Role	Reference
				Phvul.007G273400	*P. vulgaris*	7	myb transcription factor	Regulate flowering time	Shan *et al*. (2012); Liu *et al*. (2013)
				Phvul.007G274600	*P. vulgaris*	7	Protein kinase family protein	Flower development	Lehti-Shiu and Shiu (2012); Gachomo *et al*. (2014)
				Phvul.007G275400	*P. vulgaris*	7	Transducin/WD40 repeat-like superfamily protein	Flower development	Stirnimann *et al*. (2010)
				Phvul.007G278500	*P. vulgaris*	7	Pentatricopeptide repeat (PPR) superfamily protein	Leads to embryo abortion	Rahama et al. (2018)
4	D50F	100003791|F|0-8:C>T-8:C>T	Pv03:6,786..6,847	Phvul.003G001300	*P. vulgaris*	3	Pentatricopeptide repeat (PPR) superfamily protein	Leads to embryo abortion	Rahama *et al*. (2018)
				Phvul.003G005700	*G. max*	3	Endoglucanase 12-like	Regulate flowering time	Kundu and Sharma (2016); Kundu and Sharma (2018)
				Phvul.003G005500	*P. vulgaris*	3	Cytochrome P450 superfamily protein	Regulate flowering time	Liu *et al*. (2015).
				Phvul.003G002200	*P. vulgaris*	3	Myosin 1	Regulate flowering time	Peremyslov *et al*. (2011)
				Phvul.003G003800	*G. max*	3	MADS-box transcription factor 6	Regulate flowering time	Teo *et al*. (2019)
				Phvul.003G003700	*P. vulgaris*	3	Transmembrane protein	Regulate flowering time	Liu *et al*. (2012); Liu *et al*. (2018)
				Phvul.003G001500	*P. vulgaris*	3	U2 small nuclear ribonucleoprotein A	Regulate flowering time	Wang and Brendel (2006)
				Phvul.003G001000	*P. vulgaris*	3	Protein arginine methyltransferase 6	Regulate flowering time	Niu *et al*. (2007)

Days to 50% flowering (D50F).

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Role	Reference
				Phvul.003G000900	*P. vulgaris*	3	Eukaryotic aspartyl protease family protein	Flower development	Simões and Faro (2004)
				Phvul.003G000700	*P. vulgaris*	3	Serine/threonine protein phosphatase 2A	Photoperiodic control of flowering	Kim *et al*. (2002)
				Phvul.003G006700	*P. vulgaris*	3	Zinc finger (C3HC4-type RING finger) family protein	Flower development	Yang *et al*. (2014)
5	D50F	100026403|F|0-5:G>T-5:G>T	Pv02:1,894,146..1,894,211	Phvul.002G021200	*P. vulgaris*	2	LOB domain-containing protein 38	Flower development	Yang *et al*. (2017)
				Phvul.002G012500	*G. max*	2	BTB/POZ domain-containing protein	Flower development	Ha *et al*. (2004)
				Phvul.002G017700	*P. vulgaris*	2	Protein kinase superfamily protein	Flower development	Lehti-Shiu and Shiu (2012); Gachomo *et a*l. (2014)
				Phvul.002G013000	*P. vulgaris*	2	acyl-CoA-binding domain 3	Regulate flowering time	Zhu *et al*. (2021)
				Phvul.002G013700	*P. vulgaris*	2	Protein phosphatase 2C family protein	Regulate flowering time	Sugimoto *et al*. (2014); Xue *et al*. (2008)
				Phvul.002G013866	*P. vulgaris*	2	Tetratricopeptide repeat (TPR)-like superfamily protein	Regulate flowering	Wei and Han (2017)
				Phvul.002G014200	*P. vulgaris*	2	Eukaryotic aspartyl protease family protein	Flower development	Simões and Faro (2004)
				Phvul.002G014800	*P. vulgaris*	2	Cytochrome P450 superfamily protein	Regulate flowering	Liu *et al*. (2015)
				Phvul.002G015100	*P. vulgaris*	2	myb transcription factor	Regulate flowering time	Liu *et al*. (2013); Shan *et al.* (2012)

Days to 50% flowering (D50F).

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Role	Reference
				Phvul.002G016300	*P. vulgaris*	2	ubiquitin-conjugating enzyme 3	Regulate flowering time	Zhao *et al*. (2019)
				Phvul.002G017200	*P. vulgaris*	2	histone-lysine N-methyltransferase	Regulate flowering time	Gu *et al*. (2016)
				Phvul.002G018900	*P. vulgaris*	2	arginine/serine-rich splicing factor	Regulate flowering time	Yan *et al*. (2017)
				Phvul.002G020500	*P. vulgaris*	2	Transducin/WD40 repeat-like superfamily protein	Flower development	Stirnimann *et al*. (2010)
				Phvul.002G022400	*P. vulgaris*	2	Pentatricopeptide repeat (PPR) superfamily protein	Leads to embryo abortion	Rahama *et al*. (2018)
6	D50F	29422735|F|0-21:G>A-21:G>A	Pv04:1,453,687..1,453,752	Phvul.004G009809	*P. vulgaris*	4	Pentatricopeptide repeat (PPR) superfamily protein	Leads to embryo abortion	Rahama *et al*. (2018)
				Phvul.004G012600	*P. vulgaris*	4	Protein kinase superfamily protein	Floral development	Lehti-Shiu and Shiu (2012); Gachomo *et al*. (2014)
				Phvul.004G010500	*P. vulgaris*	4	Transducin/WD40 repeat-like superfamily protein	Floral development	Gachomo *et al*. (2014)
				Phvul.004G015000	*P. vulgaris*	4	F-box/RNI-like superfamily protein	Regulate flowering time	Cao *et al*. (2008)
				Phvul.004G017000	*P. vulgaris*	4	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein	Regulates flowering	Singh *et al*. (2020)
7	GFP	29420466|F|0-41:C>T-41:C>T	Pv03:36,109,432..36,109,500	Phvul.003G146400	*P. vulgaris*	3	P-loop containing nucleoside triphosphate hydrolases superfamily protein	Regulate flowering time	Liu *et al*. (2016b)
				Phvul.003G146900	*P. vulgaris*	3	4-coumarate:CoA ligase 2	Regulate flowering time	Li *et al*. (2020)

Days to 50% flowering (D50F); Grain filling period (GFP).

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Role	Reference
				Phvul.003G149000	*P. vulgaris*	3	Zinc finger protein CONSTANS-LIKE 5-like	flower development	Yang *et al*. (2014)
				Phvul.003G147200	*G. max*	3	CASP-like protein 3	Regulate flowering time/ express during grain filling	Sapkota *et al*. (2020)
				Phvul.003G149100	*P. vulgaris*	3	Protein kinase superfamily protein	Flower development	Lehti-Shiu and Shiu (2012); Gachomo *et al*. (2014)
				Phvul.003G148200	*G. max*	3	MYB transcription factor MYB92	Regulate flowering time	Shan *et al*. (2012); Liu *et al*. (2013)
				Phvul.003G150900	*G. max*	3	Ankyrin repeat-containing protein	Regulate maturity time	Sheoran *et al*. (2019)
8	NPPPL, PW	29420888|F|0-53:C>T-53:C>T	Pv08:39,988,866..39,988,934	Phvul.008G141200	*G. max*	8	chalcone synthase-like	Epressed in seeds and pod tissue	Vadivel *et al*. (2018); Wu *et al*. (2020)
				Phvul.008G158136	*G. max*	8	cellulose synthase like G1	Regulate seed weight and pod length	Lo *et al*. (2018)
				Phvul.008G158124	*G. max*	8	RNA-binding protein 1-like	Regulate seed development	Lou *et al*. (2020)
9	NLPPD, NSPPD	29422706|F|0-34:C>T-34:C>T	Pv05:8,461,497..8,461,544	Phvul.005G058700	*G. max*	5	1-aminocyclopropane-1-carboxylate oxidase homolog 1-like	Seed development	Hussain *et al*. (2020)
				Phvul.005G060000	*P. vulgaris*	5	Myb transcription factor	Regulate grain size	Zhang *et al*. (2013); Watt *et al*. (2020)
				Phvul.005G060100	*P. vulgaris*	5	Related to ubiquitin 1	Regulate seed size	
				Phvul.005G060200	*P. vulgaris*	5	Epidermal patterning factor 1	Regulate grain length	Li *et al*. (2019)

Pod weight (PW); Number of pods per plant (NPPPL); Number of locus per pod (NLPPD); Number of seeds per pod (NSPPD).

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Role	Reference
10	NLPPD, NSPPD	100009412|F|0-67:G>A-67:G>A	Pv01:21,547,835..21,547,889	Phvul.001G102600	*P. vulgaris*	1	Ubiquitin-conjugating enzyme 22	Regulate silique length and seed number	Wang *et al*. (2016)
				Phvul.001G102200	*P. vulgaris*	1	COP9 signalosome complex subunit 2	Regulate seed weight	Das *et al*. (2015); Wang *et al*, (2019)
				Phvul.001G103300	*P. vulgaris*	1	MATE efflux family protein	expressed in silique, seed and young pod development	Liu *et al*. (2016a); Lu *et al*. (2018)
11	ST	100034480|F|0-31:C>A-31:C>A	Pv02:2,303,744..2,303,815	Phvul.002G016300	*P. vulgaris*	2	Ubiquitin-conjugating enzyme 3	Reglate seed size	Li and Li (2014); Guo *et al*. (2020)
				Phvul.002G016400	*P. vulgaris*	2	UDP-glucosyltransferase family protein	Regulate grain size	Dong *et al*. (2020)
				Phvul.002G016900	*G. max*	2	Ethylene-responsive transcription factor 3-like	mediates seed size and seed weight	Jiang *et al*. (2020); Sharma *et al*. (2020)
				Phvul.002G017600	*G. max*	2	Transcription factor SPATULA-like	Reglate grain size	Liu *et al*. (2017)
				Phvul.002G019500	*P. vulgaris*	2	Cyclin-dependent kinase inhibitor family protein	Reglate seed size/weight	Ajadi *et al*. (2020)
				Phvul.002G021600	*G. max*	2	Serine/threonine-protein kinase TIO-like	Reglate seed size	Hu *et al*. (2012)
				Phvul.002G024100	*P. vulgaris*	2	Pentatricopeptide repeat (PPR) superfamily protein	Regulate grain size	Li *et al*. (2019)
				Phvul.002G022600	*P. vulgaris*	2	GDSL-like Lipase/Acylhydrolase superfamily protein	Seed development	Ma *et al*. (2018)
				Phvul.002G022800	*P. vulgaris*	2	Cytochrome P450 superfamily protein	Regulate seed/fruit size	Qi *et al*. (2017)

Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); Seed thickness (ST).

