UNIVERSITY OF CAPE COAST

GENETIC DIVERSITY AND GENOME-WIDE ASSOCIATION STUDIES OF BUSH YAM (*Dioscorea praehensilis* Benth.)

ADEYINKA SABURI ADEWUMI

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BY

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Thesis submitted to the Department of Crop Science of the School of Agriculture, College of Agriculture and Natural sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Crop Science

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature……Date…….

Name: Adeyinka Saburi Adewumi

Supervisors' Declaration

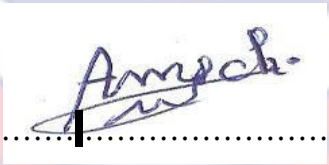
We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature…… Date…….

Name: Prof. Kingsley Joseph Taah

Co-Supervisor's Signature……. Date……..

Name: Prof. Paul Agu Asare



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Name: Dr. Paterne Angelot Agre

ABSTRACT

Bush yam (*Dioscorea praehensilis* Benth.) is a wild yam that provides food and contributes to the livelihoods of people in West Africa. Several socio-cultural, nutritional and agronomic factors impede its production and commercialization. A participatory rural survey conducted in 2019 in 23 communities across Ghana's three main growing regions revealed poor culinary quality (39.9%) and agronomic traits (20.7%) as the leading causes of decreasing productivity and abandonment. A preliminary investigation of genetic diversity among 43 bush accessions previously collected and maintained at School of Agriculture, University of cape Coast, Central Region, Ghana using 11 simple sequence repeats (SSRs) revealed low genetic diversity, suggesting a need for regional germplasm collection. Quantitative traits (15) grouped 162 accessions into best performing accessions for tuber yield and yield-related traits, best in resistance to yam mosaic virus resistance and best for post-harvest tuber quality traits. Using 24 qualitative traits, a high variation among the accessions. Potential sources of genes for yield and quality attributes in 162 accessions of *D. praehensilis* to improve the predominant yam species, *D. rotundata* was evaluated. For tuber yield (23.47 t ha^{-1}), yam mosaic virus (YMV) resistance (AUDPC=147.45) and tuber size (2.37), *D. praehensilis* accessions outperformed the best *D. rotundata* landraces. Population structure analysis based on hierarchical clustering, admixture and principal component analyses using 4,525 single nucleotide polymorphism (SNP) markers grouped *D. praehensilis* accessions into five clusters. Genome-wide association study revealed twenty-one SNPs associated with the agronomic and tuber quality traits. The identified SNPs accounted for approximately 16% of the total phenotypic variation. Gene annotation of significant SNPs identified candidate genes with functions related to growth and development of tubers, quality traits and defence mechanisms against yam mosaic virus. This study provides the first insight into the genetic diversity and marker-trait association of bush yam using farmers' indigenous knowledge, morphological and molecular approaches and genome-wide association studies.

LIST OF PUBLICATIONS

1. **Adewumi, A.S**., Taah, K.J., Asare, P.A., Adu, M.O., and Agre, P.A. (2020). Assessment of Genetic Diversity of *Dioscorea praehensilis* (Benth.) Collected from Central Region, Ghana, using Simple Sequence Repeat (SSR) Markers. *ES Journal of Agriculture and Current Research 2020: 1(1):1005*

2. **Adewumi, A.S**., Asare, P.A., Adu, M.O., Taah, K.J., Akaba, S., Mondo, J.M, and Agre, P.A. (2021). Farmers' Perceptions on Varietal Diversity, Trait Preferences, and Diversity Management of Bush Yam (*Dioscorea praehensilis* Benth.) in Ghana. *Scientific African. doi.org/10.1016/j.sciaf.2021.e00808*

3. **Adewumi, A.S**., Agre, P.A., Asare, P.A., Adu, M.O., Taah, K.J., Mondo, J.M., and Akaba, S. (2021). Exploring Bush Yam (*Dioscorea praehensilis* Benth.) as a Source of Agronomic and Quality Trait Genes in White Guinea Yam (*Dioscorea rotundata* Poir.) Breeding. *Agronomy, 12, 55. https://doi.org/10.3390/ agronomy12010055*

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KEYWORDS

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Dioscorea praehensilis

Farmers' perceptions

Genome-wide association studies

Genotyping-by-Sequencing

Participatory rural appraisal

Single nucleotide polymorphism

Tuber quality attributes

Wild yam

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DEDICATION

To my children: Abdul-Salam Adewolu, Tawakalt Adetoun, Rahmah Abidemi; my wife: Bilikis Oluwatosin, and my parents: Mr. and Mrs. Tunji Adewumi

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LIST OF ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
AUDPC	Area under Disease Progression Curve
BLUP	Best Linear Unbiased prediction
DNA	Deoxyribose Nucleic Acid
GBS	Genotyping by Sequencing
GWAS	Genome-Wide Association Studies
H^2	Broad Sense Heritability
LD	Linkage Disequilibrium
MLM	Mixed Linear Model
QTL	Quantitative Trait Loci
QTNs	Quantitative Trait Nucleotides
RAPD	Random Amplified Polymorphic DNA
RFLP	Random Fragment Length Polymorphism
SSRs	Simple Sequence Repeats
SNPs	Single Nucleotide Polymorphisms
YAD	Yam Anthracnose Disease
YMV	Yam Mosaic Virus

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CHAPTER ONE

INTRODUCTION

Background to the Study

Yams belong to the earliest monocotyledonous Dioscoreaceae family (Coursey, 1976), and are known to be extensively distributed across Africa, Asia, Oceania and South America (Darkwa et al., 2019). Yam is a food crop of economic importance in many tropical countries, mostly West Africa, South Asia and the Caribbean. Yam is one of the world's largest tuber crops (Sesay et al., 2013). It is noted that yams are a good source of essential nutrients such as fiber, carbohydrates, vitamins and vital minerals (Polycarp et al., 2012).

According to Couto et al. (2018), more than 600 known species are identified in genus *Dioscorea*, with 50-60 species being available for cultivation or wild-harvested, which can be used as food and by pharmaceutical companies for making drugs. Africa's most cultivated species are *D. alata* L., *D. bulbifera* L., *D. cayenesis* Lam. *D. esculenta* (Lour.) Burk, *D. rotundata* Poir., and *D. trifida* L. (Ile Crauford, Battey & Asiedu, 2006). The dominant wild yam species in Africa are *D. abyssinica*, *D. sagitifolia*, *D. preahensilis*, *D. liebrechstiana*, *D. mangenotiana* and *D. lecardi* (Dumont et al., 2006).

Bush yam (*Dioscorea praehensilis*), a wild or semi-cultivated yam species, is a food source that is of great importance to the welfare of more than 300 million people in tropic and sub-tropic countries (Dumont et al., 2006). *D. praehensilis* has spread across wider areas in Western, Central and Eastern Regions of Africa (Dumont et al., 2006). Bush yam (*D. praehensilis*) has been

reported to possess several advantages when compared to the known commercial species of yam. It possesses the ability to resist insect pest infestation and disease infection, can be cultivated in any environmental condition, does not require elaborate land preparation, agronomic management or additional land, as it can be established in tree crop plantations and, when mature, can remain wholesome in the soil for several months until it is harvested (Pitalounani et al., 2017). *D. praehensilis* is also high-yielding potentially much greater than the known commercial species and is a delicacy or premium yam, preferred to the commercial species, in localities where it is grown (Pitalounani et al., 2017). Moreover, the ability to flower quickly and generate many seeds makes it a good candidate for crop improvement through hybridisation (Pitalounani et al., 2017).

Statement of the Problem

Bush yam (*Dioscorea praehensilis)* is widely spread across the forest and semi-deciduous forest zones in Ghana, most especially where cocoa is cultivated, has been used as a source of food and for income generation among cocoa farmers in Ghana for ages (Aboagye, Nyadanu, Opoku-Agyeman, Owusu & Asiedu-Darko, 2015) but is presently abandoned and under serious threat of extinction. The *D. praehensilis,* in terms of production, consumption, and marketing, has been restricted to the cocoa belt primarily due to its short postharvest shelf-life (Dumont et al., 2006). The utilization of intra-species diversity among *D. praehensilis* genotypes has not been well explored and studied. Exploring the knowledge and information on the level of diversity in this crop using indigenous knowledge, morphological descriptors, molecular tools and variations in post-

harvest quality attributes is crucial to its involvement in yam breeding and improvement programmes. Therefore, this research work aimed to create a baseline information for the diversity of this yam species in terms of farmers' indigenous knowledge, morphological descriptors and molecular tools to support scientific exploitation and production as a commercial and food security crop. Some of the research problems addressed in this study are as follows:

The indigenous knowledge of the large pool of yam landraces, such as *Dioscorea praehensilis,* which is expected to be found in the yam diversity, is yet to be utilized in improving the crop. Besides, the diversity, distribution, and management of bush yam have not been studied much; thus, there is little information about the application of ethnobotanical information, agronomic and culinary characteristics for scientific research and development programmes. Several studies have been conducted on farmers' indigenous knowledge to estimate the diversity, distribution, and utilization of other species in the genus *Dioscorea* (Loko et al., 2013). Laly et al. (2019) also conducted indigenous knowledge on *Dioscorea dumeturum* using folk classifications. Application of perceptions and indigenous knowledge of farmers will provide baseline information on the genotype diversity, preferential criteria of farmers, distribution, management and utilization of bush yams (*Dioscorea praehensilis*).

The extent of intra-species diversity and phylogenic relationship in bush yam (*D. praehensilis*) germplasm have been understudied when compared to other yam species. Limited information is available in estimating the intra-species diversity of *D. praehensilis* using morphological descriptors. Several research

studies have been conducted using morphological descriptors to assess the divergence in *Dioscorea* spp, such as *D. rotundata*, *D. alata*, *D. cayenensis*, etc. Anokye, Tetteh and Otoo (2014) used morphological characterization to estimate the diversity among genotypes of water yam germplasm from Ghana. Oben, Egbe, Chuyong and Tabot (2016) used morphological descriptors to categorize Dioscorea spp. in South Western Cameroon. Sheikh and Kumar (2017) have also used morphological characterization to understand genetic diversity among Meghalayan *Dioscorea spp.* from North East India. Girma et al. (2018) also used morphological traits to redefine yam core collections in IITA. The only reported morphological characterization of *D. praehensilis* was by Djedatin et al. (2017). There is a need to explore morphological descriptors to assess the level of variations that exist among the bush yam genotypes. The application of morphological descriptors will provide a better understanding of the level of diversity in terms of agronomic and quality traits that could be used as selection criteria for good quality genotypes of bush yams in improvement programmes.

To prevent the problem of admixture among genotypes and enhance proper selection of parental materials for yam breeding programmes that may arise in the course of using morphological markers, it would be appropriate to employ molecular markers in the characterization and classification of yam genotypes. Molecular markers are more preferred to morphological traits because they are less dependent on environmental factors and epistatic interactions do not influence them. Several molecular markers, such as Isozymes (Bressan et al., 2011; Bressan et al., 2014), RAPD (Zannou et al., 2009), SSR (Siqueira,

Dequigiovanni, Corazon-Guivin, Feltran & Veasey, 2012; Mulualem et al., 2018), AFLP (Sonibare et al., 2010), RFLP (Terauchi et al., 1992), EST-SSR (Tamiru et al., 2015; Bhattacharjee et al., 2018) and SNPs (Tamiru et al., 2017; Siadjeu et al., 2018; Cormier et al., 2019a; Darkwa et al., 2020) have been employed to characterize the genetic diversity of *Dioscorea spp.* There have not been extensive studies on using molecular markers to estimate the genetic differentiation of *D. praehensilis* except as secondary materials with other yam species (Scarcelli et al., 2006; 2019). There is a need to conduct an extensive molecular characterization of this yam species using a large panel to understand the level of phylogenic relationships among the genotypes in this yam species. Application of single sequence polymorphisms (SNPs) (bi-allelic markers) based on the high throughput techniques of next-generation sequencing (NGS), such as genotyping-by-sequencing (GBS), will reveal the level of divergence that exists among the genotypes of *D. praehensilis*.

The yield of white yam (*D. rotundata*) in Ghana has been reported to be approximately 17.8 t/ha, far below the potential yield of between 40-50 t/ha (FAOSTAT, 2021). The low yield and productivity of *D. rotundata* in West Africa have been linked to yam mosaic virus (YMV) disease (Adeniji et al., 2012). As a result of their high yield potential and ability to withstand YMV severity, *D. praehensilis* accessions can serve as potential germplasm to develop new cultivars of white yam which are high-yielding and possess the ability to resist YMV infection.

Bush yams are known to be high-yielding compared to other yam species. However, there is limited information on yield potential and shelf-life even though it is said to be highly perishable (Pitalounani et al., 2017). Other undesirable characteristics of the yam include hardnening of tubers after harvesting, failure to soften after cooking as well as flesh colouring and oxidation, which have led to neglet and underutilization (Pitalounani et al., 2017). There is no information on bush yams' postharvest hardening and flesh tuber coloration and oxidation. Therefore, there is the need to screen for bush yam genotypes with superior yield and postharvest quality traits. Screening for bush yam with outstanding tuber quality traits will enhance food security, farmers' income and selections of promising genotypes for yam breeding programmes.

Agronomic and tuber quality characteristics are quantitatively inherited and controlled by many genes (polygenic). Limited information has been reported on detecting candidate genes and regions linked to the agronomic and tuber quality traits in *D. praehensilis*. Therefore, there is the need to understand the genetics of these complex traits (Rabbi et al., 2017). The application of genome-wide association studies (GWAS) is the best option to explore the variations in several beneficial alleles via the appropriate material selections and detection of the functional genes and regions linked to desired phenotypic traits. The application of GWAS has been reported in other yam species. Candidate genes linked to tuber yield and YMV severity has been reported in white yam (Agre et al., 2021a). Mondo et al. (2021) also used GWAS to detect genomic regions linked to sex determination traits in *D. alata*. GWAS have also been employed to

identify candidate genes associated with oxidative browning and dry matter content in greater yam (Gataraira et al., 2020). GWAS have been widely used on many crops to locate quantitative trait loci linked to phenotypes using linkage disequilibrium (L.D.) (Nordborg & Weigel, 2008). Thus, genetic dissection of complex traits in crops is possible using GWAS because it is a high throughput technology with a minimum cost of sequencing. Functional regions that code for desirable complex traits in crops can also be identified using GWAS (Lorenz, Hambli, & Jannink, 2010). GWAS can precisely estimate the size and direction of the allelic effects in a known locus compared to quantitative trait loci (Abdel-Shafy, Bortfeldt, Tetens, & Brockmann, 2014). GWAS have been employed in rice (Huang et al., 2010, 2012; Zhao et al., 2011) and maize (Kump et al., 2011). Genome-wide association studies have been employed in detecting candidate genes and regions linked with eleven agronomic traits in cassava (Zhang et al., 2018). GWAS has also been used in studying phenotypic traits in soybeans using SNP markers and SNP-based haplotype analysis (Contreras-Soto et al., 2017).

Objectives of the Study

This study aimed to assess the genetic diversity and detect candidate genes and regions associated with agronomic and tuber quality traits in bush yam. Specifically, the goal of this work was to:

1. estimate the perception of farmers on the diversity, distribution and management of bush yam;
2. assess the genetic diversity of bush yam accessions using simple sequence repeat (SSR) markers;

3. identify new sources for yield and YMV-resistant genes for improving *D. rotundata*;
4. determine the extent of genetic divergence among the accessions of bush yam in Ghana using morphological characterization;
5. assess the genetic diversity and phylogenic relationships among bush yam accessions using SNP markers; and
6. identify and detect candidate genes and regions linked and associated with key agronomic and tuber quality traits in bush yam accessions.

Hypotheses

1. Farmers are aware of the existence of diversity among the bush yam varieties.
2. SSR markers can indicate genetic differentiation among *D. praehensilis* accessions.
3. *D. praehensilis* possesses a gene pool that can be used to improve *D. rotundata*.
4. Morphological characterization can reveal the presence of genetic diversity and phylogenic relationships among bush yam accessions.
5. SNP markers can reveal the existence of high variability among bush yam accessions.
6. Known genes are linked to agronomic and tuber quality traits of bush yam accessions.

CHAPTER TWO

LITERATURE REVIEW

Dioscoreaceae

Yam (*Dioscore*a L. spp.) is the 4th of the crucial tuber crops on the economic scale of preference after potatoes (*Solanum tuberosum* L.), cassava (*Manihot esculenta* Crantz) and sweet potatoes (*Ipomoea batatas* (L.) Poir.) (Mengesha et al., 2013). As an essential source of dietary energy, livelihoods and income in Africa (Bekele & Bekele, 2018; Olatoye & Arueya, 2019), yam production is significant in some parts of the world. The West African yam belt accounts for over 97 per cent of global yam output (FAOSTAT, 2021). As one of the leading producers in West Africa, Ghana is rated second, with an average production of 0.47 metric tons in 2018 (FAOSTAT, 2021). Yams have cultural significance in West Africa because they represent riches and power; and they are the food of choice for several social rites and festivals (Tetteh & Saakwa, 1991). For example, yams are the food for celebrating the Asogli yam festival in Ghana (Mensah, 2013).

There are approximately 650 species in the family Dioscoreaceae (Couto et al., 2018), including the cultivated yams, *D. alata* (water yam), *D. rotundata* (white guinea yam), *D. esculenta* (Chinese or lesser yam), *D. cayenensis* (yellow guinea yam), *D. bulbifera* (aerial or bulbils yam) and *D. dumetorum* (trifoliate or bitter yam) and wild or semi-cultivated relatives, such as *D. praehensilis* (bush yam) and *D. abyssinica* (Scarcelli et al., 2019). In the West African yam belt, *D. rotundata* is commonly grown (Coursey, 1976). In Ghana, yam production is based on landraces and is mainly by smallholder farmers, predominantly in the

Northern, Brong-Ahafo and Eastern regions (Wumbei et al., 2019). In these production areas, where savannah vegetation zones are predominant, *D. rotundata* and *D. alata* are commonly grown. *Dioscorea rotundata*, however, accounts for about 80% of yams produced with an average yield of about 14.5 tons/ha (Anokye et al., 2014; Tetteh & Saakwa, 1991). The main factors influencing the choice of yam variety for production include (in order of importance) consumer taste preference, early maturity, storability, yield and availability of planting materials (Tetteh & Saakwa, 1991).

Importance of *Dioscorea* spp

Throughout the tropics, wild yam has strong ethno-medicinal benefits. The wild yam species have been used in treating several ailments such as, warts, asthma and fever (Maneenoon et al., 2008), gastritis (Kadiri et al., 2014), diarrhoea and jaundice (Dutta, 2015), tuberculosis (Sharma & Bastakoti, 2010). The mucilage from the tubers of some wild species is used as a pesticide by the Malaysian indigenous people (BurKill, 1960). In India, wild yam species are also used to make soap and shampoo to kill lice (Maneenoon et al., 2008). The tubers *D. oppositifolia* L. are used to treat swellings, scorpion stings and snake bites (Dutta, 2015). *D. hispida* Dennst. is used as an arrow poison antidote (Swarnkar & Katewa, 2008; Sahu et al., 2010).

The presence of starch and energy-supplemented metabolites in edible roots and tubers enriches the diet, and the presence of various secondary metabolites confers therapeutic characteristics. The *Dioscorea* spp is superior to many others as an essential medico-food used by about 300 million people

worldwide (Arnau et al., 2010). Yams are one of the most important sources of energy food for people in the Tropical regions (Kumar, Das, Shin, & Patra, 2017a). *Dioscorea* spp is important root and tuber crop that is very rich in dietary nutrients (Lev & Shriver, 1998).

Polyphenol chemicals are abundant in the tubers of *Dioscorea* spp (Farris et al., 2011). Antioxidant, antifungal, antimutagenic, and immunomodulatory properties have been documented for *Dioscorea* spp (Son et al., 2007). Yams are also employed in the cosmetics and pharmaceutical industries as necessary nutritional supplements. Yam tubers and other components contain several phenolic chemicals responsible for antibacterial activity (Kumar et al., 2017a). Kumar et al. (2017a) also discovered that water yam peel extracts have antifungal properties.

Wild Yam Relatives

Apart from Guinea yam or yam species of commercial values, there are wild relatives or semi-domesticated yam species such as *D. burkilliana*, *D. minutiflora*, and *D. praehensilis* (bush yam), which the subsistence or local farmers cultivate. These wild relatives or semi-domesticated yams are mainly cultivated around the forest and semi-deciduous forest zones and were previously the leading energy food for hunter-gatherers (Sato, 2006). While wild yams constitute a staple for many local farmers in Africa, they are also traditionally used as a close substitutes to white or water yam during off-seasons (Andriamparany et al., 2014). The cultivation of wild yams has been associated with specific ethnic groups in many parts of Africa, as can be seen in the case of

D. schimperiana and *D. bulbifera* for the Sheko people in Ethiopia (Hildebrand et al., 2003) and *D. semperflorens, D. mangenotiana, D. burkilliana, D. minutiflora* and *D. smilacifolia* for the Baka people in south-eastern Cameroon (Sato, 2001). For the people in these communities, the cultivation of these wild yams creates social bonds as it simultaneously shows belongingness and maintains cultural differences. However, despite the significant value of wild yams to local farmers' economic and social comfortability, the utilization of these crops remains unnoticed. The hypothesis is that there may be many complicated and, perhaps, interconnected constraints limiting the productivity, commercialization and greater use of wild yams.

In the Post-Green Revolution era, the argument is no longer about mechanized high input agriculture but rather the acceptance or not of genetically modified crops. Hence, the preservation of wild yams, crops only known and cultivated by few poor farmers in remote areas, may seem petty. However, depriving people, regardless of their wealth, location or magnitude of the cultivation, curation and consumption of their indigenous food constitutes the erosion of cultural heritage. Tinkering or an attempt to tinker with the identity claims of any people cannot be said to be trivial. Additionally, given the overexploitation of the few commercialized staples leading to loss of diversity and the high prevalence of food insecurity and poverty in many rural communities in Africa, measures to conserve biodiversity and improve wild yams' sustainability are very crucial. Moreover, there is only fragmented information on wild yams, such as *D. praehensilis*, found in the grey literature, as no extensive

studies have been conducted on the genetic diversity these yam species (Dumont et al., 2006).

D. praehensilis and its Potentials

Dioscorea praehensilis is a species of wild yam with a high potential to alleviate local food insecurity within households of peasant farmers and to be commercialized and introduced to urban dwellers. In Ghana, for example, the production of *D. praehensilis* provides subsistence and income for many peasant farmers and women who sell the produce, the majority of whom live in very remote areas. Currently, only about six yam species are commercially produced. The inclusion of *D. praehensilis* offers an avenue to prevent the risk of over-dependence on other cultivated yam species. The cultivation of *D. praehensilis* also lends itself very well to mixed-cropping and integrated farming systems. Therefore, it is one of the sure ways of providing food and dietary diversification at the community and household levels. The cultivation of *D. praehensilis* contributes to food quality and is also a way of preserving and celebrating cultural and dietary diversity in a community (Mayes et al., 2012).

The potentials of most underutilized crop species mostly exceed the performance of well-known commercial species in their genera, notably in stressful environments, such as drought stress and soil infertility where productivity is hampered (Mayes et al., 2012). In this context, the production of *D. praehensilis* bears several advantages over the known commercial yam species, especially in resource-poor environments where abiotic and biotic

stresses also abound. *Dioscorea praehensilis* is tolerant to insect infestations and disease infections compared to Guinea yams, which are severely constrained by the increased infestation of coleopteran pests, problems of leaf insect pests (leaf beetles), tuber pests, such as tuber beetles, mealy bugs, scales, diseases, such as leaf spot, leaf blight, tuber rots, yam mosaic virus and nematode infections (Braimah, Archirinah, & Adu-Mensah, 2008; Koradaa, Naskar, & Edison, 2010). Emshwiller et al. (2015) have stressed that most wild and indigenous yams as a whole and *D. praehensilis,* in particular, possess beneficial traits such as tolerance and adaptability to moisture stress, poor soils and resistance to diseases and pests. Moreover, it is environmentally friendly to cultivate *D. praehensilis,* as it does not require elaborate land preparation, agronomic management or additional land as it grows under established cocoa plantations and other mixed cropping systems. These physiognomies, among many others of *D. praehensilis,* are very crucial in promoting sustainable production through reduction in inputs, such as new lands, fertilizers and other agrochemicals.

Besides, *D. praehensilis*, when mature, can remain wholesome in the soil for several months if not harvested. If the tuber is harvested at the end of the dry season, the heads can be replanted, thus ensuring sustainability (Dumont et al., 2006). The tuber of *D. praehensilis* is also potentially much larger than that of the known commercial species (Figure 2.1). It has, for example, been reported that yields of these underutilized species could be three to seven-fold higher than that of the known commercial yam species (Treche & Guion, 1979; Brillouet et al., 1981). As an annual crop, *D. praehensilis* provides quicker outputs than other

perennial wild yams, such as *D. burkilliana* and *D. minutiflora*. Indigenous yams, on the whole, are a delicacy or premium yam, preferred to the commercial species in localities where it is grown. In fact, among the edible wild yams, *D. praehensilis* is said to be the most reliable staple food in Africa (Sato, 2006).

Yams, in general, are clonally propagated crops. They are characterized by inconsistent flowering, reduced botanic seed production and germination, as well as poor crossing success and, consequently, the yam tuber itself is the main propagule for the crop. However, intraspecific hybridisation is easily achievable for cultivated yams (Emshwiller et al., 2015), and this is an advantage to *D. praehensilis*. *Dioscorea praehensilis* flowers readily (Figure 2.2) and produces seeds profusely (Figure 2.4), making it amenable to crop improvement through hybridization (Treche & Guion, 1979; Brillouet et al., 1981). Therefore, it begs the question of why *D. praehensilis* and other wild relatives of cultivated yams have not been used in yam variety development. Many reasons have been ascribed, including intra- and inter-species level variations, poor viability of pollen, and low genetic distance within the genome leading to poor hybridizations at intra- and inter-species levels (Emshwiller et al., 2015). That notwithstanding, intraspecific hybridizations have been successfully made between commercialized cultivated yam species with *D. praehensilis* to transfer some of the beneficial traits of *D. praehensilis* to the commercialized species. The Dp2 morphotype is especially preferred for domestication and, hence, as source material or parental line for breeding *D. rutundata* cvs (Dumont et al., 2006). This hybridization is possibly due to its broader genetic diversity and adaptability scope. For example,

hybridizations have been reported successful in the crosses between *D. rutundata* and its wild relatives (*D. abyssinica* Hochst and Knuth and *D. praehensilis* Benth) (Emshwiller et al., 2015).

Figure 2. 1. Tubers of different morphotypes of *Dioscorea praehensilis*

Figure 2. 2. Female inflorescence in *Dioscorea praehensilis*

Figure 2. 3. Male inflorescence in *Dioscorea praehensilis*

Figure 2. 4. Fruit formation in *Dioscorea praehensilis*

Farmers' Perceptions of Indigenous Knowledge of *Dioscorea praehensilis*

Studies have been carried out on the indigenous knowledge system (IKS) in yams (Dansi et al., 2013; Loko et al., 2015). The IKS of a farming society, expressed in folklore form and transferred orally from one generation to the next, is integrated into food crops, yams cultivated by farmers and the community's atmosphere. It is well known that documenting and deploying historical understanding about the management and use of yams by farmers is a crucial point for enhancing farming systems and fending off the loss of biocultural diversity (Tamiru, Becker, & Maass, 2008).

Indigenous knowledge and procedures of farmers support the generation and continuous conservation of local yam diversity. Formal study hardly supports yam and its farming schemes, and its resources are underused compared to its potential (Tamiru et al., 2008). This restricted effort has resulted in under-exploitation of the potential of yam and putting its genetic resources and related IKS at constant erosion risk. Furthermore, Dansi et al. (2013) indicated that social characteristics of human groups, such as local understanding, experiences, and cultural values, contributed a crucial role in the viable management, preservation and utilisation of yam germplasm and the restoration of agro-ecosystems. Indigenous knowledge is an instrument of higher benefit and significance for the owning community and planners, policymakers and academics to design policies for agriculture conservation and the restoration of agro-ecosystems.

Farmers' indigenous knowledge has been reported on *Dioscorea praehensilis* in some studies. The indigenous knowledge has been employed in understanding the diversity and usefulness of wild edible plants in the Bullin District of North West Ethiopia. This study identified 29 wild tuberous plant species from 15 families and 24 genera, with five of them belonging to the Dioscoreaceae family (Mosissa, 2018). The species of yam documented in the District were *D. preahensilis*, *D. hisipida*, and *D. oppositiflora* (Mosissa, 2018). *D. praehensilis* has been reported to be abundant in farmers' indigenous knowledge on *Dioscorea* spp diversity conducted at the forest-savanna transition agro-ecology of Ghana (Otoo et al., 2015a). Three morphotypes of *D. praehensilis* (Katie, Otim Bale and Odono) were reported in a survey conducted on the diversity of *Dioscorea* spp in four communities in southern Ghana (Aboagye et al., 2015).

Avouhou et al. (2012) reported the influence of ethnicity, age and gender on the management and use of wild crop relatives in agricultural environments of Benin using farmers' indigenous knowledge. The younger household members within the surveyed localities are more encouraged to select and use a more restricted variety of wild edible plants for commercial activities. The gender of the household members has also contributed to the diversity management of the wild crop relatives through traditional local authorities by encouraging women to specialize in particular types of wild crop relatives (Avouhou et al., 2012).

The only comprehensive farmers' participatory survey on *D. praehensilis* was carried out in Togo (Pitalounani et al., 2017). This study identified four

morphotypes of *D. praehensilis* using flesh colours (White, Yellow, Black, and Red yams) as the criteria. In this study, 100% of the participated farmers preferred *D. praehensilis* on the basis of its high yielding and good culinary attributes.

Genetic Diversity of *D. praehensilis* using Morphological Descriptors

Morphological descriptors are those key features that can be differentiated and assessed visually by the naked eye. These characteristics include plant height, growth habit, plant vigour, flowering, intensity, tuber flesh colour, tuber shape, disease-resistant, leaf shape, leaf colour and type of flowers, sex, date of flowering and crop yield (Asfaw, 2016). These are lists of published descriptor keys that are designed for *Dioscorea* spp, which make morphological characterization highly significant. The morphological characterization is comparatively less expensive and easy to execute; it can be carried out in both insitu and exsitu (Asfaw, 2016).

Although several morphological descriptions have been carried in many species of yam, including *D. alata* (Purnomo et al., 2012; Anokye et al., 2014; Siqueira et al., 2014; Sheela, Abhilash, Asha, & Arnau, 2016; Girma et al., 2017; Agre et al., 2019; Patel et al., 2019), *D. cayenensis* Lam./ *D. rotundata* Poir. Complex (Guinea yam) (Bressan et al., 2014; Loko et al., 2015; Darkwa et al., 2020), *D. bulbifera* (Kouam et al., 2018), *D. burkilliana* (Gbadamosi et al., 2020), and *D. dumetorum* (Adaramola et al., 2016; Adeigbe et al., 2015; Oladeji et al., 2016). However, limited information is available on the characterization of *D. praehensilis* using morphological markers. The only documented morphological characterization of *D. praehensilis* was reported by (Djedatin et al., 2017).

Djedatin et al. (2017) employed 140 yam accessions comprising 19 domesticable and 18 non-domesticable *D. praehensilis*, 53 domesticable and 38 non-domesticable *D. abyssinica*, and 12 accessions of both *D. bulbifera* and *D. togoensis*. The IPGRI descriptor keys used were able to provide a clear distinction between the domesticable and non-domesticable *D. praehensilis*.

Application of Molecular Markers in *Dioscorea praehensilis* Studies

Molecular markers are tools for identifying a piece of DNA that are sources of variability across populations in the genome (Vaseeharan et al., 2013). Molecular markers, compared with the morphological markers are more stable, highly distributed throughout the genome and their applications are more important in estimating the genetic diversity and structure of most plant genomes; and environmental conditions and farm management activities have no influence on them (Kumar et al., 2010; Abdin et al., 2017). Molecular markers are helpful to crop improvement in various ways, including germplasm characterization, gene expression studies, linkage analysis, disease diagnostics, phylogenic analysis, and progeny validation in genetic hybridization (Varshney et al., 2007; Tamiru et al., 2015).

Many molecular markers have been successfully employed in the genetic diversity and characterization of yam species. A few of the markers that have been used in genetic diversity and characterization of *Dioscorea praehensilis* are discussed below.

Amplified fragment length polymorphisms (AFLPs)

In amplified fragment length polymorphisms, analysis of loci with high polymorphisms can be conducted on a single gel at a time with a single primer combination. The DNA fragment amplifications in AFLP are between 50 to 100 fragments (Mueller & Wolfenbarger, 1999). This marker is highly reliable, reproducible, and highly efficient in detecting polymorphism and has good resolution when compared with random amplified polymorphic DNA (RAPD) markers (Mignouna et al., 2003). Few studies have reported the application of AFLP markers involving *D. praehensilis*. Scarcelli et al. (2006) established the genetic nature of pre-domesticated yams in a population of 213 accessions of *Dioscorea* spp using 91 AFLP markers. In this study, 25 genotypes of *D. praehensilis* were involved. This study suggested that the wild yam relative hybridisation might be the source of new yam varieties. Genetic diversity of Guinea yam (*D. cayenensis*/*D. rotundata* complex) with their wild relative, *D. praehensilis* was conducted using two AFLP markers. The study revealed the generation of 87 polymorphic loci with five possible clusters indicating the effectiveness of AFLP markers in the yam improvement programme.

Simple sequence repeat (SSR) markers

Simple sequence repeats (SSRs) or microsatellites are molecular markers that are highly recommended due to their ability to locate specific loci in the genome, ability to cover the genome comprehensively. SSRs also have high level of polymorphism, ability to inherit co-dominantly, and ease of scoring gel bands.

These attributes make microsatellite markers the choice markers increasingly used to analyse the genetic divergence of several crop species (Zalapa et al., 2012).

SSR markers have been used in many studies on other yam species (Sosinski et al., 2000; Zalapa et al., 2012), but few are reported involving *D. praehensilis*. A genetic diversity study of 58 yam accessions consisting of 21 wild yams (*D. praehensilis* and *D. abyssinica*) and Guinea yam (*D. rotundata*/*D. cayenensis* complex) was carried out using seven microsatellite markers (Mengesha et al., 2013). Mengesha et al. (2013) found significant variations among the wild yams compared to cultivated yams. Scarcelli et al. (2017) using 12 microsatellite markers to estimate genetic diversity between 35 accessions of *D. rotundata*, 31 accessions of *D. praehensilis* and 34 accessions of *D. abyssinica* revealed the possibility of the wild yams being the hybrids between the wild and cultivated yams. Tostain et al. (2006) reported transferability of 16 microsatellite-enriched bank markers developed from *D. praehensilis*, *D. abyssinica* and *D. alata* in other *Dioscorea* spp. Chloroplast DNA microsatellite markers (cpSSRs) were used to characterise 148 accessions of *Dioscorea* spp comprising of Guinea yam (*D. rotundata*/*D.cayenensis* complex, *D. praehensilis*, *D. abyssinica*, *D. munitiflora*, *D. burkilliana*, *D. smilacifolia*, *D. togoensis*, *D. dumetorum*, *D. bulbifera*, *D. preusii*, and *D. alata* (Chaïr et al., 2005) to elucidate genetic variation in Benin. The results showed apparent similarities between *D. abyssinica* and *D. praehensilis*, and *D. rotundata*/D. *cayenensis* complex.

Single nucleotide polymorphism (SNP) markers

Single nucleotide polymorphisms (SNPs) are the most widely available sources of genetic variations in living organisms. SNPs indicate single base alterations at specific sites in the genomes (Duran et al., 2009). SNPs are co-dominant and are dispersed evenly in the plant genomes considerably (Yan et al., 2010). SNPs have been widely used like other genetic markers for several roles in improving crops, including the construction of linkage maps, genetic diversity analysis, association mapping and marker-assisted selection (MAS) (Yan et al., 2010). There has been improvement in perceptions and understanding of plant breeders about plant genetic diversity through the discovery and applications of SNP markers; hence, enhancing crop improvement programs. SNPs are used in the high-density techniques of next-generation sequencing due to their ability to generate large volumes of sequence and cost-effectiveness (Elshire et al., 2011).

Genotyping-by-sequencing (GBS) is a simple, highly multiplexed procedure, useful in studying the populations, germplasm characterization, breeding, and mapping of traits in plants and animals (Elshire et al., 2011). Several GBS applications have been reported in the study of the genetic diversity of *Dioscorea* spp, including *D. praehensilis*. Girma et al. (2014) carried out an evolution and pedigree study of guinea yams with 2,215 SNPs using the genotyping-by-sequencing platform. The investigation disclosed a tight link between the *D. praehensilis* and the two cultivated Guinea yam species (*D. rotundata* and *D. cayenensis*), but *D. burkilliana*, *D. abyssinica*, and *D. togoensis* were more distinct from the Guinea yam species (Girma et al., 2014). Whole-genome re-sequencing which is also another technique of next-generation-

sequencing (NGS) has been employed in investigating the domestication of white yam (*Dioscorea rotundata* Poir.) (Scarcelli et al., 2019). In this study, 3,570,940 SNPs were generated through the re-sequencing of 167 accessions of yam, which comprised of *D. rotundata*, *D. praehensilis*, and *D. abyssinica*. The study provided the likelihood evidence of *D. praehensilis* as the progenitors of African cultivated yam (*D. rotundata*) (Scarcelli et al., 2019).

Approaches of Enhancing the Potentials of *D. praehensilis*

Development of reference genomes

Wild plants have limited improvement when compared with their cultivated species. The potentials of these wild crop relatives, such as good nutritional values, disease tolerance and high yielding are not recognized (Michael & VanBuren, 2015). However, in the less developed parts of the world, especially among the farmers in forest zones, these wild crop species are the primary sources of diet and their genetic resources are vital to enhancing crop production (Michael & VanBuren, 2015). The advent of plant reference genomes has heralded a new era in plant genomics. Since the year 2000, more than 100 plant genomes have been sequenced, with 63 per cent of these being crop species (Michael & VanBuren, 2015). These genome sequences shed more light on crop genomes' architecture, evolution and unique characteristics, such as the persistence of essential agronomic traits following whole-genome duplication events (Michael & VanBuren, 2015). Even low-quality reference genomes can strengthen crop germplasm by reducing the cost of phenotyping and breeding cycles by using genome-wide molecular markers.