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Role	Reference
				Phvul.002G023100	*P. vulgaris*	2	Transducin/WD40 repeat-like superfamily protein	Reglate grain size	He and Ho (2018)
12	SL	29420365|F|0-55:C>G-55:C>G	Pv01:40,806,541..40,806,608	Phvul.001G153000	*P. vulgaris*	1	ovate family protein 13	regulate seed/fruit size	Ma *et al*. (2017); van der Knaap and Østergaard (2018)
				Phvul.001G152900	*P. vulgaris*	1	RING/FYVE/PHD zinc finger superfamily protein	Regulate seed length and seed width	Wang *et al*. (2020)
				Phvul.001G153400	*P. vulgaris*	1	kelch repeat F-box protein	Regulate seed size	Chen *et al*. (2013)
				Phvul.001G153700	*P. vulgaris*	1	Pentatricopeptide repeat (PPR) superfamily protein	Regulate seed size	Li *et al*. (2019)
				Phvul.001G155600	*G. max*	1	beta-carotene isomerase D27	Regulate seed length	Wang *et al*. (2020)
				Phvul.001G156500	*P. vulgaris*	1	auxin response factor 11	Regulate seed size	Li and Li (2015)
				Phvul.001G157400	*P. vulgaris*	1	E3 ubiquitin protein ligase DRIP2-like	Regulate seed size	Li and Li (2014); Choi *et al*. (2018)
				Phvul.001G157600	*G. max*	1	ethylene-responsive transcription factor 12-like	Regulate seed size	Jiang *et al*. (2020)
				Phvul.001G157900	*P. vulgaris*	1	Cytochrome P450 superfamily protein	Regulate seed/fruit size	Qi *et al*. (2017)
13	ST, SW	29420736|F|0-57:G>T-57:G>T	Pv07:40,040,935..40,041,001	Phvul.007G269900	*P. vulgaris*	7	ovate family protein 13	Regulate seed/fruit size	Ma *et al*. (2017); van der Knaap and Østergaard (2018)
				Phvul.007G270100	*P. vulgaris*	7	ubiquitin-conjugating enzyme 20	Regulate seed size	Li and Li (2014); Guo *et al*. (2020)

Seed length (SL); Seed thickness (ST); Seed width (SW).

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Role	Reference
				Phvul.007G272700	*P. vulgaris*	7	RING-H2 finger protein 2B	seed development	Kang *et al*. (2018)
				Phvul.007G273100	*P. vulgaris*	7	serine/threonine protein phosphatase 2A	Regulate grain shape	Hu *et al*. (2012); Wang *et al*. (2019)
				Phvul.007G273400	*P. vulgaris*	7	myb transcription factor	Regulate grain size	Zhang *et al*. (2013); Watt *et al*. (2020)
				Phvul.007G278500	*P. vulgaris*	7	Pentatricopeptide repeat (PPR) superfamily protein	Regulate seed size	Li *et al*. (2019)
				Phvul.007G278600	*P. vulgaris*	7	Argonaute family protein	Regulate seed size	Zhong *et al*. (2020)
				Phvul.007G279400	*P. vulgaris*	7	ARM repeat superfamily protein	Regulate seed size	Xie *et al*. (2014)
				Phvul.007G280200	*P. vulgaris*	7	ATP-binding/protein serine/threonine kinase	Regulate seed size	Hu *et al*. (2012)
14	SP	100024379|F|068:C>A-68:C>A	Pv10:41,095,551..42,095,550	Phvul.010G129300	*P. vulgaris*	10	E3 Ubiquitin ligase family protein	Regulate seed size	Li and Li (2014); Choi *et al*. (2018)
				Phvul.010G130500	*P. vulgaris*	10	Myb transcription factor	Control grain size	Watt *et al*. (2020); Zhang *et al*. (2013)
				Phvul.010G131100	*P. vulgaris*	10	Cytochrome P450 superfamily protein	Regulate seed/fruit size	Qi *et al*. (2017)
				Phvul.010G132300	*P. vulgaris*	10	RING-H2 finger protein 2B	seed development	Kang *et al*. (2018)
				Phvul.010G134200	*P. vulgaris*	10	protein kinase family protein	Regulate seed/grain size	Li *et al*. (2019)
				Phvul.010G134500	*G. max*	10	Mitochondrial import inner membrane translocase subunit TIM23-2-like	Regulate seed development	Karikari *et al*. (2019)

Shelling percentage (SP).

Table 4.16. continued

SN	Trait	Marker	Position	Gene ID	Crop	Chromosome	Encoding Product	Role	Reference
				Phvul.010G134700	*P. vulgaris*	10	Seed linoleate 9S-lipoxygenase	Regulate pod indehiscence	Di Vittori *et al*. (2020)
				Phvul.010G135050	*G. max*	10	RNA-binding protein 25-like	Regulate seed development	Lou *et al*. (2020)
				Phvul.010G136900	*P. vulgaris*	10	ATP-binding ABC transporter	Regulate seed size/wieght weight	Basu *et al*. (2019)
				Phvul.010G137100	*G. max*	10	EPIDERMAL PATTERNING FACTOR-like protein 2-like	Regulate grain length	Li *et al*. (2019)
15	SP	29420331|F|029:T>C-29:T>C	Pv06:23,488,945..23,489,010	Phvul.006G120900	*P. vulgaris*	6	Ubiquitin protein ligase 6	Regulate seed size	Xia *et al*. (2013)
				Phvul.006G121200	*P. vulgaris*	6	Protein phosphatase 2C family protein	Regulate seed weight/size	Lu *et al*. (2017)
				Phvul.006G127900	*P. vulgaris*	6	Protein kinase family protein	Regulate seed/pod weight	Gangurde *et al*. (2020)
				Phvul.006G122900	*P. vulgaris*	6	Acyl-CoA synthetase 5	Regulate seed/pod weight	Gangurde *et al*. (2020)
				Phvul.006G123000	*P. vulgaris*	6	WRKY family transcription factor	Regulate seed size	Gu *et al*. (2017); Li *et al*. (2019)
				Phvul.006G123600	*P. vulgaris*	6	Cytochrome P450 superfamily protein	Regulate seed/fruit size	Qi *et al*. (2017)
				Phvul.006G126800	*P. vulgaris*	6	Pentatricopeptide repeat (PPR-like) superfamily protein	Regulate seed size	Li *et al*. (2019)
				Phvul.006G128200	*P. vulgaris*	6	ADP-ribosylation factor GTPase-activating protein AGD12-like	Regulate seed size	Muthamilarasan *et al*. (2016)

Shelling percentage (SP).

CHAPTER 5
DISCUSSION

Knowledge of the amount of genetic variability is of utmost importance for the success of any crop improvement programme. The present study used 14 agronomic and yield traits to investigate the extent of genetic variability among 196 accessions of African yam bean and identify gene pools among the accessions. The significant differences exhibited by the accessions for all traits evaluated in this study was an indication of the genetically diverse nature of the accessions and good progress can be made in selection for the improvement of the crop for desired traits. Previous studies using fewer number of accessions (Adewale *et al*., 2010; Aremu, and Ibirinde, 2012; Adewale *et al*., 2012b, Ibirinde and Aremu, 2013; Adesoye and Ukwueze, 2015; Ojuederie *et al*., 2015; Adewale and Kehinde, 2016; Ibirinde *et al*., 2019; Aremu *et al*., 2019, Aremu *et al*., 2020a and Aina *et al*., 2020) had reported substantial variability for different traits in AYB. The significance of environment and accession × environment interaction effects for all traits indicated that the performance of accessions were influenced by environmental differences. This finding requires that selections are made for the different environments. This is in agreement with the results reported by Adewale *et al*. (2012b); Aremu *et al*. (2019) and Aremu *et al*. (2020a). The generally low CV buttresses the good level of reliability achieved in the study. Low CVs suggest of improved level of reliability (Gomez and Gomez, 1976).

The inconsistency in tuber production across the environments suggests a high influence of environmental effects on AYB tuber production. Accession TSs-121 which produced tuber in the 6 environments could be a good candidate for tuber traits improvement. The four accessions (138A, TSs-22, TSs-314, TSs-87B) that produce no tuber in any of the 6 environments could be described as non-tuber producing.

The greater magnitudes of PCV, environmental variance and the variance due to accession × environment interaction than GCV and genotypic variance suggest higher environmental influence in the expression for all traits. Similar results have been reported by Nwofia *et al*. (2014), Adewale *et al*. (2012b), Ibirinde *et al*. (2019) and Alake and

Porbeni, (2020) in their study of AYB variability. The high PCV and moderate GCV observed for both seed yield and number of pods per plant indicate the presence of high variability for these traits among AYB accessions studied, thus highlighting opportunities for improvement through effective selection. The Low GCV and PCV found for days to flowering, days to maturity, grain filling period, shelling percentage, pod length, number of seeds and locules per pod, 100-seeds weight, seed length, seed width and seed thickness showed that variability among the accessions was very low for these traits. This is in agreement with the reports of Nwofia *et al*. (2014); Adewale *et al*. (2012b); Ibirinde *et al*. (2019); Alake and Porbeni, (2020).

The moderate to high broad-sense heritability estimates observed for some traits implied the possibility of effective selection for genetic improvement of these traits. The heritability estimates are in agreement with the results reported for seed metric traits (Adewale *et al*., 2010), 100-seed weight and number of seeds per pod (Nwofia *et al*., 2014; Alake and Porbeni, 2020) as well as days to flowering and pod length (Adesoye and Ukwueze, 2015). Low broad-sense heritability values observed for seed yield per plant, days to pod maturity, grain filling period, number of pods per plant, pod weight, shelling percentage and number of locules per pod indicated the large influence of environment which could limit progress of improvement through selection. To increase the efficiency of selection with low heritability estimates, Alake and Porbeni (2020) suggested the use of genetic correlation between traits as a tool in selecting progenies with desirable attributes in crop improvement programmes.

In morphological characterisation of germplasm, data are often collected on a large number of variables, some of which could be inadequate in discriminating the germplasm evaluated. As such, PCA is used to reveal patterns of variation and eliminate redundancy in data sets (Das *et al*., 2017). In this study, the fourteen seed yield and yield-related traits significantly contribute to the first three PCs with eigenvector > 0.2which explained more than half of the variation. Traits associated with the first three PCs are more useful in differentiating the germplasms (Guei *et al*., 2005; Das *et al*., 2017). Therefore, consideration should be given to these fourteen traits for genetic improvement of the AYB germplasm. Akande (2009), Popoola *et al.* (2011), Aremu and Ibirinde (2012) and Ibirinde *et al.* (2019) had also reported these seed yield related characters as traits contributing most to the observed variations in their studied AYB germplasms studied. The PCA biplot of the first and second PCs further explain the relationship among traits and between PCs and traits. The acute angles between vectors illustrate close

relationships or high correlation among traits. The spread of the accessions in the four quadrants on account of the fourteen agronomic characteristics suggest wide diversity. This was also confirmed by the constellation plot. Cluster analysis classify accessions into separate group based on their similarity for one or more morphological traits such that heterosis can be exploited through hybridization of accessions belonging to different group (Alake and Porbeni 2020). The division of the accessions used in this study into five distinct clusters is in line with the findings of Akande (2009) and Adewale et al. (2012a). Both studies also reported the presence of five clusters in 32 and 79 GRC-IITA AYB accessions, respectively. A cross between members of clusters I and II could lead to the development of hybrids characterized by earliness, better phenological appeal and high seed yield. Also, hybridization between members of cluster V and any of those of clusters I and II could be useful in bi-parental study of genetic control of variation in seed yield and yield-related traits in AYB.