Reference genomes have been developed in some yam species (Michael & VanBuren, 2015). White Guinea yam (*D. rotundata*) reference genome sequence has been developed and released using a single diploid genotype, TDr96_F1, with a 594 Mb genome size, from which 76.4% is spread among 21 linkage groups (Tamiru et al., 2017). This reference genome has been used to develop molecular markers currently used in sex determination in *D. rotundata*. The reference genome of *D. rotundata* can be accessed on http://genome-e.ibrc.or.jp/home/ bioinformatics-team/yam. Cormier et al. (2019b) have reported the development of a reference high-density genetic map for two mapping populations of *D. alata* using the genotyping-by-sequencing platform. In this study, 1,579 polymorphic markers were generated across 20 linkage groups. A XX/XY method of sex was identified, resulting in a significant QTL for sex determination in *D. alata* (Cormier et al., 2019). Therefore, development of a reference genome for *D. praehensilis* will facilitate the identification of candidate genes and regions linked to the potential quality traits in its genome.

Genome-wide association studies for candidate gene detection

With the advent of the rapid development of genotyping and next-generation sequencing technologies and computational methods, genome-wide association studies (GWAS) are becoming effective tools for identifying genotype-phenotype variations underlying complex traits in crops (Liu & Yan, 2019; Rafalski, 2010). GWAS in crop plants typically use a long-term resource in the form of a diverse (and preferably homozygous) population that can be characterized for a variety of traits and only needs to be genotyped once, after

which specific mapping populations for specific traits or QTLs in crops can be generated (Atwell et al., 2010).

GWAS have now been widely conducted successfully in many crops, including maize, rice, sorghum and foxtail millet (Huang et al., 2010, 2012; Jia et al., 2013; Kump et al., 2011; Li et al., 2013; Morris et al., 2013; Takeda & Matsuoka, 2008; Zhao et al., 2011).

In root and tuber crops, such as cassava, GWAS have been used in detecting candidate genes linked to some key agronomic and quality traits (Esuma et al., 2016; Rabbi et al., 2017; Zhang et al., 2018; do Carmo et al., 2020). GWAS have been successfully employed in *Dioscorea* spp such as *D. alata* (Gatarira et al., 2020; Mondo et al., 2021) to detect candidate genes linked to dry matter content, oxidative browning and sex-linked traits. Agre et al. (2021a) also reported the application of GWAS in white guinea yam to identify quantitative trait nucleotides associated with tuber yield and resistance to yam mosaic virus. This indicates that GWAS can be applied in *D. praehensilis* to detect candidate genes linked to agronomic and tuber quality traits.

Metabolite profiling

Metabolites are crucial factors of plant metabolism due to their significant impact on plant biomass and architecture (Turner et al., 2016). Recent metabolomics initiatives have been aimed at improving quality, focusing on yield-related variables of crop plants (Kumar, Bohra, Pandey, Pandey, & Kumar, 2017b). Notably, the integration of metabolomics with other methods like

quantitative genetics, transcriptomics and genetic manipulation has demonstrated metabolomics' outstanding value to plant improvement (Kumar et al., 2017b). Researchers can effectively combine these modern methodologies to find the functional gene(s) and characterize significant metabolites, rank potential genes for downstream investigations and eventually, propose trait-specific markers to improve commercially essential traits (Kumar et al., 2017b).

Price, Bhattacharjee, Lopez-Montes and Fraser (2018) conducted a study to determine the diversity in carotenoid contents and other metabolites present in yam species comprising of *D. rotundata*, *D. cayenensis*, *D. alata*, *D. dumetorum*, and *D. bulbifera*. The presence of high β-carotene that can aid provitamin A bio-fortification was identified in *D. dumetorum* than in any other species. Yellow Guinea yam (*D. cayenensis*) has higher β-carotene than white Guinea yam (*D. rotundata*), but, no variation was reported between the two Guinea yam species. C25-epoxy-apocarotenoid persicaxanthin, a metabolite that has been reported to be a potential source for tuber dormancy, was identified in other species except for *D. alata* (Price et al., 2018). Dormancy is known to extend tuber storability and growth cycles for more extended periods in root and tuber crops (Price et al., 2018).

Price, Bhattacharjee, Lopez-Montes and Fraser (2017) also carried out metabolite profiling of 49 genotypes comprising four species of yam through gas chromatography-mass spectrophotometry resulting in the identification of a total of 152 metabolites from the polar extract of leaves. In order to quantify 522 yam accessions in eight *Dioscorea* species for individual sugars, catechins, phenolic

acids and saponin contents of yam flours, Lebot, Malapa and Molisalé (2019) developed high-performance thin-layer chromatography, a novel technique for rapid nutritional analysis of many samples.

Although the majority of cultivated yam species have been involved in metabolite profiling, there is a need to carry out the metabolomics of their wild relatives, especially *D. praehensilis* which has been identified to have challenges of tuber hardening as well as short shelf-life after harvesting. This will identify the secondary metabolites associated with these tuber quality traits in *D. preahensilis*.

Near-infrared reflectance spectroscopy

In root and tuber crops, the application of high throughput techniques for phenotyping in breeding and crop improvement programmes is highly significant for the early screening of larger population of genotypes for quality traits (Alamu et al., 2020). Because of current needs to select crop with superior post-harvest quality traits, it is more important to establish a quick and effective approach for determining nutritional and culinary quality attributes in crops (Cen & He, 2007).

In recent years, near-infrared reflectance spectroscopy (NIRS) has become a popular non-destructive and quick technology for evaluating crop nutritional and culinary quality. It provides more valuable information in screening the nutritional and culinary qualities of root and tuber crops to enhance accuracy in evaluation and improve the precision of selecting superior genotypes (Darkwa et al., 2019).

Several successes have been reported in applying NIRS in *Dioscorea* spp. NIRS has been successfully used in developing models for rapidly predicting physicochemical properties in Dioscorea spp (Lebot & Malapa, 2013; Alamu, Adesokan, & Maziya-Dixon, 2019; Alamu et al., 2020). Alamu et al. (2019) conducted research to develop calibration equations of high coefficient of determination ($R^2 \geq 0.80$) and high to medium coefficients of determination (R^2 = 0.5 for tannin, - 0.8 for moisture) in cross-validation for predicting moisture, ash, protein, crude fibre and tannin in *Dioscorea* spp. Lebot & Malapa (2013) also developed high calibration curves ($R^2 \geq 0.84$) for starch, sugar and protein in 265 yam accessions across seven yam species. Alamu et al. (2020) also developed calibration curves for predicting tuber quality traits in *D. alata* and *D. rotundata*, employing varying methods of sampling, such as blending, chopping and grating. Blended samples had the highest coefficient of prediction (R^2) for dry matter (0.95) and starch (0.83) while grated samples had the lowest coefficient of prediction (R^2) for dry matter (0.87) and starch (0.50).

Application of NIRS in *D. praehensilis* could lead to the development of prediction models which would be employed in determining the physicochemical properties of this yam species to ensure selection of superior genotypes to enhance yam improvement.

CHAPTER THREE

FARMERS' PERCEPTIONS ON VARIETAL DIVERSITY, TRAIT PREFERENCES, AND DIVERSITY MANAGEMENT OF BUSH YAM (*Dioscorea praehensilis* Benth.) IN GHANA

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Abstract

Bush yam (*Dioscorea praehensilis* Benth.) is an important food and cash crop species in some West and Central African countries. Unfortunately, several socioeconomic, cultural, nutritional and agronomic constraints hinder its cultivation and, thus lead to its underutilization and gradual disappearance. To effectively promote its cultivation and utilization, knowledge of its diversity, distribution, management and farmers' varietal preferences are necessary. This study, therefore, used a participatory rural appraisal survey to assess such information in 23 villages from three regions of Ghana. A total of 42 *D. praehensilis* accessions were recorded and grouped in seven classes based on the tuber flesh colour. The Shannon diversity index (H' = 1.88), equitability (0.65) and Margalef species richness (2.53) revealed the presence of moderate diversity and distribution in the surveyed regions. Farmers' variety trait preferences included mainly the early maturity (21.1%), smooth tuber texture (16.5%), stability in tuber flesh colour (7.8%), good storage aptitude (7.6%) and high tuber yield (12.8%). In contrast, *D. praehensilis* production and utilization rates

declined mainly due to poor culinary quality (39.9%) and agronomic traits (20.7%) of most accessions. Survey results showed that *D. praehensilis* is essentially an *in-situ* conserved species in Ghana (60.0%). This study provided insight on *D. praehensilis* diversity, distribution and farmers' varietal preferences in Ghana, which will guide its genetic resource conservation and breeding interventions.

Keywords: *Dioscorea praehensilis*, participatory rural appraisal survey, Ghana, farmers' preference criteria, genetic resource conservation

Introduction

Yam (*Dioscorea* spp.) is a common name for ~600 species in the *Dioscorea* genus. It is extensively cultivated in tropical and subtropical areas for its starchy underground or aerial tubers. These species provide dietary energy from direct consumption and often contain secondary metabolites used for industrial and pharmaceutical purposes (Cuoto et al., 2018; Price et al., 2020). More than 90% of the world's yam is grown in West Africa, where the crops feed 300 million people (Price et al., 2020) and contribute an essential cultural and religious significance to local communities (Obidiegwu & Akpabio, 2017).

Apart from the commercial yam species, there are edible wild and semi-domesticated yams such as *D. burkilliana*, *D. minutiflora* and *D. praehensilis*, which are grown for subsistence. These wild yams are primarily found in forest areas and are used by the farmers and rural dwellers as a staple food in filling the hunger gap during drought periods and a source of traditional medicine (Andriamparany et al., 2014; Sato, 2001; 2006). These wild yams have been

known to be cultivated and domesticated in Africa. For example, *D. schimperiana* and *D. bulbifera* in Ethiopia (Hildebrand et al., 2000); *D. semperflorens*, *D. mangenotiana*, *D. burkilliana*, *D. minutiflora*, *D. smilacifolia* and *D. smilacifolia* in south-eastern Cameroon (Sato, 2001); and *D. praehensilis* in West Africa, particularly (Dansi et al., 2013; Scarcelli et al., 2006; 2019). However, despite the critical role of wild yams in the livelihood of many tropical rural dwellers, these crops remain underutilized (Sato, 2006). The underutilization of these yam species suggests that there may be some complex and, perhaps, inter-related constraints exerting variable limitations on the productivity, processing, commercialization and hence, reduction in utilization of these wild yams. Therefore, the factors challenging wide use of wild and semi-domesticated yams should be investigated with respect to local socio-economic, cultural, technical and agro-ecological realities to guide farmer-support structures and other stakeholders involved in maintaining biodiversity or promoting widespread use of these yam species.

Among the wild yams, *D. praehensilis* has emerged as the most popular species with a significant contribution potential to food security and poverty alleviation in most rural areas of some West and Central African countries (Pitalounani et al., 2017). Bush yam tubers, like those of other yam species such as white Guinea (*D. rotundata*) and water (*D. alata*), are mostly consumed boiled. *D. praehensilis* is a perennial vine with green to purplish thorny stems that grow up to 15 meters long and are often supported by trees. The leaves are commonly cordate or sagittate in form and phyllolaxy is alternate or opposite. Bush yam

tubers are generally up to 60 cm long, with a bitter violet layer beneath the epidermis and white meat tinted with yellow (Di Giusto, Dounias, & McKey, 2017).

Despite the benefits of *D. praehensilis* and its significant contributions to addressing food insecurity among rural farmers in West Africa during lean and unfavourable periods, this species is rapidly vanishing from the ecosystem (Scarcelli et al., 2019). Moreover, there are limited research efforts to improve the production status of this yam species. No information exists on the yield potentials of this crop compared to other yam species. There is a need to conduct a germplasm collection for *D. praehensilis* to serve as the basis for developing varietal improvement programs and implementing conservation strategies.

Ghana is the second-largest producer of yams in the world after Nigeria (FAOSTAT, 2020). Its ecological conditions are suitable for the bush yam, which is harvested from the forest and grown and marketed by its inhabitants. However, there is limited information on farmers' perception of the diversity, distribution, varietal preference criteria, production constraints, seed systems, conservation methods and farm management practices of *D. praehensilis* in Ghana. The only reported indigenous knowledge on *D. praehensilis* in Ghana was conducted along with other yam species (*D. rotundata*, *D. alata*, *D. cayenensis*, *D. esculenta* and *D. dumetorum*) (Aboagye et al., 2015). This implies that local knowledge of *D. praehensilis* has not been well explored, as it was just a secondary item among investigated species. The study by Aboagye et al. (2015) only focused on the diversity and production constraints of the six yam species. No information was

reported on varietal preference criteria, seed systems, conservation methods and farm management practices of yam in Ghana. As a result, only three accessions (*Otim*, *Odonor* and *Kat*) of *D. praehensilis* were reported in that study (Aboagye et al., 2015). Pitalounani et al. (2017) grouped *D. praehensilis* accessions of Togo, a neighbouring country to Ghana, into four classes based on tuber flesh colours (white, yellow, red and black) using farmers' perceptions and indigenous knowledge. Understanding the genetic diversity, uses and distribution of orphan crops, such as *D. praehensilis,* is essential in determining what and where to conserve for sustainable utilization. Therefore, conducting an extensive survey to assemble all the relevant information related to farmers' perceptions about this yam species is crucial for guiding future genetic resource conservation, production, improvement and breeding interventions. This will serve as baseline information in understanding the extent of diversity, farmers' varietal preference criteria and diversity management of *D. praehensilis* in Ghana.

In the sub-Saharan African countries, where agriculture is the spearhead of the economy, improved crop varieties must be developed or simply discovered within the existing diversity to increase the resilience of African farming systems to rapid population growth, changes in eating habits and climate change effects. In both cases, a good knowledge of the existing yam varietal diversity and the agronomic performance of these varieties are necessary for effective interventions (Orobiyi et al., 2013). Moreover, documentation and identification of high-performing cultivars based on farmers' varietal preference criteria will provide strategies to overcome constraints affecting *D. praehensilis* production in Ghana.

This will, consequently, enhance the production and productivity of *D. praehensilis*, thereby unravelling its potential contribution to food security and poverty reduction among rural farmers. Besides, farmers' participation in identifying needs and desirable traits for plant breeding and their involvement in the varietal selection process could increase the probability of farmers' new variety adoption.

Therefore, this study aimed to contribute to the understanding of the perceptions of local farmers on *D. praehensilis* varietal diversity and preferences to guide efforts in its diversity conservation and management in Ghana. It specifically sought to: (i) investigate farmers' knowledge and perception of the diversity, distribution, conservation methods and farm management practices of *D. praehensilis* in Ghana, (ii) determine the major factors limiting the production and utilization of *D. praehensilis* in Ghana and (iii) inventory the farmers' preference criteria for *D. praehensilis* accessions in Ghana.

Materials and Methods

Description of the study area

Three administrative regions (Central, Eastern, and Western North), where *D. praehensilis* is predominantly cultivated in Ghana, were selected for this survey (Figure 3.1). The three regions are located in the southern part of Ghana. The Central Region is bordered to the north by Ashanti and Eastern regions, the Western Region to the west, the Greater Accra Region to the east, and the south by the Gulf of Guinea. The Western North Region is bounded to the west by the Côte d'Ivoire border, the Central Region in the southeast, and the Ashanti, Ahafo,

and Bono regions in the north. The Eastern Region is bordered to the east by the Lake Volta, the north by the Bono and Ahafo regions, the west by the Ashanti Region, and the south by the Central and Greater Accra regions. All the three surveyed regions are located in Ghana's deciduous forest agro-ecological zone (Table 3.1). The deciduous forest ecological zone is the largest agro-ecological zone in Ghana. It is characterized by a bimodal climate with two rainy and two dry seasons (Kemausuor, Akowuah & Ofori, 2013). Plantation crops, including cocoa, kola, coffee, oil palm, coconut, and food crops like yam, maize and cassava are the most common crops cultivated in this zone. This is why *D. praehensilis* is restricted to Ghana's woodland and cocoa farming zones, known as "kokoo ase bayèrè" (yam in a cocoa plantation).

Table 3. 1. Ecological characteristics of the studied regions of Ghana

Region	Climatic zone	Rainfall regime	Mean rainfall (mm/year)	Mean temp. (°C)	Dominant land-use systems	Major grown crops
Central	Rain forest, semi-deciduous forest and coastal savannah	Bimodal (2 rainy seasons)	800-1500	Min: 26.2 Max: 26.7	Forest and plantation crops	Cocoa, plantain, banana, cassava, maize, oil palm and coconut
Eastern	Semi-deciduous forest	Bimodal (2 rainy seasons)	1086-1500	Min: 23.5 Max: 27.0	Forest and plantation crops	Cocoa, plantain, banana, cassava, maize, oil palm and coconut
Western North	Rain forest and semi-deciduous forest	Bimodal (2 rainy seasons)	1200-1500	Min: 25.0 Max: 26.0	Forest and plantation crops	Cocoa, plantain, banana, cassava, maize, oil palm and coconut

Source: Ghana Meteorological Agency. Min: Minimum; Max: Maximum; temp.: temperature

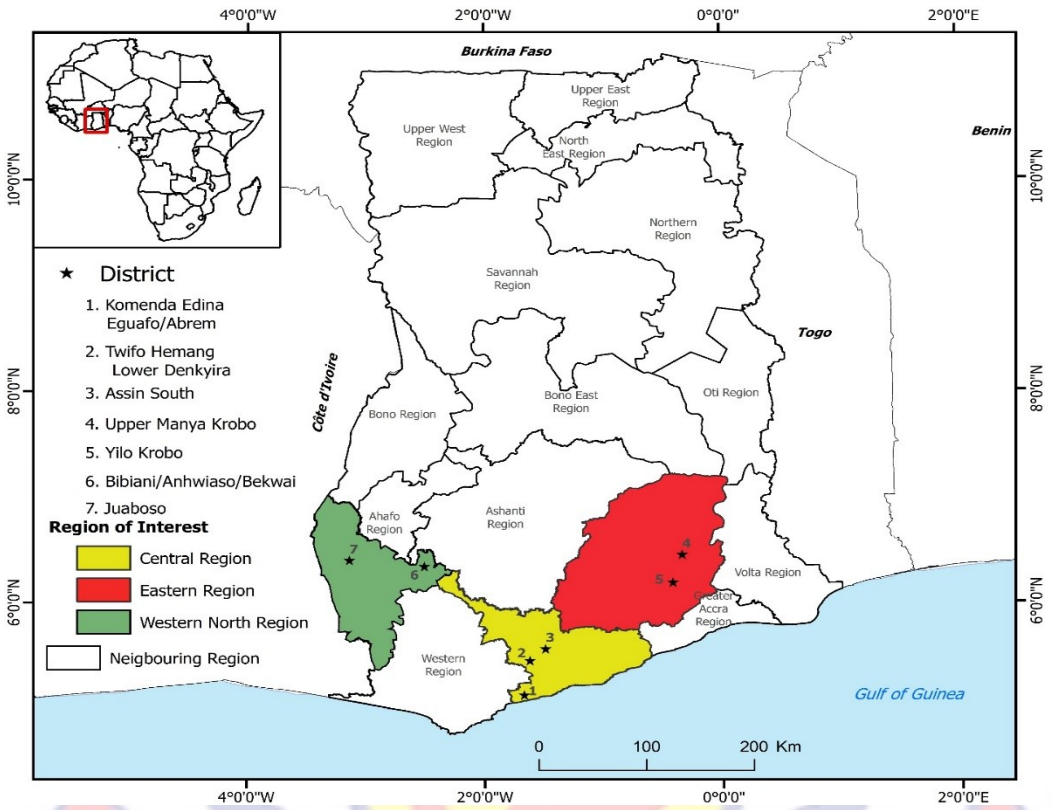

Figure 3. 1. Map of Ghana showing the geographical locations of regions and districts surveyed for *Dioscorea praehensilis* diversity.

Source: Field Survey (2019)

Sampling techniques and data collection

Prior to data collection, a pre-survey was conducted by consulting regional departments of agriculture and other resource persons (extension agents, farmers' association's representatives, local authorities, experienced persons in yam cultivation, etc.) in Central, Eastern and Western North regions, where *D. praehensilis* is mainly produced in Ghana. Discussions with these resource persons aimed at determining the major *D. praehensilis* production areas within

the selected regions of Ghana. A total of seven districts were then selected across the three target regions (Figure 3.1). Across the seven districts and, because of high coverage, 23 villages were randomly selected among those listed by resource persons as major bush yam producers from each selected district. In each selected village, a chain-referral sampling technique (also referred to as snowball sampling), which is a non-probability technique, was used to select bush yam farmers (Etikan, Alkassim & Abubakar, 2016). A chain-referral approach entailed selecting the first respondent, a well-known bush yam specialist in a community, who then offered another well-known respondents until the entire respondents required in the community (target sample size) was covered. Twenty farmers were interviewed in each community; and their fields were visited to gather information on morphological traits of bush yam plants and collect field management data. In communities where the snowball sampling technique was unable to define the target population number (less than 20), all accessible bush yam farmers were surveyed . Four hundred and thirty-seven (437) *D. praehensilis* farmers were individually interviewed across the three regions. Individual interviews with farmers were conducted by administering a pre-elaborated semi-structured questionnaire with the help of local translators.

The collected data included farmers' socio-demographic information (age, gender, education status, family size, farming experience, family income, primary occupation, non-farm income-generating activities and farm size), the *D. praehensilis* genetic resources (number of cultivars, farmers' preference criteria, and the diversity management) and reasons behind *D. praehensilis* production

decline and varietal losses and abandonment by farmers. Abandonment and varietal loss in this study were assessed by comparing the number of *D. praehensilis* accessions a farmer used to exploit and the one he/she continued using at the time of the survey. The interpretation of the socio-demographic information is provided in Table 3.2. Farmers' perceptions on the performance of the accessions based on agronomic and culinary characteristics were documented using 11 traits (yield potential, tolerance to insect pests, tolerance to diseases, flowering rate, no/less thorns on tubers, earliness to maturity, tuber flesh texture (boiled tuber texture), tuber taste after cooking, tuber flesh colour/non-oxidative browning, aroma and storage capability) (Table 3.3). A binary database was then constructed using the 11 traits, and accessions were scored "1" if performance was good for a characteristic and scored "0" otherwise.

Table 3. 2. Interpretation of socio-demographic variables used in the study

Variable	Interpretation	Scale	Score
Sex	Sex of the household head/representative	Nominal	0 = Female, 1 = Male
Age	Age of the household head/representative	Continuous/Categorical	1 = 20-29, 3 = 30-39, 5 = 40-49, 7 = 50-59, 9 = 60-69, 11 = 70-79, 13 = 80-89
Education level	Education level of the household head/representative	Ordinal	1= No formal education, 3 = Primary, 5 = Secondary, 7 = Tertiary
Primary occupation	First occupation of the household head/representative	Nominal	1 = Farming, 3 = Others
Secondary occupation	Age of the household head/representative	Nominal	0 = No, 1 = Alternative
Marital status	-	Nominal	1 = Single, 3 = Married
Family size	Number of living people under the household	Continuous/Categorical	NA
Farm size	Land owned by the head of the household	Continuous/Categorical	NA
Years of	Experience of the	Continuous/Categorical	NA

experience	household related to yam growing		
Number of yam accessions	Number of bush yam accessions grown by the household	Continuous/Categorical	NA

Table 3. 3. Meaning of agronomic and culinary traits assessed and their significance

Trait	Meaning	Significance
Yield performance	Ability of producing high yield in terms of tons per hectare	Breeding and selection
Tolerance to insect pests	Ability of yam varieties to withstand insect pest infestation	Breeding and selection
Tolerance to diseases	Ability of yam varieties to display low susceptibility to disease even in the presence of major diseases' infection	Breeding and selection
No/less thorns on tubers	Ability of the tuber skin to be free from thorns	Selection
High flowering rate	Ability of the yam variety to produce a large number of male or female flowers.	Breeding
Tuber flesh texture	Softness and smoothness of tuber after cooking	Culinary traits for selection
Tuber taste after cooking	Sweetness of tuber or blandness after cooking	Culinary traits for selection
Oxidative browning	Ability of tuber flesh to maintain its original consumer's preferred colour after cutting	Breeding and selection
Aroma	Ability of the tuber to have good smell after cooking	Culinary traits for selection

Source: Yam crop ontology (Asfaw, 2016)

Data analysis

Descriptive statistics (frequencies, means, standard deviations, minimum, maximum, etc.) were used in generating summary tables. To estimate the

influence of socio-demographic parameters (age, years of experience, farm size, family size, marital status, education level, secondary occupation) on the number of accessions maintained at each household and across the surveyed villages, Pearson's correlation analysis was performed using corrplot R package version 0.84 (Wei & Simko, 2017). Shannon Weiner, evenness or equitability (E) and Margalef species richness (d) diversity indices were computed using Paleontological statistics software (PAST) 326b version (Hammer, Harper & Ryan, 2001) to quantify the diversity of *D. praehensilis* at the village and region levels.

Analysis of variance at a 5% level of significance was used to determine the influence of gender and age categories on the diversity of *D. praehensilis* (translated by the number of accessions).

The Pearson's chi-square test was used to compare the influence of regions surveyed and farmer's gender on the practices used in *D. praehensilis* production and utilization.

To assess the distribution and concentration of each *D. praehensilis* accession across the surveyed regions, quotation frequency described by Adigoun-Akotegnon et al. (2019) was used to compute the proportion of the respondents who quoted a given morphotype based on the total number of farmers surveyed. The quotation levels for ranking were: 1 = quoted by 1–10% respondents; 2 = quoted by 11–20% respondents; 3 = quoted by 21–60% respondents, and 4 = quoted by >60% respondents.

Subject to synonymy / homonymy, a database was constructed by considering the unique accessions based on different villages and the farmers' identified agronomic and culinary traits (11 in total). A database was then developed considering the key traits and was used to generate genetic distance among the identified morphotype using R cluster package (Maechler, Rousseew, Struyf, Hubert & Hornik, 2019) implemented in R Development Core Team (2019). Generated dissimilarity matrix was then used to construct hierarchical clusters (dendrogram) using ward.2 method implemented in cluster package (R Development Core Team, 2019). The silhouette method implemented in Cluster package and FactorMinerR (R Development Core Team, 2019) were used to determine the maximum cluster number and assess the effectiveness of grouping.

Results

Socio-demographic characteristics of *D. praehensilis* farmers

A total of 437 *D. praehensilis* farmers including 159 in the Central Region, 120 in the Eastern Region and 158 in the Western North Region were surveyed. The majority (72.5%) of the respondents were male, 19.5% had no formal education, and only 4.1% had tertiary education, while 32.3% and 44.2% had primary and secondary education, respectively. The main activity of these farmers across the study areas was farming (~90%). Some farmers had non-farm income-generating activities, such as artisan, petty trading, clergy and civil services. The surveyed farmers were between 20 and 82 years old, with an average age of 47 years. The mean family size was six members, although some households accommodated up to 30 members from the extended family. The *D.*

praehensilis farm sizes in sole and intercropping systems ranged between 0.2 and 20 ha, with a mean of 2.1 ha. The farmers' experience in *D. praehensilis* cultivation ranged between 1 and 60 years with a mean of 14 years (Table 3.4).

Table 3. 4. Socio-demographic characteristics of *D. praehensilis* farmers across the surveyed regions of Ghana

Variable	Modalities	Central (n=159)	Eastern (n=120)	Western North (n=158)	Total study area (n=437)
Gender (%)	Male	66.6	80.8	72.2	72.5
	Female	33.3	19.2	27.8	27.5
Education level (%)	No formal education	11.9	23.3	24.1	19.5
	Primary	28.9	43.3	27.2	32.3
	Secondary	55.4	30.0	43.7	44.2
	Tertiary	3.8	3.3	5.1	4.1
Primary occupation (%)	Farming	83.7	91.7	91.1	88.6
	Farming and other activities	16.4	8.3	8.9	11.4
Non-farm activities (%)	None	83.7	91.7	91.1	88.6
	Artisans	5.7	5.0	4.0	4.8
	Petty trader	8.2	1.7	1.9	4.1
	Civil servant	1.9	0.8	3.2	2.1
	Student	-	0.8	-	0.2
	Clergyman	-	0.8	-	0.2
Age (years)	Average	49.4	46.4	45.	47.
	Minimum	22.0	21.0	20.0	20.0
	Maximum	82.0	75.0	77.0	82.0
Farming experience (years)	Average	13.9	14.9	13.7	14.1
	Minimum	1.0	2.0	1.0	1.0
	Maximum	50.0	60.0	40.0	60.0

Family size	Average	6.1	6.1	6.8	6.4
	Minimum	1.0	1.0	1.0	1.0
	Maximum	30.0	15.0	25.0	30.0
Farm size (ha)	Average	1.9	1.0	3.1	2.1
	Minimum	0.2	0.2	0.4	0.2
	Maximum	10.0	20.0	20.0	20.0

Genetic resources of *D. praehensilis* across the surveyed areas

Subject to synonymy/homonymy, the number of *D. praehensilis* accessions cultivated in the surveyed villages varied from 5 to 14. The highest number of accessions (14) was recorded at Sutapong village,, located in the Eastern Region, while the lowest (5) was recorded at Aburansa and Frami villages, located in the Central Region (Table 3.5).

The diversity index means of 1.88, 0.65 and 2.53 were recorded for Shannon Weiner index (H'), evenness/equitability (E) and Margalef species richness (d), respectively, across the study areas (Table 1). Esuom Manya village in the Eastern Region recorded the highest H' (2.41) and equitability (0.93) among the villages surveyed. In contrast, Aburansa village in the Central Region recorded the lowest H' (1.05), and Komfokrom recorded the lowest index for equitability (0.53). The Highest Margalef morphotype richness (3.55) was observed in the village Sutapong in the Eastern Region, while the lowest (1.17) was observed in Aburansa village in the Central Region. Across the regions, the highest diversity indices (H'=3.22, equitability = 0.84, and Margalef species richness = 6.87) were recorded in Eastern Region while the lowest (H'=2.66,

equitability (E) = 0.75, and Margalef species richness (d) = 4.25) were recorded in the Central Region.

There was no significant difference in the number of accessions cultivated by either male or female respondents, as well as the age categories across the surveyed regions (Table 3.6). An average of two *D. praehensilis* accessions were cultivated by male and female farmers irrespective of the age categories.

The relationship between the socio-demographic characteristics and the number of accessions cultivated by *D. praehensilis* farmers in the study area is presented in Figure 3.2. Family income, family size, farm size, years of experience in *D. praehensilis* farming and education level positively influenced the number of accessions cultivated by the farmers. In contrast, gender, age, primary occupation and marital status had a negative impact on the number of accessions grown by the farmers.

Table 3. 5. Diversity of *D. praehensilis* across surveyed regions in Ghana

Region	Village	Number of accessions (S)	Shannon (H')	Evenness/ Equitability	Margalef Species Richness (d)
Central Region	Aburansa	5	1.05	0.57	1.17
	Komfokrom	11	1.77	0.53	2.64
	Kwametah	7	1.45	0.61	1.63
	Frami	5	1.19	0.66	1.21
	Nyameani	7	1.37	0.56	1.70

	Watreso	8	1.56	0.60	1.83
	Achiase	10	1.84	0.63	2.60
	Asamankese	9	1.59	0.54	2.20
	Manso	6	1.33	0.63	1.43
	Mean		2.66	0.75	4.25
Eastern	Dzaman	10	1.62	0.50	2.25
Region	Esuom	12	2.41	0.93	2.52
	Manya Nsutapong	9	1.78	0.66	2.11
	Brukum	13	2.01	0.57	3.21
	Klo Agogo	11	1.78	0.54	2.71
	Sutapong	14	2.35	0.75	3.55
	Mean		3.22	0.84	6.87
Western	Anhwiaso	7	1.66	0.75	1.43
North	Dominibo	12	2.37	0.89	2.55
Region	No. 2				
	Adupri	11	2.09	0.74	2.35
	Adobawura No. 1	12	2.07	0.66	2.76
	Naama	10	1.96	0.71	2.26
	Nyetina	10	1.99	0.73	2.27
	Adwumam	8	1.61	0.63	1.78
	Juaboso	7	1.36	0.56	1.48
	Mean		2.90	0.79	5.07

Averall mean	1.88	0.65	2.53

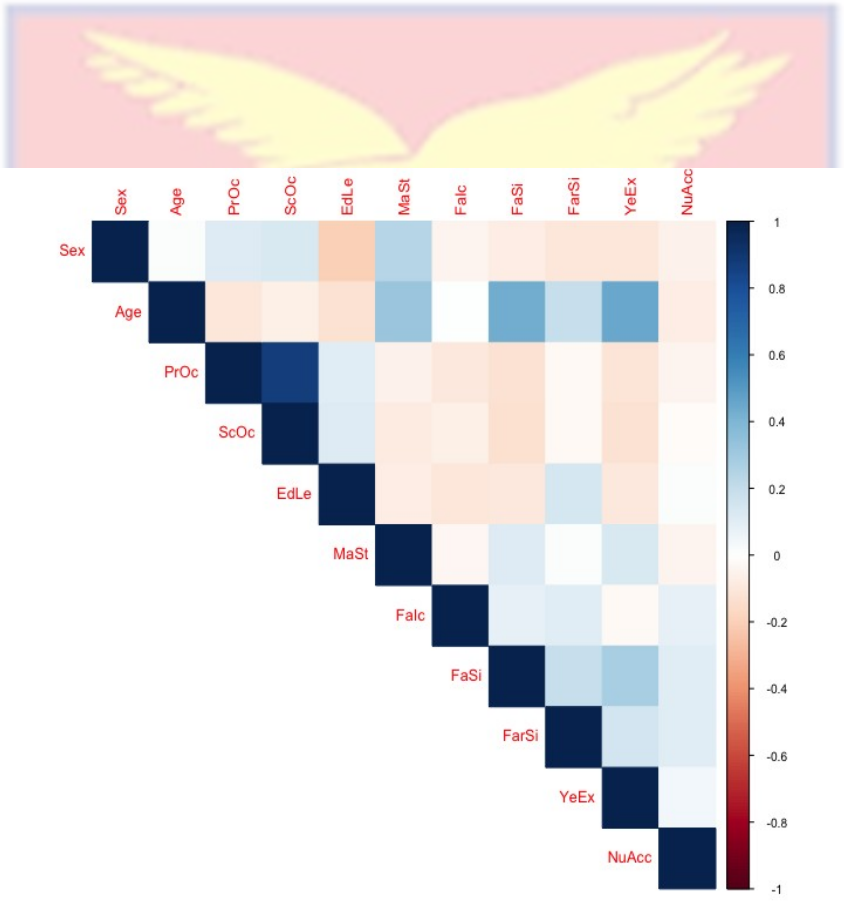

Figure 3. 2. Correlation coefficients between the numbers of accessions maintained at household and socio-demographic parameters. *NuAcc: Number of accessions, YeEx: Years of experience, FarSi: Farm size, FaSi: Family size, FaIc: Family income levels, MaSt: Marital status, EdLe: Education level, ScOc: Secondary occupation, PrOc: Primary occupation*

Table 3. 6. Diversity of *D. praehensilis* accessions on the basis of gender and age category of the respondents

Disaggregated categories	Modalities	Average accessions	Standard deviation	Range of accessions	*p*-value

Gender	Male	2.26	1.32	1-7	0.28
	Female	2.12	1.09	1-5	
Age categories	20-29	2.00	1.20	1-5	0.078
	30-39	2.43	1.33	1-7	
	40-49	2.41	1.39	1-7	
	50-59	2.02	1.12	1-5	
	60-69	2.12	1.11	1-5	
	70-79	2.06	1.25	1-5	
	80-89	1.00	0.00	1-1	

Spatial distribution of *D. praehensilis* across the surveyed regions in Ghana

A total of 42 *D. praehensilis* accessions were collected across the surveyed regions and varied morphologically in tuber flesh colour (Table 3.7). The accessions were grouped into seven classes based on the tuber flesh colour: white, yellow, cream, purple, red, brown and black flesh colour (Figure 3.3, Table 3.7). The distribution of these accessions based on flesh colour varied across the surveyed regions. White, yellow, red, and black flesh coloured yams were found in all the regions. Cream and purple flesh coloured yams were found only in Central and Eastern regions. The most predominantly cultivated classes across the surveyed regions were yams with white and yellow tuber flesh colours.

The Eastern Region had the highest recorded number of accessions (29), while the lowest was recorded in the Central Region (17). The spatial distribution of the accessions revealed that the accessions *Fufuw, Akoko-angoa, Memen* and *Kat* were the most cultivated in the Central Region. *Futaa, Yumu, Kungwɔ zɔ, Kokoasobayere, Mamsoso, Kat*, and *Asebayere* were mostly cultivated in the Eastern Region. In contrast, *Afo, Akyekyere, Asebayere, Tumtum, Kokoasobayere, Mamsoso, Ngani, Fufuw, Akoko-angoa, Bobeyere* and *Fowking* were the most cultivated *D. praehensilis* accessions in the Western North Region. Some

accessions were mostly concentrated in each of the regions. For example, *Adongo, Esiam* and *Ɔtse dɛ wim* were concentrated in the Central region; *Chamachron, Cherimanche, Keke, Wo su, Tsu tsu, Odonor* and *Obobi* in the Eastern Region; and *Apubayere, Bootan, Kwah, Nkam,* and *Tolege* in the Western North Region. Some accessions were found across the three surveyed regions. These included *Akyekyere*, *Asebayere*, *Kat*, *Kokoasobayere*, *Mamsoso,* and *Ngani*.

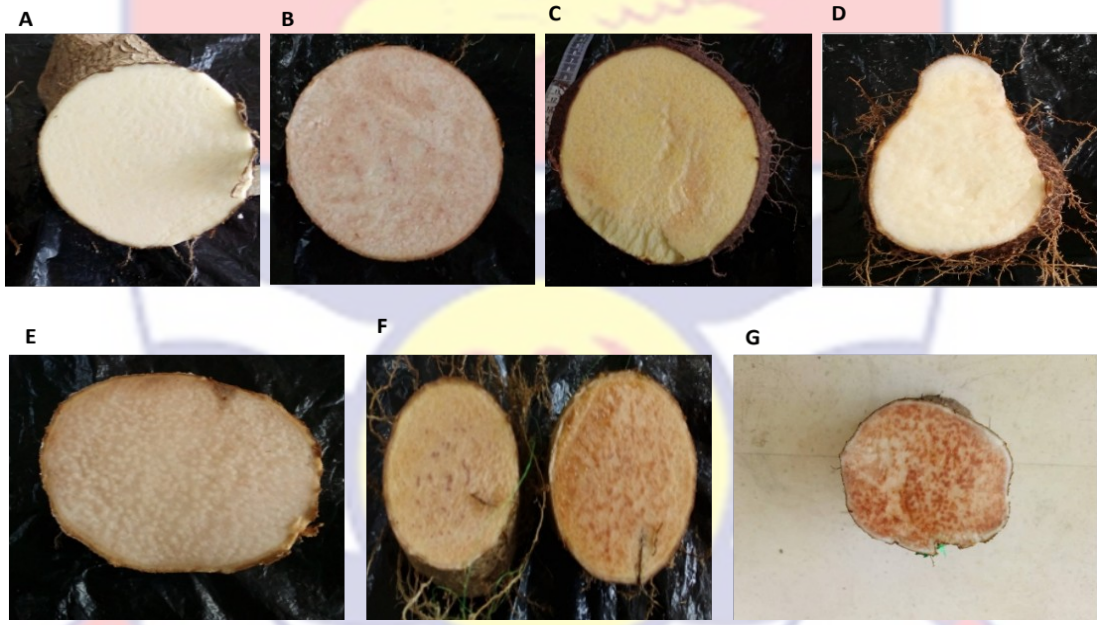

Figure 3. 3. Differences in tuber flesh colour among *D. praehensilis* accessions. A: White (*Odonor*); B: Purple (*Ewuruku*); C: Yellow (*Mamsoso*); D: Cream (*Sika a ɔhowa*); E: Black (*Tumtum*); F: Brown (*Bredum*); G: Red (*Tsutsu*)

Table 3. 7. Tuber flesh colour and spatial distribution of 42 *D. praehensilis* accessions inventoried across the study areas

Tuber flesh colour	*D. praehensilis* accessions	Concentrations of accessions		
		Central Region	Eastern Region	Western North Region
Black	Tumtum	1	1	2
	Yumuu	-	-	-
	Ɔtse dɛ wim	1		-
	Wo su	-	1	-
Brown	Bredum	1	1	1

Table 3. 7. Continued

Cream	Sika a ɔhowa	1	1	-
Red	Memen	2	-	1
	Tsutsu	-	1	-
Purple	Ewuruku	1	-	-
	Kɔkwa ɔhwa	-	1	-
White	Afo	-	1	2
	Akyekyere	1	1	2
	[illegible]bayere	-	-	1
	[illegible]ayere	1	1	4
	[illegible]bita	-	1	1
	Kat	2	2	1
	Chamachron	-	1	-
	Fowking	-	1	2
	Keke	-	1	-
	Kokoasebayere	1	2	2
	Nkam	-	-	1
	Obobi	-	1	-
	Odonor	-	1	-
	Otimbale	-	1	1
	Pone	1	1	1
	Tolege	-	-	1
	Fufuw	4	-	2
	Futaa	-	3	-
Yellow	Adongo	1	-	-
	Ayangaro	-	1	1
	Bayere	-	1	1
	Bobede	-	1	1
	Bobeyere	-	1	2
	Bootan	-	-	1
	Cherimache	-	1	-
	Esiam	1	-	-
	Kpaku	-	1	-
	Kwah	-	-	1
	Mamsoso	1	2	2
	Ngani	1	1	2
	Akoko-angoa	4	-	2
	Kungwɔ zɔ	-	3	-
Total /region		17	29	25

1 = quoted by 1–10% respondents; 2 = quoted by 11–20% respondents; 3 = quoted by 21–60% respondents; 4 = quoted by ≥ 60% respondents

Constraints in production and utilization of *D. praehensilis* accessions across the studied regions of Ghana

The challenges associated with the declining production and utilization of *D. praehensilis* accessions in Ghana are presented in Table 4.8. These include poor tuber culinary and quality characteristics (39.9%), among which fast tuber flesh oxidation (22.7%), poor post-harvest shelf-life (10.8%), fibrous tuber texture (5.0%) and poor taste (1.4%) were the most mentioned. Agronomic constraints contributed up to 20.7% of the factors limiting the production and utilization of *D. praehensilis* by surveyed farmers. Among these agronomic constraints, prolonged dormancy (7.6%), low productivity (4.8%), difficulty in harvesting (3.9%), non-adaptation to poor soil fertility (1.4%) and lack of sufficient quality seeds (3.0%) were the most important bottlenecks faced by the bush yam farmers. Marketing challenges were mainly the low market values and the lack of organized markets (14.9%) for bush yam. Abiotic (bush burning and drought susceptibility), biotic (insect pests and diseases) and socio-cultural (loss of cultural values and introduction of new yam species) challenges represented 10.5, 11.0 and 3.2 %, respectively.

Table 3. 8. Challenges limiting production and utilization of *D. praehensilis* in Ghana

Challenge categories	Factors	% of responses
Culinary and quality traits	High tuber flesh oxidation	22.7
	Short post-harvest shelf-life	10.8
	High fibrous (boiled) tuber texture	5.0

	Poor taste	1.4
	Sub-total	39.9
Agronomic	Prolonged dormancy	7.6
traits	Low productivity	4.8
	Difficulty in harvesting	3.9
	Lack of quality seed	3.0
	Non-adaptation to low soil fertility	1.4
	Sub-total	20.7
Marketing	Low market value	12.1
	Lack of organized markets	2.8
	Sub-total	14.9
Biotic	Insect pests	8.9
	Diseases	2.1
	Sub-total	11.0
Abiotic	Bush burning	5.5
	ility to drought stress	5.0
	Sub-total	10.5
Socio-cultural	Introduction of new yam species	2.3
	Loss of cultural values	0.9
	(ceremonies and yam festivals)	
	Sub-total	3.2

Farmers' preference criteria of *D. praehensilis* accessions and utilization in the studied regions of Ghana

Twelve (12) criteria grouped into three categories (agronomic, culinary, and economic characteristics) were perceived by the farmers as the most important traits across the study areas (Table 3.9). These categories accounted for 51.6, 43.2, and 5.5% of the responses from *D. praehensilis* farmers, respectively. Among the 12 cited as preferred criteria, "early maturity" in the category of agronomic quality traits was the most preferred (21.1%), while "readily available during the dry season" in the category of economic characteristics was the least of the preferred criteria (0.9%). However, the farmers' preferences varied from one region to another. Early maturity (27.4%) was the most preferred criterion in the Central Region, followed by smooth tuber texture (14.5%) and good tuber flesh colour (14.5%). The most preferred criteria in the Eastern Region were early maturity (17.9%), followed by good taste (17.2%). The most preferred criteria in the Western North Region were smooth tuber texture (19.6%) and early maturity (19.0%). Across the studied areas, 92.9% of the surveyed farmers utilized *D. praehensilis* for direct consumption (subsistence), 6.1% commercialized it and less than 1% used it for medicinal purposes (Table 3.12).

Perceptions of farmers on agronomic and culinary traits of *D. praehensilis*

The perception of local farmers on agronomic and culinary characteristics of bush yam is presented in Figure 3.4. More than 90% of the respondents reported high yield potential, high flowering rate and good aroma of *D. praehensilis*. About 70% of the respondents reported high tolerance of *D.*

praehensilis to insect pests and diseases. On the other hand, 37% of the respondents reported good storage capability of *D. praehensilis*. Thirty-two percent (32%) of the respondents reported no or less thorns of *D. praehensilis*. Less than 30% of the respondents reported smooth tuber texture, earliness to maturity, non-oxidising tuber flesh and good taste of *D. praehensilis*.

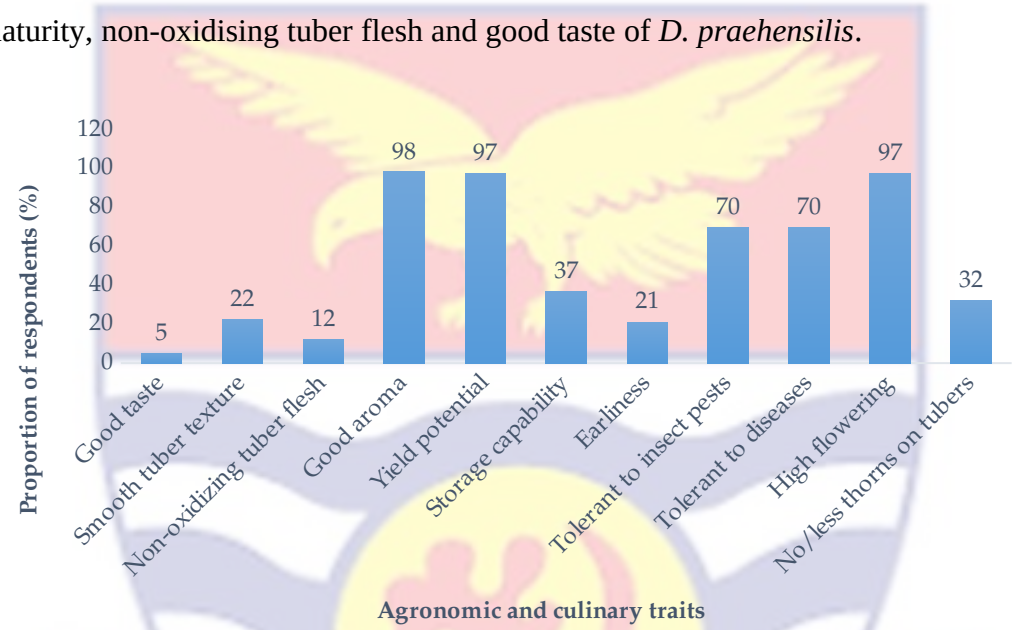

Figure 3. 4. Perceptions of farmers on the agronomic and culinary traits of *D. praehensilis* in Ghana

Perceptions of age categories on the preferential criteria for *D. praehensilis* accessions

The perception of different age categories of respondents on their preferences for culinary, quality and market traits of *D. praehensilis* is presented in Table 3.10. For respondents in the age category of 40-49 years, majority preferred good aroma (41.7%), non-oxidising tuber flesh (41.2%), high market value (40%), smooth tuber texture of boiled yam (31.9%), good taste (29.6%) and early maturity (26.1%), while respondents in the age category of 50-59 years preferred attributes such as readiness during off-season (50%), high yield

potential (30.4%), and high poundability (25%). For age category of 30-39 years old, 30% preferred high market value, 26.4% smooth tuber texture of boiled yam and 25% high poudability.

Table 3. 9. Farmers' varietal preference criteria and their importance across Ghanaian regions

Category	Preference criteria	% respondents across surveyed areas	% respondents		
			Central	Eastern	Western North
Culinary and quality traits	Smooth tuber texture	16.5	14.5	14.9	19.6
	Good taste	12.4	11.3	17.2	9.5
	Non-oxidizing tuber flesh	7.8	14.5	5.2	5.0
	High poundability	3.7	8.1	0.0	3.4
	Good aroma	2.8	0.8	3.7	3.4
	Sub-total	**43.2**	**49.2**	**41.0**	**40.8**
Agronomic traits	Early maturity	21.1	27.4	17.9	19.0
	High productivity	12.8	13.7	16.4	9.5
	Tolerance to insect pests and diseases	10.1	5.7	12.7	11.2
	High in-soil storage aptitude	7.6	2.4	4.5	13.4
	Sub-total	**51.6**	**49.2**	**51.5**	**53.1**
Economic characteristics	High market value	4.6	1.6	6.0	5.6
	Readily available during off season	0.9	0.8	1.5	0.6
	Sub-total	**5.5**	**2.4**	**7.5**	**6.2**

Table 3. 10. Perceptions by age categories on the preferential criteria for *D. praehensilis* accessions

Preference criteria	Age categories (%)						
	20-29	30-39	40-49	50-59	60-69	70-79	80-89
Good aroma	0.0	25.0	41.7	33.3	0.0	0.0	-
Non-oxidizing tuber flesh	8.8	17.6	41.2	17.6	11.8	2.9	-
High market value	10.0	30.0	40.0	20.0	-	-	-
Smooth tuber texture (boiled tuber)	6.9	26.4	31.9	23.6	11.1	1.4	-
Good taste	9.3	20.4	29.6	14.8	14.8	7.4	3.7
Early maturity	9.8	22.8	26.1	23.9	14.1	3.3	-
High yield potential	8.9	21.4	25.0	30.4	12.5	1.8	-
Readily available during off-season	-	-	25.0	50.0	25.0	-	-
High poundability	25.0	25.0	12.5	25.0	12.5	0.0	-

Conservation techniques by *D. praehensilis* farmers in the studied regions of Ghana

Two conservation techniques, *in-situ* and *ex-situ* techniques, were reported in the study area. The *in-situ* conservation technique involved retaining mature tubers in the mounds until the time was suitable for harvest and sale. The farmer's location significantly influenced ($p < 0.001$) conservation techniques adopted by bush yam farmers (Table 3.11). *In situ* conservation technique was used by 60.0% *D. praehensilis* farmers. These farmers conserved mature tubers in the mounds for a period of 1-3 months (34.1%), 4-6 months (20.8%), 7-9 months (2.3%), and 10-12 months (2.8%) (Table 3.11). *Ex-situ* conservation techniques accounted for 40.0% of the farmers' responses. In *ex-situ* conservation techniques, nine (9) methods were reported. Conservation in traditional huts (10.1%) was the most preferred *ex-situ* conservation. Another popular method was storage in rooms on

bare ground (8.7%), while the least popular *ex-situ* conservation practice was storage in basket (0.5%) (Table 3.11). The widely used *in-situ* conservation technique was pre-storage in the mounds for 1-3 months irrespective of the location – Central (34%), Eastern (42.5%) and Western North (23.4%). The gender of bush yam farmers had no significant relationship ($p > 0.05$) with the conservation techniques adopted. The *in-situ* conservation was used by 60.8% female farmers and 58.0% male farmers. On the other hand, *ex-situ* conservation techniques were practised by 42% male farmers vs 39.2% female farmers. The most commonly used in-*situ* technique irrespective of the farmer's gender was 1-3 month storage in the mound, 36.7% women vs 30.9% men. The most commonly used *ex-situ* conservation technique among women was storage in the room on bare-ground (12.5%), while men primarily used storage in traditional hut (8.8%).

Table 3. 11. Traditional conservation techniques of *D. praehensilis* based on regions and gender of the respondents

Conservation techniques		Disaggregated by category of respondents						
		Regions (%)				Gender (%)		
		Central (159)	Eastern (120)	Western North (158)	Overall (437)	Male (317)	Female (120)	Overall (437)
In-situ (Months)	1-3	34.0	42.5	23.4	34.1	30.9	36.7	34.1
	4-6	21.4	5.8	33.5	20.8	22.1	20.0	20.8
	7-9	1.9	2.5	2.5	2.3	2.2	2.5	2.3
	10-12	2.5	2.5	2.5	2.8	2.8	1.7	2.8
	Sub-total	59.7	53.3	62.0	60.0	58.0	60.8	60.0
		($\chi^2 = 74.8$, $p < 0.001$)				$\chi^2 = 14.8$, $p > 0.05$)		
Ex-situ	Traditional huts	9.4	5.0	10.1	10.1	8.8	7.5	10.1
	Room on bare ground	8.8	11.7	6.3	8.7	7.3	12.5	8.7
	On wooden platforms	10.1	5.8	2.5	6.2	6.3	5.8	6.2
	Under shed	3.1	9.2	7.0	5.2	7.6	2.5	5.2
	Heaps	2.5	5.8	5.1	3.4	4.4	4.2	3.4
	Open pile	1.3	2.5	4.4	2.0	3.2	1.7	2.0
	Holes	3.8	2.5	2.5	1.8	3.2	2.5	1.8
	Sacks	0.6	3.3	0.0	2.1	0.6	2.5	2.1
	Baskets	0.6	0.8	0.0	0.5	0.6	0.0	0.5
	Sub-total	40.3	46.7	38.0	40.0	42.0	39.2	40.0
		($\chi^2 = 31.0$, $p < 0.05$)				($\chi^2 = 11.0$, $p > 0.05$)		

Harvesting frequency and criteria adopted by farmers for estimating time to harvest *D. praehensilis* based on farmers' region and gender

The harvesting frequency significantly varied with farmers' location (χ^2 = 64.0, $p < 0.001$) (Table 3.12). Harvested twice was the most practised in the Central (69.2%) and Western North regions (74.1%), while harvested once was most popular (57.5%) in the Eastern Region. The gender of the *D. praehensilis* farmers had also significantly influenced the harvesting frequency ($\chi^2 = 8.8$, $p < 0.05$) (Table 3.12). Double harvesting was the most practised irrespective of the farmer's gender – 72.5% females and 58.4% males.

Farmers cultivating *D. praehensilis* adopted different criteria to determine when the tubers were mature and ready for harvesting (Table 3.12). A significant relationship ($\chi^2 = 124.5$, $p < 0.001$) was observed between the farmers' region and the criteria used to estimate harvest time. Time from planting to harvest was the most used criterion in Central (39% of respondents) and Eastern (35% of respondents) regions. Approximately 35% of the farmers across the areas surveyed reported that browning of the leaves and time of planting to harvesting is the primary criteria they used in determining the maturity of the tubers. Approximately 24% believed that cracks in mounds or ridges signified maturity. ~7% used senescence of inflorescence to determine the maturity of tubers. In comparison, 3.2% adopted wilting of vine tips to determine the tuber maturity. There was no significant relationship between the farmers' gender and the criteria for estimating the time to harvest ($\chi^2 = 3.0$, $p > 0.05$). Time from planting to harvest was the major criteria adopted by both females (36.7%) and males

(34.7%), while the least used was wilting of vine tips, females (1.7%) and males (3.8%).

Practices used in *D. praehensilis* production based on region and farmers' gender

No significant relationship was established between the farmers' region and the adopted cropping system in *D. praehensilis* cultivation ($\chi^2 = 4.6$, $p > 0.05$) (Table 3.12). The intercropping was predominant (89.2%). Sole cropping accounted for only 10.8% of *D. praehensilis* farms (Table 3.12). The same trend was reported across the regions with 93.1, 82.5, and 91.1 % of intercropped farms in Central, Eastern and Western North regions, respectively (Table 3.12). The relationship between the farmers' gender and the cropping system was not significant ($\chi^2 = 1.14$, $p > 0.05$) (Table 3.12). About 90.2% of males and 86.7% of females practised intercropping system for *D. praehensilis* cultivation, while 9.8% males and 13.3% females practised the sole cropping system.

More than 80% of *D. praehensilis* farms were under cocoa plantations. The remaining farms (~14%) were grown with cassava, plantain, cocoyam, banana, maize and vegetables.

The majority (~14%) of the *D. praehensilis* farmers who intercropped bush yam with other crops (cassava, cocoyam, banana, maize, and vegetables) used live stakes in supporting the crops for proper foliar and tuber development (Table 3.12). The most popular tree species that used as live stakes were *Newbouldia laevis* (Neem, Sesemansa or Nyabatso), mahogany, *Albizia zygia*, *Milicia excelsa*, *Gliricidia sepium* (Gliricidia) and *Leucaena leucocephala*

(Leucanea).

There was a significant relationship between the farmers' region and sources of planting materials/yam seeds ($\chi^2 = 31.9$, $p < 0.001$) (Table 3.12). Exchange with neighbouring farmers was the most practised means of getting *D. praehensilis* planting materials across the three regions: Central (83%), Eastern (74.2%), and Western North (55.1%). The farmer's gender did not influence how they acquired yam seeds ($\chi^2 = 3.94$, $p > 0.05$) (Table 3.12). For instance, 72.5% male and 65.8% female farmers practised mostly exchange from the neighbouring farmers to acquire germplasm. The main source of *D. praehensilis* planting materials in Ghana was the exchange with neighbouring farmers (70.5%). In comparison, markets and collection in wild environments accounted for 27.7 and 1.8 %, respectively (Table 3.12).

A significant correlation was observed between the farmers' region and the final utilization/destination of harvested *D. praehensilis* ($\chi^2 = 25.8$, $p < 0.001$) (Table 3.12). Across the studied regions, the majority of farmers used *D. praehensilis* as a source of food (for direct family consumption): Central (98.7%), Eastern (83.3%) and Western North (94.3%). On the other hand, 15.8% of farmers from Eastern, 5.1% from Western North and 1.3% among Central Region farmers utilized harvested *D. praehensilis* yams for commercial purposes. In comparison, only ~1% of farmers used *D. praehensilis* as a source of medicine. The farmer's gender had no relationship with the final destination of harvested *D. praehensilis* ($\chi^2 = 0.95$, $p > 0.05$) (Table 3.12). Approximately 93.0% of female and male farmers used harvested *D. praehensilis* for direct food consumption. In

comparison, ~7.0 female and 6.0% male farmers utilized the crop for commercial purposes; and only ~1% among males utilized it for medicinal purposes.

Relationship among collected Ghanaian's *D. praehensilis* accessions

The relationship of the culinary and agronomic characteristics among the 42 collected *D. praehensilis* accessions is presented in Figure 3.5. Cluster analysis partitioned the accessions into three groups (Figure 3.6). G1 comprised of accessions with high yield, high flowering rate, good taste, no/less tuber flesh oxidation (no or less change in tuber flesh colour), tolerant to pests and diseases but late maturing with short tuber shelf-life and hard tuber flesh texture. G2 comprised of accessions with high yielding ability, high flowering rate, good taste, and smooth tuber flesh texture, but late-maturing with high tuber flesh oxidation, presence of thorns on tubers and short tuber shelf-life. G3 comprised of accessions with high yielding potential and high flowering rate, tolerant to pests and diseases, but late maturing with poor taste, hard tuber flesh texture, high tuber flesh oxidation, presence of thorns on tubers, and short shelf-life.

Table 3. 12. Practices in *D. praehensilis* farming based on farmers' region and gender

		Regions (%)				Gender (%)		
Practice	Modality	Central (159)	Eastern (120)	Western North (158)	Overall (437)	Male (317)	Female (120)	Overall (437)
Harvesting frequency	Once	23.3	57.5	15.2	29.8	33.8	19.2	29.7
	Twice	69.2	37.5	74.1	62.2	58.4	72.5	62.2
	More than twice	7.5	5.0	10.8	8.0	7.9	8.3	8.0
		$\chi^2 = 64.0, p < 0.001$)				$\chi^2 = 8.8, p < 0.05$)		
Criteria for harvest time estimation	Time of planting to harvesting	39.0	35.0	31.6	35.2	34.7	36.7	35.2
	Browning of leaves	34.6	33.3	24.1	30.4	31.2	28.3	30.4
	Cracking of mounds	24.5	0.0	41.1	23.8	22.4	27.5	23.8
	Senescence of inflorescence	1.9	22.5	1.3	7.3	7.9	5.8	7.3
	Wilting of vine tips	0.0	9.2	1.9	3.2	3.8	1.7	3.2
		$\chi^2 = 124.5, p < 0.001$)				$\chi^2 = 3.0, p > 0.05$)		
Cropping system	Intercropping	93.1	82.5	91.1	89.5	90.2	86.7	89.2
	Sole cropping	6.9	17.5	8.9	10.5	9.8	13.3	10.8
		$\chi^2 = 4.6, p > 0.05$)				$\chi^2 = 1.14, p > 0.05$)		
Staking		12.5	26.6	5.7	13.9			
Sources of seeds	Farmers' exchange	83.0	74.2	55.1	70.5	72.2	65.8	70.5
	Market	15.1	25.0	42.4	27.7	26.5	30.8	27.7
	Wild	1.9	0.8	2.5	1.8	1.3	3.3	1.8
		$\chi^2 = 31.9, p < 0.001$)				$\chi^2 = 3.14, p > 0.05$)		
Utilization of bush yam	Consumption	98.7	83.3	94.3	92.9	93.4	92.5	92.9
	Commercial	1.3	15.8	5.1	6.6	6.3	7.5	6.6
	Medicine	0.0	0.8	0.6	0.5	0.6	0	0.5
		$\chi^2 = 25.8, p < 0.001$)				$\chi^2 = 0.95, p > 0.05$)		

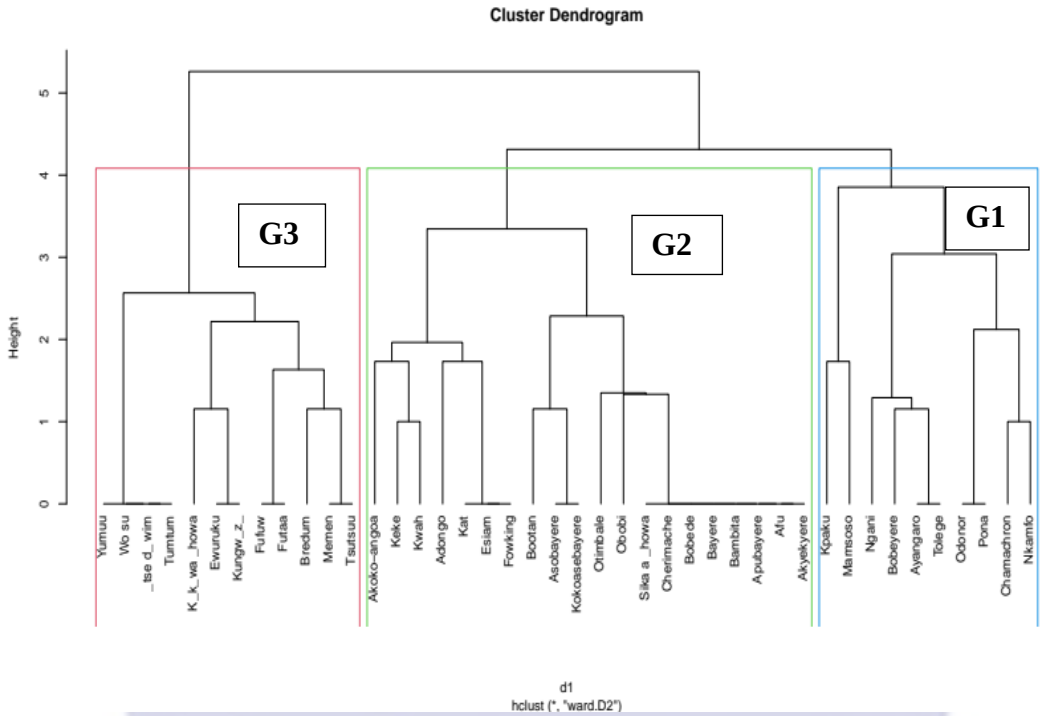

Figure 3. 5. Dendrogram classifying *D. praehensilis* accessions from Ghana into 3 groups (blue (G1), green (G2) and red (G3)) based on agronomic and culinary traits using ward method.

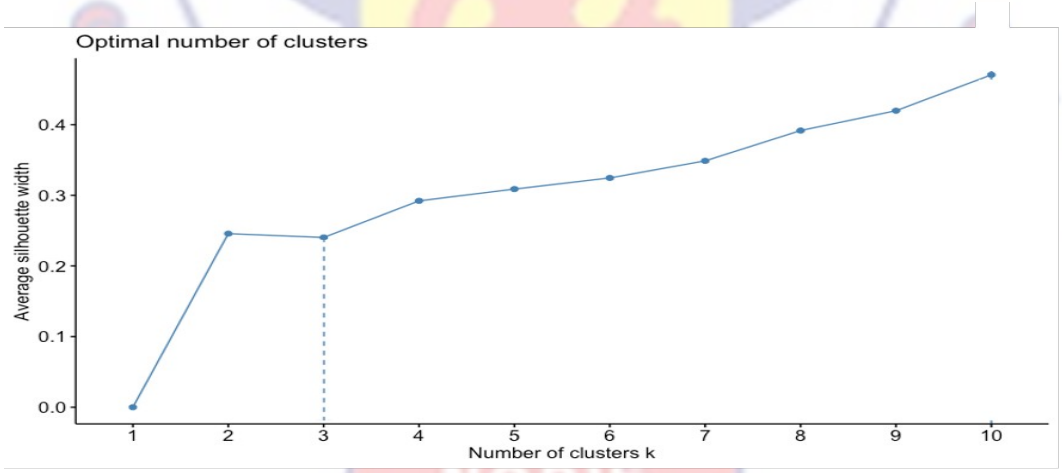

Figure 3. 6. Partitioning of 42 accessions of *D. praehensilis* into 3 groups using Silhouette method

Discussion

Diversity and distribution of *D. praehensilis* in studied regions of Ghana

Tuber flesh colour was the only criterion used by Ghanaian farmers in grouping *D. praehensilis* accessions. In total, across the surveyed regions, 42 *D. praehensilis* accessions were identified and grouped into seven (7) classes based on the tuber flesh colours (white, yellow, red, black, purple cream, and brown). This study reported 42 accessions rather than three (3) accessions (*Otim, Odonor and Kat*) reported by Aboagye et al. (2015) and also added three (3) flesh colour classes (purple, cream, and brown) compared to 4 flesh colour classes reported in Togo (Pitalounani et al., 2017). Therefore, this study has found more *D. praehensilis* accessions in Ghana than reported in Togo.

The diversity of 42 *D. praehensilis* accessions named by the farmers across the 23 surveyed villages may be facing the challenges of over- or under-estimation since the same local name may be given to different accessions (synonyms) or different local names may be given to the same morphotype (homonyms) (Adoukonou-Sagbadja et al., 2007). It is not certain that all the 42 accessions of the *D. praehensilis* collected from the study areas were genetically distinct. For instance, Adewumi et al. (2020) reported the possibility of duplication in *D. praehensilis* planting materials due to the linguistic polymorphism in the Central Region of Ghana. This possibility of duplication has also been reported for Guinea yam accessions in Ghana (Otoo, Akromah, Kololesnikova-Allen, & Asiedu, 2009), in Ethiopia (Tamiru, Becker, & Maass, 2008) and Benin (Loko et al., 2013), and in *D. dumetorum* (Adigoun-Akotegnon

et al., 2019; Laly et al., 2019). Detection of duplicate accessions using phenotypic and molecular characterization is a prerequisite in identifying true-to-type accessions for efficient management of crop genetic resources (Adewumi et al., 2020). This study encourages further studies using molecular markers to complement morphological *D. praehensilis* diversity analysis.

We employed different diversity indices to assess the *D. praehensilis* diversity in the study areas. Shannon diversity index, equitability and Margalef species richness indices revealed moderate genetic diversity among *D. praehensilis* accessions across the surveyed regions. This could be attributed to favourable agro-ecological conditions, such as high and well-distributed rainfall patterns and moderate relative humidity, conducive to the growth and development of most yam species, including *D. praehensilis*. There is also a high diversity in varietal preferences, which partly explains a large number of accessions among bush yam farmers in Ghana. Moderate diversity of *D. praehensilis* was previously reported in Togo, a neighbouring country to Ghana, where different accessions were recorded (Gnamkoulamba, Tchala, Tostain, & Soumana, 2002; Pitalounani et al., 2017). Therefore, selecting or breeding clones combining multiple traits is a valuable option in promoting the wide and commercial production of *D. praehensilis* yam in Ghana. These superior clones could be competitive and easier to multiply (as they are limited in number) compared to current clones and, thus, benefit a high uptake among Ghanaian farmers.

This study revealed positive relationships between the number of used *D. praehensilis* accessions and some farmers' socio-demographic characteristics such as farm size, years of farming experience, family size and education level. Having large farms and family sizes, farmers are offered the opportunity to test multiple options compared to those with limited plot size and family labour. Besides, *D. praehensilis* being mostly intercropped with cash crops such as cocoa, it is logical that they are found in larger plots. It is difficult to find sufficient seed of a single yam clone as claimed by Ghanaian farmers; this could have explained the multitude of accessions of *D. praehensilis* grown on large farms. Long experience in *D. praehensilis* cultivation could have enabled the farmers to familiarize themselves with the promising accessions combining good adaptation and tuber quality traits. The education level could have increased awareness of efficient cultivation techniques and marketing opportunities for bush yam.

The results of this study corroborate the finding of Andriamparany et al. (2014), who reported a strong influence of some socio-economic characteristics, such as family size and education level, on production and use of wild yams and medicinal plants in south-western Madagascar.

Challenges associated with production, marketing and seed systems of *D. praehensilis* in Ghana

From this study, poor culinary quality traits (poor post-harvest shelf-life, fast tuber flesh oxidation, poor tuber texture and poor taste) were the major challenges affecting the production and marketing of *D. praehensilis* in Ghana. These challenges make farmers abandon the production of *D. praehensilis* for other yam species, such as *D. rotundata* (mainly Puna, Dente, Punjo and Labroko

varieties) and *D. alata* (Afase variety), which are good in making preferred recipes such as yam balls, fufu, yam chips, mashed yam (mpotonpoto). Pitalounani et al. (2017) also reported the influence of poor culinary quality on the abandonment (and production decline) of *D. praehensilis* in Togo. Poor culinary quality was also one of the factors responsible for the genetic erosion of *D. rotundata* and *D. cayenensis* as reported by Dansi et al. (2013). Our study identified varieties (accessions) with good culinary quality and agronomic traits, which could be the starting point for selecting most the suitable *D. praehensilis* clones to promote wide cultivation and use in genetic resource conservation and improvement programmes. By identifying traits constraining extensive production of this yam species, our study would also guide breeding programs to develop adapted novel yam varieties that could meet Ghanaian farmers' expectations.

Inadaptability to poor soil fertility and dry areas was also mentioned as a factor causing the decline in the production and utilization of *D. praehensilis* in Ghana. According to the farmers, *D. praehensilis* requires more water and soil fertility for optimum growth. Thus, with the irregular rainfall observed in some of the cultivation areas, especially in the Central Region, it was not surprising that the diversity of this crop has dropped. Bush burning and increased frequency of bush fires reported by farmers had also contributed to the loss of *D. praehensilis* diversity in the studied areas. Some farmers adopt bush burning to trap bush meats in the forest areas where *D. praehensilis* is predominant during the dry season. Therefore, a diversity preservation program should discourage bush fire to slow down the *D. praehensilis* genetic erosion. A clear policy on inventory,

identification, research and conservation of *D. praehensilis* germplasm should be formulated by relevant decision-makers in Ghana to serve as a basis for promoting this underutilized yet important species of yam.

Most *D. praehensilis* farmers obtained their planting materials through informal seed exchanges with neighbouring farmers from this study. This encourages the distribution of *D. praehensilis* planting materials across the regions, resulting in wide distribution and concentration of some *D. praehensilis* accessions such as *Akyekyere*, *Asɔbayere*, *Tumtum*, *Kat*, *Kokoasobayere*, *Mamsoso* and *Ngani*, across the surveyed areas. Seed yams that serve as planting materials have been reported by the farmers to be inadequate and insufficient due to lack of appropriate storage facilities, resulting in the loss of a large amount of planting materials before the next planting season in addition to the absence of market for seed yam supply. The seed production system for bush yam is not well developed in Ghana. Minisett, bioreactor, hydroponic and aeroponic seed yam production techniques can be used to develop viable seed yam supply systems for multiplying and distributing the seed yams of superior clones to the farmers. These techniques have been successfully employed in developing seed yam supply systems for white yam (*D. rotundata*) (Aighewi, Asiedu, Maroya, & Balogun, 2015). Pests such as beetles, grasshoppers, yam beetles, rodents and caterpillars are associated with *D. praehensilis* in this study area and contributed to the decline in production of *D. praehensilis* and abandonment of most susceptible accessions in the study areas. Larvae of *Lilioceris latipennis* have been reported to cause serious destruction to the growing shoot apex of the *D.*

praehensilis plant (Di Giusto et al., 2017). Screening for susceptibility to insect and disease attacks in *D. praehensilis* using morphological and molecular tools will provide more insight into insect pest and disease resistance in *D. praehensilis*.

Farmers' varietal preferences for *D. praehensilis* clones in Ghana

A farmer's preference criterion plays an important role in guiding breeding and crop improvement programs' priorities and facilitates improved varieties adoption rates by farmers (Zavinon et al., 2018). According to the farmers, the demand for *D. praehensilis* as a staple food is deficient among the rural dwellers due to its late maturity as a result of prolonged dormancy and poor culinary quality traits. Thus, the farmers need to have cultivars with good agronomic traits (early maturity, high in-soil storage aptitude, big tuber size, high productivity, insect pest and disease tolerance) and culinary quality traits (good taste, smooth tuber flesh texture, good tuber flesh colour, high tensile strength and good aroma). *D. praehensilis* was reported to be a high yielding yam species in Ghana. This corroborates the report of the high yielding ability of *D. praehensilis* in Togo (Pitalounani et al., 2017). Pitalounani et al. 2017 reported the need to improve its culinary and tuber quality traits to enhance its market demands. Application of high throughput phenotyping and cost-effective near-infrared reflectance spectroscopy (NIRS) that has been reported to be useful in screening and detecting physico-chemical properties linked to tuber flesh quality traits in *D. alata* and *D. rotundata* (Alamu et al., 2019; 2020; Lebot & Malapa, 2013) can be employed in *D. praehensilis* to select desirable cultivars with good culinary

attributes. Since the market opportunity of *D. praehensilis* is low compared to popular yam species, such as *D. alata* and *D. rotundata*, its improvement program to enhance culinary quality and other traits should be gradual. The first stage would be collecting and screening existing materials for agronomic and culinary traits with the active participation of *D. praehensilis* farmers. Superior clones would then be extensively multiplied using rapid propagation/regeneration techniques (such as minisett, aeroponic, and other adequate yam seed multiplication techniques) to make seeds readily available for farmers. *D. praehensilis* farmers' support structures should be parallelly identifying market opportunities (i.e., export, processing, etc.) for probable high production volumes from promoting the species. It is only when the production has increased, the market is well-developed, and the seed system is established, then, preliminary and cost-effective breeding activities could be initiated for *D. praehensilis* yam in Ghana and West Africa.

Utilization, cultural practices and conservation techniques of *D. praehensilis* in Ghana

In this study, geographical regions greatly influenced the practices used in *D. praehensilis* production and utilization. For all the villages surveyed, *D. praehensilis* was more exploited for subsistence than commercial purposes. Like other yam species, such as white Guinea (*D. rotundata*) and water (*D. alata*) yams, the tubers of bush yam are mainly consumed boiled (Ampesi) with vegetable soups. According to most farmers surveyed, bush yam can also be pounded into fufu and consumed with soups, such as light, palm nut and

groundnut soups as for other cultivated yam species. In the off-season (lean season) when the popularly cultivated yams (*D. rotundata* and *D. alata*) are out of stock, *D. praehensilis* is been used in filling hunger gaps by the local farmers (Tostain, Allomasso, & Sokpon, 2002). *D. praehensilis* is also reported as source of valuable starch due to its high solubility, high density and high water absorption capacity (Songuimondenin et al., 2018). *In situ* conservation technique is mostly preferred by *D. praehensilis* farmers in the majority of the surveyed villages because of the belief and perception that this conservation method preserves the culinary qualities of the tubers. The farmers in the surveyed areas also mentioned several other conservation techniques (Table 3.11). Tostain et al. (2002) have reported the conservation of *D. praehensilis* in agroforestry under mango and neem trees (*Azardiracta indica*) as live stake. To enhance the production and cultivation of *D. praehensilis* in Ghana, screening this crop for all the conservation techniques mentioned by the farmers and selecting those that can effectively preserve *D. praehensilis* for more extended periods are necessary.

From this study, Ghanaian farmers generally cultivated *D. praehensilis* in cocoa plantations. *D. praehensilis* can survive in the cocoa plantations due to its ability to form cataphyll stems and branches which climb cocoa trees until they reach sunlight conditions and form leaf crowns which cover the canopy vegetation (Di Giusto et al., 2017). Some farmers also intercrop it with other staples, such as cassava, cocoyam, and sweet potatoes. *Dioscorea praehensilis* has been reported to be often associated with *D. dumetorum* in a study conducted in Benin (Tostain et al., 2002). In this study, some farmers also reported the sole

cultivation of *D. praehensilis* like other cultivated yam species in the surveyed villages.