In this study, genotypic correlation coefficient was higher than the corresponding phenotypic correlation coefficient for nearly all traits especially seed yield with low broad-sense heritability, indicating the inherent nature of the association among the traits. The positive and significant genotypic correlation between seed yield on the one hand and days to flowering, days to pod maturity, grain filling period, number of pods per plant, pod weight, shelling percentage, number of locules per pod, number of seeds per pod, 100-seeds weight, seed width and seed thickness on the other, showed that these traits can be considered as an index for indirect selection to improve seed yield in AYB. These results are consistent with the findings of previous workes on the relationship between seed yield and number of pods per plant (Ibirinde and Aremu, 2013), seed yield and 100-seed weight (Alake and Porbeni, 2020) as well as seed yield on the one hand and grain filling period and number of seeds per pod (Aremu *et al*., 2019) in AYB. Similarly, the significant negative genotypic correlation between seed yield and pod length is in agreement with the findings of Osuagwu *et al*. (2014) that longer pods may not necessarily translate to more seed yield in AYB because of longer seed lenght. The positive and significant associations among the yield-related traits indicated the possibility to simultaneously improve the traits.

Path coefficient analysis is a standardized partial regression statistical technique that partition correlation coefficients into direct and indirect effects, in such a way that the most important traits relating to yield is known. In the present study, the residual value of 0.30 suggests that the collective effect of the thirteen yield-related traits on seed yield

is 0.70. Due to the positive direct effects of days to pod maturity, pod weight, shelling percentage, number of seeds per pod, 100-seed weight and seed thickness on seed yield, special importance should be placed on the genetic improvement of these traits to improve seed yield through indirect section. Nwofia *et al*. (2014) reported similar effect of 100-seeds weight on seed yield of AYB, while Aremu *et al*. (2019) identified number of seeds per pod and days to maturity as parts of the first order predictor variables of seed yield. Days to flowering, grain filling period, number of pod per plant, number of locules per pod and seed width that had negative direct effect on seed yield, had significant positive genotypic correlation coefficient with it. This is due to their positive indirect effects through other traits. For instance, days to flowering and grain filling period had a high positive indirect effect on seed yield via days to maturity, suggesting that selection for these traits would be effective and hence influence seed yield and days to maturity indirectly.

The YIS was used in this study as a means of identifying stable accessions with good mean seed yield performance. Environment significantly explain 53.43% of the total treatment sum of squares indicating that environmental diversity caused most (above average) of the observed variation in seed yield. The higher magnitude of accessions × environment interaction sum of square compared to that of accession indicate the presence of accession difference across the environment and a crossover genotype × environment interaction for seed yield. Accessions TSs-119, TSs-101, 138A, TSs-4, TSs-157A, and TSs-61 ranked most desirable, integrating stability with high mean seed yield. Similar result had been reported by Aremu *et al*. (2020b) for TSs-61. Accessions TSs-143, TSs-280, 138A, TSs-84, TSs-69, TSs-157A, TSs-119, 151B, TSs-361and TSs-22B with the lowest ASV were the most stable of the 196 accessions studied across the six environments. Aremu *et al*. (2020b) also reported that TSs-69 as one of the most stable accession for seed yield while Adewale and Kehinde (2016) reported TSs84 as the most stable accession for 100-seeds weight in four environments. Accession TSs-143 which was the most stable is not the most desirable because of its low mean seed yield. Accessions TSs-421 and TSs-195 that had the highest mean seed yield per plant, were not the most desirable accessions because of their high ASV. This further buttress the fact that stable genotypes do not necessarily give the best performance and high yielding genotypes are not always stable. Accessions like TSs-104, TSs-363, TSs-29, TSs-278, TSs-19, TSs-443 and TSs-11 were low yielding and less stable, hence , they are least desirable.

Association analyses between specific phenotypes and genotypes within a genome is an important step towards the discovery of genes controlling the traits (Mwadzingeni *et al*., 2017; Adewale *et al*., 2020). Of the 195 accessions used in this study, 137 had been previously utilized for conducting GWAS for nutritional traits (Oluwole *et al*., 2020). Several significant SNPs were found to be associated with the studied traits. In this present study, all fourteen agronomic traits followed normal distribution confirming their fitness for used in GWAS analysis. The model fitness for the GWAS was also confirmed by the Q-Q plots. The alignment of observed and expected P-values in the Q-Q plots for all the measured traits indicates that spurious associations as a result of population structure and familial relatedness were largely corrected. The random distribution of genotypes on the PCA plot suggest the absence of population structure in the AYB population used. The significant markers associated with seed yield and yield-related traits in each and combined location analysis is the first report of GWA analysis for seed yield related traits in AYB germplasms. The contribution of all the significant markers to the phenotypic variation ranged between 5.8 to 14.4 % suggesting that these markers could be useful for marker-assisted selection for AYB improvement. Lack of significant markers for traits such seed yield and detection of few significant markers in other traits in the combine location analysis could be as a result of their low heritability estimate. Low broad-sense heritability resulting from strong genotype × environment interaction in genotype accessed in multi-environments limits the power of association (Alqudah *et al*., 2020). The exact chromosomes in AYB on which the significant makers were located is unknown due to lack of reference genome. The whole-genome sequencing project of AYB is currently ongoing at the Alliance for Accelerated Crop Improvement in Africa (ACACIA) (Paliwal, 2020). Identification of more than one significant marker for a specific quantitative trait further confirms their polygenic and complex nature. Lack of SNPs overlap for the same trait in the three locations indicated the divergent nature of the locations. The observed pleiotropic markers could be useful in simultaneous improvement of correlated traits. The 17 significant markers that were consistent in one location and combine location analysis for the same traits could be referred to as putative candidate makers. The 15 significant markers found in *Phaseolus vulgaris* genome at a location close to genes whose encoding proteins had been reported to regulate the traits they are associated with in AYB, can also be referred to as putative candidate makers. Makers associated with days to flowering for instance are located close to genes having secretory carrier membrane protein (SCAMP) family protein, Myosin 1

and Protein kinase superfamily protein as their encoding proteins. Secretory carrier membrane protein (SCAMP) family protein is involve in *Arabidopsis* pollen tube growth (Wang *et al*., 2010), while Myosin 1 and protein kinase superfamily protein regulates flowering time in *Arabidopsis* (Ojangu *et al*., 2012; Gachomo *et al*., 2014). Ovate family protein 13 which is the protein for one of the genes located close to markers associated with seed size is known to regulate fruit shape in tomato and seed size in rice (Ma *et al*., 2017). Also, chalcone synthase-like the encoding protein of the gene Phvul.008G141200 which is located close to a marker (29422706|F|0-34:C>T-34:C>T) associated with AYB pod traits is known to have it expression in soybean seeds and pod tissue (Wu *et al*., 2020). Ankyrin repeat-containing protein which regulate maturity time in Indian spring wheat (Sheoran *et al*., 2019) is an encoding protein of a gene (Phvul.003G150900) found close to marker (29420466|F|0-41:C>T-41:C>T) associated with grain filling period.

Though significant marker-trait associations were detected in this study, the result serves as a foundation for genetic understanding of putative makers underlying yield and related traits in AYB. Validation using several mapping populations is required before the identified makers could be targeted by plant breeders in marker-assisted selection to accelerate the genetic improvement of AYB for yield and related traits.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary and conclusions

The investigation involving 196 accessions of African yam bean was undertaken to ascertain the extent of variability for agronomic and seed yield traits, the genetic basis of these variations at genome wide level and the relative importance of some yield-related traits to seed yield.

Findings from this study are summarised below:

I. Genetic variation exists among the 196 African yam bean accessions used in this study for seed yield and yield related traits.

II. The high phenotypic coefficient of variation and moderate genotypic coefficient of variation observed for seed yield and number of pods per plant suggest that the diversity among the lines evaluated could be effectively exploited for genetic improvement of the traits.

III. Moderate to high broad-sense heritability estimates obtained for seed length, seed width, seed thickness, 100-seeds weight, days to flowering, pod length and number of seeds per pods indicates that the environmental influence was low on these characters, and therefore easy to improve genetically through direct selection.

IV. All 14 seed yield and yield-related traits significantly differentiated the 196 African yam bean accessions used. Hence, these traits should be considered for genetic improvement of these accessions.

V. Outstanding accessions that can be used in future breeding programme for seed yield and related traits improvement and bi-parental studies of quantitative trait loci underlining genetic variation in seed yield and yield-related traits were identified.

VI. Higher magnitude of genotypic and phenotypic correlation for most traits especially seed yield per plant with low broad-sense heritability, indicate inherent relationship that can be used in seed yield improvement through selection.

VII. When selecting for improved seed yield in African yam bean, days to pod maturity, pod weight, shelling percentage, number of seeds per pod, 100-seed weight and seed thickness are traits that should be included in the selection base index.

VIII. The presence of accession × environment interaction produced a different rank in order of accessions under different environments, thus selections should be made for the different environments where the study was carried out.

IX. Accessions such as TSs-119, TSs-101, 138A, TSs-4, TSs-157A, and TSs-61 which combined superior mean seed yield with stability can be considered in future breeding programme for seed yield improvement.

X. Several Single Nucleotide Polymorphisms markers were significantly associated with seed yield and yield-related traits. The 15 significant markers found on the *Phaseolus vulgaris* genome could be regarded as putative candidate markers and could be used for seed yield and yield-related traits improvement in AYB.

6.2 Recommendations

It is recommended that efforts should be directed towards the validation of significant makers identified in this study using several mapping populations before they can be targeted by plant breeders in maker-assisted selection to accelerate genetic improvement of African yam bean for seed yield and related traits.

Moreover, in the context of legumes, this study demonstrated that *Phaseolus vulgaris* is the closest reference to AYB. Therefore, it is highly recommended for future research on AYB Association mapping.

6.3 Contributions to knowledge

1. A wide genetic variation for seed yield and related traits exists among the African yam bean accessions used.
2. Five phenotypic clusters of accessions that can be used in future improvement programmes and bi-parental studies were identified.
3. Accessions TSs-119, TSs-101, 138A, TSs-4, TSs-157A and TSs-61 combined high seed yield with yield stability, this study present them for subsequent utilization.
4. Shelling percentage, 100-seed weight, days to pod maturity, pod weight, seeds per pod and seed thickness were identified as selection criteria for increased seed yield in African yam beans.