According to the farmers in the surveyed villages, the common cultural practices included mounding, staking and weeding. Farmers also reported the use of live stakes, such as cocoa trees, mahogany and some local trees in supporting the stems of the crop because of its crawling and climbing nature. This allows *D. praehensilis* to climb to the top of the trees to receive sunlight to enhance its tuber initiation and development (Dumont, Dansi, Vernier, & Zoundihèkpon, 2006). Staking elevates shoots above the soil surface, permits better leaf exposure and reduces mutual shading of leaves, thus, enhancing the plant's photosynthetic capacity and ultimately leading to improved yield. Live staking, where vines of the yams are elevated up from the ground level on growing trees, has many advantages over dead stakes. Farmers reported that live staking was especially useful for *D. praehensilis* which can remain in one place for decades, giving one early and late harvest per year.

Role of farmers' gender and age on the diversity and practices engaged in *D. praehensilis* production in Ghana

No significant effect of the farmers' gender and age was found on the diversity and practices engaged in *D. praehensilis* farming. This indicates that gender and age play no role in selecting these crop varieties, cultivation, conservation techniques, and utilization in Ghana. Besides, there was equal participation of men and women, young and old, in the production of bush yam across the surveyed regions. This supports the findings of Haleegoah (2018), who

reported that the roles regarded as men's activities in the yam production system, such as seed yam cutting, planting and field maintenance, are currently performed by women and youths. Traditionally, female spouses did not own yam farms, but male spouses now allocate a portion of yam farms to females for seed yam production (Haleegoah, 2018). Although more men were involved in *D. praehensilis* due to its association with a male crop (cocoa), behaviours, perceptions and practices were not influenced by the farmer's gender.

Conclusions

This study revealed a moderate *D. praehensilis* diversity across the surveyed regions in Ghana. The diversity and distribution were high in the Eastern and Western North regions compared to the Central Region. Farmers preferred early maturing and highly productive varieties with good tuber size and tolerance to insect pests and diseases, and good culinary qualities. This study showed poor culinary tuber quality traits were the major cause of declining production and abandonment of some *D. praehensilis* accessions in Ghana. This study also revealed that the utilization of *D. praehensilis* is primarily for filling the hunger gaps in lean seasons and that farmers rely on informal exchange for planting materials. The establishment of a genetic resource conservation program to maintain diversity among *D. praehensilis* accessions and gradual development of an effective clonal selection, seed delivery system and breeding programs to meet farmers' preference criteria could increase *D. praehensilis* production and utilization in Ghana.

CHAPTER FOUR

ASSESSMENT OF GENETIC DIVERSITY OF *Dioscorea praehensilis* (Benth.) COLLECTED FROM CENTRAL REGION, GHANA, USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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Abstract

In Ghana, *Dioscorea praehensilis* (Benth.) is a wild yam cultivated species, especially in the cocoa grown regions. Recently, *D. praehensilis* has been reported to contribute to the food security of the small households in the forest zones of tropical Africa. This study evaluated the genetic diversity of 43 *D. praehensilis* collected from three regions in Ghana using simple sequence repeat (SSR) markers. Using 11 SSR markers, 99 alleles were generated, with an average of 8.48 alleles per locus. The mean gene diversity was 0.81, the mean polymorphic information content was 0.82 and the mean Shannon information index was 1.94. Principal coordinate analysis (PCoA) revealed a contribution of 40.16% of the first three coordinate axes and grouped the 43 genotypes into two groups, while hierarchical cluster using UPGMA revealed the presence of 3 main clusters. Molecular variance (AMOVA) revealed low genetic diversity among the three populations. This study revealed the need to conduct extensive germplasm collection across the country where *D. praehensilis* are cultivated as a source of food to obtain the genetic baseline information for its proper utilization in breeding programmes as a source of novel genes.

Keywords: Genetic diversity, Ghana, *Dioscorea praehensilis*, SSR markers, Wild yam

Introduction

Yams (*Dioscorea* spp.) are significant food crops in West Africa, where they contribute actively to food security and poverty alleviation of the local farmers (Dansi et al., 2013). Ghana is one of the highest yams producing nations after Nigeria (FAOSTAT, 2017). The genus *Dioscorea* spp comprises about 650 species (Cuoto et al., 2018). Only 50-60 species are available for cultivation and wild-harvest (Cuoto et al., 2018). Africa's most cultivated species are *D. alata* L., *D. bulbifera* L., *D. cayenesis* Lam, *D. esculenta* (Lour.) Burk, *D. rotundata* Poir and *D. trifida* L. (Craufurd, Battley, Ile, & Asiedu, 2006). The major wild yam species in Africa are *D. abyssinica*, *D. sagitifolia*, *D. preahensilis*, *D. liebrechstiana*, *D. mangenotiana* and *D. lecardi* (Dumont, Dansi, Vernier, & Zoundjihékpon, 2005).

Bush yam (*Dioscorea praehensilis*), which is one of the wild yams serves as a source of food and contributes greatly to the welfare of people in West Africa (Bhattacharjee et al., 2011). This species has a wide geographical range in Africa and occurs throughout the Western, Central, and Eastern parts of the continent (Dumont et al., 2005). Bush yam (*Dioscorea praehensilis)* is an edible wild yam that is mostly found around cocoa plantations in Ghana, has been known to fill the hunger gaps (food and income security) among cocoa farmers in Ghana for ages but is currently known to be disappearing (Anokye, Tetteh, & Otoo 2014). The genetic variability level in these yam species has been underutilized and understudied. Thus, there is

the need to explore molecular markers for genotype characterization to determine the genetic variability level in *D. praehensilis*.

Molecular markers, such as Random Amplified Polymorphic DNA (RAPD) (Zannou et al., 2009), simple sequence repeat (SSR) (Mengesha et al., 2013; Nascimento et al., 2013; Scarcelli et al., 2013; Silva et al., 2014; Siqueira et al., 2014; Otto, Anokye, Asare, & Tetteh, 2015; Tewodros, Firew, Hussein, Endale, & Beyene, 2018) and Amplified Fragment Length Polymorphism (AFLP) (Tamiru, Becker, & Maass, 2009; Sonibare, Asiedu, & Albach, 2010; Rivera-Jiménez et al., 2011), among others had been employed to assess the genetic variability of *Dioscorea spp.*

SSR markers are widely known due to their locus specificity, comprehensive genome coverage, elevated degree of polymorphism, co-dominant inheritance and convenience for simple automated scoring (Sosinski et al., 2000; Zalapa et al., 2012), thereby making them being increasingly used as the marker of choice in diversity analysis of different crop species. Several studies have been conducted on some other species of yam. Loko et al. (2016) reported high genetic diversity among 64 yam landraces in Benin using microsatellite markers. Otoo et al. (2015b) observed variability among 49 accessions of *D. alata* in Ghana using SSR markers. Silva et al. (2016) observed little spatial structure and a considerable level of variability in *D. bulbifera* using SSR markers.

Some researchers had conducted genetic diversity on *Dioscorea* spp using molecular markers, but few genotypes of *D. praehensilis* were involved. Bekele

(2014) has reported genetic variability in *Dioscorea* spp in Ethiopia using simple sequence repeat markers (SSRs), but only 5 genotypes of *D. praehensilis* were included.

There has been no rigorous study of the genetic diversity and genetic relationships currently in the collections of *D. praehensilis* using molecular markers. This research, therefore, aims at evaluating genetic variability in *Dioscorea praehensilis* genotypes using simple sequence repeat (SSR) markers and to understand the population structure in *D. praehensilis* for proper utilization.

Materials and Methods

Collection site and leaf sampling procedure

The germplasm studied consisted of three populations of *D. praehensilis* (Awo, Nyame, and Tetteh). The populations have 17, 12, and 14 genotypes, making 43 *D. praehensilis* genotypes (Table 4.1). These populations were maintained in a farmer's field at Amasamkrum village, Anomabo District, Central Region, Ghana (5° 37.8"N, 1° 33.3"E). Three-piece leaf samples were detached from each genotype and placed inside covered plastic containers containing 10g of silica gel to remove moisture and prevent the degradation of DNA from the leaves. The leaf samples were later transferred into Ziploc bags for preservation.

Table 4. 1. List of *D. praehensilis* accessions evaluated for SSR analysis

S/No	Sample	Pop	S/No	Sample	Pop
1	Awo1	Awo	23	Nyame2	Nyame
2	Awo10	Awo	24	Nyame3	Nyame
3	Awo11	Awo	25	Nyame5	Nyame

4	Awo12	Awo	26	Nyame6	Nyame
5	Awo13	Awo	27	Nyame7	Nyame
6	Awo14	Awo	28	Nyame8	Nyame
7	Awo15	Awo	29	Nyame9	Nyame
8	Awo16	Awo	30	Tetteh1	Tetteh
9	Awo17	Awo	31	Tetteh10	Tetteh
		Awo	32	Tetteh11	Tetteh
		Awo	33	Tetteh12	Tetteh
12	Awo3	Awo	34	Tetteh13	Tetteh
13	Awo4	Awo	35	Tetteh14	Tetteh
14	Awo6	Awo	36	Tetteh15	Tetteh
15	Awo7	Awo	37	Tetteh2	Tetteh
16	Awo8	Awo	38	Tetteh3	Tetteh
17	Awo9	Awo	39	Tetteh4	Tetteh
18	Nyame1	Nyame	40	Tetteh5	Tetteh
19	Nyame10	Nyame	41	Tetteh6	Tetteh
20	Nyame11	Nyame	42	Tetteh7	Tetteh
21	Nyame13	Nyame	43	Tetteh9	Tetteh
22	Nyame15	Nyame			

Table 4. 1. Continued

DNA extraction, quality and purity check procedures

DNA was extracted from leaves of all the 43 genotypes using a modified cetyltrimethylammonium bromide (CTAB) procedure (Doyle & Doyle, 1990). The concentrations and quality of DNA were determined by 1% agarose gel electrophoresis and DNA gel image was viewed using a UV light gel documentation system (Aplegen). The DNA concentrations were estimated by measuring the absorbance at 260nm (A_{260}) and 280nm (A_{280}) in a Gene Quant pro spectrophotometer (Amersham Bioscience, Piscataway, NJ, USA). DNA Purity or quality was determined by calculating the absorbance ratio at 260 nm and absorbance at 280 nm (A_{260}/A_{280}).

Polymerase chain reaction (PCR) procedure

To determine the genetic diversity among the genotypes of *D. praehensilis*, 11 SSR primers were used (Table 4.2). DNA samples were diluted to a working solution of 50 ng/µl and were subjected to PCR reaction. Primer optimization was done initially to identify the best annealing temperature using the first eight genotypes, and a gradient protocol for optimizing PCR was used. The PCR cocktail had 10µl of the reagents (Ultra-pure water at 4.34µl, 10x NH4 (Reaction buffer) at 1µl, 50mM MgCl2 at 0.4µl, 25mM dNTPs at 0.2µl, DMSO at 1µl, 25ng/µl Forward primer at 0.5µl, 25ng/µl Reverse primer at 0.5µl, 5 U/ml Taq polymerase at 0.06µl and 50ng/µl DNA template at 2µl). The polymerase chain reaction followed an optimized program with an initial denaturation at 94^{o}C for 3 min; denaturation at 94^{o}C for 1 min; annealing depending on the primers for 1 min; extension at 72^{o}C for 1 min; final extension at 72^{o}C for 10 min; and hold at 4^{o}C until the PCR products were removed from the thermocycler. The polymerase chain reaction products were electrophoresed on 2% agarose gel (2g agarose powder + 100ml 0.5X TBE buffer) with 1µg/ml ethidium bromide and ran in an electrophoresis tank containing 0.5X TBE buffer at 100V for 1.5 hours. Gel photographs were captured using a UV illuminator gel documentation system (Aplegen) and saved as TIFF images for easy uploading for gel analysis.

Gel Analysis and molecular data analysis

Gel images were analyzed using Bio-Rad image lab analysis software (version 6.0). The gel images were loaded into the software to generate the molecular size of the amplification in base pairs of the respective markers. fifty

base pair (50bp) DNA molecular ladder (Biolab) was used to estimate the molecular sizes of the DNA fragments. Where no amplification was detected, it was recorded as 0.

Genetic Analysis in Excel (GenAlEx) software version 6.503 (Peakall & Smouse, 2012) was used in estimating the number of different alleles (Na), number of effective alleles (Ne), Shannon Information Index (I), number of observed heterozygosity (Ho), number of expected heterozygosity (He), fixation index (F), allelic pattern across the populations and percentage of polymorphic loci (%P) across the three populations and the eleven SSR markers. Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) was also computed via distance matrix using the GenAlEx software version 6.503 (Peakall & Smouse, 2012). The significance for AMOVA was determined at 9999 permutations. Major allelic frequency and polymorphism information Content (PIC) were estimated using PowerMarker software version 3.25. Cluster analysis was carried out using the unweighted pair group method with arithmetic mean (UPGMA) trees in Powermarker software version 3.25. The dendrogram was then generated using Molecular Evolutionary Genetics Analysis (MEGA-X) version 10.0.5.

Table 4. 2. Information of the 11 SSR markers used in this study

Marker name	Marker sequence	Annealing Temp.°C	Observed marker size (bp)
YM16	F 5'- TGA AGA GAA TGT TGA GAT CGT ACC -3' R 5'- TAT CCG GCC CTC TCA TTG G -3'	56	87-180
YM18	F 5'- GAC ATT GGG GAT CTC TTA TCA T -3' R 5'- TAG CAG CAG TAA CGT TAA GGA A -3'	48	259-304
YM25	F 5'- GAT GGA GAT GAG GAG GCC G -3' R 5'- TTC GAA GCC AGA GCA AGT G -3'	57	197-269

	F 5'- TCC AGC TCT TTA GCA CAG G -3'		
YM27	R 5'- AGG AGC ATA GGC AAC AAG C -3'	55	215-236
	F 5'- CCA CAA CTA AAA ACA CAT GGA C -3'		
YM30	R 5'- GTG GTA GGG TGT GTA GCT TCT T -3'	49	212-250
	F 5'- AAG CCT AGT CGA TGG GTG G -3'		
YM31	R 5'- TGC TGT TCC AAC TTC CAA GC -3'	51	207-294
	F 5'- GCC TTG TTT TGT TGA TGC TTC G -3'		
YM43	R 5'- CCA GCC CAC TAA TCC CTC C -3'	52	178-225
YM44	F 5'- CGC AAC CAG CAA AGG ATT TA -3'	49	138-293
	R 5'- ATT CTG TCT CTC AAA ACC CCT -3'		
	F 5'- TGG GGT GAG AGA GTA AGT GG -3'		
	A CCG GGG ATC TTC TTG C -3'	52	116-146
	G CCC TTG GGA TGT AGG G -3'		
YM50	R 5'- CAT CCC CGT TGT ATC CTG C -3'	52	184-296
	F 5'- AGT GGT GCT GTA GTA ACT GGA A -3'		
YM61	R 5'- CAT GAC TAC CTT TCC TCA ATC A -3'	50	217-290

Table 4. 2. Continued

F=Forward Primer, R=Reverse Primer, bp = base pairs

Results

Genetic diversity of 43 *D. praehensilis* genotypes collected from Central Region based on 11 SSR primers

The polymorphism and allelic variation of the 11 SSR primers used to assess the genetic diversity among 43 *D. praehensilis* genotypes are presented in Table 4.3. A total of 99 alleles were generated using the 11 SSR primers in this study. The number of different alleles generated by each primer ranged from 5 to 13, with an average of 8.48 alleles per locus. The highest and lowest number of alleles was detected for primers YM18 and YM61, respectively. Major allele frequency ranged from 0.29 in YM30 and YM49 to 0.50 in YM18 and YM44. The observed heterozygosity per primer ranged from 0.00 to 0.50 with an average of 0.10, while the expected heterozygosity (gene diversity) per primer ranged from 0.58 in YM44 to 0.91 for YM61 with an average of 0.81. Polymorphism

information contents (PIC) ranged from 0.7 in YM18 to 0.83 in YM30, YM31, YM49 and YM61, with an average value of 0.82.

Genetic diversity within and among 43 *D. praehensilis* genotypes based on the populations

Genetic diversity within and among 43 *D. praehensilis* genotypes based on the populations is presented in Table 4.4. The average inbreeding coefficient (F) recorded was 0.89, with the Nyame population having the lowest value of 0.84 while the Awo population had the highest value of 0.92. The highest Shannon's Information Index (I) of 2.36 was recorded in the Awo population, while the lowest value of 1.41 was recorded in the Tetteh population. The mean Shannon's Information Index (I) recorded was 1.94.

Table 4. 3. Polymorphism and allelic variations of 11 SSR primers among 43 *D. praehensilis* genotypes

Markers	N	Na	Ne	Ho	He	MAF	PIC
YM30	10.00	9.00	8.38	0.00	0.85	0.29	0.88
YM43	9.33	6.00	4.11	0.00	0.72	0.33	0.78
YM31	9.67	9.00	8.61	0.00	0.86	0.31	0.88
YM18	7.00	4.67	4.01	0.00	0.73	0.50	0.70
YM16	9.67	9.00	8.54	0.00	0.88	0.40	0.79
YM27	8.33	7.00	6.39	0.00	0.83	0.31	0.86
YM50	9.67	8.00	7.23	0.00	0.83	0.31	0.88
YM61	9.67	13.33	11.48	0.50	0.91	0.38	0.83
YM25	8.67	11.33	9.96	0.42	0.87	0.29	0.88
YM49	10.00	8.67	7.91	0.00	0.87	0.50	0.73
YM44	7.00	7.33	6.71	0.11	0.58	0.36	0.83
Total	99.00	93.33	83.33	1.03	8.92	3.98	9.05
Mean	9.00	8.48	7.58	0.09	0.81	0.36	0.82
SE	0.21	0.21	0.19	0.01	0.01	0.02	0.02
SD	0.69	0.70	0.63	0.04	0.03	0.08	0.06

N=Number of alleles per locus, Na=Number of different alleles, Ne=number of effective alleles, Ho=Observed heterozygosity, He=Expected heterozygosity, MAF=Major allele frequency, PIC=Polymorphism information content, SE=Standard error, SD=Standard deviation

Table 4. 4. Genetic diversity within and among the 43 *D. praehensilis* genotypes based on the populations

Pop	N	Na	Ne	I	Ho	He	F	%P
Awo	13.45	11.73	10.27	2.36	0.07	0.89	0.92	100
Nyame	8.55	8.64	7.75	2.05	0.14	0.85	0.84	100
Tetteh	5.00	5.09	4.71	1.41	0.07	0.69	0.91	91
Total	27.00	25.45	22.73	5.82	0.28	2.43	2.68	291
Mean	9.00	8.48	7.58	1.94	0.09	0.81	0.89	97
SE	0.40	0.40	0.36	0.06	0.02	0.02	0.02	1.75
SD	0.69	0.70	0.63	0.11	0.04	0.03	0.04	3.00

N=Number of genotypes per population, Na=Number of different alleles, Ne=number of effective alleles, I=Shannon's information index, Ho=Observed heterozygosity, He=Expected heterozygosity, F=Inbreeding coefficient, content, %P=Percentage of polymorphic loci, SE=Standard error, SD=Standard deviation

A high percentage of polymorphism was recorded among individuals in all three populations, Awo population (100%), Nyame population (100%) and Tetteh population (91%). The level of genetic diversity observed among the populations and within individuals was very low, although high genetic diversity was observed among individuals. Nyame population recorded observed heterozygosity values of 0.14 while Awo and Tetteh populations recorded 0.07 each.

The allelic analysis across populations revealed the existence of variability among individuals within populations with the highest heterozygosity (0.89) recorded among individuals in the Awo population, while the lowest heterozygosity (0.69) was recorded among individuals in the Tetteh population (Figure 4.1).

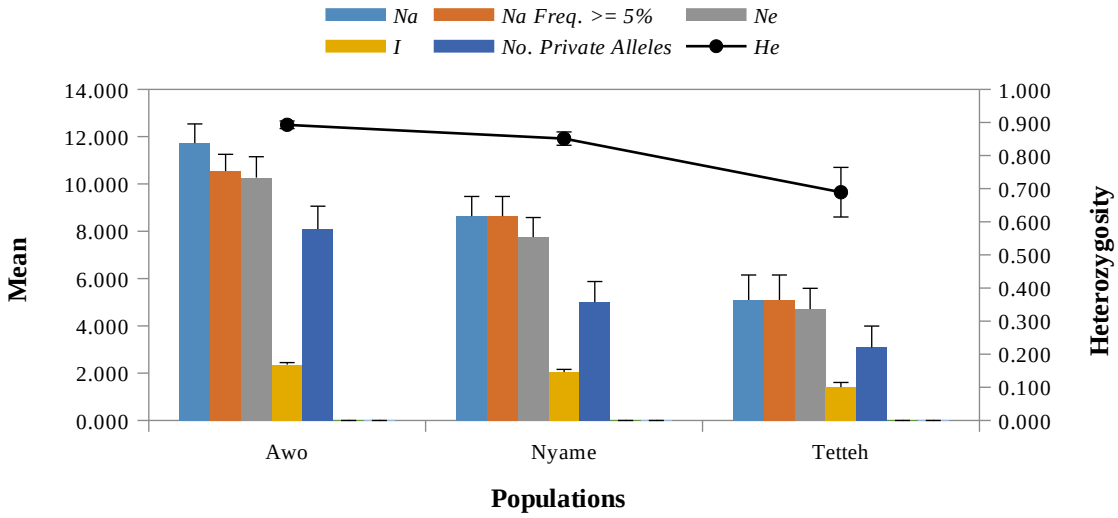

Figure 4. 1. Allelic patterns across three populations of *D. praehensilis*

Na=Number of different alleles, Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%, Ne = No. of Effective Alleles, I = Shannon's Information Index, No. Private Alleles = No. of Alleles Unique to a Single Population

Population differentiation and genetic structure

The molecular variance analysis (AMOVA) based on the fixation index (Fst) values indicated that among populations and within individuals accounted for the least percentage variation of 7% each, while among individuals accounted for the most percentage variation of 86%. Low genetic diversity was observed among the three different populations and within genotypes of the same population. (Table 4.5). Fst value of 0.066 observed in this study indicates low genetic differentiation among the three populations. Fis value of 0.93 indicates high heterozygote deficits, which may be due to non-random mating within the populations.

The principal coordinates analysis revealed a contribution of 40.16% of the first three coordinates and classified the 43 genotypes evaluated into two groups (Table 4.6). Group I comprised 28 mixtures of genotypes from the three populations and group II also comprised 15 genotypes originating from three populations; Awo, Nyame, and Tetteh (Figure 4.2).

The Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis grouped the 43 genotypes of *D.praehensilis* into three clusters (A, B, and C) (Figure 4.3). Cluster A was further grouped into six sub-clusters (I, II, III, IV, V and VI) (Figure 4.3). Sub-cluster I contained 22 genotypes in which five belonged to the Awo population, five from the Nyame population and 12 from the Tetteh population. Sub-cluster II consisted of six genotypes in which four belonged to the Awo population while two belonged the Nyame population. Sub-clusters III, IV and V contained two genotypes, each belonging to the Awo population and one belonging to the Nyame population. Sub-cluster VI consisted of six genotypes in which three, two and one belonged to the Awo, Nyame and Tetteh population, respectively. Cluster B consisted of two genotypes in which one each belonged to Awo and Tetteh populations. Cluster C contained one genotype belonging to the Nyame population.

Table 4. 5. Analysis of molecular variance of 43 *D. praehensilis* classified to 3 populations

Source	Df	SS	MS	Est. Var.	% Variation
Among Pops	2	35.147	17.573	0.315	7
Among Indiv	40	344.935	8.623	4.143	86
Within Indiv	43	14.500	0.337	0.337	7
Total	85	394.581		4.796	100
F-Statistics	Value	P(≥0.001)			
Fst	0.066	0.001			

Fis	0.925	0.001
Fit	0.930	0.001

df = Degree of freedom, SS = Sum of Square, MS = Mean of Square, Est. Var. = Estimated Variance, Fst = Total genetic differentiation, Fis = Inbreeding coefficient, Fit = Inbreeding Coefficient, P = Probability

Table 4. 6. Eigenvalues and % variations observed at the first three axes of the PCoA

Axis No.	Eigen Value	% Variation	Cum %
1	182.35	24.90	24.90
2	67.68	9.24	34.15
3	44.04	6.01	40.16

Cum % = Cumulative Percentage

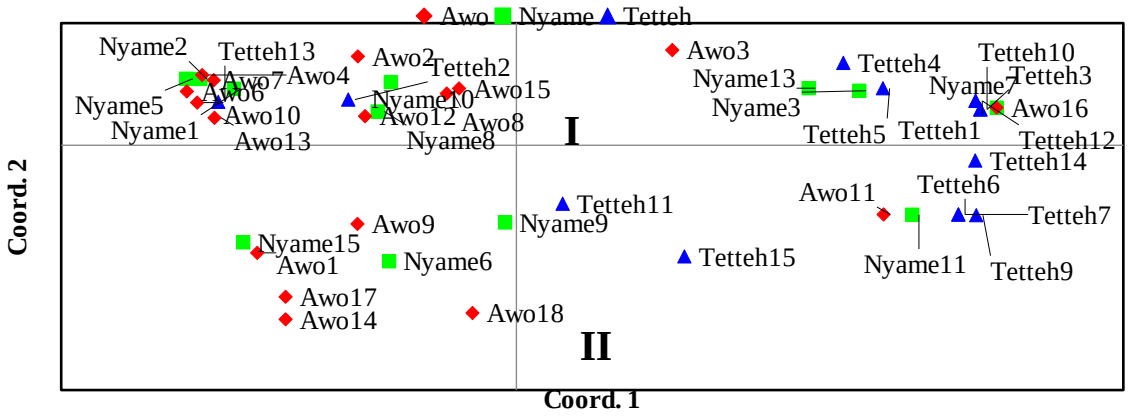

Figure 4. 2. Principal coordinates analysis (PCoA) for genetic variability among 43 *D. praehensilis* genotypes

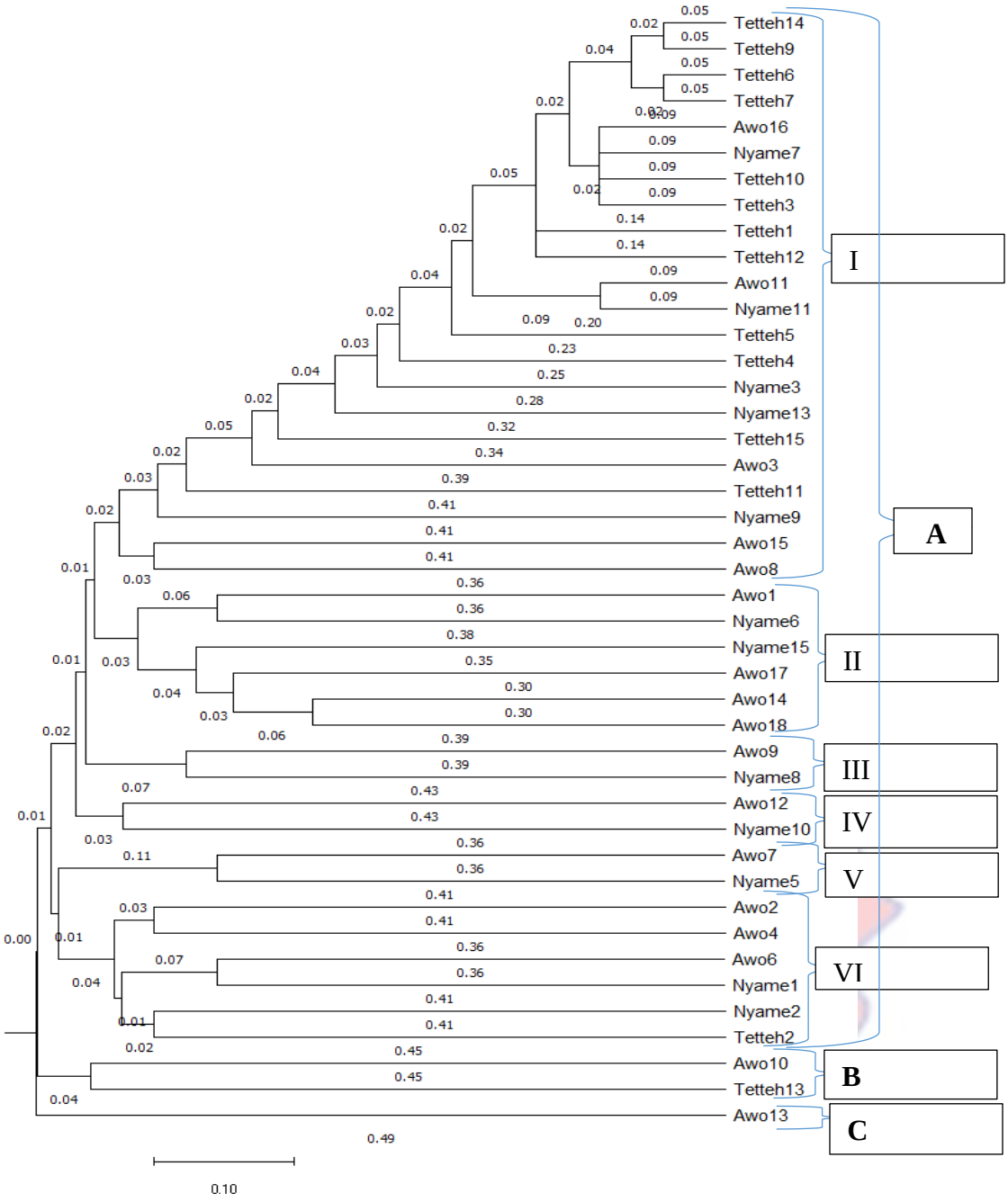

Figure 4. 3. Genetic similarities among 43 genotypes of *D. praehensilis* based on 11 SSR primers using Euclidian similarity coefficients with UPGMA Clustering

Discussion

Simple sequence repeat markers have been extensively employed in accessing genetic diversity among other yam species (Tewodros et al., 2018; Loko et al., 2016; Silva et al., 2015; Otoo et al., 2015). In this present study, the genetic diversity within and between populations was analyzed using 11 SSR markers in three populations of *D.praehensilis*.

The range and mean of alleles detected per primer in this study (4.67-13.33 and 8.48) were similar to what was reported by Loko et al. (2016) when using SSR markers to understand the genetic diversity and relationship among guinea yam germplasm in Benin Republic. The mean value of 3.3 reported by Silva et al. (2015) on genetic diversity of *D. bulbifera* using SSR markers was lower than the value from the present study. Siqueira et al. (2014) also reported a mean value of 5.1 on *D. alata* using 12 SSR primers. The high alleles detected in this study indicates the effectiveness of SSR markers that were used in this study.

It has been reported that high informative markers should have polymorphic information contents (PIC) greater than 0.5 and lower than 0.95 (Paal et al., 2013). The average PIC value (0.82) detected in this study indicates that the 11 SSR markers are highly informative and useful for detecting genetic variability among *Dioscorea* spp. Silva et al. (2014) also reported high average PIC for nine SSR markers in the study conducted on *D. rotundata* and *D. cayenensis*.

The average gene diversity (observed heterozygosity) of 0.09 obtained in this study was very low compared to what had been reported by several authors

who have worked on different species of yam. Loko et al. (2016) reported a very high observed heterozygosity (0.72) in guinea yam with 13 SSR markers. Otoo et al. (2015) also detected a high average observed heterozygosity (0.77) when categorizing water or greater yams using SSR markers.

The mean expected heterozygosity, which is a measure of genetic variation in a population, was very high in this present study (0.81) compared to what was reported on *Dioscorea* spp from Ethiopia (Tewodros et al., 2018). The high mean gene diversity in this present study might be due to the high reproducible ability of the SSR primers used.

The mean Shannon index (I) of 1.94 reported in this study is higher than the mean Shannon index value of 1.22 reported for Tanzanian sweet potato genotypes (Ngailo, Shimelis, Sibiya, Amelework, & Mtunda, 2016). A mean Shannon index value of 0.45 has also been reported in Ethiopia on diversity studies of *Dioscorea* spp landraces (Tewodros et al., 2018). Bekele (2014) reported a mean Shannon index (0.49) when conducting research on the molecular genetic diversity of yam germplasm collections from Ethiopia. Higher Shannon information indices have been reported on guinea yam and water yam genotypes (Abebe et al., 2013). The mean Shannon information index reported in this study indicates low genetic diversity among the populations assessed.

The mean inbreeding coefficient observed in this study was high (0.8) compared to 0.24 reported by Tewodros et al. (2014). A high inbreeding coefficient indicates the movement of genetic materials between and within the

populations resulting in a large number of duplications, which implies low genetic diversity among the evaluated populations.

The percentage polymorphic loci range from 91% for the genotypes in the Tetteh population to 100% in Awo and Nyame populations. A percentage polymorphic of 97% was observed in this study which was higher than 58.6% reported by Tewodros et al. (2018). Bekele (2014) also reported percentage polymorphic loci of 81.21%, which was lower than the value reported in this study. The higher value of percentage polymorphic loci is an indication of the close relatedness of the genotypes in each of the studied populations.

The high variation attributed to individuals in the populations and low variability accounted for among the populations is as a result of high gene flow among the evaluated population which was due to exchange of planting materials by the farmers across the regions of plant materials resulting to low population genetic diversity observed among farmers. High population genetic diversity (Abebe et al., 2013; Marie, Ngwe, & Simon, 2015; Tewodros et al., 2018) and low population genetic diversity (Zhigang et al., 2014) have been reported for several species of yam.

The fixation index (Fst), which is the measure of the difference in allele frequency between populations, was low (0.066). This implies that there was movement and sharing of genetic materials among the farmers, i.e. there is weak genetic differentiation among populations.

Principal coordinate analysis (PCoA) revealed low genetic diversity among the 43 genotypes of *D. praehensilis*. were distributed into all the four

groups in which they were grouped. The closeness of the genotypes to each other in the two groups implies high gene flow due to exchange of the planting materials among the farmers, resulting to duplication of the genetic materials.

This study observed that the clusters were not far from one another. The weak genetic differentiation among the clusters may be attributed to low genetic distances among the populations. The weak population structure that exists among the populations allows the movement of genes resulting in effective communication between one another. Close similarities of these genotypes among the populations indicate a wide distribution of the genotypes of bush yam within this community. This is in agreement with Al Salameen et al. (2018), who agued that pairs of populations geographically close to each other would be more genetically similar because their seeds or pollen easily migrate within short distances. The absence of barriers has allowed the movement of genetic materials within the locality resulting in low genetic distances among genotypes within the populations.

Conclusions

Low genetic diversity and structural differences were observed among the evaluated genotypes in this study. A high level of similarity was observed among the genotypes assessed which might be due to functional seed network or exchange of planting materials among the farmers within the study locality. This could be due to the planting of identical genotypes which were given different varietal names based on the locality. Further studies needed to be conducted

involving many more regions and localities where bush yams are being cultivated to provide the baseline information of genetic diversity in this yam species.

CHAPTER FIVE

EXPLORING THE BUSH YAM (*Dioscorea praehensilis* Benth.) AS A SOURCE OF AGRONOMIC AND QUALITY TRAIT GENES IN WHITE GUINEA YAM (*Dioscorea rotundata* Poir.) BREEDING

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Abstract

Yam (*Dioscorea* spp.) is an important food security crop in the tropics and subtropics. However, it is characterized by a narrow genetic base within cultivated and breeding lines for tuber yield, disease resistance and postharvest traits, hindering yam breeding progress. This study employed 162 accessions of *D. praehensilis* as a source of genes to improve the major yam species, *D. rotundata*. Significant differences were observed for assessed traits ($p < 0.05$), with *D. praehensilis* accessions out-performing the best *D. rotundata* landraces for tuber yield (23.47 t ha^{-1}), yam mosaic virus (YMV) resistance (AUDPC=147.45), plant vigour (2.43) and tuber size (2.73). The study revealed significant genotypic (GCV) and phenotypic (PCV) coefficients of variance for tuber yield, YMV severity score and tuber flesh oxidation. We also had a medium to high broad-sense heritability (H^2) for most of the traits, except for the dry matter content and tuber flesh oxidation. This study identified some promising *D. praehensilis* accessions for traits, such as high yield potential (WNDpr76, CDpr28, CDPr7, EDpr14 and WNDpr63), resistance to YMV (WNDpr76, CDpr7, EDpr14, CDpr28 and EDpr13), high dry matter content (WNDpr76, CDpr28 and WNDpr24), low tuber flesh oxidation (WNDpr76, CDpr5, WNDpr31, CDpr40 and WNDpr94) and high number of tubers per plant (WNDpr76, CDpr7, CDpr68, CDpr29 and CDpr58). Therefore, these accessions could be employed in breeding programmes to improve the white Guinea yam by broadening its genetic base.

Keywords: Wild relative, *D. praehensilis*, *D. rotundata*, inter-specific crosses, yield traits, post-harvest quality, resistance genes.

Introduction

Yam (*Dioscorea* spp.) is an important food security crop in sub-Saharan Africa, particularly in West Africa, where it accounts for over 95 per cent of world yam production, and approximately 300 million people rely on its cultivation and trade for food and money (Asiedu & Sartie, 2010; Sesay et al., 2013; Alabi et al., 2019; FAOSTAT, 2021). In West Africa, Nigeria, Ghana, Côte d'Ivoire, and Benin were the leading producers with ~50, 8.3, 7.2, and 3.1 million tons, respectively, in 2019 (FAOSTAT, 2021). In these countries, yam provides carbohydrates, proteins, essential minerals, vitamins and lipids and it is significantly involved in the local people's social, economic and religious lives (Zannou et al., 2004; Obidiegwu & Akpabio, 2017). The annual yam yield in Ghana is ~17.8 t ha^{-1}, far below its potential (40–50 t ha^{-1}) (FAOSTAT, 2021).

Several factors, mostly abiotic (e.g., poor soil fertility and drought stress) and biotic stresses (e.g., insect pests and diseases, such as yam mosaic virus (YMV), yam anthracnose disease (YAD) and yam nematodes), are responsible for the low productivity of cultivated yam species in West Africa (Thouvenel & Dumont, 1975; Adeniji et al., 2012; Frossard et al., 2017; Matsumoto, Ishikawa, Asfaw & Asiedu, 2021; Agre et al., 2021). Unfortunately, yam is mostly produced by resource-poor farmers who can hardly afford alternative control measures (external farm inputs) such as application of inorganic fertilizer and pesticides and use of irrigation. Developing and deploying improved varieties, combining high yield potential and abiotic and biotic stress resistance, is the most cost-effective and practical way of raising yields in farmer fields in West Africa.

Dioscorea rotundata, also referred to as African yam or white Guinea yam, is the most cultivated yam species in West Africa. Along with *D. cayenensis* and *D. alata*, they represent more than 95% of produced yam worldwide (Asiedu & Sartie, 2010; Agre et al., 2021a). Farmers' and consumers' preferences for a white Guinea yam varieties depend on key traits such as high tuber yield potential, low tuber flesh oxidation/browning, reduced tuber flesh hardening, high dry matter content, and tolerance YMV and YAD (Agre et al., 2021). Accordingly, the genetic improvement of this yam species will have a tremendous impact on food security and poverty alleviation if varieties combining these preferred traits are developed and distributed to the predominantly resource-poor farmers of West Africa. Such effort implies that donor parents for each trait are identified within the yam primary and secondary gene pools. Thus, knowing genetic diversity and ease of gene flow among and within yam species is vital before designing an inter-specific breeding programme. Based on previous reports, yam breeders have been using a narrow genetic base in developing new varieties for agronomic traits, such as resistance to YMV, tuber flesh oxidation and colour, dry matter content and tuber flesh hardness, which has resulted in slow progress and low genetic gain in past years (Agre et al., 2021a). This is partly due to the vegetative propagation (planting tubers) used for yam cultivation since its domestication. This clonal propagation gradually reduced genetic diversity, making plants more susceptible to diseases and more challenging to remove undesirable mutations from germplasms (Sugihara et al., 2021). Broadening the genetic base of existing yam (*Dioscorea* spp.) breeding

populations is crucial for increasing the variability and the chance of finding more promising accessions. Wild relatives are potential sources of resistance, adaptation and quality trait genes for yam breeding programmes and, therefore, a better understanding of their genetic variability is crucial for maximum impact (Dempewolf et al., 2017; Lebot et al., 2019b).

The genus *Dioscorea* consists of ~600 yam species, of which eight are grown in West Africa, where *D. cayenensis* and *D. rotundata* are native and predominant species (Couto et al., 2018; Darkwa et al., 2020). These two native species emerged from the domestication of wild yams, mainly *D. praehensilis*, *D. burkilliana*, and *D. abyssinica* (Scarcelli et al., 2019; Sugihara et al., 2020; Sugihara et al., 2021). Therefore, these wild yam species related to the cultivated species constitute a vast reservoir of genetic resources that can be exploited to improve the white Guinea yam. Besides, in the era of changing climate, the diversity offered by wild species might provide alternative forms of valuable genes, which could be fundamental in the production of cultivars that are resilient to current and future climatic and edaphic conditions (Bhandari, Bhanu, Srivastava, Singh & Shreya, 2017). Wild relatives of cultivated yams might also be the sources of key agronomic and tuber quality traits, which can be introgressed as beneficial alleles to improve white Guinea yam and, thus, broaden its genetic base for breeding in West Africa.

Bush yam (*D. praehensilis*) is an edible semi-cultivated wild yam species that is predominantly used by local farmers in the forest zones of West African countries, such as Nigeria, Ghana, Benin and Togo, as a source of food during

lean seasons (Pitalounani et al., 2017; Adewumi et al., 2021). This species has a high yield potential, tolerance to insect pests and diseases, longer in-soil storage aptitude and the ability to flower and set fruits profusely (Adewumi et al., 2021). Besides, spontaneous and controlled hybridizations of this species with the white Guinea yam have been reported in West Africa (Mondo et al., 2020; Agre et al., 2021b). Thus, this species is a promising candidate for broadening the genetic base of the white Guinea yam and increasing the genetic gain for critical traits.

Therefore, this study aimed to explore the potential of *D. praehensilis* as a new source for key agronomic and tuber quality traits in white Guinea yam breeding programmes. Specifically, this study sought to: (i) identify *D. praehensilis* accessions with superior performance for nine agronomic and tuber quality traits and (ii) estimate the variance components and broad-sense heritability of those traits.

Materials and Methods

Experimental site

The experiment was conducted between December 2019 and November 2020 at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast, Ghana (5°07´7.6´´N, 1°17´18.9´´W, and at 15 m above the sea level). This University is located in the Central Region of Ghana, with a semi-deciduous forest and coastal savannah climatic zones with a bimodal rainfall pattern. During the study period, the annual rainfall was 1246.2 mm. The average minimum and maximum temperatures were 24.2 and 28.7°C, respectively. The average relative humidity for this period was 75.7%. The soil of the experimental site was sandy

loam with a slightly acidic pH (6.72), 1.31% organic carbon, 754.6 µg/g available phosphorus and 0.081cmol/kg potassium.

Plant materials

A panel of 174 yam accessions, including 162 *D. praehensilis* accessions and 12 *D. rotundata* landraces (serving as checks), were used in the study (Table 5.1). *Dioscorea praehensilis* panel comprised of 72, 24 and 66 accessions collected from Ghana's Central, Eastern and Western North regions, respectively. At the same time, the most preferred *D. rotundata* landraces were sourced from the local markets in Ghana.

Table 5. 1. List of *D. praehensilis* and *D. rotundata* accessions used in the study and their sources of collection

S/ No	Accession No	Species	Sex	Region of collection
1	CDpr1	*D. praehensilis*	Female	Central
2	CDpr10	*D. praehensilis*	Male	Central
3	CDpr11	*D. praehensilis*	Male	Central
4	CDpr12	*D. praehensilis*	Male	Central
5	CDpr13	*D. praehensilis*	Male	Central
6	CDpr15	*D. praehensilis*	Male	Central
7	CDpr16	*D. praehensilis*	Male	Central
8	CDpr17	*D. praehensilis*	Not flowering	Central
9	CDpr18	*D. praehensilis*	Male	Central

Table 5. 1. Continued

10		*D. praehensilis*	Female	Central
11	CDpr22	*D. praehensilis*	Male	Central
12	CDpr23	*D. praehensilis*	Not flowering	Central
13	CDpr24	*D. praehensilis*	Male	Central
14	CDpr25	*D. praehensilis*	Female	Central
15	CDpr26	*D. praehensilis*	Monoecious male	Central
16	CDpr27	*D. praehensilis*	Monoecious female	Central
17	CDpr28	*D. praehensilis*	Male	Central
18	CDpr29	*D. praehensilis*	Male	Central
19	CDpr3	*D. praehensilis*	Male	Central
20	CDpr33	*D. praehensilis*	Male	Central
21	CDpr34	*D. praehensilis*	Male	Central
22	CDpr35	*D. praehensilis*	Male	Central
23	CDpr37	*D. praehensilis*	Male	Central
24	CDpr4	*D. praehensilis*	Monoecious male	Central
25	CDpr40	*D. praehensilis*	Male	Central
26	CDpr41	*D. praehensilis*	Male	Central
27	CDpr43	*D. praehensilis*	Female	Central
28	CDpr44	*D. praehensilis*	Male	Central
29	CDpr45	*D.*	Female	Central

Table 5. 1. Continued

		praehensilis		
30	CDpr46	*D. praehensilis*	Male	Central
31	CDpr47	*D. praehensilis*	Monoecious male	Central
32	CDpr48	*D. praehensilis*	Female	Central
33	CDpr49	*D. praehensilis*	Male	Central
34	CDpr5	*D. praehensilis*	Monoecious male	Central
35	CDpr50	*D. praehensilis*	Female	Central
36	CDpr51	*D. praehensilis*	Male	Central
37	CDpr52	*D. praehensilis*	Female	Central
38	CDpr53	*D. praehensilis*	Male	Central
39	CDpr54	*D. praehensilis*	Female	Central
40	CDpr55	*D. praehensilis*	Male	Central
41	CDpr56	*D. praehensilis*	Male	Central
42	CDpr57	*D. praehensilis*	Male	Central
43	CDpr58	*D. praehensilis*	Female	Central
44	CDpr59	*D. praehensilis*	Male	Central
45	CDpr6	*D. praehensilis*	Male	Central
46	CDpr60	*D. praehensilis*	Female	Central
47	CDpr61	*D. praehensilis*	Male	Central
48	CDpr62	*D. praehensilis*	Male	Central

Table 5. 1. Continued

49	CDpr64	*D. praehensilis*	Monoecious male	Central
50	CDpr66	*D. praehensilis*	Not flowering	Central
51	CDpr68	*D. praehensilis*	Male	Central
52	CDpr69	*D. praehensilis*	Female	Central
53	CDpr7	*D. praehensilis*	Male	Central
54	CDpr70	*D. praehensilis*	Female	Central
55	CDpr72	*D. praehensilis*	Male	Central
56	CDpr73	*D. praehensilis*	Male	Central
57	CDpr74	*D. praehensilis*	Female	Central
58	CDpr75	*D. praehensilis*	Monoecious male	Central
59	CDpr76	*D. praehensilis*	Male	Central
60	CDpr77	*D. praehensilis*	Female	Central
61	CDpr79	*D. praehensilis*	Male	Central
62	CDpr8	*D. praehensilis*	Male	Central
63	CDpr81	*D. praehensilis*	Male	Central
64	CDpr83	*D. praehensilis*	Male	Central
65	CDpr85	*D. praehensilis*	Male	Central
66	CDpr87	*D. praehensilis*	Monoecious male	Central

Table 5. 1. Continued

67	-	*D. praehensilis*	Male	Central
68	CDpr9	*D.*	Female	Central

		praehensilis		
69	CDpr90	*D. praehensilis*	Female	Central
70	Dp/Asamankese/Assin/C/002	*D. praehensilis*	Male	Central
71	Dp/Asamankese/Assin/C/009	*D. praehensilis*	Not flowering	Central
72	Dp/Asesewa/UP/E/001	*D. praehensilis*	Not flowering	Eastern
73	Dp/UP/E/001	*D. praehensilis*	Male	Eastern
74	EDpr1	*D. praehensilis*	Male	Eastern
75	EDpr13	*D. praehensilis*	Male	Eastern
76	EDpr14	*D. praehensilis*	Male	Eastern
77	EDpr15	*D. praehensilis*	Male	Eastern
78	EDpr17	*D. praehensilis*	Not flowering	Eastern
79	EDpr2	*D. praehensilis*	Monoecious male	Eastern
80	EDpr20	*D. praehensilis*	Female	Eastern
81	EDpr21	*D. praehensilis*	Male	Eastern
82	EDpr22	*D. praehensilis*	Female	Eastern
83	EDpr23	*D. praehensilis*	Not flowering	Eastern
84	EDpr24	*D. praehensilis*	Monoecious male	Eastern
85	EDpr3	*D. praehensilis*	Male	Eastern

Table 5. 1. Continued

86		*D. praehensilis*	Male	Eastern
87	EDpr5	*D. praehensilis*	Monoecious male	Eastern

88	EDpr6	*D. praehensilis*	Male	Eastern
89	EDpr7	*D. praehensilis*	Female	Eastern
90	EDpr8	*D. praehensilis*	Male	Eastern
91	EDpr9	*D. praehensilis*	Not flowering	Eastern
92	Kaati	*D. praehensilis*	Not flowering	Eastern
93	Odonor big	*D. praehensilis*	Not flowering	Eastern
94	Ologojo	*D. praehensilis*	Not flowering	Eastern
95	Otim	*D. praehensilis*	Male	Eastern
96	PGR/20/002	*D. praehensilis*	Not flowering	Eastern
97	WNDpr1	*D. praehensilis*	Male	Western North
98	WNDpr10	*D. praehensilis*	Female	Western North
99	WNDpr11	*D. praehensilis*	Not flowering	Western North
100	WNDpr13	*D. praehensilis*	Male	Western North
101	WNDpr15	*D. praehensilis*	Male	Western North
102	WNDpr18	*D. praehensilis*	Female	Western North
103	WNDpr2	*D. praehensilis*	Male	Western North
104	WNDpr21	*D. praehensilis*	Male	Western North

Table 5. 1. Continued

105	-	*D. praehensilis*	Male	Western North
106	WNDpr23	*D. praehensilis*	Female	Western North
107	WNDpr24	*D.*	Female	Western

		praehensilis		North
108	WNDpr29	*D. praehensilis*	Male	Western North
109	WNDpr3	*D. praehensilis*	Male	Western North
110	WNDpr30	*D. praehensilis*	Male	Western North
111	WNDpr31	*D. praehensilis*	Male	Western North
112	WNDpr32	*D. praehensilis*	Monoecious male	Western North
113	WNDpr33	*D. praehensilis*	Male	Western North
114	WNDpr34	*D. praehensilis*	Male	Western North
115	WNDpr35	*D. praehensilis*	Female	Western North
116	WNDpr36	*D. praehensilis*	Female	Western North
117	WNDpr39	*D. praehensilis*	Male	Western North
118	WNDpr4	*D. praehensilis*	Not flowering	Western North
119	WNDpr41	*D. praehensilis*	Not flowering	Western North
120	WNDpr42	*D. praehensilis*	Male	Western North
121	WNDpr44	*D. praehensilis*	Not flowering	Western North
122	WNDpr45	*D. praehensilis*	Male	Western North
123	WNDpr46	*D. praehensilis*	Male	Western North

Table 5. 1. Continued

124	-	*D. praehensilis*	Female	Western North
125	WNDpr5	*D. praehensilis*	Not flowering	Western North
126	WNDpr54	*D. praehensilis*	Male	Western North

127	WNDpr56	*D. praehensilis*	Male	Western North
128	WNDpr57	*D. praehensilis*	Male	Western North
129	WNDpr59	*D. praehensilis*	Male	Western North
130	WNDpr6	*D. praehensilis*	Male	Western North
131	WNDpr60	*D. praehensilis*	Male	Western North
132	WNDpr63	*D. praehensilis*	Female	Western North
133	WNDpr65	*D. praehensilis*	Male	Western North
134	WNDpr66	*D. praehensilis*	Female	Western North
135	WNDpr67	*D. praehensilis*	Not flowering	Western North
136	WNDpr68	*D. praehensilis*	Male	Western North
137	WNDpr69	*D. praehensilis*	Male	Western North
138	WNDpr7	*D. praehensilis*	Female	Western North
139	WNDpr71	*D. praehensilis*	Male	Western North
140	WNDpr72	*D. praehensilis*	Female	Western North
141	WNDpr74	*D. praehensilis*	Male	Western North
142	WNDpr75	*D. praehensilis*	Male	Western North

Table 5. 1. Continued

143		*D. praehensilis*	Male	Western North
144	WNDpr77	*D. praehensilis*	Male	Western North
145	WNDpr79	*D. praehensilis*	Male	Western North
146	WNDpr8	*D.*	Female	Western

		praehensilis		North
147	WNDpr81	*D. praehensilis*	Male	Western North
148	WNDpr82	*D. praehensilis*	Female	Western North
149	WNDpr83	*D. praehensilis*	Male	Western North
150	WNDpr84	*D. praehensilis*	Female	Western North
151	WNDpr86	*D. praehensilis*	Female	Western North
152	WNDpr87	*D. praehensilis*	Female	Western North
153	WNDpr88	*D. praehensilis*	Female	Western North
154	WNDpr89	*D. praehensilis*	Female	Western North
155	WNDpr9	*D. praehensilis*	Male	Western North
156	WNDpr91	*D. praehensilis*	Male	Western North
157	WNDpr92	*D. praehensilis*	Not flowering	Western North
158	WNDpr93	*D. praehensilis*	Male	Western North
159	WNDpr94	*D. praehensilis*	Male	Western North
160	WNDpr96	*D. praehensilis*	Female	Western North
161	WNDpr97	*D. praehensilis*	Male	Western North
162	WNDpr98	*D. praehensilis*	Male	Western North
163	Dr_Alata_Puna	*D. rotundata*	Male	Greater Accra
164	Dr_Asana_North	*D. rotundata*	Female	Greater Accra
165	Dr_Dente	*D. rotundata*	Male	Greater Accra

166	Dr_Durben	*D. rotundata*	Female	Greater Accra
167	Dr_Kpanjol	*D. rotundata*	Male	Greater Accra
168	Dr_Mutwu	*D. rotundata*	Male	Central
169	Dr_Nyaminti	*D. rotundata*	Male	Greater Accra
170	Dr_Puna_Central	*D. rotundata*	Male	Central
171	Dr_Puna_Female	*D. rotundata*	Female	Greater Accra
172	Durban	*D. rotundata*	Not flowering	Central
173	Olodo-1	*D. rotundata*	Male	Central
174	Puna	*D. rotundata*	Male	Central

Experimental design and field management

The experiment was conducted in a simple lattice design with two replicates. The field layout was generated using "Agricolae" package in R software (de Mendiburu, 2021). Each replicate comprised 18 incomplete blocks with 10 experimental units (plots) as the block size. In each replicate, the experimental unit comprised 3 m long ridges containing three plants at 1m spacing between and within rows. The planting setts were pre-treated using 70 g Mancozeb (80% WP) as fungicide and 75 ml of cypermetrin (25% EC) as an insecticide in 10 L of tap water to prevent soil-borne fungi and insect pests from spoiling the setts after planting. The tuber setts from the same accession were labelled properly in net bags and dipped into the solution for 10 min, and left in a shaded place for 24 hours to allow the cut surface to dry. Hand weeding using hoe was carried out when necessary to reduce the weed competition.

Data collection

Data were collected on traits of economic significance to yam farmers and consumers. Assessed traits included YMV and YAD severity scores recorded monthly from two to six months after sprout emergence, plant vigour was assessed four months after sprout emergence, the number of tubers per plot and tuber size were recorded at harvest, while tuber yield (per hectare) was determined one year after planting. Tuber dry matter content, tuber flesh oxidation and tuber flesh hardness were determined at post-harvest. All these traits were assessed using the yam crop ontology recommendations (Asfaw, 2016).

The plot yield was extrapolated to the yield in tons per hectare using the following formula:

$$TTYH = \frac{TTWP \times 10}{PLS} \quad (5.1)$$

Where: TTWP is the total tuber yield per plot, and PLS is the plot size.

The dry matter content was determined by chopping 100 g of fresh tuber flesh into small pieces and then oven-dried at 105°C for 24 h till a constant weight was achieved. The percentage dry matter content was then estimated as:

$$\%\ dry\ matter\ content = ¿\ \frac{Dry\ tuber\ flesh\ weight\ (g)}{Wet\ tuber\ flesh\ weight\ (g)} \times 100 \quad (5.2)$$

The intensity of tuber flesh oxidation (colour change or browning of cut tuber flesh) was assessed immediately the surface was cut and exposed to air (0 min) and 60 min after cutting, using a Chroma (colourimeter) meter (CR-400, Ke5onica Minolta, Japan), and the (L*) lightness, (a*) red/green coordinate, (b*)

yellow/blue coordinate values were recorded. A reference of white and black porcelain tiles was used to calibrate the Chroma meter before each reading. The delta (colour difference) (**Δ**E*) between all the three coordinates was calculated using the following formulas:

$$\Delta E^{¿} = \left(L¿¿¿ + a^{¿} + b^{¿} \right)^{1/2} ¿ \quad (5.3)$$

Oxidative browning = FΔE* - IΔE* (5.4)

Where: F**Δ**E* is the colour change and I**Δ**E* is the initial colour change

The area under the disease progression curve (AUDPC), a valuable quantitative summary of disease intensity or severity for YMV and YAD over time, was estimated using the trapezoidal method (Campbell & Madden, 1990). This method discretizes the time variable and calculates the average disease intensity or severity between each pair of adjacent time points:

$$AUDPC = \sum_{i=1}^{N} \left(\frac{y_{i+yi+1}}{2} \right) \left(t_{i+1} - t_i \right) \quad (5.5)$$

Where: N is the number of observations, y_i is the disease severity at i^{th} observation, t_i is the time at i^{th} observation.

Tuber flesh hardness was assessed with a 6.00 mm probe digital penetrometer. Tuber samples of 1 cm thickness and ~5 cm diameter were prepared from each genotype/accession, and the probe was pressed into the tuber. The force necessary for its penetration into the tuber was considered an indicator of the hardness of the tuber. Three measurements were taken per accession, the average was calculated and the data were expressed in Newtons.

The number of tubers harvested per plot was hand-counted and recorded at harvesting.

Data on plant vigour were collected two months after the emergence of the sprouts using the rating scale: 1 = weak (75% of the plants or all the plants in a plot are small, few leaves and thin vine); 2 = medium (intermediate or normal); 3 = vigorous (75% of the plants or all the plants in a plot are robust with thick vine and leaves very well-developed or with abundant foliage).

Data on tuber size was collected at harvest using the rating scale: 1 = small (less than 15 cm length); 2 = medium (between 15 and 25 cm length); 3 = big/large (more than 25 cm length).

Data analysis

A linear mixed model (LMM) for simple lattice design was employed in performing the analysis of variance (ANOVA) through *lm* function in R package (R Development Core Team, 2019). The linear model used was as follows:

$$Y_{hijk} = \mu + S_h + G_i + R_j + B_k + \varepsilon_{hijk} \quad (5.6)$$

Where: Y_{hijk} = value of the observed quantitative trait in block k and replicate j, μ = population mean; S_h = effect of the h^{th} species, Gi = effect of the i^{th} genotype; Rj = effect of the j^{th} replicate (superblock); B_k = effect of the k^{th} incomplete block within the j^{th} replicate; and ε_{ijk} = experimental error.

Species and accessions were considered random effects, while replicates and blocks were considered fixed effects. Expected mean squares (EMS) from ANOVA using *lmerTest* and *lme4* in the R package (R Development Core Team, 2019) were employed to estimate the variance components for each trait. Broad-

sense heritability (H^2b), phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) were calculated using the values derived from respective variance components. Broad-sense heritability (H^2b) was classified as low (<30%), medium (30–60 %) and high (>60%), according to Johnson et al. (Johnson, Robinson & Comstock, 1955). Based on Deshmukh, Basu & Reddy (1986), phenotypic and genotypic coefficients of variation greater than 20 % are regarded as high. In contrast, values between 10 and 20 % are medium, and values less than 10% are considered low.

The broad-sense heritability (H^2b) was estimated using the following formula:

$$H^2b = \left(\frac{\delta_g^2}{\delta_g^2 + \delta_{p/n}^2}\right) \times 100 \quad (5.7)$$

The phenotypic coefficient of variance (PCV) was determined by:

$$PCV = ¿\ \frac{\sqrt{\delta_p^2}}{Grand\,mean} \times 100 \quad (5.8)$$

The genotypic coefficient of variance (GCV) was calculated as follows:

$$GCV = ¿\ \frac{\sqrt{\delta_g^2}}{Grand\,mean} \times 100 \quad (5.9)$$

In these formulas, δ^2_g is the genotypic variance, δ^2_p is the phenotypic variance explained as the residual, and *n* is the number of replications.

Descriptive statistics, such as the calculations of means, standard deviations, minimum and maximum values and coefficients of variation, were employed to describe variations in key agronomic and tuber quality traits of *D. praehensilis*

and *D. rotundata*. Degrees of association among assessed traits were determined using Pearson's correlation coefficient in R (R Development Core Team, 2019). The association between traits was visualized using corrplot R package version 0.84 (Wei & Simko, 2017).

The principal component analysis (PCA) was carried out using the packages, Factoextra and FactoMineR in R (R Development Core Team, 2019). Hierarchical cluster analysis was generated using Pheatmap and Ward.2 methods implemented in Cluster package in R (R Development Core Team, 2019). To calculate the optimal number of clusters and assess grouping efficacy, the silhouette approach implemented in Cluster package and FactoMineR (R Development Core Team, 2019) were employed. FactoMineR in the R package was also used to generate biplots to determine the position of the key agronomic and tuber quality traits of *D. praehensilis* and *D. rotundata* collections. Path coefficient analysis was conducted using lavaan and semPlot in the R package, considering the tuber yield and dry matter content as response variables. A path diagram was constructed to depict the direct effect of key agronomic and tuber quality traits on tuber yield and dry matter content to determine which traits can be adopted for indirect selection.

Results

Variability in key agronomic and tuber quality traits of *D. praehensilis* and *D. rotundata*

Analysis of variance for species revealed significant differences ($p < 0.05$) for most of the parameters evaluated, except the dry matter content, tuber flesh oxidation and the number of tubers per plot (Table 5.2). Significant differences (p

< 0.05) were also observed among the accessions within species for all the traits, except the tuber flesh oxidation (Table 5.2). *Dioscorea praehensilis* obtained significantly high tuber yield (23.47 t ha^{-1}), low YMV severity score (AUDPC=147.45), high plant vigour (2.43) and large tuber size (2.73) compared to *D. rotundata* (Table 5.2). No significant variations were observed in dry matter content and tuber flesh oxidation between the two yam species. However, higher maximum values for dry matter content (41.96%) and the number of tubers per plot (~6.00) were recorded for *D. praehensilis*, while *D. rotundata* had a better tuber flesh hardness score (39.00) (Table 5.3).

Coefficients of variation (CV) ranged from 0.27% for YAD severity score to 89.67% for the tuber flesh oxidation. High CVs were recorded for traits, such as tuber yield, tuber flesh oxidation and the number of tubers per plot. In contrast, low CVs were recorded for dry matter content, plant vigour, tuber size, YMV severity, YAD severity and tuber flesh hardness (Table 5.3).

Table 5. 2. Variation due to random effects of agronomic and tuber quality traits in *D. praehensilis* and *D. rotundata* accessions

		Mean Squares								
Sources of variation	DF	Tuber yield ($t\ ha^{-1}$)	DMC (%)	YMV	YAD	TBOXI	TBHard (N)	PLNV	TBRSZ	NTP
Replicate	1	946.61*	323.41*	0.65^{NS}	1.28×10^{-24}*	288.63^{NS}	0.15^{NS}	0.003^{NS}	0.003^{NS}	7.47*
Block	14	194.37^{NS}	9.97^{NS}	1704.20*	271.42*	112.12^{NS}	12.21*	0.44*	0.16*	1.28^{NS}
Species	1	1824.46*	31.19^{NS}	602.71*	1814.16*	238.16^{NS}	2395.04*	2.06*	1.38*	0.69^{NS}
Genotype	172	640.93*	19.58*	1861.65*	553.71*	92.37^{NS}	2.59*	0.54*	0.68*	1.65*
Residual	159	209.96	12.62	0.64	1.91×10^{-24}	81.98	0.37	0.01	0.01	0.74

DMC = Dry matter content, YMV = Yam mosaic virus, YAD = Yam anthracnose disease, TBOXI = Tuber flesh oxidation, TBHard = Tuber flesh hardness, PLNV = Plant vigour, TBRSZ= Tuber size, NTP = Number of tubers per plot, SD = Standard deviation, CV = Coefficient of variation. df = degree of freedom, NS = Non-significant, * = Significant at *p*-value < 0.05.

Table 5. 3. Mean variations in key agronomic and tuber quality traits of *D. praehensilis* and *D. rotundata* accessions

Species	Tuber yield (t ha^{-1}) ±SD	DMC (%) ±SD	YMV±SD	YAD±SD	TBOXI± SD	TBHard (N) ±SD	PLNV ±SD	TBRSZ± SD	NTP± SD
D. praehensilis	23.47± 18.53^{a}	32.83± 3.16^{a}	147.45± 31.00^{b}	267.78± 16.48^{a}	-10.36± 7.13^{a}	50.76± 1.15^{a}	2.43± 0.52^{a}	2.73± 0.61^{a}	1.89± 0.93^{a}
Min.	1.67	21.90	135.00	210.00	-35.30	39.60	1.00	1.00	1.00
Max.	123.00	41.96	270.00	315.00	4.43	53.55	3.00	3.00	5.50
D. rotundata	16.39± 10.23^{b}	34.00± 2.82^{a}	157.50± 40.70^{a}	260.00± 23.35^{b}	-6.47± 4.25^{a}	40.10± 1.42^{b}	1.92± 0.29^{b}	2.25± 0.45^{b}	1.71± 0.69^{a}
Min.	7.67	28.20	135.00	210.00	-13.08	39.00	1.00	2.00	1.00
Max.	44.34	37.14	270.00	270.00	-0.55	41.03	2.00	3.00	2.50
CV (%)	63.40	10.79	0.53	0.27	89.67	1.22	4.94	4.35	45.90

Means followed by the same superscripts are not significantly different using HSD test at $p<0.05$; SD: Standard deviation.
DMC = Dry matter content, YMV = Yam mosaic virus, YAD = Yam anthracnose disease, TBOXI = Tuber flesh oxidation, TBHard = Tuber flesh hardness, PLNV = Plant vigour, TBRSZ= Tuber size, NTP = Number of tubers per plot, SD = Standard deviation.

Genetic variability and broad-sense heritability of agronomic and tuber quality traits in *D. praehensilis* and *D. rotundata*

Phenotypic and genotypic variance components, phenotypic and genotypic coefficients of variation and broad-sense heritability of agronomic and tuber quality traits in *D. praehensilis* and *D. rotundata* accessions are presented in Table 5.4. Genotypic coefficients of variation (GCV) ranged from 5.8 to 66.3 % for tuber flesh hardness and tuber yield, respectively. Phenotypic coefficients of variation ranged from 4.8 to 93.5 % for YAD severity and tuber flesh oxidation, respectively. Broad-sense heritability (H^2) varied between 4.9 and 99.9 %. High H^2 (>60%) was observed in YMV severity, YAD severity, tuber flesh hardness, plant vigour and tuber size. Moderate H^2 (30–60 %) was observed in tuber yield and the number of tubers per plot, while low H^2 (<30%) was observed in dry matter content and tuber flesh oxidation.

Table 5. 4. Genetic variability and broad-sense heritability in *D. praehensilis* and *D. rotundata* accessions

	Genetic parameters				
Traits	δ^2_g	δ^2_p	GCV (%)	PCV (%)	H^2 (%)
Tuber yield (t ha^{-1})	229.6	435.4	66.3	91.3	52.7
DMC (%)	4.0	16.6	6.1	12.4	24.1
YMV	994.8	995.4	21.4	21.4	99.9
YAD	162.0	164.0	4.8	4.8	98.8
TBOXI	4.4	89.2	20.8	93.5	4.9
TBHard (N)	8.5	8.9	5.8	6.0	95.5
PLNV	0.3	0.3	21.9	22.6	93.3
TBRSZ	0.3	0.5	21.2	24.7	73.9
NTP	0.5	1.2	37.6	58.5	41.3

DMC = Dry matter content, YMV = Yam mosaic virus, YAD = Yam anthracnose disease, TBOXI = Tuber flesh oxidation, TBHard = Tuber flesh hardness, PLNV = Plant vigour, TBRSZ= Tuber size, NTP = Number of tubers per plot, δ^2_g = Genotypic variance, δ^2_p = Phenotypic variance, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, H^2b = Broad-sense heritability.

Principal component analysis of the key agronomic and tuber quality traits

The first three principal components accounted for 53.76% of the genotypic variations. The first principal component (PC1) accounted for 23.51% of the total variation and correlated positively with tuber yield, the number of tubers per plot, tuber size, plant vigour, tuber hardness, YAD severity and dry matter content, but it was negatively associated with tuber flesh browning/oxidation and YMV severity (Table 5.5; Figure 5.1). The accessions that contributed positively to the PC1 were: WNDpr76, WNDpr63, CDpr7, EDpr14, CDpr58, WNDpr15, CDpr28, CDpr11, WNDpr79 and EDpr13 (Figure 5.2). The traits that positively contributed to the second principal component (PC2) were tuber flesh oxidation, dry matter content and YAD severity, while YMV severity and tuber flesh hardness contributed negatively to PC2 (Figure 5.1). Accessions such as CDpr50, WNDpr89, Dente, Puna_Central, WNDpr4, Olodo-1, Durban, Dp_Asesewa_UP_E_001, Dp_UP_E_001, WNDpr1, CDpr81, Puna, CDpr23, CDpr1, CDpr54, WNDpr8, CDpr24, WNDpr59, WNDpr41, CDpr75, TDr_Durben, TDr_Mutwu, CDpr10, Dr_Kpanjol, WNDpr56, TDr_Nyaminti, TDr_Asana_North, CDpr85, TDr_Alata_Puna, WNDpr9, WNDpr10 and Dp_Asamankese_Assin_C_002 were positively associated with the PC2 (Figure 5.2). The variations at the third principal component (PC3) were positively associated with tuber flesh hardness, YAD severity and plant vigour, while YMV severity, tuber yield and number of tubers per plot had a negative contribution (Table 5.5).

Table 5. 5. Principal component analysis and contributions of agronomic and tuber quality traits on the variability

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Yield	0.0041	-0.8613	-0.5015	0.0667	-0.0207	0.0171	-0.0355	-0.0156	0.0015
DMC	-0.0162	-0.0257	0.0094	0.1242	0.8464	-0.5164	-0.0111	-0.0188	-0.0059
YMV	0.9992	-0.0096	0.0268	0.023	0.0104	-0.008	0.0018	-0.0002	0.0006
YAD	-0.0263	-0.5051	0.8598	-0.0626	0.0018	0.0295	0.0035	-0.0026	-0.0012
TBOXI	-0.0232	0.0268	0.0886	0.9768	-0.1813	-0.0611	0.0062	-0.0031	-0.0084
TBHard	0.0014	-0.0205	0.0073	-0.1467	-0.4997	-0.8516	0.0122	-0.0353	-0.0414
PLNV	-0.0011	-0.0049	-0.0001	0.0027	-0.0154	-0.0412	0.1198	0.0835	0.9883
TBRSZ	0.0001	-0.0128	-0.0029	0.0009	-0.003	-0.0371	-0.1019	0.9913	-0.073
NTP	-0.0015	-0.0301	-0.0219	-0.0006	0.0176	0.0068	0.9868	0.092	-0.1269
Eigenvalue	2.115	1.516	1.207	0.994	0.876	0.765	0.712	0.557	0.258
Variance (%)	23.505	16.843	13.412	11.040	9.736	8.504	7.913	6.184	2.864
Cumulative (%)	23.505	40.348	53.760	64.800	74.535	83.040	90.952	97.136	100.000

DMC = Dry matter content, YMV = Yam mosaic virus, YAD = Yam anthracnose disease, TBOXI = Tuber flesh oxidation, TBHard = Tuber flesh hardness, PLNV = Plant vigour, TBRSZ= Tuber size, NTP = Number of tubers per plot

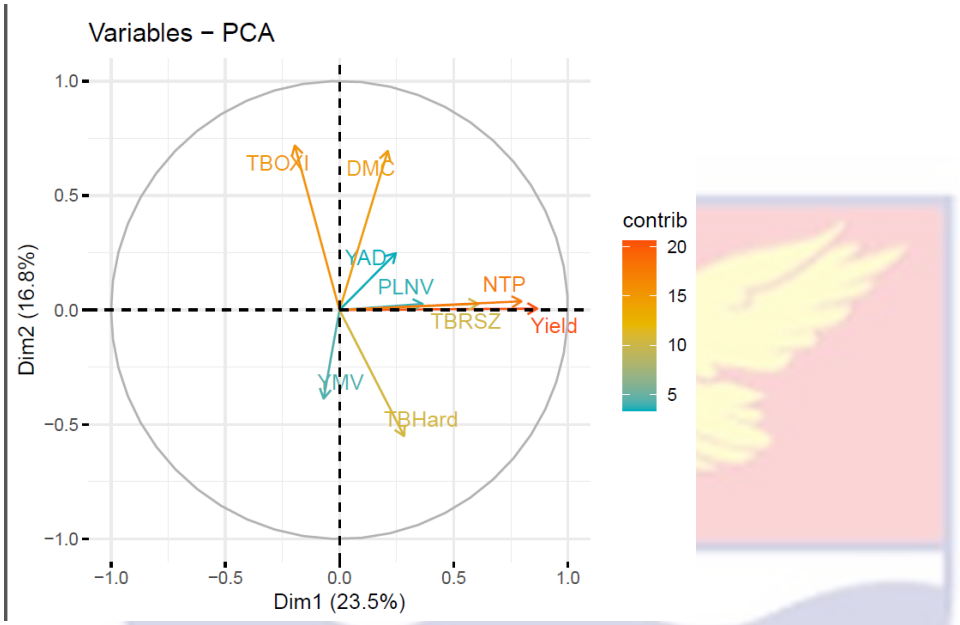

Figure 5. 1. Contributions of agronomic and tuber quality traits to PC1 and PC2.

DMC = Dry matter content, YMV = Yam mosaic virus, YAD = Yam anthracnose disease, TBOXI = Tuber flesh oxidation, TBHard = Tuber flesh hardness, PLNV = Plant vigour, TBRSZ= Tuber size, NTP = Number of tubers per plot. Dim1 = PC1; Dim2 = PC2

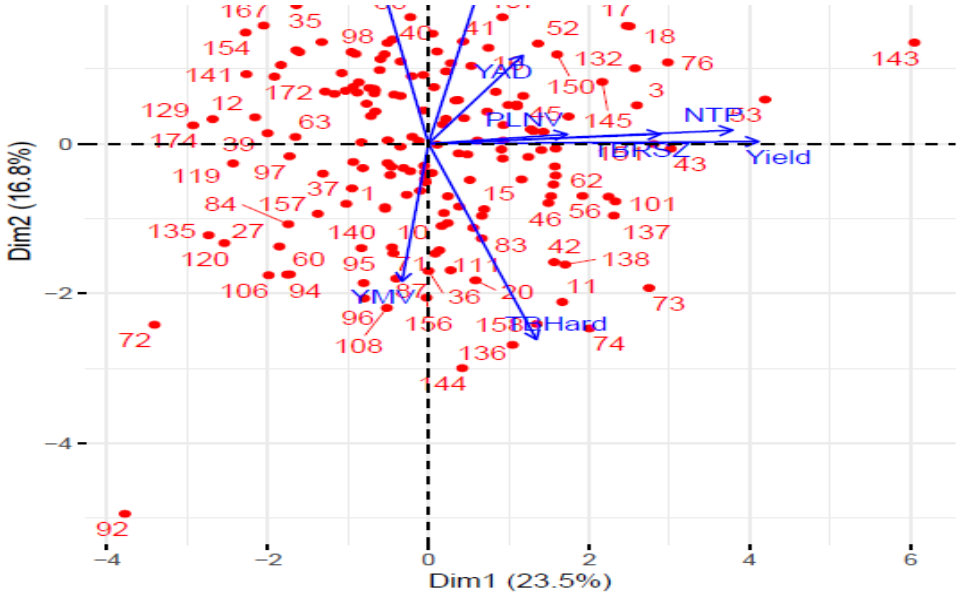

Figure 5. 2. PCA-Biplot of key agronomic and tuber quality traits for 174 accessions of *D. praehensilis* and *D. rotundata*

DMC = Dry matter content, YMV = Yam mosaic virus, YAD = Yam anthracnose disease, TBOXI = Tuber flesh oxidation, TBHard = Tuber flesh hardness, PLNV = Plant vigour, TBRSZ= Tuber size, NTP = Number of tubers per plot

Relationships among agronomic and tuber quality traits

We observed significant correlations among evaluated traits (Figure 5.3). Tuber yield (t ha^{-1}) had significant positive correlations with tuber size (r = 0.38; $p < 0.001$), number of tubers per plot (r = 0.72; $p < 0.001$) and plant vigour (r = 0.16; $p < 0.05$). However, the tuber yield showed significant negative relationship with tuber flesh oxidation (r = -0.13; $p < 0.05$). Tuber yield showed positive but not significant relationship with the dry matter content (r = 0.12) and the YAD severity (r = 0.11). Dry matter content had significant negative correlation with YMV severity (r = -0.16; $p < 0.05$) and tuber flesh hardness (r = -0.17; $p < 0.05$),

but showed significant positive correlation (r = 0.23; $p < 0.01$) with tuber flesh oxidation.