5. Maker-trait associations previously not reported in African yam bean germplasm for seed yield and agronomic traits were found.

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APPENDICES

Appendix 1. List of 196 African yam bean landraces used in this study

S/N	Accession	Country	S/N	Accession	Country	S/N	Accession	Country
1	104B	Unknown	34	TSs-13	Nigeria	67	TSs-217	Unknown
2	119A	Unknown	35	TSs-133	Nigeria	68	TSs-22	Nigeria
3	138A	Unknown	36	TSs-136	Nigeria	69	TSs-224	Unknown
4	151B	Nigeria	37	TSs-137	Nigeria	70	TSs-22A	Unknown
5	159A	Unknown	38	TSs-138	Nigeria	71	TSs-22B	Unknown
6	23C	Unknown	39	TSs-14	Nigeria	72	TSs-23	Nigeria
7	30A	Unknown	40	TSs-143	Nigeria	73	TSs-23C	Unknown
8	30B	Unknown	41	TSs-144	Nigeria	74	TSs-24	Nigeria
9	44C	Unknown	42	TSs-148	Nigeria	75	TSs-249	Unknown
10	55A	Unknown	43	TSs-15	Nigeria	76	TSs-255	Unknown
11	56A	Unknown	44	TSs-150	Nigeria	77	TSs-26	Nigeria
12	59B	Unknown	45	TSs-151A	Unknown	78	TSs-266	Unknown
13	60B	Unknown	46	TSs-151B	Unknown	79	TSs-268	Unknown
14	61A	Unknown	47	TSs-153	Nigeria	80	TSs-269	Unknown
15	62B	Unknown	48	TSs-155	Nigeria	81	TSs-273	Unknown
16	63A	Unknown	49	TSs-156A	Nigeria	82	TSs-274	Unknown
17	7A	Unknown	50	TSs-157	Nigeria	83	TSs-275	Unknown
18	89A	Unknown	51	TSs-157A	Unknown	84	TSs-276	Unknown
19	TSs-1	Nigeria	52	TSs-159A	Unknown	85	TSs-277	Unknown
20	TSs-10	Nigeria	53	TSs-16	Nigeria	86	TSs-278	Unknown
21	TSs-101	Nigeria	54	TSs-161	Unknown	87	TSs-28	Nigeria
22	TSs-104	Nigeria	55	TSs-162	Unknown	88	TSs-280	Unknown
23	TSs-10A	Nigeria	56	TSs-166	Unknown	89	TSs-282	Unknown
24	TSs-11	Nigeria	57	TSs-168	Unknown	90	TSs-285	Unknown
25	TSs-111	Nigeria	58	TSs-186	Unknown	91	TSs-287	Unknown
26	TSs-113	Nigeria	59	TSs-19	Nigeria	92	TSs-289	Unknown
27	TSs-115	Nigeria	60	TSs-192	Unknown	93	TSs-29	Nigeria
28	TSs-116	Nigeria	61	TSs-195	Unknown	94	TSs-293	Unknown
29	TSs-119	Nigeria	62	TSs-1A	Unknown	95	TSs-294	Unknown
30	TSs-119A	Unknown	63	TSs-201	Unknown	96	TSs-296	Unknown
31	TSs-12	Nigeria	64	TSs-2015-06	Unknown	97	TSs-297	Unknown
32	TSs-120	Nigeria	65	TSs-212	Unknown	98	TSs-298	Unknown
33	TSs-121	Nigeria	66	TSs-216	Unknown	99	TSs-299	Unknown

TSs - Tropical *Sphenostylis stenocarpa*

Appendix 1. continued

S/N	Accession	Country	S/N	Accession	Country	S/N	Accession	Country
100	TSs-3	Nigeria	133	TSs-378	Unknown	166	TSs-59	Nigeria
101	TSs-30	Nigeria	134	TSs-38	Nigeria	167	TSs-5A	Unknown
102	TSs-301	Unknown	135	TSs-39A	Unknown	168	TSs-6	Nigeria
103	TSs-302	Unknown	136	TSs-3A	Unknown	169	TSs-60	Nigeria
104	TSs-304	Unknown	137	TSs-4	Nigeria	170	TSs-60B	Unknown
105	TSs-307	Unknown	138	TSs-42	Nigeria	171	TSs-61	Nigeria
106	TSs-309	Unknown	139	TSs-421	Nigeria	172	TSs-62	Nigeria
107	TSs-31	Nigeria	140	TSs-422	Nigeria	173	TSs-62B	Nigeria
108	TSs-311	Unknown	141	TSs-424	Nigeria	174	TSs-63	Nigeria
109	TSs-312	Unknown	142	TSs-427	Nigeria	175	TSs-63A	Unknown
110	TSs-313	Unknown	143	TSs-433	Nigeria	176	TSs-66	Bangladesh
111	TSs-314	Unknown	144	TSs-434	Nigeria	177	TSs-66A	Unknown
112	TSs-317	Unknown	145	TSs-435	Nigeria	178	TSs-67	Bangladesh
113	TSs-32	Nigeria	146	TSs-437	Nigeria	179	TSs-68	Ghana
114	TSs-320	Unknown	147	TSs-438	Nigeria	180	TSs-69	Nigeria
115	TSs-326	Unknown	148	TSs-44	Nigeria	181	TSs-6A	Unknown
116	TSs-33	Nigeria	149	TSs-440	Nigeria	182	TSs-6B	Unknown
117	TSs-331	Unknown	150	TSs-441	Nigeria	183	TSs-7	Nigeria
118	TSs-333	Unknown	151	TSs-442	Nigeria	184	TSs-7A	Unknown
119	TSs-337	Unknown	152	TSs-443	Nigeria	185	TSs-8	Nigeria
120	TSs-338	Unknown	153	TSs-445	Nigeria	186	TSs-82	Nigeria
121	TSs-34	Nigeria	154	TSs-44C	Unknown	187	TSs-84	Nigeria
122	TSs-357	Unknown	155	TSs-45	Nigeria	188	TSs-84A	Unknown
123	TSs-358	Unknown	156	TSs-450	Nigeria	189	TSs-86	Nigeria
124	TSs-361	Unknown	157	TSs-46	Nigeria	190	TSs-87	Nigeria
125	TSs-363	Unknown	158	TSs-47	Nigeria	191	TSs-87B	Unknown
126	TSs-365	Unknown	159	TSs-48	Nigeria	192	TSs-90	Nigeria
127	TSs-366	Unknown	160	TSs-49	Nigeria	193	TSs-92	Nigeria
128	TSs-367	Unknown	161	TSs-5	Nigeria	194	TSs-93	Nigeria
129	TSs-368	Unknown	162	TSs-51	Nigeria	195	TSs-96	Nigeria
130	TSs-369	Unknown	163	TSs-55	Nigeria	196	TSs-98	Nigeria
131	TSs-371	Unknown	164	TSs-56	Nigeria			
132	TSs-377	Unknown	165	TSs-56A	Unknown			

TSs - Tropical *Sphenostylis stenocarpa*

Appendix 2. Means of the 196 accessions of African yam bean evaluated during the 2018 and 2019 cropping season in three agro-ecologies of Nigeria

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
104B	97.69	153.06	55.25	6.83	30.61	45.56	21.19	12.63	11.89	18.43	14.78	7.74	6.07	6.01
119A	86.83	152.94	66.11	9.51	36.66	40.75	20.60	11.66	10.89	23.96	16.41	8.23	6.29	6.14
138A	86.00	146.79	61.21	8.34	35.26	47.40	20.27	13.55	12.82	24.34	16.81	8.39	6.44	6.36
151B	90.78	146.56	55.78	6.99	29.62	49.04	20.14	11.83	10.95	23.84	15.94	7.96	6.32	6.35
159A	87.56	157.39	69.83	7.97	27.67	35.89	21.16	11.45	10.75	19.27	12.90	7.59	5.84	5.53
23C	91.50	150.78	59.28	6.96	24.92	40.26	20.13	11.58	10.51	21.82	11.93	7.80	6.31	5.98
30A	87.94	152.39	64.22	5.71	21.95	36.70	20.63	10.57	9.74	19.81	9.87	8.27	6.25	5.94
30B	84.00	144.06	60.00	10.80	36.64	43.69	20.81	12.33	11.51	21.28	16.58	7.91	6.26	6.11
44C	88.39	151.17	62.78	8.40	30.99	45.94	21.58	11.65	10.37	22.21	13.62	8.16	6.28	6.33
55A	86.22	151.17	64.89	9.58	36.48	46.76	20.96	12.83	11.97	22.21	17.08	8.24	6.54	6.19
56A	91.00	154.83	63.78	9.60	33.93	40.99	20.57	11.9	10.93	21.00	16.71	8.36	6.23	6.02
59B	88.39	153.00	64.61	11.03	40.38	46.95	19.91	11.93	11.31	23.24	20.51	7.82	6.05	5.91
60B	86.67	159.72	73.06	14.54	43.45	43.27	20.55	13.34	12.48	18.80	18.85	8.02	6.21	5.98
61A	88.44	156.11	67.61	10.48	34.55	41.95	21.30	12.58	11.35	17.77	17.41	7.73	5.83	5.50
62B	91.89	161.33	69.28	9.21	33.19	44.09	22.79	12.68	11.48	21.69	14.76	8.39	6.37	6.16
63A	91.17	155.61	64.44	10.52	35.75	46.97	20.71	12.55	11.81	19.29	17.52	7.64	6.23	5.92
7A	94.33	156.89	62.56	8.02	32.68	42.82	20.42	11.42	10.77	22.24	15.93	7.88	5.93	5.63
89A	86.00	139.93	57.21	6.69	20.08	45.69	19.93	12.85	12.14	20.14	10.81	8.08	6.10	5.65
TSs-1	84.22	153.39	69.17	10.79	30.69	42.33	17.95	11.41	10.66	17.42	14.76	7.55	6.10	5.99
TSs-10	87.67	147.61	59.94	8.47	27.92	44.61	21.36	12.68	11.62	19.89	12.54	7.80	6.04	5.83
TSs-101	87.33	155.61	68.28	13.42	48.33	49.32	22.56	14.43	13.4	23.47	24.06	8.32	6.41	6.26
TSs-104	86.11	144.89	58.75	6.15	20.83	42.49	22.01	11.96	10.55	23.94	8.83	8.17	6.34	6.16
TSs-10A	86.50	152.61	66.11	12.59	37.18	48.83	20.94	11.71	11.02	20.16	18.20	8.01	5.97	5.59
TSs-11	90.67	152.61	62.22	8.83	22.32	40.56	19.51	12.92	12.02	16.31	9.94	7.27	5.88	5.82
TSs-111	90.94	150.28	59.33	7.36	28.71	44.13	20.21	12.24	11.51	19.64	15.42	7.58	6.05	6.03