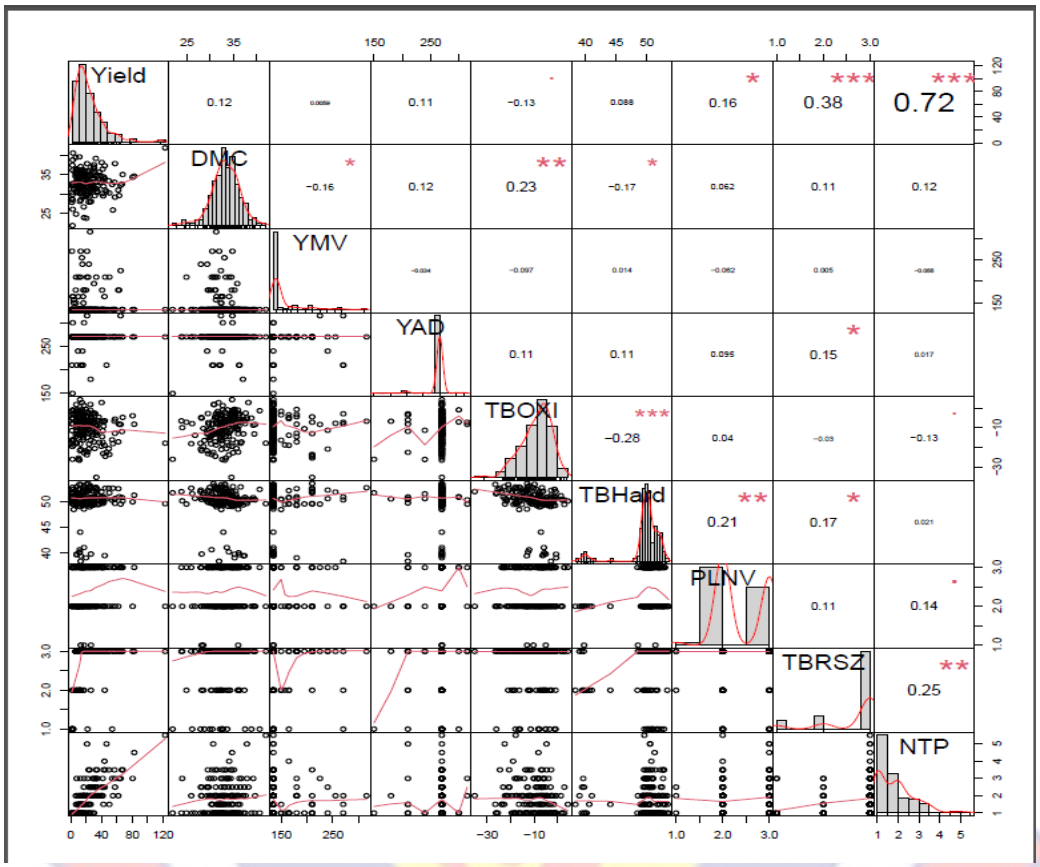

Figure 5. 3. Correlation coefficients among agronomic and tuber quality traits.

DMC = Dry matter content, YMV = Yam mosaic virus, YAD = Yam anthracnose disease, TBOXI = Tuber flesh oxidation, TBHard = Tuber flesh hardness, PLNV = Plant vigour, TBRSZ= Tuber size, NTP = Number of tubers per plot. Significance level: "$p < 0.05$" = *; "$p < 0.01$" = **; "$p < 0.001$" = ***.

Hierarchical clustering on principal components of *D. praehensilis* and *D. rotundata* accessions

Hierarchical clustering based on agronomic and quality traits performance grouped the accessions of *D. praehensilis* and *D. rotundata* into three groups (Figure 5.4). Cluster 3 had the highest number of accessions (79), while cluster 1 had the lowest number (39). Hierarchical clustering revealed significant variation

in the distribution of *D. praehensilis* and *D. rotundata* accessions among the clusters (Table 5.6). Cluster 1 comprised of accessions of *D. praehensilis* and *D. rotundata* that possessed low tuber yield (12.19 t ha^{-1}), high dry matter content (33.14%), highly susceptible to YMV, high YAD resistance, moderate tuber flesh oxidation, minimal tuber flesh hardness and moderate plant vigour, a low number of tubers per plot and small tuber size (Table 5.6; Figure 5.4). Among members of cluster 1, we had: Puna, Nyaminti, Puna_Central, Olodo-1, Asana, Dente, Alata_Puna, Nyamint, Durben, Mutwu and CDpr54 as the accessions with low tuber flesh hardness and CDpr50, WNDpr50, WNDpr74, Dente, Puna, WNDpr4, Olodo, WNDpr10, WNDpr9 and Alata-Puna as the accessions with high dry matter content. The Cluster 2 consisted of *D. praehensilis* accessions characterized by high tuber yield (30.91 t ha^{-1}), high dry matter content (33.81%), high resistance to YMV, moderate resistance to YAD, low tuber flesh oxidation, high tuber flesh hardness, high plant vigour, large tuber size and high number of tubers per plot (Table 4.6; Fig. 4.4). Among cluster 2 members, we had: WNDpr76, CDpr28, CDpr7, WNDpr63, EDpr14, CDpr58, WNDpr15, CDpr11 and WNDpr79 as superior accessions with high yielding ability, WNDpr76, CDpr7, WNDpr63, EDpr14, CDpr58, CDpr28, CDpr11, WNDpr79, EDpr13 and WNDpr10 as the accessions with high resistance to YMV, WNDpr76, WNDpr88, CDpr28, CDpr29, WNDpr24, CDpr6, WNDpr84, CDpr48, WNDpr36, CDpr34 and CDpr5 as the top accessions with high dry matter content, WNDpr87, WNDpr36, WNDpr31, WNDpr94, WNDpr21, WNDpr40, WNDpr76, CDpr5, CDpr6 and WNDpr34 were the top selected accessions for low tuber flesh

oxidation and WNDpr76, CDpr29, CDpr7, CDpr73, CDpr58, CDpr79, CDpr11, EDpr14, CDpr68 and EDpr6 were the accessions with high number of tubers per plot. Cluster 3 contained *D. praehensilis* accessions that were characterized by low or no tuber flesh oxidation, high tuber flesh hardness, high susceptibility to YMV and YAD, moderate tuber yield, large tuber size, moderate plant vigour and number of tubers per plot and moderate dry matter content (Table 5.5, Figure 5.4). Of the members of this group, we had: WNDpr68, Cdpr51, Otim, WNDpr29, EDpr1, CDpr33, WNDpr93, WNDpr7, WNDpr49 and WNDpr19 as top accessions with low or no tuber flesh oxidation.

Table 5. 6. Description of clusters of *D. praehensilis* and *D. rotundata* accessions

Traits	Cluster 1±SD (39)	Cluster 2±SD (56)	Cluster 3±SD (79)	*F*-value
Tuber yield (t ha^{-1})	12.19±12.70^{c}	**30.91±22.14^{a}**	22.69±14.20^{b}	14.11***
Dry matter content (%)	**33.14±3.67^{ab}**	**33.81±2.60^{a}**	32.17±3.08^{b}	4.76**
Yam mosaic virus	145.77±31.15^{a}	**137.68±10.36^{b}**	155.60±39.03^{a}	5.66*
Yam anthracnose disease	**256.15±31.42^{b}**	270.54±4.01^{a}	270.38±8.65^{a}	11.94***
Tuber flesh oxidation	-8.17±6.04^{b}	**-9.43±6.59^{ab}**	**-11.52±7.56^{a}**	3.42*
Tuber flesh hardness (N)	**47.44±5.07^{b}**	50.47±0.95^{a}	50.98±1.31^{a}	25.30***
Plant vigour	2.23±0.48^{b}	**2.96±0.19^{a}**	2.09±0.36^{c}	107.15***
Tuber size	1.79±0.73^{b}	**2.96±0.19^{a}**	2.95±0.22^{a}	133.64***
Number of tubers per plot	1.55±0.86^{b}	**2.25±1.03^{a}**	1.77±0.78^{b}	8.15***

Significance level: "$p < 0.05$" = *; "$p < 0.01$" = **; "$p < 0.001$" = ***. Means followed by the same superscripts are not significantly different using

the least significant difference (LSD) test at 5% *p*-value threshold; SD: Standard deviation.

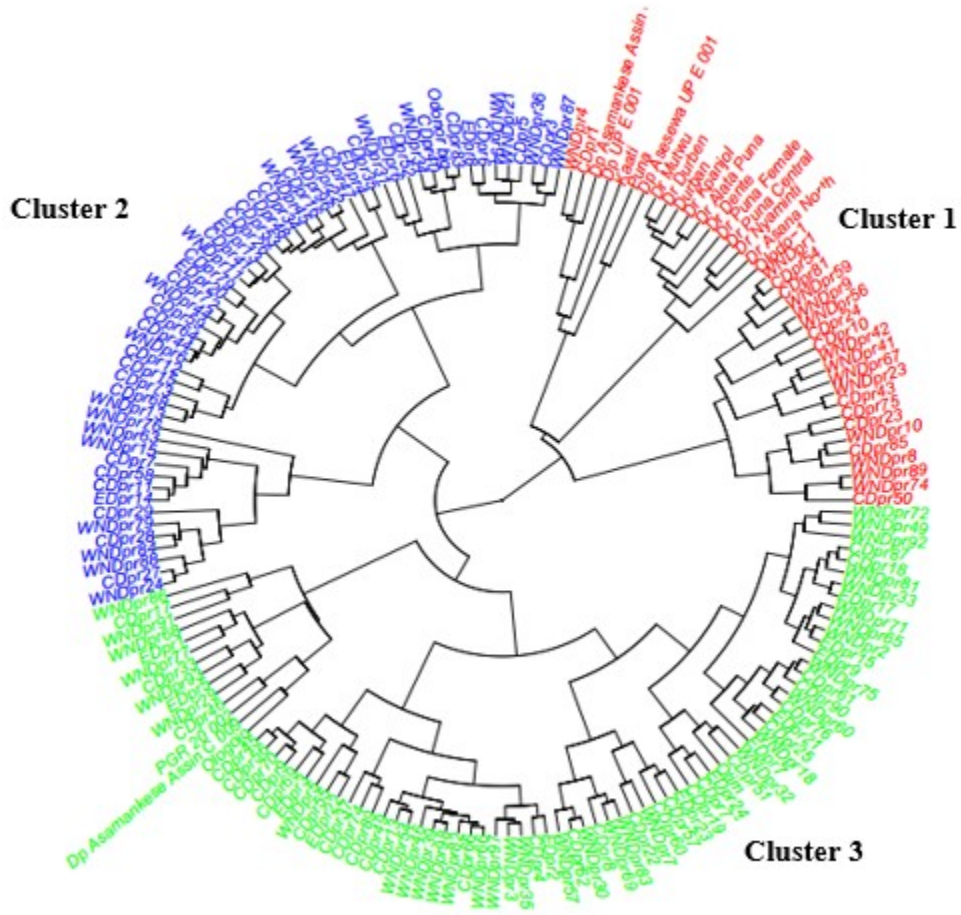

Figure 5. 4. Hierarchical dendrogram showing grouping patterns of *D. praehensilis* and *D. rotundata* accessions using nine key agronomic and tuber quality traits based on the Gower dissimilarity matrix.

Path coefficient analysis among assessed traits of *D. praehensilis* and *D. rotundata*

The number of tubers per plot and tuber size had high positive (r = 0.67) and moderate positive (r = 0.21) direct path effects, respectively, on tuber yield per hectare (Figure 5.5). Dry matter content (r = 0.09), YAD severity (r = 0.08), plant vigour (r = 0.04) and YMV severity (r = 0.03) recorded low but positive direct path effects on tuber yield. The tuber flesh oxidation had low and negative direct

path effect (r = -0.03) on tuber yield (Figure 5.5). In addition, tuber yield (r = 0.24) and tuber flesh oxidation (r = 0.22) had positive moderate path effects on dry matter content, while tuber flesh hardness had low negative direct path effect (r = -0.13) on dry matter content (Figure 5.5).

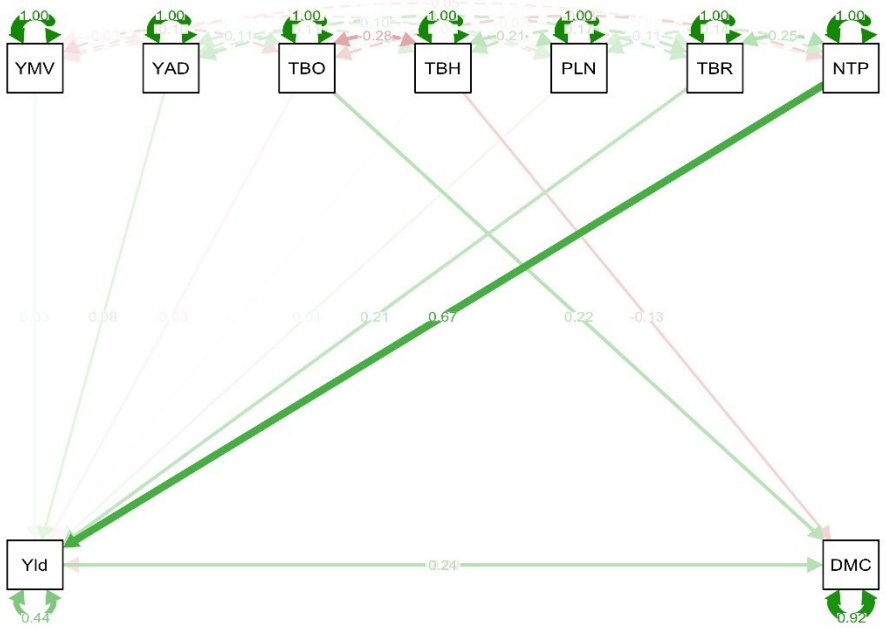

Figure 5. 5. Path coefficient analysis between response and independent yam variables.

DMC = Dry matter content, YMV = Yam mosaic virus, YAD = Yam anthracnose disease, TBOXI = Tuber flesh oxidation, TBHard = Tuber flesh hardness, PLNV = Plant vigour, TBRSZ= Tuber size, NTP = Number of tubers per plot.

Discussion

Variability in key agronomic and tuber quality traits and potential of *D. praehensilis* as a source of genes for *D. rotundata* breeding

White yam production has been constrained by pests and diseases and poor postharvest tuber quality. Unfortunately, breeding populations had shown a narrow genetic base for those traits. Identifying new sources of genes for high

yield potential, disease and pest resistance and good post-harvest tuber quality traits is a prerequisite to developing varieties acceptable by farmers, consumers and other end-users. Compared to the cultivated yam varieties, little information is available on the genetic potential of wild yam relatives (Padhan, Mukherjee, Mohanty, Lenka, & Panda, 2019). The high CVs observed for several traits, including tuber yield, YMV, tuber flesh oxidation and the number of tubers per plant, are indicative of the impact of the environment on these traits. Anokye et al. (2014) also recorded high CVs for yield traits among Ghanaian water yam (*D. alata*). Such wide range in trait values and attendant high CVs could serve as a basis for selection in breeding programmes.

The findings from this study revealed the existence of a vast genetic variation in the assessed agronomic and tuber quality traits between *D. praehensilis* and *D. rotundata*. High tuber yield observed for *D. praehensilis* compared to *D. rotundata* indicates that *D. praehensilis* could be used to improve the yield potential of the white Guinea yam. Currently, the white Guinea yam yield is ~20% of its attainable yield (40 t ha^{-1}) (Bassey & Akpan, 2015; FAOSTAT, 2021) and the bush yam could be explored in bridging this yield gap. The high yield of *D. praehensilis* in this study corroborates the findings by Pitalounani et al. (2017) and Adewumi et al. (2021), who reported high yields after a participatory rural appraisal survey in Togo and Ghana, respectively. Wild yam relatives have also been reported to produce higher yields when compared to the cultivated varieties (Padhan et al., 2019). The wide range recorded in the agronomic and tuber quality traits are indicative that these traits provide an opportunity for the selection of

superior accessions that can be used for hybridization in yam breeding programmes (Kouam et al., 2018).

From this study, *D. praehensilis* showed more resistance to the YMV when compared with *D. rotundata*. This agrees with the study outcomes of Ayisah, Mawussi, Tchaniley and Aziadekey (2020), who reported high resistance in *D. praehensilis* in Togo. The high resistance to YMV suggests the existence of resistance genes in the genetic resources of *D. praehensilis* (Ayisah et al., 2020) which can be exploited in *D. rotundata* breeding.

Tuber quality traits are also important traits in the selection and breeding of superior yam varieties (Padhan & Pandan, 2018). The *D. rotundata* local varieties showed better performance in tuber quality attributes (dry matter content, tuber flesh oxidation and tuber hardness) than the *D. praehensilis* accessions. However, some *D. praehensilis* accessions also had comparable tuber quality attributes. The poor tuber quality attributes of *D. praehensilis* have been reported as the major hindrance associated with its disappearance (genetic erosion) from agrosystems in Ghana (Adewumi et al., 2021). White yam breeders should, therefore, look for alternative sources of genes for these quality traits.

Genetic parameters and broad-sense heritability of assessed traits

High GCV and PCV (>20%) were observed in some of the evaluated traits, such as tuber yield, YMV, tuber flesh oxidation, plant vigour, tuber size and number of tubers per plant. This indicates high selection intensity, which can be imposed on these critical traits of superior accessions in future yam breeding programmes. High GCV and PCV recorded for tuber yield agreed with the

previous results obtained in the research conducted on the advanced breeding population of white yam (Norman et al., 2021). High H^2b (>60%) recorded in this study for traits such as YMV, YAD, tuber flesh hardness, and plant vigour indicates a high correspondence between phenotypic and genotypic variance and hence, increased response to selection. Our results are in agreement with the finding of Bhattacharjee et al. (Bhattacharjee et al., 2018) and Agre et al. (2021a), who reported high broad-sense heritability for YAD in water (*D. alata*) yam and YMV in white yam, respectively.

Correlation coefficients, principal components, path coefficients, and hierarchical clusters among assessed traits of *D. rotundata* and *D. praehensilis*

Accessions with high dry matter content, large tuber size, and a high number of tubers per plant could be selected for when breeding for improved yield. This was exemplified in the positive correlations between tuber yield and dry matter content, large tuber size and the high number of tubers per plot (Figure 5.3). This corroborates the finding of Agre et al. (2019), who found a positive correlation between total tuber weight, tuber shape and the number of tubers per plant in a panel of water yam. The negative correlation between tuber yield and tuber flesh oxidation suggests that the selection for accessions with high tuber yield could simultaneously reduce enzymatic flesh oxidation. In the present study, no significant correlation was observed between tuber yield and the severity of the two major yam diseases (YMV and YAD).

From our correlation analysis, the positive correlation between dry matter content and tuber flesh oxidation indicates that selection for accessions with high

dry matter content will not be affected by increased tuber flesh oxidation. Desirable significant negative correlation observed between dry matter content and YMV severity suggests that the selection of high dry matter content cultivars could reduce the severity of YMV or alternatively, any YMV control measure will help improve yam dry matter content. Weak association of YMV severity with other evaluated traits has also been reported by Asfaw et al. (2021) in a study on early generations of the breeding population of white yam.

The key agronomic and tuber quality traits that best discriminated the 174 accessions of *D. praehensilis* and *D. rotundata* were those resolved on PC1. These traits, including tuber yield, number of tubers per plant, tuber size, plant vigour, tuber hardness, YAD severity, dry matter content, and tuber oxidation, could be utilized in evaluating genetic diversity among related *Dioscorea* spp. Agre et al. (2019; 2021b) and Siadjeu, Toukam, Bell and Nkwate (2015) have reported these traits' significant contribution in discriminating yam accessions.

The direct path effects of some of these traits on tuber yield could be utilized for indirect selection in yam breeding programmes to enhance the genetic gain in white Guinea yam. Tewodros, Firew, Shimelis and Endale (2020) reported significant direct path coefficients between dry matter content and tuber weight in a study conducted on Ethiopian yam accessions.

The hierarchical clustering in this study revealed similarities among accessions that were grouped in the same cluster. Scarcelli et al. (2019), who had argued that *D. praehensilis* was the most likely ancestor of white Guinea yam, this is supported by the clustering of *D. praehensilis* and *D. rotundata* accessions

in cluster 1 (Fig. 5.4). From our hierarchical clustering, *D. praehensilis* accessions showed outstanding performance for attributes, such as tuber yield, resistance to YMV, tuber size, plant vigour, tuber flesh oxidation and number of tubers per plant, while *D. rotundata* landraces were best for attributes like tuber flesh hardness and resistance to YAD. Crosses between promising accessions of *D. praehensilis* and *D. rotundata* using *D. rotundata* accessions as female parents could result in the development of improved cultivars of white Guinea yam with outstanding performance in important traits like tuber yield, resistance to yam mosaic virus, and some post-harvest tuber quality attributes.

Conclusions

This study explored 162 accessions of *D. praehensilis* and 12 landraces of *D. rotundata* to identify new sources for key agronomic and tuber quality traits to improve white Guinea yam by broadening its genetic base. We observed wide variations between the two yam species in terms of tuber yield, dry matter content, resistance to YMV and YAD, tuber flesh hardness, plant vigour, number of tubers per plant, and tuber size. We also observed significant relationships among some traits, which can be useful for indirect selection. Cluster analysis revealed three groups with contrasting characteristics. This study identified some accessions of *D. praehensilis* with outstanding tuber yield, resistance to YMV, dry matter content, tuber flesh oxidation, tuber size, number of tubers per plant and plant vigour. These accessions could be explored in breeding programmes to improve white Guinea yam for those traits. Further characterization of *D. praehensilis* germplasm is required with high throughput molecular markers to

refine parental selection before designing cross-combinations. Combined assessment of these germplasm collections using descriptor keys and molecular markers would provide more insight into the genetic diversity of *D. praehensilis* accessions and their effective use as a source of genes to improve white Guinea yam.

CHAPTER SIX

EFFECTIVENESS OF MORPHOLOGICAL DESCRIPTORS IN DISCRIMINATING AMONG BUSH YAM (Dioscorea praehensilis Benth.) ACCESSIONS

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Abstract

Bush yam (*Dioscorea praehensilis* Benth.) is a semi-domesticated yam species and a valuable source of yield, adaptation and resistance trait genes. However, it is at risk of extinction due to inadequate research (on its genetic diversity and conservation) and poor post-harvest tuber quality characteristics. This study utilised 15 quantitative and 24 qualitative traits to differentiate among 162 accessions of *D. praehensilis*. Assessed qualitative traits showed significant variations among the bush yam accessions. Shannon diversity and evenness indices revealed high genetic diversity for most qualitative traits. Analysis of variance (ANOVA) revealed significant ($p < 0.05$) differences across the growing seasons. The 2020 season recorded superior accessions for yam mosaic virus severity (AUDPC = 147.45), tuber yield (23.47 tons/ha) and tuber yield per plant (2.04 kg/plant), while 2021 season recorded superior accessions for dry matter content (35.19%) and tuber flesh oxidation (-16.32). Significant variation ($p < 0.05$) was also observed among the accessions for all the evaluated quantitative traits. High broad-sense heritability values (>60%) were observed for most of the measured quantitative traits except yam mosaic severity response (30-60%). Cluster analysis identified three clusters, with Cluster 3 having superior accessions for tuber yield-related traits. In contrast, Cluster 1 contained the best performing accessions for resistance to yam mosaic virus (YMV) severity. These accessions could be utilised in the yam breeding scheme to improve the bush yam genetic base.

Keywords: *D. praehensilis*, bush yam, genetic parameters, quantitative traits, qualitative traits

Introduction

Yam *(Dioscorea* spp.) is a popular tropical and subtropical food crop. It is widely grown in West Africa, contributing significantly to food security and poverty reduction (Asiedu & Sartie, 2010; Alabi et al., 2019). West Africa accounts for more than 95% of global yam output, with Nigeria, Ghana, Côte d'Ivoire and Benin leading (FAOSTAT, 2021). Yam is an essential element of people's socio-cultural, economic and religious lives in West Africa, providing carbohydrates, proteins, minerals, vitamins and lipids to the human diet (Obidiegwu et al., 2020).

The genius *Dioscorea* consists of ~600 species of which the African yam complex, *Dioscorea cayenensis - Dioscorea rotundata,* is the most cultivated (Couto et al., 2018). According to Tamiru et al. (2008), the species forming this complex have emerged from a long domestication process of wild yams, mainly *D. praehensilis*, *D. burkilliana* and *D. abyssinica*. Genetic studies further support this hypothesis (Mignouna & Dansi, 2003; Chaïr et al., 2005; Scarcelli et al., 2019; Sugihara et al., 2020).

Dioscorea praehensilis is an edible semi-cultivated wild yam species that is mostly used to ease food insecurity among local farmers in the proximity of forest zones in West African nations, such as Nigeria, Ghana, Benin and Togo during lean seasons (Pitalounami et al., 2017; Adewumi et al., 2021). *D. praehensilis* has a high yield potential, insect pest and disease tolerance, in-soil

storage ability and the capacity to blossom and fruit profusely, making it ideal for hybridization (Adewumi et al., 2021).

Despite these significances, the economic values of *D. preahensilis* have not been fully realized due to the oxidative browning and hardening of tuber flesh a few days after harvesting, resulting in poor utilization and under-exploitation of its potential. As a result, there are no improved varieties for commercialization. The germplasm collection and estimation of morphological divergence in *D. praehensilis* compared with widely cultivated and utilized yam species, such as *D. rotundata* and *D. alata*, are partial and not comprehensive, especially in Ghana. These factors have resulted in rapid genetic erosion and the risk of extinction of this valuable yam species (Adewumi et al., 2021). The information on genetic structure, diversity, tuber culinary quality traits, cropping system, agronomic practices and production statistics of *D. praehensilis* is highly deficient. Assessing its genetic divergence is paramount for conservation, selection and breeding purposes. To avoid genetic erosion and improve *D. praehensilis*, there is a need to unlock the genetic potential of bush yam genetic resources for identifying bush yam accessions with high yielding attributes, tolerant to yam mosaic virus and superior post-harvest tuber qualities that farmers and consumers prefer. This can be achieved through the implementation of efficient germplasm collection from different regions and its conservation and diversity analysis.

The first step in germplasm assessment is to detect the desirable traits of interest using morphological descriptors. In studying genetic variation in plants,

agro-morphological traits have been extensively used (Govindaraj et al., 2015). Morphological markers involved the visual assessment of traits, such as flower sex, tuber shape, leaf shape, leaf colour, growth habits and tuber colour. Morphological characterization is affordable compared with molecular assessment (Govindaraj et al., 2015). These marker traits are frequently prone to phenotypic plasticity; on the other hand, this encourages the evaluation of genetic divergence in the presence of environmental variation, which cannot be ignored when genotypic variation is taken into account. These markers are very important, and they are required to separate adult plants from genetic contamination in the field, such as flower and leaf colour variations (Govindaraj et al., 2015). Morphological markers are significant in describing and establishing links among cultivated crop varieties and accessions that are not genetically profiled (Plazas et al., 2014). Morphological characterization also aids in selecting desirable cultivars through the estimation of the heritability of the measured traits. Heritability affects the size of the selection method, making it a useful tool for improving a specific characteristic and predicting the genetic gain from selection, and measuring the comparative effect of genes (Umar, Ado, Aba, & Bugaje, 2014).

Several comprehensive research studies have been carried out on *Dioscorea* spp using morphological descriptors (Norman, Tongoona, & Shanahan, 2011; Efisue, 2016; Oben, Egbe, Chuyong, & Tabot, 2016; Sheikh & Kumar, 2017). On individual yam species, many authors (Anokye et al., 2014; Girma et al., 2017; Agre et al., 2019; Patel, Desai, & Ahlawat, 2019) reported the application of morphological markers in assessing genetic diversity in *D. alata*.

Also, in Guinea yam (*D. rotundata and D. cayenensis*), morphological traits have been employed in evaluating genetic variability (Silva et al., 2017; Darkwa et al., 2020). The application of morphological descriptors has also been employed in *D. bulbifera* (Kouam et al., 2018). Also, genetic diversity among 140 accessions of *Dioscorea trifidia* was conducted in the Municipality of Caapiranga, in the Central Amazon Region of Brazil, using 64 morphological descriptors (Beyerlein & Pereira, 2018). However, little information is available in the application of morphological traits to evaluate the genetic diversity of *D. praehensilis*. Hence, a comprehensive analysis of the phenotypic diversity of *D. praehensilis* germplasm indigenous to Ghana may be critical for identifying and developing *D. praehensilis* with economically valuable traits and for the conservation and utilization of the gerplasm.

The objectives of the present study were to (i) assess genetic diversity and differentiation in qualitative and quantitative traits among *Dioscorea praehensilis* accessions in Ghana, (ii) assess variance components, heritability and clustering pattern of *D. praehensilis* based on quantitative traits and (iii) identify desirable phenotypic groups for future bush yam improvement. The results from this study should facilitate *D. praehensilis* germplasm collection, conservation and selection of desirable accessions for future breeding and improvement programmes.

Materials and Methods

Experimental site

The study was conducted at the Teaching and Research Farm of the School of Agriculture, the University of Cape Coast, Ghana (5°07´7.6´´N, 1°17

´18.9´´W; 15 m above sea level), located in the Central Region with semi-deciduous forest and coastal savannah ecozones. The trial was conducted under field conditions during the 2020 and 2021 growing seasons. The annual rainfall for the experiment period was 1,246.2 mm for the 2020 season and 1,170.2 mm for the 2021 season; the average maximum and minimum temperatures for the 2020 season were 27.9 and 26.9° C, while that for the 2021 season were 28.6 and 25° C, respectively. The average relative humidity values for the 2020 and 2021 seasons were 75.7 %, and 81.2 %, respectively. The soil on this experimental site is sandy loam with a pH of 6.72, organic carbon (1.31%), available phosphorus (754.6 ug/g) and potassium (0.081 cmol/kg).

Plant materials and experimental design

Planting materials from 162 accessions of *D. praehensilis* comprising 71 accessions collected from the Central Region, 25 from the Eastern Region, and 66 from the Western North Region (Figure 6.1) were used. These accessions were collected from bush yam farmers during a germplasm collection survey conducted in the 2019 harvest season. Details including accession codes and regions of the collection, are presented in Table 5.1. The experiment was laid out using a simple lattice design in two replicates. The field layout was generated using R software's "Agricolae" package (R Development Core Team, 2019). Each replicate comprised 17 incomplete blocks with 10 experimental units as the block size. In each replicate, the experimental units comprised 3 m long ridges containing three plants with 1m intra- and inter-row spacing. The recommended cultural practices, such as weeding, were implemented during the growing seasons.

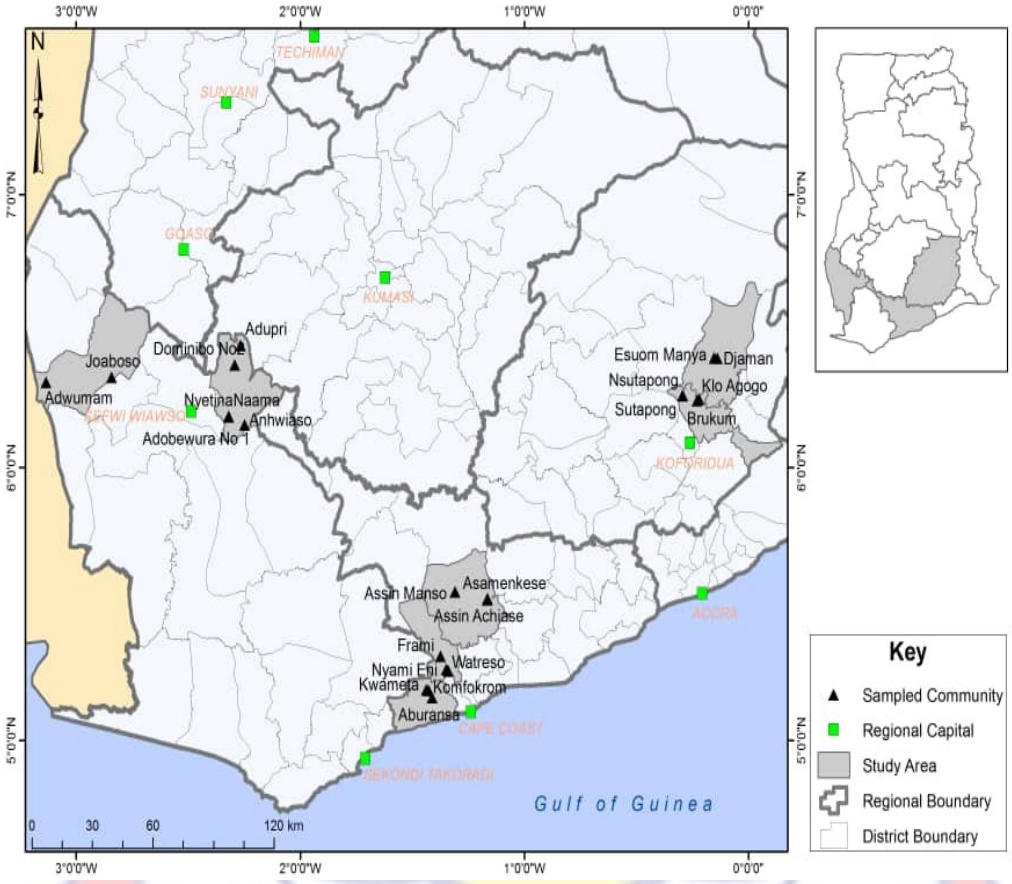


Figure 6. 1. Map showing the locations of collection of *D. praehensilis* accessions

Source: Germplasm Collection (2019)

Table 6. 1. *Dioscorea praehensilis* accessions used in the study and respective source of collection

Accession No	Source	District	Region
CDpr66	Achiase	Assin south	Central
CDpr68	Achiase	Assin south	Central
CDpr69	Achiase	Assin south	Central
CDpr70	Achiase	Assin south	Central
CDpr72	Achiase	Assin south	Central
CDpr73	Achiase	Assin south	Central
CDpr74	Achiase	Assin south	Central
CDpr75	Achiase	Assin south	Central
CDpr76	Achiase	Assin south	Central
CDpr77	Achiase	Assin south	Central
CDpr79	Achiase	Assin south	Central
CDpr81	Achiase	Assin south	Central
CDpr56	Asamankese	Assin south	Central
CDpr57	Asamankese	Assin south	Central
CDpr58	Asamankese	Assin south	Central
CDpr59	Asamankese	Assin south	Central
CDpr60	Asamankese	Assin south	Central
CDpr61	Asamankese	Assin south	Central
CDpr62	Asamankese	Assin south	Central
CDpr64	Asamankese	Assin south	Central
CDpr17	Frami	THLD	Central
CDpr18	Frami	THLD	Central
CDpr19	Frami	THLD	Central
CDpr22	Frami	THLD	Central
CDpr23	Frami	THLD	Central
CDpr24	Frami	THLD	Central
CDpr25	Frami	THLD	Central
CDpr26	Frami	THLD	Central
CDpr27	Frami	THLD	Central
CDpr28	Frami	THLD	Central
CDpr29	Frami	THLD	Central
CDpr33	Frami	THLD	Central
CDpr43	Konfokrom	KEEA	Central
CDpr44	Konfokrom	KEEA	Central
CDpr45	Konfokrom	KEEA	Central
CDpr46	Konfokrom	KEEA	Central

Table 6. 1. Contiuned

CDpr47	Konfokrom	KEEA	Central
CDpr48	Konfokrom	KEEA	Central
[illegible]	Konfokrom	KEEA	Central
[illegible]	Konfokrom	KEEA	Central
CDpr51	Konfokrom	KEEA	Central
CDpr52	Konfokrom	KEEA	Central
CDpr53	Konfokrom	KEEA	Central
CDpr54	Konfokrom	KEEA	Central
CDpr55	Konfokrom	KEEA	Central
CDpr34	Konfokrom	KEEA	Central
CDpr35	Konfokrom	KEEA	Central
CDpr37	Konfokrom	KEEA	Central
CDpr40	Konfokrom	KEEA	Central
CDpr41	Konfokrom	KEEA	Central
CDpr83	Manso	Assin south	Central
CDpr85	Manso	Assin south	Central
CDpr87	Manso	Assin south	Central
CDpr89	Manso	Assin south	Central
CDpr90	Manso	Assin south	Central
CDpr1	Nyame Eni	THLD	Central
CDpr3	Nyame Eni	THLD	Central
CDpr4	Watreso	THLD	Central
CDpr5	Watreso	THLD	Central
CDpr6	Watreso	THLD	Central
CDpr7	Watreso	THLD	Central
CDpr8	Watreso	THLD	Central
CDpr9	Watreso	THLD	Central
CDpr10	Watreso	THLD	Central
CDpr11	Watreso	THLD	Central
CDpr12	Watreso	THLD	Central
CDpr13	Watreso	THLD	Central
CDpr15	Watreso	THLD	Central
CDpr16	Watreso	THLD	Central
Dp/Asamankese/Assin/C/002	Asamankese	Assin south	Central
Dp/Asamankese/Assin/C/009	Asamankese	Assin south	Central
EDpr13	Asesewa	Upper Manya Krobo	Eastern
EDpr14	Asesewa	Upper Manya Krobo	Eastern
EDpr15	Asesewa	Upper Manya Krobo	Eastern

Table 6. 1. Contiuned

	Brukum	YiloKrobo	Eastern
	Brukum	YiloKrobo	Eastern
EDpr23	Brukum	YiloKrobo	Eastern
EDpr24	Brukum	YiloKrobo	Eastern
EDpr1	Djaman	Upper Manya Krobo	Eastern
EDpr2	Djaman	Upper Manya Krobo	Eastern
EDpr3	Djaman	Upper Manya Krobo	Eastern
EDpr4	Djaman	Upper Manya Krobo	Eastern
EDpr5	Djaman	Upper Manya Krobo	Eastern
EDpr6	Djaman	Upper Manya Krobo	Eastern
EDpr7	Djaman	Upper Manya Krobo	Eastern
EDpr8	Esuom Manya	Upper Manya Krobo	Eastern
EDpr9	Esuom Manya	Upper Manya Krobo	Eastern
EDpr17	Nsutapong	Upper Manya Krobo	Eastern
EDpr20	Nsutapong	Upper Manya Krobo	Eastern
PGR/20/002	SARI-Plant Genetics	Bunso	Eastern
Dp/Asesewa/UP/E/001	Asesewa	Upper Manya Krobo	Eastern
Dp/UP/E/001	SARI-Plant Genetics	Bunso	Eastern
Odonor big	Djaman	Upper Manya Krobo	Eastern
Kaati	Djaman	Upper Manya Krobo	Eastern

Table 6. 1. Contiuned

Otim	Djaman	Upper Manya Krobo	Eastern
Ologojo		Upper Manya Krobo	
	Djaman		Eastern
WNDpr56	Adobawura	Bekwai	Western North
WNDpr57	Adobawura	Bekwai	Western North
WNDpr59	Adobawura	Bekwai	Western North
WNDpr60	Adobawura	Bekwai	Western North
WNDpr63	Adobawura	Bekwai	Western North
WNDpr65	Adobawura	Bekwai	Western North
WNDpr66	Adobawura	Bekwai	Western North
WNDpr67	Adobawura	Bekwai	Western North
WNDpr68	Adobawura	Bekwai	Western North
WNDpr6	Anhwiaso	Bibiani	Western North
WNDpr7	Anhwiaso	Bibiani	Western North
WNDpr8	Anhwiaso	Bibiani	Western North
WNDpr9	Anhwiaso	Bibiani	Western North
WNDpr10	Anhwiaso	Bibiani	Western North
WNDpr11	Anhwiaso	Bibiani	Western North
WNDpr13	Anhwiaso	Bibiani	Western North
WNDpr15	Anhwiaso	Bibiani	Western North
WNDpr18	Anhwiaso	Bibiani	Western North
WNDpr21	Anhwiaso	Bibiani	Western North
WNDpr22	Anhwiaso	Bibiani	Western North
WNDpr23	Anhwiaso	Bibiani	Western North
WNDpr24	Anhwiaso	Bibiani	North
WNDpr1	Dominibo	Bekwai	Western

Table 6. 1. Contiuned

WNDpr2	Dominibo	Bekwai	North Western North
WNDpr3	Dominibo	Bekwai	Western North Western
WNDpr4	Dominibo	Bekwai	North Western
WNDpr5	Dominibo	Bekwai	North Western
WNDpr29	Adupri	Bibiani	North Western
WNDpr30	Adupri	Bibiani	North Western
WNDpr31	Adupri	Bibiani	North Western
WNDpr32	Adupri	Bibiani	North Western
WNDpr33	Adupri	Bibiani	North Western
WNDpr34	Adupri	Bibiani	North Western
WNDpr35	Adupri	Bibiani	North Western
WNDpr36	Adupri	Bibiani	North Western
WNDpr39	Adupri	Bibiani	North Western
WNDpr41	Adupri	Bibiani	North Western
WNDpr42	Adupri	Bibiani	North Western
WNDpr44	Adupri	Bibiani	North Western
WNDpr45	Adupri	Bibiani	North Western
WNDpr46	Adupri	Bibiani	North Western
WNDpr49	Adupri	Bibiani	North Western
WNDpr54	Adupri	Bibiani	North Western
WNDpr96	Juaboso	Juaboso	North Western
WNDpr97	Juaboso	Juaboso	North Western
WNDpr98	Juaboso	Juaboso	North Western
WNDpr81	Naama	Bekwai	North Western
WNDpr82	Naama	Bekwai	North

Table 6. 1. Contiuned

WNDpr83	Naama	Bekwai	Western North
WNDpr84	Naama	Bekwai	Western North
WNDpr86	Naama	Bekwai	Western North
WNDpr87	Naama	Bekwai	Western North
WNDpr88	Naama	Bekwai	Western North
WNDpr89	Naama	Bekwai	Western North
WNDpr69	Nyentina	Bekwai	Western North
WNDpr71	Nyentina	Bekwai	Western North
WNDpr72	Nyentina	Bekwai	Western North
WNDpr74	Nyentina	Bekwai	Western North
WNDpr75	Nyentina	Bekwai	Western North
WNDpr76	Nyentina	Bekwai	Western North
WNDpr77	Nyentina	Bekwai	Western North
WNDpr79	Nyentina	Bekwai	Western North
WNDpr91	Adwumam	Juaboso	Western North
WNDpr92	Adwumam	Juaboso	Western North
WNDpr93	Adwumam	Juaboso	Western North
WNDpr94	Adwumam	Juaboso	Western North

Data collection

Using the field book software application (Rife and Poland, 2014), data were collected on 15 quantitative traits (Table 6.2) and 24 qualitative traits (Table 6.3) according to the standard operating protocol for yam performance evaluation trial (Asfaw, 2016) and yam trait ontology available on yambase (www.yambase.org). The area under the disease progression curve (AUDPC) for

yam mosaic virus (YMV) severity, yield per hectare, dry matter content, tuber flesh oxidation intensity and tuber flesh hardness were evaluated as described below.