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Appendix 2. continued

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
TSs-113	88.83	153.61	64.78	10.67	37.83	42.75	21.19	11.75	10.99	20.62	18.92	8.09	6.16	5.98
TSs-115	92.39	150.67	58.28	8.53	27.39	45.38	19.63	10.56	9.66	22.91	12.64	8.62	6.02	5.75
TSs-116	91.88	147.36	56.93	8.98	30.30	47.52	20.43	12.48	11.72	19.21	15.11	8.51	6.19	5.90
TSs-119	86.07	145.50	59.64	11.90	45.90	52.03	21.22	14.48	13.65	22.05	24.66	8.43	6.25	6.07
TSs-119A	85.11	152.72	67.61	9.90	37.26	44.73	21.90	12.42	11.76	23.48	18.89	8.63	6.69	6.61
TSs-12	88.72	151.17	62.44	12.25	43.69	40.81	20.01	11.53	10.46	21.02	21.29	8.01	6.24	5.85
TSs-120	96.94	162.00	65.06	7.16	24.69	45.65	20.07	12.03	10.85	21.96	13.29	8.10	6.53	6.30
TSs-121	88.50	152.33	63.83	11.08	33.82	40.32	23.23	13.82	12.99	22.55	14.80	8.07	6.33	6.24
TSs-13	92.47	143.07	52.64	7.59	28.14	48.84	21.22	13.31	12.05	21.29	14.19	8.23	6.14	5.71
TSs-133	87.17	155.39	68.22	11.71	45.01	47.29	19.91	12.44	11.96	22.12	24.09	8.05	6.44	6.43
TSs-136	87.83	151.50	63.67	10.85	36.28	44.09	21.13	12.74	11.81	20.80	16.49	7.89	6.42	5.95
TSs-137	89.00	154.78	65.78	8.71	31.91	48.80	19.01	12.65	11.88	19.72	16.79	7.69	6.28	6.38
TSs-138	87.83	150.44	62.61	6.99	31.05	42.92	21.50	12.94	12.24	23.28	14.82	8.14	6.23	6.11
TSs-14	86.88	149.53	64.93	10.14	36.65	44.04	20.04	13.34	11.93	18.06	18.49	7.83	6.05	5.78
TSs-143	89.22	149.06	59.83	7.59	23.55	42.14	20.23	11.08	9.87	20.60	11.10	8.32	5.99	5.70
TSs-144	89.22	153.61	64.39	10.32	34.61	45.45	19.77	12.25	11.41	20.44	18.34	7.87	6.37	6.23
TSs-148	88.67	151.78	63.11	8.50	34.05	47.04	20.04	13.53	12.49	23.11	17.83	7.87	6.39	6.13
TSs-15	87.11	155.28	67.89	10.29	32.62	48.16	19.72	12.21	11.33	21.37	14.04	7.64	6.24	6.11
TSs-150	91.39	150.50	59.11	8.65	30.41	47.16	18.97	13.8	13.42	18.22	16.14	7.38	6.15	6.06
TSs-151A	89.89	155.67	65.78	9.03	33.01	39.75	22.77	11.62	10.11	25.06	13.16	8.87	6.71	6.73
TSs-151B	86.63	144.00	59.86	10.28	39.70	48.46	20.44	14.16	13.66	20.04	20.15	7.99	6.12	5.76
TSs-153	101.39	162.61	61.22	8.94	36.56	45.15	22.43	14.55	13.05	19.23	20.32	7.93	6.25	6.15
TSs-155	91.22	145.94	54.72	10.45	43.39	43.71	20.55	13.53	12.52	20.46	21.54	7.65	6.20	6.09
TSs-156A	89.94	150.17	60.22	7.72	26.48	45.69	19.85	14.05	13.37	17.61	13.51	7.26	6.04	5.88
TSs-157	88.11	154.67	66.56	11.87	44.15	42.83	21.12	13.66	12.46	20.31	20.46	7.88	6.22	5.97

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Appendix 2. continued

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
TSs-157A	91.17	150.94	59.78	11.83	38.29	46.47	21.50	12.43	11.2	19.79	18.59	7.95	6.11	6.09
TSs-159A	87.28	153.83	66.56	8.16	23.76	43.40	20.56	11.46	10.77	21.54	10.40	8.12	6.24	6.08
TSs-16	88.33	147.00	58.50	9.33	30.18	43.01	21.03	13.37	12.55	18.92	12.54	7.78	6.22	6.12
TSs-161	92.17	152.78	60.56	9.92	30.03	45.01	21.29	12.17	11	21.76	13.39	8.19	6.07	5.47
TSs-162	90.89	159.06	68.17	16.28	49.40	43.50	19.94	12.91	11.81	21.54	21.02	8.28	6.48	5.93
TSs-166	87.78	155.39	67.61	11.49	39.80	40.92	22.14	14.06	12.48	20.00	17.72	7.91	5.97	5.86
TSs-168	88.28	154.06	66.22	9.29	34.52	42.47	21.43	11.78	10.8	22.56	16.69	8.07	6.40	6.37
TSs-186	93.22	146.00	52.78	9.43	33.91	48.04	21.39	13.63	12.53	18.05	17.35	7.92	6.06	5.68
TSs-19	94.56	163.00	68.13	6.38	27.06	35.27	20.54	11.19	10.58	19.52	13.41	8.00	6.27	6.28
TSs-192	88.94	143.87	55.87	11.82	45.41	43.74	21.32	13.1	12.23	20.55	22.62	8.04	6.17	5.83
TSs-195	91.00	151.39	60.39	14.33	56.90	46.42	19.96	12.85	11.66	21.50	27.54	7.81	6.11	5.96
TSs-1A	85.50	154.50	69.00	9.78	29.67	47.06	20.11	13.07	12	19.58	14.50	7.84	6.26	6.12
TSs-201	87.78	159.67	71.78	8.92	31.88	39.53	21.24	11.5	10.34	21.85	11.72	8.28	6.28	5.99
TSs-2015-06	90.22	149.28	58.50	9.12	28.93	50.45	22.97	13.64	12.14	18.74	14.73	8.18	6.24	5.72
TSs-212	88.67	152.61	63.94	7.32	27.89	46.50	22.66	14.59	13.38	20.68	13.62	8.06	6.26	6.19
TSs-216	88.89	156.83	67.94	6.99	24.44	42.14	21.26	12.75	11.93	23.45	10.66	8.12	6.22	6.09
TSs-217	92.72	151.83	59.11	4.09	18.25	46.72	21.43	14.17	12.58	17.54	8.45	7.96	6.05	5.48
TSs-22	88.17	150.06	61.89	10.77	32.12	47.03	19.86	11.92	11.26	17.50	12.30	7.58	5.95	5.81
TSs-224	90.44	151.50	61.06	9.08	38.07	45.16	19.87	11.78	11.22	22.67	17.93	7.80	6.19	5.99
TSs-22A	88.44	145.11	56.67	6.66	26.36	44.44	19.06	10.94	10.33	17.72	13.17	7.61	5.58	5.66
TSs-22B	91.11	152.50	61.39	8.91	29.12	39.94	18.63	11.82	11.06	17.60	13.50	7.56	5.94	5.72
TSs-23	87.44	157.11	69.67	12.99	44.36	43.90	21.13	12.62	11.97	21.29	19.42	7.90	6.13	5.98
TSs-23C	86.50	145.44	58.89	10.32	33.73	49.04	20.53	12.65	11.16	21.10	16.70	8.16	6.20	5.92
TSs-24	85.72	156.44	70.72	14.57	44.57	40.74	22.82	13.48	12.47	21.02	16.47	8.22	6.39	5.94
TSs-249	91.28	157.94	66.67	8.34	29.31	42.80	21.69	13.55	12.01	19.22	14.19	7.94	6.11	5.68

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Appendix 2. continued

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
TSs-255	87.17	157.06	69.89	11.69	45.14	42.74	20.74	12.56	11.73	20.73	18.81	8.09	6.24	5.85
TSs-26	89.50	153.78	64.28	9.63	34.52	44.51	20.75	11.95	11.2	22.24	15.63	8.30	6.37	6.07
TSs-266	87.56	154.28	66.72	8.97	31.61	43.90	22.23	12.15	11.35	22.93	14.15	8.29	6.37	5.94
TSs-268	88.50	154.44	65.94	9.32	30.22	46.78	19.82	12.15	11.04	19.52	15.84	8.30	6.20	5.98
TSs-269	84.89	156.00	71.11	7.38	23.62	52.57	20.28	12.86	12.36	19.63	13.16	7.73	6.02	5.77
TSs-273	87.83	153.06	65.22	6.76	28.76	32.62	22.04	14.18	12.78	19.96	10.55	7.96	6.34	6.03
TSs-274	91.44	146.39	54.94	8.29	29.97	42.17	21.51	12.78	11.3	20.07	15.14	8.08	6.19	5.96
TSs-275	87.61	155.00	67.39	11.24	33.02	36.12	20.49	11.76	10.46	19.14	13.85	8.16	6.06	5.69
TSs-276	88.39	146.94	58.56	8.45	35.79	38.19	22.13	11.66	11.09	19.83	15.57	7.74	6.07	5.98
TSs-277	92.67	157.67	65.00	6.72	23.89	42.93	21.75	11.5	10.23	21.33	10.42	8.38	6.30	5.94
TSs-278	88.17	155.06	66.89	8.29	28.01	38.27	21.81	12.81	11.73	23.44	11.10	8.35	6.31	6.11
TSs-28	85.31	146.93	62.40	7.37	28.11	44.00	20.24	13.51	12.4	20.61	12.89	7.92	6.14	5.97
TSs-280	89.61	153.06	63.44	8.91	39.34	41.49	21.91	12.67	12.39	19.18	16.15	7.92	6.13	5.90
TSs-282	87.72	151.22	63.50	14.09	39.12	45.85	21.00	11.79	10.75	18.84	17.24	7.84	6.05	5.74
TSs-285	88.00	150.78	62.78	11.48	37.72	46.65	21.84	13.67	12.49	20.78	16.93	8.13	6.02	5.76
TSs-287	87.44	155.72	68.28	11.61	34.91	42.01	20.90	11.73	10.82	20.94	14.19	8.25	6.31	5.95
TSs-289	85.00	149.00	64.00	9.38	31.52	37.53	18.17	11.35	10.7	18.06	10.21	8.01	6.05	5.84
TSs-29	87.20	140.80	53.47	8.24	32.56	37.74	22.36	13.34	12.35	20.29	8.99	8.13	6.14	5.88
TSs-293	88.83	159.11	70.28	9.19	36.24	42.23	20.28	12.51	11.47	19.47	16.52	7.57	6.05	5.97
TSs-294	87.06	154.39	67.33	10.21	31.93	46.29	20.07	11.69	10.84	20.16	14.79	8.05	6.23	5.90
TSs-296	87.67	159.17	71.50	11.41	35.59	43.61	19.80	13.29	11.99	19.36	16.77	7.52	6.11	6.12
TSs-297	91.61	160.39	67.11	9.96	34.69	44.68	23.40	12.35	11.23	20.39	16.41	7.95	6.32	6.01
TSs-298	92.94	160.39	67.44	7.92	25.03	48.39	20.14	13.68	12.29	20.88	12.37	7.95	6.08	5.48
TSs-299	89.22	151.78	62.56	8.32	30.71	40.16	21.26	12.49	11.74	19.91	12.57	8.17	6.00	5.89
TSs-3	88.56	158.50	69.94	8.79	28.35	40.77	18.85	11.3	10.29	18.81	12.42	7.65	6.03	5.71

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Appendix 2. continued

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
TSs-30	87.61	152.06	64.44	9.50	27.81	42.15	19.22	10.87	9.95	19.28	12.99	7.92	6.09	5.83
TSs-301	87.56	159.22	71.67	9.94	34.58	42.00	20.05	11.17	10.11	20.21	16.34	7.78	5.89	5.66
TSs-302	86.56	154.44	67.89	9.98	31.30	42.37	21.91	12.94	12.22	19.61	14.49	7.93	6.07	5.71
TSs-304	92.72	154.39	61.58	6.72	24.39	33.59	21.36	11.02	10.12	19.13	10.01	7.58	5.92	5.68
TSs-307	89.11	143.93	57.67	7.77	35.43	41.31	21.98	14.06	13.49	22.43	16.24	8.07	6.36	6.00
TSs-309	87.61	149.22	61.56	6.28	17.71	43.91	20.01	10.46	9.91	19.05	7.31	8.15	6.07	5.87
TSs-31	85.72	153.33	67.56	6.05	22.95	32.35	21.95	12.42	12.23	18.78	8.93	7.82	6.05	5.93
TSs-311	94.50	153.33	58.83	7.42	27.05	40.08	21.23	11.63	11.05	18.90	13.03	7.90	5.98	5.65
TSs-312	90.83	154.00	63.11	5.88	20.13	45.63	19.86	11.81	11.22	18.96	8.69	8.05	6.15	5.86
TSs-313	89.44	146.56	57.11	8.85	28.80	46.19	21.16	12.1	11.45	20.83	13.51	7.83	6.44	6.11
TSs-314	94.17	153.00	58.83	7.76	27.39	34.45	21.36	12.88	11.6	20.33	11.89	8.16	6.39	6.10
TSs-317	89.17	154.72	65.56	8.68	33.02	43.97	19.71	12.2	11.07	21.29	14.62	7.96	6.17	5.97
TSs-32	86.72	157.39	70.67	11.30	28.44	45.14	20.27	12.59	11.47	18.57	13.60	7.79	5.86	5.57
TSs-320	86.25	156.17	69.92	7.15	25.92	38.39	19.21	11.76	10.75	19.26	12.17	7.56	5.99	5.82
TSs-326	90.06	146.67	56.61	6.70	24.56	37.04	19.89	12.49	11.44	18.21	9.42	7.74	6.10	5.79
TSs-33	86.44	156.50	70.06	8.80	32.74	47.83	21.76	12.9	12.1	23.04	16.80	8.03	6.34	6.07
TSs-331	91.28	147.61	56.33	6.41	22.19	42.94	20.18	11.64	10.24	21.01	11.34	8.23	6.17	5.95
TSs-333	90.06	145.33	55.28	6.63	23.93	40.49	22.19	12.84	11.21	20.11	10.89	7.61	6.01	5.54
TSs-337	91.72	162.11	70.39	11.02	37.19	50.37	18.62	11.33	10.5	23.09	22.12	7.79	6.38	6.09
TSs-338	87.72	158.69	70.97	8.40	28.71	42.22	20.55	11.22	10.53	22.01	12.55	8.21	6.54	6.28
TSs-34	87.83	159.11	71.28	8.66	33.05	37.11	21.18	11.49	10.45	19.94	12.00	8.20	6.06	5.89
TSs-357	92.61	152.11	59.50	6.18	27.59	44.44	20.48	12.89	11.82	23.38	13.64	8.65	6.43	6.20
TSs-358	91.50	149.83	58.33	6.83	26.04	45.52	22.70	12.61	11.78	20.26	12.33	7.98	6.18	6.03
TSs-361	92.89	155.19	62.33	7.39	28.06	49.65	21.58	14.56	13.6	20.02	15.09	7.57	6.16	6.18
TSs-363	92.44	147.22	54.78	7.62	28.04	46.42	20.25	12.9	11.5	20.49	14.52	8.09	6.13	5.84

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Appendix 2. continued

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
TSs-365	96.83	154.17	57.33	11.29	42.50	51.62	19.89	12.56	11.88	21.68	23.61	7.53	6.35	6.43
TSs-366	87.56	155.00	67.44	11.08	33.95	47.35	19.68	11.38	10.33	20.98	17.50	8.10	6.20	5.92
TSs-367	90.06	149.72	59.67	8.99	27.25	35.99	21.26	11.79	10.72	19.14	11.71	8.11	6.12	5.72
TSs-368	89.17	154.78	65.56	7.55	28.67	39.69	22.78	11.64	10.37	16.20	12.46	7.70	5.86	5.50
TSs-369	86.22	147.22	61.00	11.74	47.84	46.41	20.28	11.59	10.68	21.10	25.08	8.11	6.43	6.05
TSs-371	90.67	151.94	61.28	7.76	26.76	40.83	20.20	11.36	10.64	21.39	11.48	8.11	6.24	6.06
TSs-377	86.06	152.44	66.39	7.01	20.86	46.77	20.04	12.73	12.24	18.96	10.74	7.53	6.09	5.85
TSs-378	86.89	149.83	62.94	9.45	32.78	38.41	19.75	11.63	10.89	21.15	15.51	7.99	6.19	5.88
TSs-38	89.56	152.89	63.28	8.20	30.58	42.69	20.44	11.96	11.14	19.45	15.24	7.80	5.91	5.58
TSs-39A	91.67	153.00	61.33	10.51	27.00	45.37	18.67	11.88	10.76	20.05	11.33	7.45	6.17	6.01
TSs-3A	85.28	153.39	68.11	9.34	31.07	38.63	21.09	12.53	11.54	17.81	12.21	7.84	6.05	5.73
TSs-4	88.44	158.61	70.17	12.27	42.41	43.62	21.02	13.09	11.88	22.68	19.78	7.92	6.38	6.27
TSs-42	85.61	153.94	68.33	8.24	33.21	47.94	19.77	11.34	10.33	20.16	17.29	7.96	6.13	6.00
TSs-421	91.06	160.00	68.94	9.51	53.07	44.43	20.50	11.9	11.11	22.74	31.63	8.39	6.42	6.17
TSs-422	86.94	151.83	64.89	9.84	29.97	46.14	18.65	12.58	12.09	17.65	12.72	7.49	5.91	5.64
TSs-424	89.41	149.27	62.60	11.28	39.78	48.97	20.77	12.8	11.79	23.33	20.59	8.20	6.52	6.27
TSs-427	91.83	155.00	63.17	10.29	38.71	40.63	21.33	12.81	11.28	19.29	17.43	7.98	6.22	5.98
TSs-433	86.61	153.83	67.22	9.26	26.55	46.66	19.77	11.35	10.89	19.63	12.75	7.62	6.10	5.97
TSs-434	88.25	151.69	63.44	7.29	25.77	35.89	19.28	11.21	10.29	22.10	10.24	8.11	6.20	5.74
TSs-435	90.17	150.94	60.78	8.54	27.72	44.36	21.30	11.15	10.13	17.78	13.44	7.40	5.88	5.93
TSs-437	88.83	149.28	60.56	7.98	32.81	41.66	20.03	11.4	10.03	19.92	15.38	7.72	6.22	6.23
TSs-438	85.28	148.00	62.72	7.04	28.59	39.93	21.02	11.91	11.5	18.96	12.39	7.67	5.97	5.86
TSs-44	89.91	152.38	62.47	8.52	34.44	40.47	19.33	13.28	11.88	20.94	12.94	8.13	6.49	6.16
TSs-440	90.33	147.67	57.33	9.69	40.02	43.28	19.21	11.49	10.84	20.31	18.64	8.01	6.42	5.97
TSs-441	92.78	158.44	65.67	8.37	33.71	45.58	20.45	12.56	11.97	24.28	16.00	7.75	6.38	6.41

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Appendix 2. continued

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
TSs-442	86.67	155.22	68.56	10.78	33.92	41.42	21.05	11.94	11.02	20.07	13.99	7.82	6.09	6.08
TSs-443	88.39	153.33	64.94	6.21	22.26	36.39	22.82	11.88	11.46	19.94	7.46	8.20	6.44	6.16
TSs-445	86.94	152.67	65.72	8.17	38.07	44.59	20.33	12.54	11.76	20.23	20.02	8.06	6.06	5.88
TSs-44C	88.56	151.33	62.78	7.94	35.46	47.37	18.46	12.03	10.05	20.76	18.85	7.97	6.43	6.36
TSs-45	87.44	153.22	65.78	8.20	30.32	45.81	18.94	11.43	10.88	21.92	14.17	8.02	6.31	6.23
TSs-450	90.94	147.86	59.93	11.35	36.46	47.50	22.11	13.16	12.18	21.55	17.07	8.22	6.09	5.84
TSs-46	90.83	161.00	70.17	7.99	31.62	58.67	18.98	11.23	10.22	21.75	17.22	7.67	6.21	6.43
TSs-47	95.28	154.33	59.06	5.83	20.23	37.45	20.92	12.92	12.23	18.97	8.54	7.77	5.86	5.58
TSs-48	88.44	153.39	64.94	12.92	45.06	45.79	20.06	13.13	12.03	18.32	21.42	7.73	5.96	5.83
TSs-49	90.44	159.78	69.33	10.39	29.74	40.20	18.91	11.56	10.07	18.94	11.72	7.66	5.97	5.83
TSs-5	86.39	145.94	59.56	8.80	29.63	54.03	20.27	11.48	9.88	24.56	15.03	8.28	6.51	6.30
TSs-51	88.44	150.67	62.22	9.39	29.86	41.79	21.62	12.03	11.1	20.19	12.75	7.92	5.99	5.81
TSs-55	87.72	154.78	67.06	9.52	39.66	45.31	21.17	12.73	11.58	20.64	17.79	8.03	6.56	6.34
TSs-56	87.06	150.61	63.56	10.90	36.06	44.61	21.05	12.17	11.56	22.67	17.00	8.28	6.24	6.21
TSs-56A	90.78	159.11	68.33	8.45	31.64	48.29	20.32	13.13	12.06	23.25	15.44	8.23	6.29	6.25
TSs-59	88.60	148.86	61.14	5.55	20.53	41.86	18.11	11.88	10.88	20.21	9.00	7.94	6.10	5.80
TSs-5A	87.72	152.72	65.00	7.78	30.75	47.89	20.72	11.33	10.43	24.98	14.94	8.54	6.60	6.37
TSs-6	86.61	153.78	67.17	7.35	24.24	40.02	18.26	10.12	9.32	20.68	10.63	8.05	6.27	6.09
TSs-60	86.61	151.28	64.67	9.79	32.88	46.14	21.01	12.24	11.37	18.65	14.89	7.89	6.06	5.73
TSs-60B	89.67	153.28	63.61	12.38	36.48	46.30	20.96	12.88	12.1	19.56	17.34	7.82	6.25	5.94
TSs-61	87.33	158.28	76.50	10.71	41.69	46.55	20.53	12.42	11.6	22.31	18.52	8.22	6.29	6.15
TSs-62	88.56	162.39	73.83	6.57	21.47	46.08	20.05	11.18	10.59	19.49	10.14	7.99	6.29	5.94
TSs-62B	90.94	155.72	64.78	7.65	26.49	44.86	20.38	12.51	11.63	24.31	11.75	8.52	6.39	6.14
TSs-63	87.28	152.39	65.11	10.21	34.98	42.63	21.63	13.06	12.34	20.89	15.08	8.00	6.27	6.03
TSs-63A	87.39	153.94	66.56	15.72	51.05	39.76	20.38	11.66	10.79	20.97	22.52	7.56	6.20	5.89

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST).