The AUDPC, a valuable quantitative summary of disease intensity or severity for YMV over time, was estimated using the trapezoidal method (Campbell and Madden, 1990). This method discretizes the time variable and calculates the average disease intensity or severity between each pair of adjacent time points:

$$AUDPC = \sum_{i=1}^{N} \left(\frac{y_{i+yi+1}}{2} \right) \left(t_{i+1} - t_i \right) \quad (6.1)$$

Where: N is the number of observations, y_i is the disease severity at i^{th} observation, t_i is the time at i^{th} observation.

Total tuber yield per hectare (Yield) was calculated using the following formula:

$$Yield = \frac{TBWP \times 10}{PLS} \quad (6.2)$$

Where: TBWP is the total tuber yield per plot and PLS is the plot size.

The dry matter content was determined by chopping 100 g of fresh tuber flesh into small pieces and then oven-dried at 105°C for 24 h till a constant weight was achieved. The percentage dry matter content was then estimated as:

$$\%\ dry\ matter\ content\ (DMC) = ¿\ \frac{Dry\ tuber\ flesh\ weight\ (g)}{Wet\ tuber\ flesh\ weight\ (g)} \times 100 \qquad (6.3)$$

The intensity of tuber flesh oxidation (colour change or browning of cut tuber flesh) was assessed immediately the surface was cut and exposed to air (0 min) and 60 min after cutting using a Chroma (colourimeter) meter (CR-400, Konica Minolta, Japan). The (L*) lightness, (a*) red/green coordinate, (b*) yellow/blue coordinate values were recorded. A reference of white and black porcelain tiles was used to calibrate the Chroma meter before each reading. The colour change (**Δ**E*) between all the three coordinates was calculated using the following formula:

$$\Delta E^{¿} = (L¿¿¿ + a^{¿} + b^{¿})^{1/2}¿ \qquad (6.4)$$

Oxidative browning (TBOxi) = FΔE* −¿ IΔE* (6.5)

Where: F**Δ**E* is the final colour change and I**Δ**E* is the initial colour change.

Tuber flesh hardness was assessed with a 6.00 mm probe digital penetrometer. Tuber samples of 1 cm thickness and ~5 cm diameter were prepared from each accession and the probe was pressed into the tuber. The force necessary for its penetration into the tuber was considered an indicator of the hardness of the tuber. Three measurements were taken per accession, the average was calculated, and the data were expressed in Newton.

Table 6. 2. Description of quantitative traits evaluated

Quantitative traits	Unit	Abbreviation
Days to first sprout emergence	Days	DFSE
Stem internode length	cm	STIL
Stem diameter of mature plant	mm	SDMP
Number of internodes to first branching	Count	NIFB
Tuber yield per plot	kg	TBWP
Tuber yield per plant	kg	TBPL
Number of plants harvested per plot	Count	NPLP
Number of tubers per plot	Count	NTBP
Tuber length	cm	TBL
Tuber width	cm	TBW
Dry matter content	%	DMC
Tuber flesh oxidation		TBOxi
Tuber flesh hardness	N	TBHard
Yield	Tha^{-1}	Yield
Yam mosaic virus severity		YMV

Table 6. 3. Descriptions of qualitative traits evaluated

Qualitative traits	Abbreviation	Collection time	Scale and description
Spine On Sprout	SPNSP	20 days after emergence	0 = Absent; 1 = Present
Spine Base Color	SPBCOL	20 days after emergence	0 = Absent; 1 = Present
Plant Vigor	PLNV	2 months after emergence	1 = Weak (75% of the plants or all the plants in a plot are small, few leaves and thin vine); 2 = Medium (Intermediate or normal); 3 = Vigorous (75% of the plants or all the plants in a plot are robust with thick vine and leaves very well developed or with abundant foliage)
Spines On Stem Above Base	SPNSB	5 - 6 months after emergence	0 = Absent; 1 = Few; 2 = Many
Spine On Stem Base	SPNASB	5 - 6 months after emergence	0 = Absent; 1 = Few; 2 = Many
Leaf Shape	LFSHP	5 - 6 months after emergence	1 = Ovate; 3 = Cordate; 5 = Sagittate; 7 = Hastate
Stem Color	STMCO	5 - 6 months after emergence	1 = Green; 2 = Purplish green; 3 = Brownish green; 4 = Dark brown; 5 = Purple
Mature Leaf Color	MLFCO	5 - 6 months after emergence	1 = Yellowish; 2 = Pale green; 3 = Dark green; 4 = Purplish green; 5 = Purple
Leaf Density	LFDE	5 - 6 months after emergence	1 = Low; 2 = medium; 3 = High
Immature Leaf Color	ILFCO	30 days after emergence	1 = Yellowish; 2 = Pale green; 3 = Dark green; 4 = Purplish green; 5 = Purple

Flowering Intensity	FLRI	More than 50% of plants in a plot flowered	0 = No bud (No inflorescence and not flowering at all); 1 = Aborted bud (Presence of small or rudimentary inflorescences/flowers that can show an abortion or abscission point at the joint of the pedicel); 3 = Low (Flowering is scarce with the presence of few flowers (buds, flower buds, flowers, fruits,and flower abscissions) per inflorescence and per plant (Less than 10 inflorescences per plant); 5 = Moderate (Flowering is moderate with some flowers (buds, flower buds, flowers, fruits) per inflorescence and per plant (10−29 inflorescences per plant); 7 = Profuse (Profuse flowering with many more flowers (buds, flower buds, flowers, fruits) per inflorescence and per plant (30−50 inflorescences per plant); 9 = Extremely profuse (Extremely profuse flowering with abundant flowers (buds, flower buds, flowers, fruits) per inflorescence and per plant (More than 50 inflorescences per plant)
Inflorescence Type	INFT	At flowering	1 = Spike; 2 = Raceme; 3 = Panicle
Sex	SEX	At flowering	0 = Not flowering; 1 = Female; 2 = Male; 3 = Female and male (predominantly female); 4 = Male and female (predominantly male)
Tuber shape	TBRS	At harvesting	1 = Round/Spherical; 2 = Oval; 3 = Cylindrical; 5 = Irregular
Tuber size	TBRSZ	At harvesting	1 = small (less than 15 cm length); 2 = Medium (between 15 and 25 cm length); 3 = Big (more than 25 cm length)
Tuber surface texture	TBRST	At harvesting	1 = Smooth; 2 = Rough
Thorniness of tuber	TBRT	At harvesting	0 = Absent; 1 = Present

Table 6. 3. Continued

Intensity of thorns on tuber	ITTTS	At harvesting	0 = Absent; 3 = Few; 7 = Many
Root on tuber surface	RTS	At harvesting	0 = Absent; 1 = Few; 2 = Many
Crack on tuber surface	CTS	At harvesting	0 = Absent; 1 = Few; 2 = Many
Tuber flesh color	TRFC	At harvesting	1 = White; 2 = Creamy white; 3 = Yellow; 4 = Purplish; 5 = Purplish white; 6 = Creamy; 7 = Brownish white; 8 = Deep purple; 9 = Orange
Flesh oxidation Intensity	INTOXD	At harvesting	0 = No oxidation; 1 = Slightly oxidized; 3 = Highly oxidized
Flesh oxidation color	FOXDC	At harvesting	1 = Grey; 2 = Purple; 3 = Orange; 4 = Brown

Data analysis

To assess differences in qualitative traits among *D. praehensilis* accessions, descriptive statistics such as frequency calculations and bar charts were used. Shannon diversity and equitability indices were used to estimate diversity among the qualitative traits using Paleontological statistics software (PAST) 326b version (Hammer et al., 2001). The lme4 program in R software was used to perform analysis of variance (ANOVA) using a linear mixed model (LMM) fitted across the seasons (R Core Team, 2019). The linear model used was as follows:

$$Y_{ijk} = \mu + G_h + S_i + (G_h * S_i) + Ri_j + B_k + \varepsilon_{hijk,} \quad (6.6)$$

where $Yijk$ = value of the observed quantitative trait; μ = population mean; G_h = effect of the *h*th accessions; S_i = effect of the *i*th growing seasons; (G_h * S_i) is the accessions x season interaction associated with accession h and season I; R_{ij} = effect of the *j*th replicate (superblock) in seasons ith; B_k = effect of the *k*th incomplete block within the *j*th replicate; and $\varepsilon hijk$ = experimental error. Accessions were considered fixed effects, while growing seasons, replicates and blocks were considered random effects. The means between growing seasons were compared using the least significant difference (LSD) test at *p*-value threshold of 0.05. The variations in the quantitative traits of *D. praehensilis* accessions were assessed using descriptive statistics, such as means, standard deviations, minimum and maximum values and coefficients of variation. Pearson correlation coefficients in R (R Development Core Team, 2019) were used to

assess relationships among quantitative traits. Corrplot in R package version 0.84 (Wei & Simko, 2017) was used to visualize the relationships among traits. The packages, ggplots, FactoMineR and Factoextra, in R (R Development Core Team, 2019) were used to evaluate the contributions of quantitative traits using principal component analysis (PCA). Pheatmap and Ward.2 methods implemented in the Cluster package in R (R Development Core Team, 2019) were employed to generate a hierarchical cluster. To estimate the maximum cluster number and measure the effectiveness of grouping, the silhouette approach implemented in the Cluster package and FactoMinerR (R Development Core Team, 2019) was used. FactoMineR in R package (R Development Core Team, 2019) was used to generate Biplot to determine the position of the qualitative and quantitative traits of *D. praehensilis* accessions. The variance components for each quantitative trait were estimated from the expected mean square (EMS) in the analysis of variance using *lmerTest* and *lme4* in the R package (R Core Team, 2019). The broad-sense heritability and genotypic and phenotypic coefficients of variation were calculated based on the estimated variance components as follows:

$$H^2b = \left(\frac{\delta_g^2}{\delta_g^2 + \delta_{p/n}^2}\right) \times 100 \quad (6.7)$$

$$\text{Phenotypic coefficient of variance } (PCV) = ¿ \frac{\sqrt{\delta_p^2}}{Grand\,mean} \times 100 \quad (6.8)$$

$$\text{Genotypic coefficient of variance } (GCV ¿ = ¿ \frac{\sqrt{\delta_g^2}}{Grand\,mean} \times 100 \quad (5.9)$$

Where δ^2_g is the genotypic variance and δ^2_p is the phenotypic variance. Shabanimofrad et al. (2013) and Robinson et al. (2015) categorized the estimated values of PCV and GCV as 0% - 10% for low, 10% - 20% for intermediate and greater than (≥20%) for high. Broad sense heritability (h^2b) was categorized as 0–29% for low, 30–60% for intermediate and greater than 60% as high.

Results

Variation among the qualitative traits assessed

The frequency distribution of qualitative traits assessed is summarized in Figures 6.2 and 6.3 (A &B). The study revealed 53.7% of the accessions showed intermediate plant vigour, 45.5% showed vigorous plant vigour, while 1.2% showed low plant vigour. The accessions had immature leaf colour of purplish-green (38.3%), green (32.7%) and dark green (29.0%). We observed 60.5% of the accessions had cordate leaf shape, 37% had sagittate leaf shape, while 2.5% of the accessions had hastate leaf shape. Most of the evaluated accessions had intermediate leaf density (58.6%), 40.1% had high leaf density and 1.2% had low leaf density. We observed that 42% of the accessions had profuse flowering intensity, 40.7% had extremely profuse, 5.6% had moderate flowering intensity and 0.6% had aborted buds, while 11.1% did not flower. The majority of the accessions that produced flowers had spike inflorescence type (88.9%). The majority of the accessions (56.8%) were males, 24.1% were females, 6.8% were monoecious males, and 0.6% were monoecious females. Among the accessions, 75.3% had cylindrical tuber shape, 20.4% had irregular tuber shape, and 4.3% had oval tuber shape. Most of the evaluated accessions had big tuber sizes (82.1%).

White tuber flesh colour (88.3%) was observed mostly among the accessions, while some had yellow flesh colour (11.1%) and purple flesh colour (0.6%). The intensity of oxidation varied among the accessions, with 44.4% showing no tuber oxidation, 37.7% showing high oxidation and 17.9% showing slight tuber flesh oxidation.

Shannon and evenness diversity indices of the evaluated qualitative traits

The estimates of Shannon (H) and evenness (E) diversity indices for 24 qualitative traits are presented in Table 6.4. The Shannon diversity index ranged from 4.34 for the intensity of tuber thorniness to 5.07 for mature leaf colour and leaf density. No variation was observed for hairs on sprouts (0.00). The evenness diversity index ranged from 0.86 for flesh oxidation colour to 1.00 for spines on the sprout, sprout base colour, hairs on the sprout, inflorescence types and intensity of tuber thorniness.

Table 6. 4. Shannon and evenness diversity indices of the evaluated qualitative traits

Traits	Shannon (H)	Evenness (E)
SPNSP	5.03	1.00
SPBCOL	4.43	1.00
HAIRSP	0.00	1.00
PLNV	5.06	0.98
SPNSB	5.05	0.96
SPNASB	5.05	0.97
LFSHP	5.05	0.96
STMCO	4.98	0.90
MLFCO	5.07	0.99
LFDE	5.07	0.98
ILFCO	5.05	0.96

FLRI	4.95	0.98
INFT	4.97	1.00
SEX	4.89	0.93
TBRS	5.06	0.97
TBRSZ	5.06	0.97
TBRST	5.03	0.94
TBRT	4.35	0.99
ITTTS	4.34	1.00
RTS	4.92	0.98
CTS	4.93	0.90
TRFC	4.98	0.90
INTOXD	4.41	0.91
FOXDC	4.35	0.86

Table 6. 4. Continued

SPNSP = spine on sprout; SPBCOL = spine base colour; HAIRSP = hair on sprout; PLNV = plant vigour; SPNSB = spines on spine base; SPNASB = spines above stem base; LFSHP = leaf shape; STMCO = stem colour; MLFCO = mature leaf colour; LFDE = leaf density; ILFCO = immature leaf colour; FLRI = flowering intensity; INFT = inflorescence type; SEX = plant sex; TBRS = tuber shape; TBRSZ = tuber size; TBRST = tuber texture; TBRT = tuber intensity; ITTTS = intensity of tuber thorniness; RTS = roots on tuber surface; CTS = cracks on tuber surface; TRFC = tuber flesh colour; INTOXD = intensity of oxidation; FOXDC = flesh oxidation colour

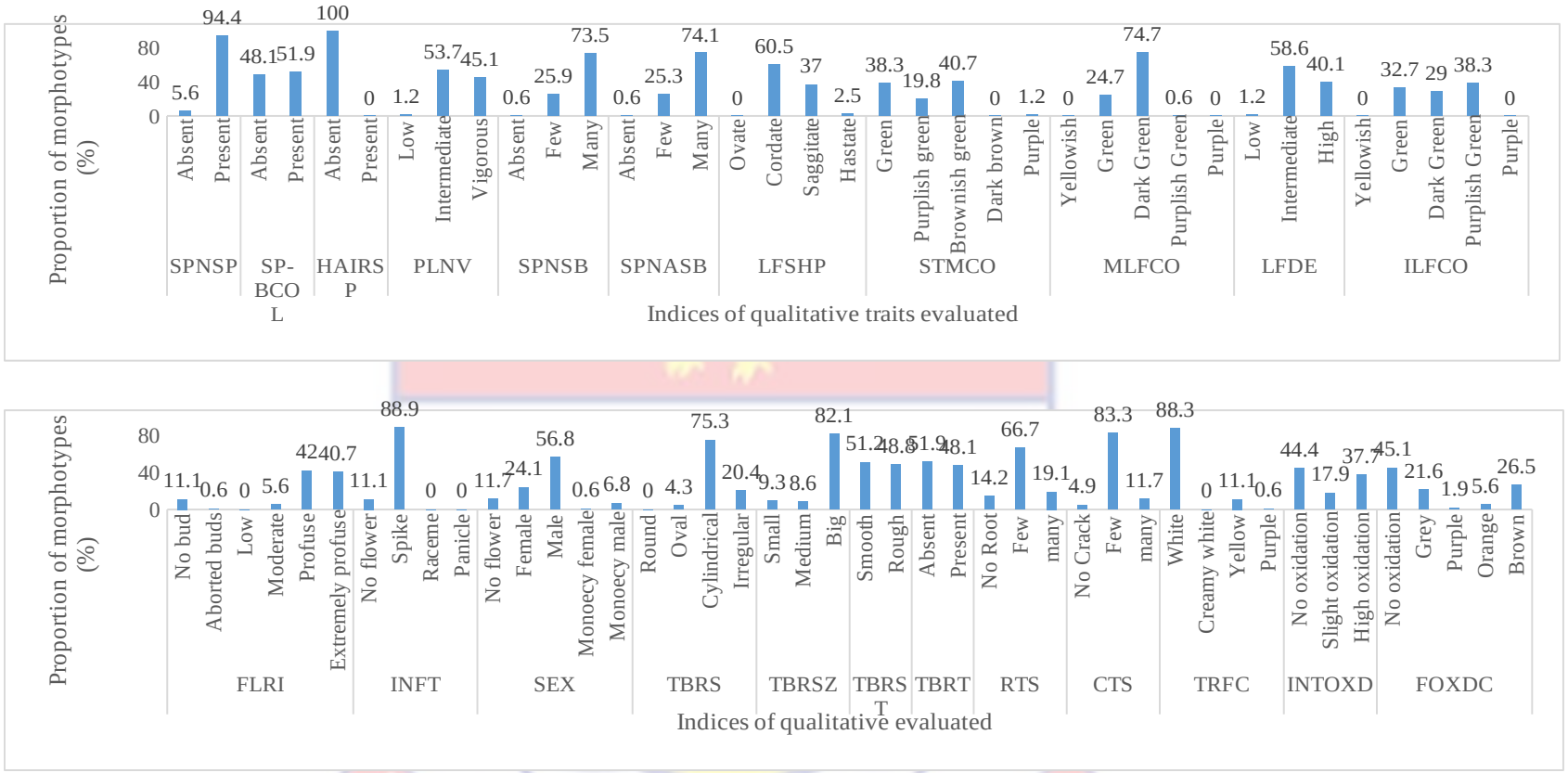


Figure 6. 2. Frequency distribution of the qualitative traits evaluated among the *D. praehensilis* accessions

SPNSP = spine on sprout; SPBCOL = spine base colour; HAIRSP = hair on sprout; PLNV = plant vigour; SPNSB = spines on spine base; SPNASB = spines above stem base; LFSHP = leaf shape; STMCO = stem colour; MLFCO = mature leaf colour; LFDE = leaf density; ILFCO = immature leaf colour; FLRI = flowering intensity; INFT = inflorescence type; SEX = plant sex; TBRS = tuber shape; TBRSZ = tuber size; TBRST = tuber texture; TBRT = tuber intensity; RTS = roots on tuber surface; CTS = cracks on tuber surface; TRFC = tuber flesh colour; INTOXD = intensity of oxidation; FOXDC = flesh oxidation colour

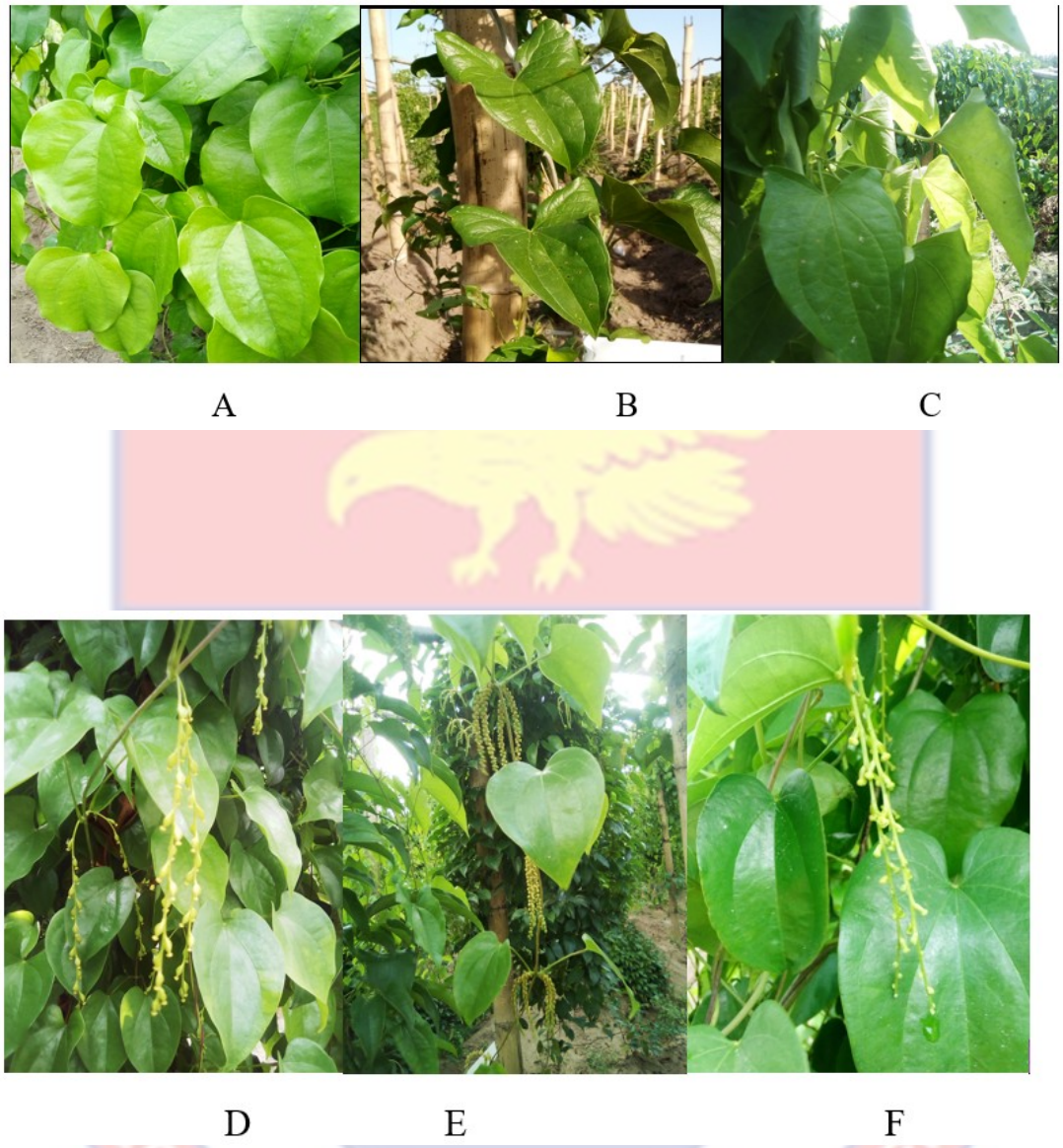


Figure 6. 3A. Some leaf and inflorescence features of *D. praehensilis* accessions

Leaf shape (A: Cordate; B: Hastate; C: Saggitate); Plant sex (D: Female; E: Male; F: Monoecious plant); Tuber flesh colour: (G: Yellow; H: White; I: Purple)

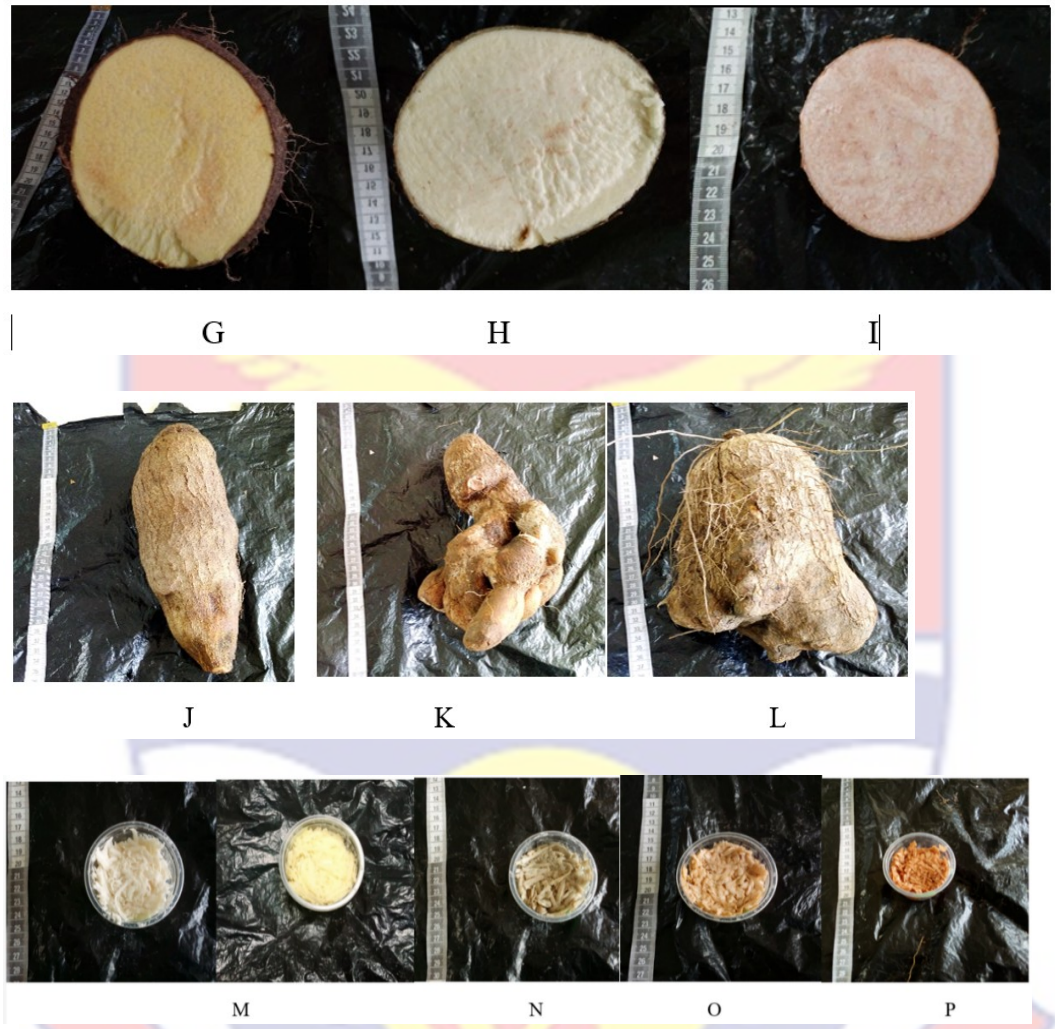


Figure 6. 3B. Some Tuber qualitative features of *D. praehensilis* accessions

Tuber shape: (J: Cylindrical; K: Irregular; L: Oval); Tuber oxidation colour: (M: No oxidation (white and yellow); N: Grey; O: Brown; P: Orange

Variation in quantitative traits among *D. praehensilis* accessions across two growing seasons

The combined analysis of variance revealed significant differences for replicate, block, season, accession and interaction between season and accession mean squares for most of the traits evaluated (Table 6.5). The effect of seasons was significant ($p \leq 0.05$) for most of the traits being assessed, except for the

number of plants harvested per plot and the number of tubers per plant (Table 6.5). The effect of accessions was highly significant ($p \leq 0.05$) for all the evaluated quantitative traits (Table 6.5). The effect of interaction between seasons and accessions was significant ($p \leq 0.05$) for days to first sprout emergence, tuber flesh hardness and tuber yield (Table 6.5).

Higher significant values were recorded in the 2020 season for traits, such as days to first sprout emergence (74.15 days), stem diameter (3.85 mm), stem internode length (16.29 cm), tuber yield per plot (3.52 kg), tuber yield per plant (2.04 kg), tuber yield (23.47 tons/ha) and response to YMV severity (AUDPC = 147.45) (Table 6.6), while higher performance was recorded in 2021 season for traits, such as dry matter contents (35.19%), the number of internodes (~3.00), tuber flesh hardness (50.95 N), tuber flesh oxidation (-16.32), tuber length (42.21 cm) and tuber width (29.26 cm) (Table 6.6). No significant variations were observed in the number of tubers per plant and tubers per plot across the seasons (Table 6.6).

The overall mean, range and coefficients of variation of the evaluated quantitative traits across the seasons are presented in Table 6.7. The tuber yield ranged from 1.30 to 140.00 tons/ha across the seasons, with a mean value of 15.78 tons/ha. The response to YMV severity varied from 135.00 320.00 with an average of 149.40. The average dry matter recorded across the seasons was 34.01%, ranging from 17.84 to 50.49%. Tuber flesh oxidation varied from -46.71 to 7.77, with an average of -13.34. In contrast, tuber flesh hardness ranged from 48.43 to 55.26 N with an average of 50.86 N. The coefficients of variation varied

from 0.25% for tuber flesh hardness to 68.73% for tuber yield.

Genotypic coefficients of variation, phenotypic coefficients of variation and broad-sense heritability of quantitative traits of *D. praehensilis* across two seasons

High genotypic coefficients of variation (GCV) (≥20%) were observed in most of the evaluated quantitative traits except for stem internode length (16.81%), dry matter contents (9.23%), tuber flesh hardness (0.40%) and response to YMV severity (0.21%) (Table 5.8). High phenotypic coefficients of variation (PCV) (≥20%) were recorded in all the evaluated quantitative traits except in dry matter content (11.46%), flesh tuber hardness (0.65%) and response to YMV (0.40%) (Table 6.8). High H^2 (>60%) was observed for all the evaluated quantitative traits except response to YMV, which revealed intermediate H^2 (30-60%) (Table 6.8).