Appendix 2. continued

ACCESSION	D50F	DPM	GFP	NPPPL	PW	SP	PDL	NLPPD	NSPPD	HSW	SY	SL	SW	ST
TSs-66	90.11	154.22	64.11	12.62	41.26	41.19	21.05	11.49	10.96	20.63	17.25	8.48	6.08	5.99
TSs-66A	90.44	157.61	67.17	10.56	40.22	41.45	20.87	13.2	12.29	21.54	17.42	8.39	6.17	5.87
TSs-67	88.39	152.06	63.67	7.81	27.66	37.03	20.42	11.27	9.73	22.34	10.84	8.10	6.08	5.89
TSs-68	86.11	154.11	68.00	10.78	32.78	45.37	19.48	11.4	10.51	19.78	15.53	7.96	6.08	5.75
TSs-69	91.89	161.50	69.61	8.05	29.40	46.24	22.47	11.28	9.91	21.79	13.85	8.48	6.35	6.12
TSs-6A	88.67	152.06	63.39	8.72	34.28	40.53	20.84	12.03	11.64	18.38	15.44	7.88	5.91	5.84
TSs-6B	89.44	154.72	65.28	8.88	40.48	45.46	22.09	12.53	11.84	20.45	19.63	7.82	5.98	5.73
TSs-7	87.11	153.11	66.00	8.39	26.66	41.34	20.54	10.68	9.14	20.50	12.03	8.07	6.37	6.17
TSs-7A	89.17	155.00	65.83	9.26	35.36	45.19	22.10	12.33	11.28	22.57	16.09	8.21	6.33	6.17
TSs-8	86.28	148.39	67.67	9.86	36.66	42.47	21.78	13.63	12.87	20.46	17.79	7.72	6.22	6.27
TSs-82	89.44	149.17	59.72	7.39	23.30	35.12	20.33	11.3	10.29	16.97	9.17	7.79	5.77	5.49
TSs-84	86.22	155.00	68.78	9.18	32.84	41.22	20.85	13.01	11.93	19.49	14.30	8.02	6.14	6.05
TSs-84A	92.11	149.83	57.72	14.07	48.82	54.40	20.76	13.37	12.81	23.05	26.18	8.23	6.46	6.18
TSs-86	93.89	156.61	62.72	8.16	25.95	51.97	18.48	11.94	11.55	23.60	13.89	8.39	6.51	6.42
TSs-87	90.28	157.67	67.39	9.10	32.96	49.87	20.46	13.32	12.57	21.83	17.31	8.09	6.26	6.12
TSs-87B	92.94	154.89	61.94	8.65	32.89	47.11	21.35	13.18	12.67	20.64	17.12	7.80	6.04	6.12
TSs-90	83.89	153.39	69.50	8.04	20.76	50.55	18.95	10.85	10.63	19.63	9.52	7.75	6.13	5.63
TSs-92	89.78	151.06	61.28	10.06	34.41	41.59	21.23	12.36	11.55	20.27	14.94	8.43	6.22	5.85
TSs-93	93.11	156.83	63.72	8.42	35.74	47.95	22.34	13.78	12.93	19.63	17.37	8.35	5.97	5.59
TSs-96	85.72	154.11	68.39	12.48	39.29	46.31	20.25	15.49	14.46	18.59	17.70	7.95	6.43	6.26
TSs-98	89.89	155.50	65.61	7.85	27.83	42.76	21.67	11.99	11.17	20.69	12.85	8.11	6.33	6.14
MIN.	83.89	139.93	52.64	4.09	17.71	32.35	17.95	10.12	9.32	16.20	7.31	7.26	5.58	5.47
MAX.	101.39	163.00	76.50	16.28	56.90	58.67	23.40	15.49	14.46	25.06	31.63	8.87	6.71	6.73
MEAM	89.11	153.03	64.05	9.23	32.52	43.8	20.69	12.35	11.42	20.58	15.19	7.98	6.19	5.97
SEM	0.24	0.47	0.40	0.12	0.50	0.24	0.07	0.07	0.07	0.08	0.27	0.01	0.01	0.01
CV(%)	6.72	5.68	15.45	21.56	56.43	13.12	11.37	7.98	8.56	18.24	64.62	5.89	6.06	7.76

Days to flowering (D50F); Days to pod maturity (DPM); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Pod length (PDL); Seed yield (SY); Number of locules per pod (NLPPD); Number of seeds per pod (NSPPD); 100-seeds weight (HSW); Seed length (SL); Seed width (SW); Seed thickness (ST). Standard error of the mean (SEM).

Appendix 3. Cluster history of 196 accessions of African yam bean traits evaluated during the 2018 and 2019 cropping season in three agro-ecologies of Nigeria

Number of Clusters	Distance	Leader	Joiner	Number of Clusters	Distance	Leader	Joiner
195	0.9148	TSs-294	TSs-366	162	1.45275	TSs-10	TSs-299
194	0.9512	TSs-3	TSs-49	161	1.46849	TSs-14	TSs-60
193	0.9913	TSs-23	TSs-255	160	1.47249	TSs-157	TSs-23
192	1.0385	TSs-136	TSs-63	159	1.47645	TSs-22	TSs-422
191	1.0559	63A	TSs-60B	158	1.49618	TSs-143	TSs-331
190	1.0619	23C	TSs-371	157	1.49975	119A	TSs-56
189	1.0633	TSs-266	TSs-7A	156	1.52975	61A	TSs-32
188	1.0668	TSs-56A	TSs-87	155	1.53032	56A	TSs-92
187	1.0868	TSs-299	TSs-51	154	1.53268	62B	TSs-297
186	1.1089	56A	TSs-26	153	1.55052	30B	TSs-23C
185	1.1315	TSs-434	TSs-67	152	1.5769	TSs-30	TSs-433
184	1.1325	TSs-294	TSs-68	151	1.63858	TSs-445	TSs-6B
183	1.1961	TSs-38	TSs-6A	150	1.64709	TSs-16	TSs-28
182	1.2382	TSs-216	TSs-278	149	1.64948	TSs-269	TSs-377
181	1.252	TSs-285	TSs-450	148	1.65368	TSs-113	TSs-287
180	1.276	TSs-274	TSs-363	147	1.65664	TSs-44C	TSs-45
179	1.2855	TSs-201	TSs-34	146	1.66702	TSs-133	TSs-4
178	1.2867	TSs-293	TSs-296	145	1.67666	TSs-266	TSs-33
177	1.3084	TSs-268	TSs-317	144	1.68029	TSs-155	TSs-192
176	1.3106	TSs-150	TSs-156A	143	1.68887	TSs-116	TSs-274
175	1.3137	TSs-10A	TSs-282	142	1.69992	TSs-277	TSs-69
174	1.3276	119A	TSs-168	141	1.70599	44C	TSs-159A
173	1.3364	55A	TSs-55	140	1.72407	TSs-6	TSs-7
172	1.3415	TSs-280	TSs-427	139	1.73042	TSs-361	TSs-87B
171	1.3533	TSs-442	TSs-84	138	1.74154	63A	TSs-157A
170	1.3781	59B	TSs-224	137	1.75224	TSs-3	TSs-320
169	1.3792	TSs-15	TSs-1A	136	1.80126	TSs-138	TSs-148
168	1.3917	TSs-294	TSs-42	135	1.80417	TSs-116	TSs-13
167	1.3965	TSs-137	TSs-144	134	1.80683	TSs-311	TSs-367
166	1.4094	TSs-302	TSs-3A	133	1.80835	30A	TSs-309
165	1.4116	TSs-159A	TSs-98	132	1.81445	TSs-378	TSs-437
164	1.4137	TSs-113	TSs-66	131	1.8185	TSs-312	TSs-59
163	1.418	TSs-357	TSs-62B	130	1.84	TSs-276	TSs-438

Appendix 3. continued

Number of Clusters	Distance	Leader	Joiner	Number of Clusters	Distance	Leader	Joiner
129	1.84665	TSs-11	TSs-22B	96	2.21163	44C	TSs-338
128	1.86122	TSs-249	TSs-298	95	2.27011	TSs-119	TSs-151B
127	1.86785	TSs-302	TSs-442	94	2.29928	TSs-120	TSs-86
126	1.86805	TSs-12	TSs-369	93	2.3147	TSs-30	TSs-90
125	1.87904	TSs-268	TSs-294	92	2.3174	TSs-314	TSs-44
124	1.88494	55A	TSs-424	91	2.31998	159A	TSs-3
123	1.88692	7A	TSs-161	90	2.34402	TSs-10A	TSs-48
122	1.92425	60B	TSs-157	89	2.35397	60B	TSs-166
121	1.92469	TSs-313	TSs-358	88	2.35657	TSs-368	TSs-435
120	1.92607	TSs-137	TSs-15	87	2.3635	119A	TSs-266
119	1.93148	TSs-10	TSs-38	86	2.37757	TSs-11	TSs-22
118	1.97058	TSs-111	TSs-39A	85	2.39893	TSs-217	TSs-47
117	1.97091	56A	TSs-113	84	2.41114	TSs-29	TSs-307
116	1.9729	TSs-304	TSs-82	83	2.4429	TSs-249	TSs-93
115	1.97964	TSs-136	TSs-8	82	2.48561	TSs-304	TSs-311
114	1.98579	159A	TSs-301	81	2.4985	TSs-337	TSs-46
113	2.02309	TSs-133	TSs-61	80	2.52141	23C	TSs-434
112	2.03698	TSs-5	TSs-5A	79	2.55185	TSs-273	TSs-443
111	2.05354	TSs-326	TSs-333	78	2.55826	60B	TSs-24
110	2.07263	63A	TSs-280	77	2.56794	TSs-195	TSs-63A
109	2.07977	TSs-166	TSs-66A	76	2.57959	30A	TSs-6
108	2.08419	TSs-14	TSs-445	75	2.60052	55A	TSs-119A
107	2.10415	TSs-121	TSs-212	74	2.6136	151B	TSs-313
106	2.11408	TSs-1	TSs-289	73	2.66938	89A	TSs-16
105	2.12138	TSs-186	TSs-2015-06	72	2.77914	TSs-186	TSs-285
104	2.12156	TSs-12	TSs-440	71	2.79015	44C	TSs-216
103	2.12317	TSs-441	TSs-56A	70	2.79527	104B	TSs-361
102	2.13309	138A	TSs-138	69	2.79717	TSs-137	TSs-44C
101	2.13477	TSs-273	TSs-31	68	2.81658	TSs-121	TSs-136
100	2.13569	TSs-115	TSs-143	67	2.85427	TSs-1	TSs-30
99	2.14575	TSs-201	TSs-275	66	2.85899	TSs-11	TSs-111
98	2.19318	23C	TSs-378	65	2.88535	62B	TSs-277
97	2.19855	TSs-10	TSs-276	64	2.89745	TSs-10A	TSs-14

Appendix 3. continued

Number of Clusters	Distance	Leader	Joiner	Number of Clusters	Distance	Leader	Joiner
63	2.91175	TSs-101	TSs-84A	30	4.05284	159A	TSs-1
62	2.91545	TSs-19	TSs-62	29	4.31955	119A	56A
61	2.92106	61A	TSs-302	28	4.3223	TSs-10	TSs-304
60	2.92167	59B	TSs-12	27	4.37982	62B	TSs-19
59	2.9265	151B	TSs-104	26	4.38237	151B	30B
58	2.98077	TSs-304	TSs-368	25	4.51635	TSs-101	TSs-133
57	2.99687	61A	TSs-293	24	4.77272	138A	TSs-121
56	3.0572	TSs-133	TSs-162	23	4.87654	TSs-137	TSs-337
55	3.10146	TSs-120	TSs-357	22	4.94098	104B	TSs-153
54	3.15712	30B	89A	21	5.03573	TSs-10	TSs-217
53	3.20377	TSs-119	TSs-155	20	5.23169	151B	TSs-116
52	3.2302	119A	44C	19	5.35245	60B	63A
51	3.2868	TSs-217	TSs-326	18	5.50891	119A	62B
50	3.32382	TSs-115	TSs-312	17	5.59326	TSs-120	TSs-151A
49	3.38437	138A	55A	16	5.62667	159A	TSs-11
48	3.39486	63A	TSs-10A	15	5.90814	61A	7A
47	3.50006	TSs-133	TSs-421	14	5.96392	59B	TSs-119
46	3.53541	30B	TSs-29	13	6.41852	60B	61A
45	3.53602	104B	TSs-150	12	6.48144	119A	TSs-273
44	3.53797	TSs-116	TSs-186	11	6.97979	59B	TSs-101
43	3.55127	TSs-11	TSs-22A	10	7.84864	119A	TSs-120
42	3.5563	23C	30A	9	8.0488	159A	TSs-10
41	3.56199	7A	TSs-249	8	8.09484	138A	59B
40	3.56739	TSs-137	TSs-268	7	8.67858	119A	TSs-137
39	3.60558	TSs-19	TSs-201	6	8.69886	104B	151B
38	3.66692	TSs-120	TSs-441	5	8.71857	159A	23C
37	3.85048	TSs-153	TSs-365	4	11.5359	138A	60B
36	3.85416	TSs-151A	TSs-5	3	12.2638	104B	138A
35	3.87238	TSs-121	TSs-96	2	14.6654	104B	119A
34	3.87523	23C	TSs-115	1	19.2892	104B	159A
33	3.88687	TSs-273	TSs-314				
32	3.99513	59B	TSs-195				
31	4.04867	61A	TSs-269				

Appendix 4. Members of five clusters generated based on 14 agronomic traits

CLUSTERS	MEMBER
I	104B, 151B, 30B, TSs-104, TSs-116, TSs-13, TSs-150, TSs-153, TSs-156A, TSs-16, TSs-186, TSs-2015-06, TSs-23C, TSs-274, TSs-28, TSs-285, TSs-29, TSs-307, TSs-313, TSs-358, TSs-361, TSs-363, TSs-365, TSs-450, TSs-87B
II	138A, 55A, 59B, TSs-101, TSs-119, TSs-119A, TSs-12, TSs-133, TSs-136, TSs-138, TSs-148, TSs-151B, TSs-155, TSs-162, TSs-192, TSs-195, TSs-212, TSs-224, TSs-369, TSs-4, TSs-421, TSs-424, TSs-440, TSs-55, TSs-61, TSs-63, TSs-36A, TSs-8, TSs-84A, TSs-96
III	60B, 61A, 63A, 7A, TSs-10A, TSs-14, TSs-157, TSs-157A, TSs-161, TSs-166, TSs-23, TSs-24, TSs-249, TSs-255, TSs-269, TSs-280, TSs-282, TSs-293, TSs-296, TSs-298, TSs-302, TSs-32, TSs-377, TSs-3A, TSs-427, TSs-442, TSs-445, TSs-48, TSs-60, TSs-60B, TSs-66A, TSs-6B, TSs-84, TSs-93
IV	119A, 44C, 56A, 62B, , TSs-113, TSs-120, TSs-137, TSs-137, TSs-144, TSs-15, TSs-151A, TSs-159A, TSs-168, TSs-19, TSs-1A, TSs-201, TSs-216, TSs-26, TSs-266, TSs-268, TSs-273, TSs-275, TSs-277, TSs-278, TSs-287, TSs-294, TSs-297, TSs-31, TSs-314, TSs-317, TSs-33, TSs-337, TSs-338, TSs-34, TSs-357, TSs-366, TSs-42, TSs-44, TSs-441, TSs-443, TSs-44C, TSs-45, TSs-46, TSs-5, TSs-56, TSs-56A, TSs-5A, TSs-62, TSs-62B, TSs-66, TSs-68, TSs-69, TSs-7A, TSs-86, TSs-87, TSs-92, TSs-98
V	159A, 23C, 30A, , TSs-1, TSs-10, TSs-11, TSs-115, TSs-143, TSs-217, TSs-22, TSs-22A, TSs-22B, TSs-276, TSs-289, TSs-299, TSs-3, TSs-30, TSs-301, TSs-304, TSs-309, TSs-311, TSs-312, TSs-320, TSs-326, TSs-331, TSs-333, TSs-367, TSs-368, TSs-371, TSs-378, TSs-38, TSs-39A, TSs-422, TSs-433, TSs-437, TSs-438, TSs-47, 49, TSs-51, TSs-59, TSs-6, TSs-67, TSs-6A, TSs-7, TSs-82, TSs-90

Appendix 5. Nucleotide sequence of fifteen significant markers found on *Phaseolus vulgaris*

SN	Trait	Markers	Sequence
1	D50F	100003301|F|0-12:C>T-12:C>T	TGCAGAACTTCCTGGAGATTGGGTTTCCATGGGTCACAGCACTCAATGGCTTTGGTATTCTGTATATGC
2	D50F	100005571|F|0-66:C>A-66:C>A	TGCAGCATCTCTCTTTGGAATCAGAGAAGAGGATCAGAATCAAATGAAGCAGCAGCATTCCTCAACAAC
3	D50F	29420809|F|0-38:T>A-38:T>A	TGCAGGCTCTCATTTTAGCTGAGTCTATGGTTTCCATGAATGGAGAAGATTGGTTGGTTAGCCAAGTAA
4	D50F	100003791|F|0-8:C>T-8:C>T	TGCAGACATTGCCTTACATCACGCACCTTTCGATTCAATGTCAAATTCATAGATGACCACTGTAGACTG
5	D50F	100026403|F|0-5:G>T-5:G>T	TGCAGTTGTTTGTTCATACCATAAGATAAGTCAAACAGTACTAGGTAATAACCACGGCAGGGAACAGAC
6	D50F	29422735|F|0-21:G>A-21:G>A	TGCAGCACCAGTGGCATGCTTAGTTAGTGAATTCATCAAAACATTCAAAATGCTCTAAAGATCAAATTT
7	GFP	29420466|F|0-41:C>T-41:C>T	TGCAGCACCTGCCTCCATGAACAGTTTGCAGGTGAAGAAACTCGATTCCCCACAAAACAAGAAAGTTTC
8	NPPPL, PW	29420888|F|0-53:C>T-53:C>T	TGCAGGTCCTCGCTCGAAAACGATTCCTCCACGAAATTCGAAAGCCATTCTAGTTCCGCTATGTCATCA
9	NLPPD, NSPPD	29422706|F|0-34:C>T-34:C>T	TGCAGAGATACCTTACGAAGCACTAAGAGTGACATGCATTTCTTGGTTGATGAACAAGAAAAACAGTGG
10	NLPPD, NSPPD	100009412|F|0-67:G>A-67:G>A	TGCAGTTTTGGTAATGCGATTTTTTATGAACGCTTTACAACTTGTTTATTTGGGAAAATCATGCTAGAT
11	ST	100034480|F|0-31:C>A-31:C>A	TGCAGTCTGCACTGCATAAAGAAAATACAATAATCATTGCGTTTCACAGCAAAAGCCTCATCTTCAATT
12	SL	29420365|F|0-55:C>G-55:C>G	TGCAGATGCGTAATCAAGATACTACTCGAGCATAACCCAAGTGATAAGGACATGAGGAAGAGATTTAGC
13	SW, ST	29420736|F|0-57:G>T-57:G>T	TGCAGGAAGCTATCTTTTGTTTCAATCAGGCAAAGGCTGTGGTTCTTGTTATGAGGTTCCAATCAAAAT
14	SP	100024379|F|0-68:C>A-68:C>A	TGCAGAACACTGTATAAAATAATGTTTGGTAATGTTGGAATCAAAAACTCAGAACGGGTCTAATATTAA
15	SP	29420331|F|0-29:T>C-29:T>C	TGCAGATACAGGAGATCGCTGGAAACTTGCCACTGAGCTCGTTCCAGATGACATGCCTGCAAAAGGAAC

Days to flowering (D50F); Grain filling period (GFP); Number of pods per plant (NPPPL); Pod weight (PW); Shelling percentage (SP); Number of locus per pod (NLPPD); Number of seeds per pod (NSPPD); Seed length (SL); Seed width (SW); Seed thickness (ST)