Table 6. 5. Mean squares of quantitative traits of *D. praehensilis* accessions across two seasons

Traits	Rep	Blo	Acc	Season	Acc:Season	Error
DFSE (Days)	9127.51*	318.55*	1902.53*	28979.15*	158.04*	86.79
DMC (%)	302.67*	17.45*	43.08*	901.41*	3.91ns	6.32
NIFB (Count)	113.33*	2.00ns	4.61*	33.11*	0.28ns	1.46
NPPL (Count)	0.11ns	0.55*	1.08*	0.44ns	0.29ns	0.32
NTP	0.014ns	1.34*	2.34*	0.91ns	0.54ns	0.62

Table 6. 5. Continued

SDMP (mm)	9.59*	0.49^{ns}	2.71*	117.32*	0.45^{ns}	0.75
SINL (cm)	18.35^{ns}	12.49^{ns}	33.06*	399.16*	1.97^{ns}	10.24
TBHard (N)	0.18^{ns}	3.39*	5.25*	6.05*	0.15*	0.07
TBL (cm)	2.45^{ns}	118.19*	345.29*	6385.18*	47.85^{ns}	60.00
TBOXI	884.18*	159.34*	261.83*	5782.89*	24.30^{ns}	40.96
TBW (cm)	457.32*	51.08*	264.57*	91.00*	1.71^{ns}	14.41
TBWP (kg/plot)	0.55^{ns}	7.11*	18.68*	190.95*	2.49^{ns}	3.11
TWPL (kg/plant)	3.08*	0.92^{ns}	4.02*	55.95*	0.44^{ns}	0.62
Yield (tha^{-1})	168.85^{ns}	280.42*	549.32*	38043.94*	186.12*	117.64
YMV	147.35*	1285.61*	3707.12*	2552.22*	0.10^{ns}	0.96

NPLP = number of plant per plot; NTP = number of tuber per plant; TBL = tuber length; TBW = tuber width; TBWP = tuber yield per plot; TWPL = tuber yield per plant; Yield = tuber yield per hectare; DMC = dry matter content; TBOXI = tuber flesh oxidation; TBHARD = tuber flesh hardness; DFSE = days to first sprout emergence; NIFB = number internodes to first branching; SDMP = stem diameter of mature plant; STIL = stem internode length; YMV = yam mosaic virus; Rep: Replicate; Block: Blo; Accession: Acc;

Table 6. 6. Variability among the evaluated quantitative traits across two growing seasons

	Seasons		
Traits	2020	2021	LSD (p≤0.05)
DFSE (Days)	74.15±23.24	87.51±24.84	1.44
DMC (%)	32.83±4.09	35.19±3.81	0.39
NIFB (Count)	2.70±1.28	3.16±1.61	0.19
NPPL (Count)	1.61±0.74	1.55±0.69	1.07
NTP (Count)	1.89±1.12	1.81±0.93	0.12
SDMP (mm)	3.85±1.10	2.99±1.05	0.13
SINL (cm)	16.29±4.29	14.72±3.05	0.49
TBHard (N)	50.76±1.17	50.95±1.25	0.04
TBL (cm)	35.87±12.19	42.21±10.55	1.20
TBOXI	-10.37±9.67	-16.32±9.96	0.99
TBW (cm)	28.70±8.95	29.26±8.39	0.59
TBWP (kg/plot)	3.52±3.19	2.43±1.92	0.27
TWPL (kg/plant)	2.04±1.44	1.46±0.88	0.12
Yield (tons/ha)	23.47±21.30	8.09±6.39	1.68
YMV	147.45±30.96	151.43±30.96	0.15

NPLP = number of plant per plot; NTP = number of tuber per plant; TBL = tuber length; TBW = tuber width; TBWP = tuber yield per plot; TWPL = tuber yield per plant; Yield = tuber yield per hectare; DMC = dry matter content; TBOXI = tuber flesh oxidation; TBHARD = tuber flesh hardness; DFSE = days to first sprout emergence; NIFB = number internodes to first branching; SDMP = stem diameter of mature plant; STIL = stem internode length; YMV = yam mosaic virus

Table 6. 7. Mean performance of accessions for the evaluated quantitative traits

Traits	Overall Mean	Minimum	Maximum	CV (%)
DFSE (Days)	80.83	20.00	163.00	11.53
DMC (%)	34.01	17.84	50.49	7.39
NIFB (Count)	2.93	1.00	12.00	41.18
NPPL (Count)	1.58	1.00	3.00	35.89
NTP (Count)	1.85	1.00	9.00	42.76
SDMP (mm)	3.40	1.00	7.50	25.40
SINL (cm)	15.50	7.70	41.50	20.64
TBHard (N)	50.86	48.43	55.26	0.52
TBL (cm)	39.04	12.00	97.00	19.84
TBOXI	-13.34	-46.71	7.77	47.97
TBW (cm)	29.08	11.00	63.00	13.05
TBWP (kg/plot)	2.97	0.11	21.00	59.34
TWPL (kg/plant)	1.75	0.11	10.00	44.91
Yield (tons/ha)	15.78	1.30	140.00	68.73
YMV	149.40	135.00	320.00	0.65

NPLP = number of plant per plot; NTP = number of tuber per plant; TBL = tuber length; TBW = tuber width; TBWP = tuber yield per plot; TWPL = tuber yield per plant; Yield = tuber yield per hectare; DMC = dry matter content; TBOXI = tuber flesh oxidation; TBHARD = tuber flesh hardness; DFSE = days to first sprout emergence; NIFB = number internodes to first branching; SDMP = stem diameter of mature plant; STIL = stem internode length; YMV = yam mosaic virus

Table 6. 8. Variance components, GCV, PCV and broad-sense heritability for quantitative traits among *D. praehensilis* accessions

Traits	$\delta^2 g$	$\delta^2 p$	GCV (%)	PCV (%)	H^2 (%)
DFSE (Days)	442.15	529.66	26.01	28.47	91.37
DMC (%)	9.85	15.19	9.23	11.46	80.53
NIFB (Count)	0.98	1.82	33.79	46.04	73.38
NPPL (Count)	0.2	0.5	28.30	44.75	63.25
NTP (Count)	0.45	1.02	36.26	54.59	66.42
SDMP (mm)	0.54	1.12	21.61	31.13	69.44
SINL (cm)	6.79	13.68	16.81	23.86	70.45
TBHard (N)	0.042	0.109	0.40	0.65	62.07

TBL (cm)	73.58	129.34	21.97	29.13	75.42
Table 6. 8 Continued	.72	94.91	57.93	73.03	79.32
TBW (cm)	64.8	74.64	27.68	29.71	93.18
TBWP (kg/plot)	4.02	6.82	67.51	87.93	76.78
TWPL (kg/plant)	0.89	1.41	53.91	67.85	79.45
Yield (tons/ha)	91.47	204.66	60.61	90.66	66.85
YMV	0.096	0.356	0.21	0.40	51.93

NPLP = number of plant per plot; NTP = number of tuber per plant; TBL = tuber length; TBW = tuber width; TBWP = tuber yield per plot; TWPL = tuber yield per plant; Yield = tuber yield per hectare; DMC = dry matter content; TBOXI = tuber flesh oxidation; TBHARD = tuber flesh hardness; DFSE = days to first sprout emergence; NIFB = number internodes to first branching; SDMP = stem diameter of mature plant; STIL = stem internode length; YMV = yam mosaic virus

Principal component analysis of the evaluated quantitative traits among the *D. praehensilis* accessions across two growing seasons

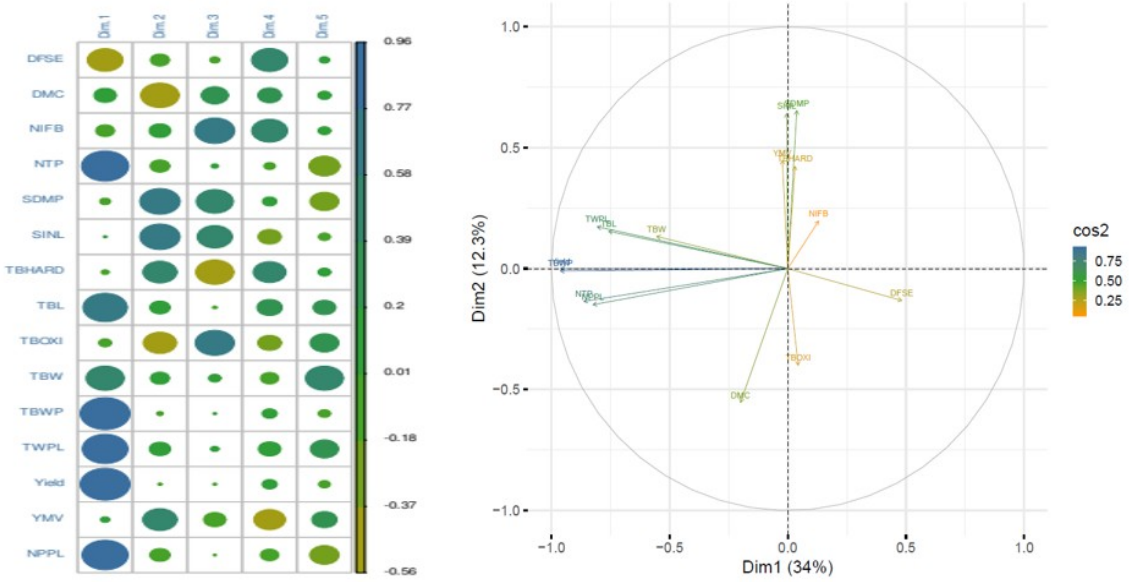
The first five principal components with Eigenvalues greater than one accounted for ~71% of the total variation in *D. praehensilis* accessions evaluated across the two seasons (Table 6.9). Principal component one (PC1) explained ~34% of the total variation and showed high positive association with traits such as number of plants harvested per plot (r = 0.82), number of tubers per plant (r = 0.86), tuber length (r = 0.76), tuber width (r = 0.56), tuber yield per plant (r = 0.81), tuber yield per plot (r = 0.96) and tuber yield per hectare (r = 0.96) and negatively correlated with days to first sprout emergence (r = -0.49) (Figure 6.4A). The second principal component (PC2) contributed 12.38% of the total variation and correlated positively with traits, such as stem diameter (r = 0.63), stem internode length (r = 0.62), tuber flesh hardness (r = 0.45) and response to YMV severity (r = 0.46) and negatively correlated with dry matter content (r = -0.57) and tuber flesh oxidation (r = -0.43) (Figure 6.4A). Approximately 11% of

the total variation was detected in the third principal component (PC3) and positively correlated with number of internodes (r = 0.61), stem diameter (r = 0.53), stem internode length (r = 0.48), dry matter content (r = 0.28) and tuber flesh oxidation (r = 0.59), but showed negative relationship with tuber flesh hardness (r = -0.59) and response to YMV severity (r = -0.19) (Figure 6.4A). The fourth principal component (PC4) accounted for 7.30% of the total variation and positively correlated to days to first sprout emergence (r = 0.50), number of internodes (r = 0.50) and tuber flesh hardness (r = 0.41), but negatively correlated with response to YMV severity (r = -0.39) (Figure 6.4A). The PC5 contributed 6.4% of the total variation and correlated positively to tuber width (r = 0.55), tuber flesh oxidation (r = 0.32) and tuber yield per plant (r = 0.31), but negatively correlated to number of plants per plot (r = -0.33) and number of tuber per plant (r = 0.36) (Figure 6.4A). The influence of the traits on principal components and the level of correlation between one another is presented in Figure 6.4B. The lesser the angle between two traits indicates a higher and positive correlation (tuber yield per hectare and tuber yield per plant); when the angle between two traits is 90^{o}, no correlation exists between them. The correlation is negative when the angle is more than 90^{o} to near 180^{o} (tuber flesh oxidation and tuber flesh hardness).

Table 6. 9. Eigenvectors and first five principal components of quantitative traits evaluated among *D. praehensilis* accessions

Traits	PC1	PC2	PC3	PC4	PC5
DFSE (Days)	**0.214**	-0.097	0.029	**-0.487**	-0.054
DMC (%)	-0.089	**-0.407**	**-0.229**	**-0.203**	-0.082
NIFB (Count)	0.058	0.144	**-0.474**	**-0.471**	-0.048
NTP (Count)	**-0.382**	-0.100	-0.009	0.049	**0.364**
SDMP (mm)	0.017	**0.480**	**-0.383**	-0.072	**0.324**
SINL (cm)	-0.002	**0.471**	**-0.347**	0.188	0.053
TBHARD (N)	0.014	**0.311**	**0.448**	**-0.394**	-0.076
TBL (cm)	**-0.336**	0.113	0.007	**-0.237**	-0.186
TBOXI	0.019	**-0.294**	**-0.476**	0.195	**-0.322**
TBW (cm)	**-0.246**	0.098	-0.050	0.115	**-0.557**
TBWP (Kg)	**-0.426**	-0.007	0.002	-0.079	0.054
TWPL (kg)	**-0.357**	0.127	-0.013	-0.183	**-0.308**
Yield (tons/ha)	**-0.426**	0.000	0.009	-0.079	0.049
YMV	-0.010	**0.328**	**0.157**	**0.365**	**-0.291**
NPPL (Count)	**-0.365**	-0.110	0.005	0.124	**0.329**
Eigenvalue	2.259	1.360	1.287	1.047	1.000
Variance (%)	34.050	12.380	11.060	7.270	6.410
Cumulative variance (%)	34.050	46.430	57.490	64.760	71.170

NPLP = number of plant per plot; NTP = number of tuber per plant; TBL = tuber length; TBW = tuber width; TBWP = tuber yield per plot; TWPL = tuber yield per plant; Yield = tuber yield per hectare; DMC = dry matter content; TBOXI = tuber flesh oxidation; TBHARD = tuber flesh hardness; DFSE = days to first sprout emergence; NIFB = number internodes to first branching; SDMP = stem diameter of mature plant; STIL = stem internode length; YMV = yam mosaic virus
PC1 to PC5 indicate Principal Components 1 to 5



A **B**

Figure 6. 4. A: Plot showing the total contribution of variables in accounting for the variability in the principal components. B: Plot showing the total contribution of variables in accounting for the variability in PC1 and PC2.

NPLP = number of plant per plot; NTP = number of tuber per plant; TBL = tuber length; TBW = tuber width; TBWP = tuber yield per plot; TWPL = tuber yield per plant; Yield = tuber yield per hectare; DMC = dry matter content; TBOXI = tuber flesh oxidation; TBHARD = tuber flesh hardness; DFSE = days to first sprout emergence; NIFB = number internodes to first branching; SDMP = stem diameter of mature plant; STIL = stem internode length; YMV = yam mosaic virus. Dim1 to Dim5 indicate Principal Components 1 to 5

Correlation coefficient analysis of the evaluated qualitative traits among the *D. praehensilis* accessions across two seasons

The results of the relationships among the evaluated quantitative traits across the two seasons are presented in Figure 6.5. Tuber yield per hectare was

significantly positively correlated with tuber yield per plot, tuber yield per plant, number of tubers per plant, number of tubers per plot, dry matter content, tuber length and tuber width ($p \leq 0.001$), but negatively correlated with days to first sprout emergence ($p \leq 0.001$) (Figure 6.5). Significant negative correlations were observed between days to first sprout emergence and yield component traits ($p \leq 0.001$). Dry matter content was found to be positively correlated with tuber flesh oxidation ($p \leq 0.01$) and tuber yield component traits ($p \leq 0.05$) but negatively correlated with tuber flesh hardness ($p \leq 0.01$), response to YMV severity ($p \leq 0.05$) and stem diameter and stem internode length ($p \leq 0.05$). Tuber flesh hardness significantly negatively correlated with tuber flesh oxidation ($p \leq 0.001$).

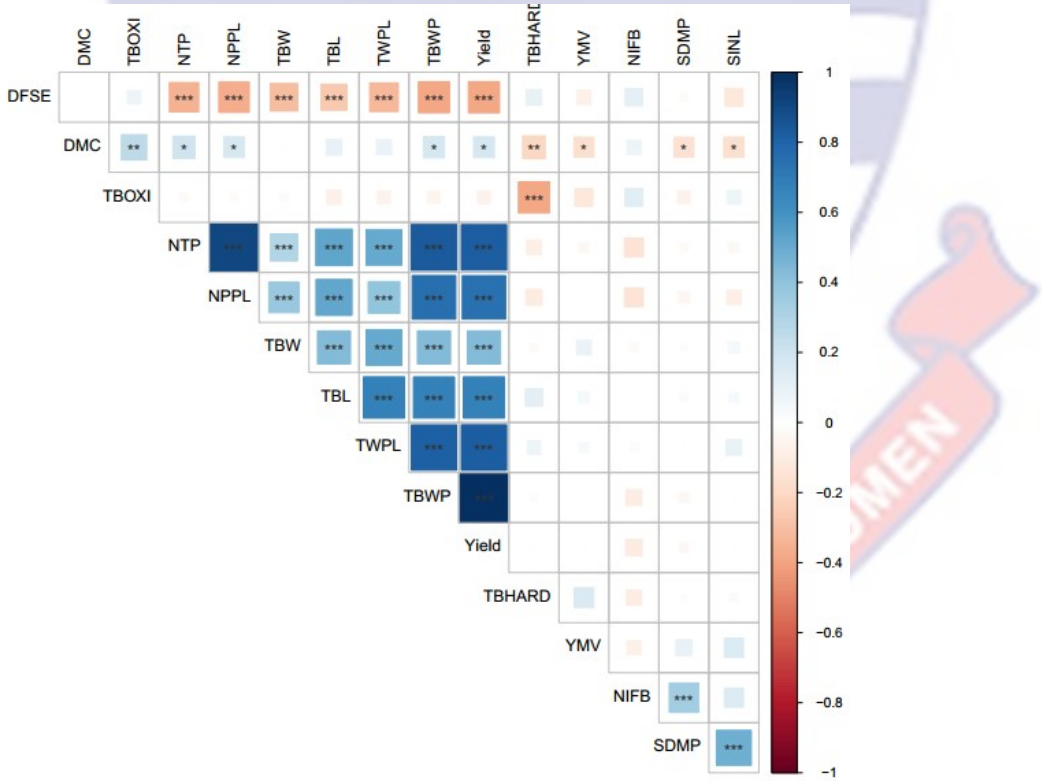


Figure 6. 5. Relationship among the evaluated 15 quantitative traits

NPLP = number of plant per plot; NTP = number of tuber per plant; TBL = tuber length; TBW = tuber width; TBWP = tuber yield per plot; TWPL = tuber yield per plant; Yield = tuber yield per hectare; DMC = dry matter content; TBOXI =

tuber flesh oxidation; TBHARD = tuber flesh hardness; DFSE = days to first sprout emergence; NIFB = number internodes to first branching; SDMP = stem diameter of mature plant; STIL = stem internode length; YMV = yam mosaic virus. * = ($p \leq 0.05$); ** = ($p \leq 0.01$); *** = ($p \leq 0.001$)

Hierarchical clustering among the *D. praehensilis* accessions based on 15 quantitative traits

Hierarchical cluster based on Gower dissimilarity coefficient using 15 quantitative traits grouped the 162 accessions of *D. praehensilis* into three clusters, and each cluster accounted for 114, 32 and 16 accessions, respectively (Figure 6.6). Cluster analysis revealed significant discrimination among the clusters (Table 6.10). Cluster I was characterized by accessions with longer days to first sprout emergence, moderate tuber yield component traits, high dry matter content, low tuber flesh oxidation and low tuber flesh hardness, with a high response to yam mosaic virus severity. The accessions in this category include CDpr1, CDpr10, CDpr40, Otim, EDpr3, EDpr13, EDpr20, WNDpr10, WNDpr18 and WNDpr33 (Figure 6.6). Cluster II consisted of accessions that were characterised by late days to first sprout emergence, low response to yam mosaic virus severity, low tuber yield component attributes, high dry matter content, low tuber flesh oxidation, low tuber flesh hardness and high number of internodes. The accessions grouped in this cluster include CDpr46, CDpr57, Dp/Asamankese/Assin/009, Kaati, Odonor big, PGR/20/002 and WNDpr77 (Figure 6.6). Cluster III comprised of accessions characterized by earliness to sprout emergence, high values for tuber yield component attributes, high dry matter content, moderate response to YMV severity, low tuber flesh oxidation, and low tuber flesh hardness. Examples of accessions in this category are

CDpr24, WNDpr76, CDpr8, EDpr14, CDpr7, WNDpr15, EDpr1, CDpr28, Dp/UP/E/001 and WNDpr93.

Table 6. 10. Description of cluster groups of *D. praehensilis* accessions

Traits	Cluster I (114)	Cluster II (32)	Cluster III (16)	LSD (p≤0.05)	F-value
DFSE	79.21±1.91	95.09±3.61	**63.83±5.10**	10.39	13.72***
DMC	34.11±0.31	33.20±0.59	34.94±0.83	1.70	1.62NS
NIFB	2.80±0.10	**3.52±0.19**	2.69±0.26	0.54	6.17*
NPPL	1.60±0.04	1.20±0.08	**2.27±0.11**	0.23	29.98***
NTP	1.83±0.06	1.29±0.11	**3.09±0.16**	0.32	45.32***
SDMP	3.31±0.08	3.73±0.14	3.60±0.20	0.41	3.81*
SINL	15.24±0.27	16.19±0.51	16.01±0.73	1.48	1.60NS
TBHard	50.78±0.11	51.15±0.21	50.79±0.30	0.60	1.27NS
TBL	39.50±0.77	32.09±1.45	**49.65±2.05**	4.18	25.00***
TBOxi	-12.75±0.76	-14.48±1.46	-15.31±2.07	4.21	1.05NS
TBW	29.65±0.72	24.12±1.36	**34.98±1.93**	3.92	11.63***
TBWP	2.79±0.14	1.36±0.26	**7.49±0.37**	0.76	93.22***
TWPL	1.69±0.07	1.11±0.14	**3.49±0.20**	0.41	48.38***
YMV	**141.61±2.67**	172.33±5.04	159.50±7.12	14.52	115.61***
Yield	14.76±0.74	6.95±1.40	**40.71±1.98**	4.04	99.69***

NPLP = number of plant per plot; NTP = number of tuber per plant; TBL = tuber length; TBW = tuber width; TBWP = tuber yield per plot; TWPL = tuber yield per plant; Yield = tuber yield per hectare; DMC = dry matter content; TBOXI =

tuber flesh oxidation; TBHARD = tuber flesh hardness; DFSE = days to first sprout emergence; NIFB = number internodes to first branching; SDMP = stem diameter of mature plant; STIL = stem internode length; YMV = yam mosaic virus

The bold values indicate superior traits at each cluster

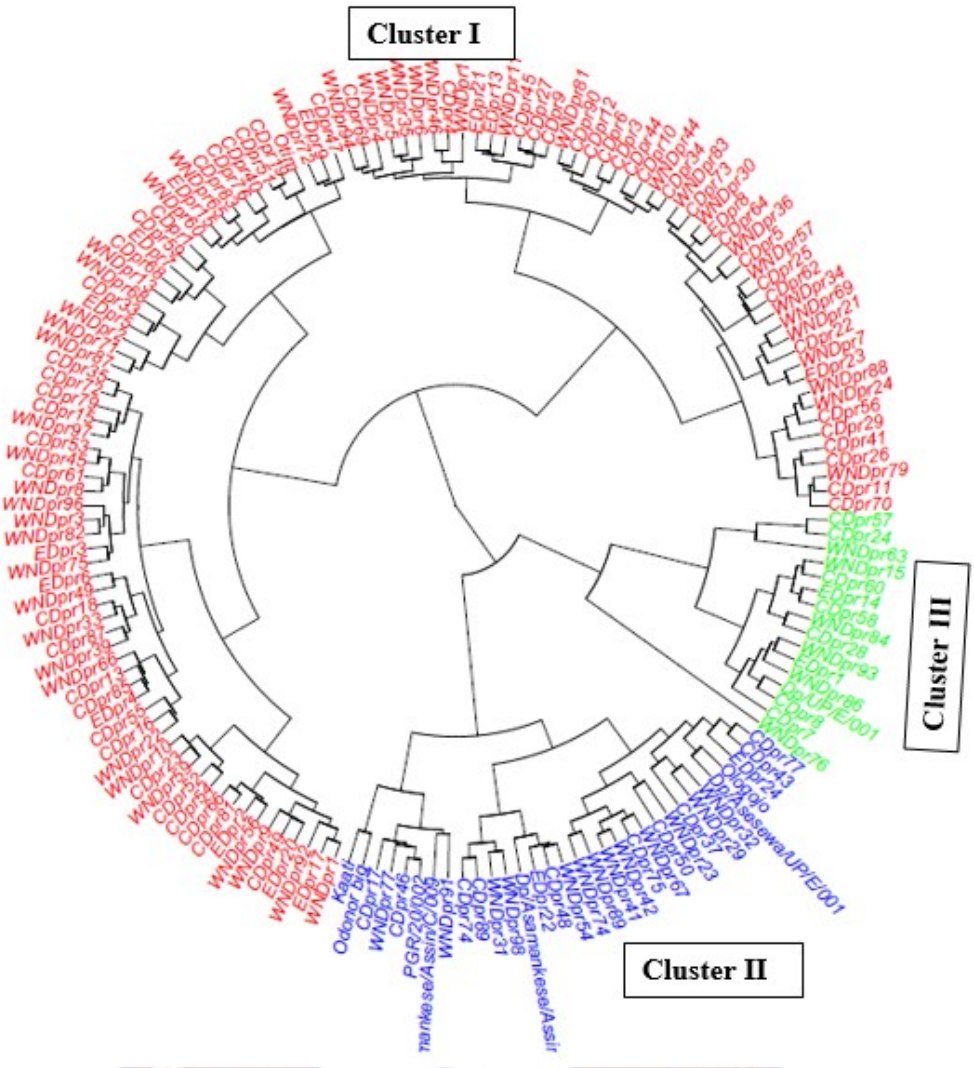


Figure 6. 6. Hierarchical clustering showing grouping patterns of *D. praehensilis* accessions using 15 quantitative traits based on Gower dissimilarity coefficient

Discussion

Genetic diversity based on qualitative and quantitative traits

The estimation of genetic diversity is an integral part of crop improvement and management; and usage of plant genetic resources. In this study, we

employed 24 qualitative and 15 quantitative traits to assess variations among 162 accessions of bush yam collected from three different regions in Ghana.

Qualitative traits evaluated revealed the existence of significant variations among the bush yam accessions. This study grouped the tuber flesh colour into white, yellow and purple, indicating the presence of genetic diversity among the evaluated bush yam accessions. The presence of different categories for plant sex (no flower, female, male, monoecious female and monoecious male), leaf shape (cordate, sagittate and hastate), tuber flesh oxidation and tuber shape (cylindrical, round and irregular) indicates the presence of high genetic variability among bush yam accessions. In this study, the observed stem colours of *D. praehensilis* were brownish green, green, purple and purplish green. This agrees with findings by Djedatin et al. (2017) on a study conducted on two wild yam species in Benin. The variations observed among qualitative traits are in accordance with Kouam et al. (2018), who observed significant diversity among aerial yam accessions using 23 qualitative traits. The observed diversity among the qualitative traits evaluated could be a result of long term selection by farmers, environmental effects and mating system of the crop. Yam is a dioecious crop and has a high ability for cross-pollination, which results in considerable diversity affecting the genetic identity of a population (Beyene et al., 2021). Shannon diversity and evenness indices revealed significant diversity in the qualitative traits evaluated among bush yam accessions. This indicates that these qualitative traits could be very useful in discriminating among *Dioscorea* spp. Shannon diversity index was

employed to differentiate variation in five yam species using 20 qualitative traits in China (Cao et al., 2021). High diversity indices indicate relatively high level diversity and evenly distribution of germplasm across the collection regions.

The traits used in this study significantly differed among the bush yam accessions collected from the three regions of Ghana using 15 quantitative traits. The large mean differences among the accessions for measured quantitative traits across seasons are indications of genetic variability for most of the traits among the test accessions of *D. praehensilis*. In this present study, the growing seasons influenced all the evaluated quantitative traits due to variation in soil moisture content, which was due to variation in rainfall patterns. This confirms that morphological descriptors are influenced by changes in environmental conditions. However, accession x season interaction effects were found to be lower than accession effects and season effects. High coefficients of variation (CV > 20) observed in some of the quantitative traits, especially the yield component traits, indicate huge and readily available genetic differentiation in *D. praehensilis*. Kouam et al. (2018) reported high coefficients of variation for tuber yield components in a study conducted on *D. bulbifera* accessions. Similar observations of high genetic variability using quantitative traits have been reported in other yam species such as *D. alata* (Agre et al., 2019), *D. rotundata* (Darkwa et al., 2020) and *D. dumetorum* (Adeigbe et al., 2015; Siadjeu et al., 2015). High coefficients of variation were also reported in tuber yields of some yam species in a study conducted in China to unlock the genetic relationships among *Dioscorea* spp. (Cao et al., 2021). These high variations in quantitative trait values and high

CVs are indications that these traits could be used as the basis for selection in the yam breeding programmes. The knowledge of existing variability and degree of association between quantitative traits is paramount for selecting superior accessions for yam breeding programmes.

Genetic coefficients and broad-sense heritability among evaluated quantitative traits

The high GCV (>20%) and high broad-sense heritability (>60%) observed for all the yield-related traits and tuber quality traits suggest high selection pressure, which could be enforced on these traits for future breeding activities. GCV coupled with heritability estimates, offers the best information about the extent of progress that can be expected from selection (Matsumoto et al., 2021). In contrast, low GCV recorded for dry matter content and YMV severity response implies that this trait can only be improved using selection methods that are not under the influence of environmental factors. Norman et al. (2021) and Asfaw et al. (2021) have reported high GCV and PCV for tuber yield and low GCV and PCV for dry matter content in studies conducted on advanced and early generation breeding populations of *D. rotundata*, respectively. Padhan et al. (2019) also reported high GCV and PCV for tuber yield in a study conducted on India's wild and cultivated yam species. High H^2 observed in yield-related traits is an indication that these traits could be improved through natural selection for superior accessions. The result from this study corroborates the findings of Agre et al. (2021a), who reported high H^2 in tuber yield in a study conducted on the elite populations of *D. rotundata*. The higher the broad-sense heritability, the higher the genetic variance linked with additive and non-additive gene effects

(Norman et al., 2021). Hence, these traits could be employed for selection of bush yam accessions.

Principal components among evaluated traits

In this study, the yield-related attributes, such as tuber yield per hectare, tuber yield per plot, tuber yield per plant, number of tubers harvested per plant, number of plants harvested per plot, tuber length and width contributed significantly to the principal component 1 (PC1). This result agrees with Agre et al. (2019) and Darkwa et al. (2020), who reported a significant contribution of these tuber related traits to PC1 in a study on a panel of winged yam and accessions of white yam. Agre et al. (2021b) have reported the effectiveness of yield-related attributes in differentiating accessions of *Dioscorea* spp. Hence, these traits could be employed to screen for superior accessions in yam improvement programmes.

Relationship among evaluated quantitative traits

Highly significant and positive correlations observed among yield-related traits in this study imply that indirect selection could be adopted for significantly correlated traits. The significant positive correlations observed among yield-related traits have been reported by Asfaw et al. (2021) and Padhan et al. (2019). In the present study, YMV severitydid not correlated with yield-related traits. In addition, significant positive correlations between internode length, number of internodes before first branching and stem diameter agree with the findings by Tewedros et al. (2020), who reported a significant positive correlation between

internode length and the number of internodes per vine. Furthermore, significant negative correlations between days to first sprout emergence and tuber yield-related traits indicate that days to sprout emergence do not influence the tuber yield. Sartie et al. (2012) also reported a significant negative correlation between tuber weight and days to sprout emergence. A significant positive correlation observed in this study between dry matter and the number of tubers per plant agreed with the findings of Sartie et al. (2012) in a study on eight white yam landraces.

Clustering pattern of *D. praehensilis* accessions based on quantitative traits

Three clusters constructed based on the 15 quantitative descriptors indicate the extent of genetic differentiation among *D. praehensilis* accessions collected from the three regions of Ghana. Cluster 3 was the most promising group because of the superior tuber yield attributes, high resistance to YMV severity and low tuber flesh oxidation. Cluster 1 also had some promising accessions for resistance to YMV severity and high dry matter content. Additionally, all accessions in cluster 3 have the potential for low tuber flesh hardness, low tuber flesh oxidation and high dry matter content. Initiation of hybridization processes among the promising accessions of *D. praehensilis* could lead to the development of varieties that could meet the farmers and consumers' preference criteria of better post-harvest tuber quality traits such as low tuber flesh oxidation, low tuber flesh hardness and high yield.

Conclusions

This study explored the potential of 39 morphological descriptors to assess the degree of genetic diversity among 162 accessions of *D. praehensilis*. Results showed that improving bush yam for yield-related traits and post-harvest tuber quality could be achieved through exploring genetic diversity using quantitative and qualitative traits. Effectively, differentiated qualitative traits, such as plant sex, leaf shape, leaf colour, tuber shape, tuber size, tuber flesh colour and tuber flesh oxidation were observed among *D. praehensilis* accessions. Diversity indices using quantitative traits also revealed high diversity among the evaluated accessions. High significant genetic variation was observed for traits, such as YMV severity, tuber yield-related traits, days to first sprout emergence, tuber flesh oxidation intensity and tuber flesh hardness across the collection regions and among the accessions. In addition, high GCV and PCV and moderate H^2 were observed among key traits related to tuber yield. Cluster analysis grouped the accessions into three clusters with different attributes. Cluster 3 contained the best performing accessions for tuber yield-related traits. In contrast, cluster 1 produced the best performing resistance to yam mosaic virus (YMV) severity. All three clusters contained good accessions with post-harvest tuber quality attributes and dry matter content. These accessions such as CDpr1, CDpr10, CDpr40, Otim, EDpr3, EDpr13, EDpr20, WNDpr10, WNDpr18 and WNDpr33 were identified for their ability to withstand yam mosaic severity, and accessions such as CDpr24, WNDpr76, CDpr8, EDpr14, CDpr7, WNDpr15, EDpr1, CDpr28, Dp/UP/E/001 and WNDpr93 were identified for their superior tuber yield attributes. These accessions could be employed in a yam breeding scheme to improve bush yam

and the genetic base. Further assessment of these bush yam accessions with high throughput molecular markers is necessary to confirm the results from this study. The combined application of morphological descriptors and molecular markers will elucidate further understanding of the genetic diversity of *D. praehensilis*.

CHAPTER SEVEN

DIVERSITY OF BUSH YAM (*Dioscorea praehensilis* Benth.) ACCESSIONS FROM GHANA USING SNP MARKERS

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Abstract

Bush yam (*Dioscorea praehensilis* Berth.) is a semi-cultivated yam species in West, East and Central Africa. Although bush yam is essential for conservation and breeding initiatives, limited information on its molecular diversity studies is available. This study used 4,525 single nucleotide polymorphism (SNP) markers, generated by the genotyping-by-sequencing (GBS) method, to dissect the genetic diversity among 133 bush yam accessions from Ghana. Hierarchical clustering, admixture and principal component analyses all grouped the *D. praehensilis* accessions into five clusters. Diversity indices and the analysis of molecular variance (AMOVA) revealed high genetic diversity within regions, resulting in a high level of gene flow among the collection regions. This study provided insight into Ghana's bush yam genetic diversity and opened avenues for conservation and genetic improvement efforts in breeding programmes.

Keywords: *Dioscorea praehensilis*, admixture, population structure, genetic diversity, SNP markers, genotyping-by-sequencing.

Introduction

Yam (*Dioscorea* spp.) is a multispecies crop (~600 species) that ranks fourth among root and tuber crops worldwide and second to cassava in West Africa. It has an estimated global production of 74.3 million tons, from nine million hectares in 2019 (FAOSTAT, 2021). Yam is an important crop that plays a crucial role in alleviating food insecurity and poverty in tropical and subtropical areas. It provides high nutritional value in carbohydrates, proteins, fats, fibres, essential minerals and vitamins (Obidiegwu et al., 2020).

Bush yam (*Dioscorea praehensilis* Benth) is one of the wild relatives or semi-cultivated yam species that is native to West (Nigeria, Ghana, Benin and Togo) and Central Africa (Cameroon and the Democratic Republic of Congo) (Pitalounani et al., 2017; Scracelli et al., 2019). Bush yam is potentially high yielding with good agronomic characteristics (Pitalounani et al., 2017; Adewumi et al., 2021). Bush yam has been reported to be utilized to alleviate the problem of food security among the local farmers in countries like Togo (Pitalounani et al., 2017). As the world's population grows, so is the demand for food; and food crops

are threatened by poor culinary attributes, poor resistance to insect pests as well as diseases and poor resilience to climatic changes (Ray et al., 2013; Okoro et al., 2019). This necessitates the introduction and selection of crop wild relatives (CWRs) closely related to the cultivated ones, including all direct crop descendants. These CRWs are sources of important traits such as biotic and abiotic tolerance and high yield traits, which are essential for crop improvement due to their relatedness (Hajjar & Hodgkin, 2007).

Despite these important features, *D. praehensilis* has not been inadequately researched in yam producing countries like Ghana (Adewumi et al., 2021). Poor post-harvest tuber quality attributes, such as severe tuber flesh browning oxidation and hardening after harvest, are the leading factors of its neglect (Pitalounani et al., 2017). It is, therefore, necessary to select and develop new *D. praehensilis* cultivars with enhanced tuber flesh colour and soft texture to boost their production and wide use among farmers. Variations within germplasm are necessary for the successful development of high-performance cultivars (Govindaraj et al., 2015; Ajala et al., 2019). Thus, evaluating genetic variability and similarity among accessions and populations is a prerequisite for breeding programmes to achieve any breeding objective. This information is critical in the choice of parental lines and the detection of genotypes containing key alleles for breeding goals (Chen et al., 2017).

Furthermore, categorizing genetic variability is essential to effective germplasm management and development of core collection of germplasm representing maximum diversity in a small number of accessions (Kumar et al.,

2016). Assessing and understanding genetic diversity within breeding lines, cultivars, landraces and wild relatives) is critical for identifying genes that control beneficial biological processes, which can then be logically employed to improve the existing varieties resulting in enhanced agricultural productivity and sustainable agricultural practices (Onda & Mochida, 2016). Furthermore, investigating diversity in natural plant populations allows researchers to understand better genetic exchange or gene flow within and between populations (Schaal et al., 1998).

Molecular markers have been used in assessing genetic diversity among *Dioscorea* spp. Some of these markers are isozymes (Bressan et al., 2011; Bressan et al., 2014), random amplified polymorphic DNA (RAPD) markers (Zannou et al., 2009), random fragment length polymorphism (RFLP) markers (Terauchi et al., 1992), amplified fragment length polymorphism (AFLP) markers (Sonibare et al., 2010), and simple sequence repeat (SSR) markers (Siqueira et al., 2012; Otoo et al., 2015; Silva et al., 2016; Arnau et al., 2017; Mulualem et al., 2018). However, few studies and information have been reported to use molecular markers to understand the genetic diversity, population structure and relationship among accessions of *D. praehensilis* from Ghana. Simple sequence repeat (SSR) markers have been employed to evaluate genetic diversity in *D. praehensilis* (Djedatin et al., 2017; Adewumi et al., 2020); however, PCR amplification of genomic DNA using SSR markers, on the other hand, can result in sequence artefacts due to errors in Taq DNA polymerase activity and the formation of chimeric and heteroduplex molecules (Brakenhoff et al., 1991; Cline et al., 1996;

Acinas et al., 2005). Artefact generation, particularly in the case of highly polymorphic SSR markers, can complicate allele size calling (Kulibaba & Liashenko, 2016). Alleles of the same size can have distinct sequences (Tsykun et al., 2017). This can also impact the quality of genotyping data (Yu et al., 2021). Amplified fragment length polymorphic (AFLP) markers have also been used for diversity studies in *D. praehensilis* (Scarcelli et al., 2006). This marker system has the disadvantages of low discriminating powers, low accuracy, low genotyping effort, low development effort and high cost (Vignal et al., 2002). These marker systems were found to be poorly distinguishing the diversity within *D. praehensilis* or they were complex and not cost effective to use (Vignal et al., 2002). Moreover, application of high throughput genome-wide variant detection paired with cost-effective SNP assay like genotyping-by-sequencing (GBS) (Davey et al., 2011), should provide better information on the diversity nature, as well as population structure of *D. praehensilis*.

GBS is an outstanding tool for studying genomic diversity (Elshire et al., 2011; Fu et al., 2014). Lower copy regions can be targeted more efficiently with an appropriate restriction enzyme(s) by avoiding repetitive regions of genomes and simplifying computational challenges such as alignment problems in species with high levels of genetic diversity (Elshire et al., 2011). Single nucleotide polymorphism (SNP) markers are markers which arise as a result of point mutations (Vignal et al., 2002; Clarke et al., 2016). SNPs are bi-allelic in nature, with greater genotyping accuracy; high co-dominance and the ability to allow a larger number of markers (tens of thousands or more) to be screened in less time (

Vignal et al., 2002; Clarke et al., 2016). They should be utilized in a large panel of accessions to reveal the actual genetic variation in the crop species. The high heritability nature of SNPs also makes them the markers of choice for studying genetic diversity and phylogeny in crop species when compared with other markers (Vignal et al., 2002; Clarke et al., 2016). Genotyping by sequencing (GBS) paired with SNP markers has been reported to be effective for diversity studies in yam species, such as white Guinea yam (Bhattacharjee et al., 2020), greater yam (Agre et al., 2019; Sharif et al., 2020), and trifoliate yam (Siadjeu et al., 2018). Limited information is available on using SNP markers to evaluate genetic diversity, population structure and relationships among *D. praehensilis* accessions from Ghana. The only available studies evaluated *D. praehensilis* and other species, such as *D. rotundata*, *D. cayenensis*, *D. abyssinica*, *D. togoensis*, *D. burkilliana* and *D. mangenotiana* (Girma et al., 2014; Scarcelli et al., 2019). This indicates that the genetic diversity in *D. praehensilis* has been underexplored, as it was just a secondary component among the species evaluated.

In this study, we used single nucleotide polymorphism (SNP) markers from the GBS platform to assess the genetic divergence in a panel of 162 *D. praehensilis* (bush yam) accessions collected from three regions of Ghana. The study objectives were to: (i) assess the genetic diversity among *D. praehensilis* accessions from three regions of Ghana using SNP markers, and (ii) understand the genetic structure and differentiation of *D. praehensilis* along a regional gradient.

Materials and Methods

Description of germplasm

One hundred and sixty-two (162) accessions of *D. praehensilis* which were germplasm collections of 2019 across three predominant regions (Central, Eastern and Western North) of cultivation in Ghana, were used in this study. Seventy-two (72) of these accessions were collected from the Central Region, 25 from the Eastern Region and 66 from the Western North Region, as indicated in (Table 6.1; Chapter 6).

DNA extraction and genotyping

Young fresh leaves from a single plant for each accession were collected from a field experiment trial conducted between December 2019 and November 2020 at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast, Ghana, located in the Central Region with semi-deciduous forest and coastal savannah ecozones. The experimental location was on latitude 5°07´7.6´´N and longitude 1°17´18.9´´W based on Global Positioning System (GPS). About 15g of silica gel in small white plastic containers were used to dry 5g of leaves for three days and later preserved in Ziploc bags. Dried leaf samples of 4 mm diameter for each genotype were collected into three 96-deep well sample collection plates. DNA extraction, library preparation, genotyping-by-sequencing (GBS) and SNP marker development were conducted at Intertek AgriTech in Sweden.

The DNA was extracted using a technique developed by Intertek-AgriTech (http://www.intertek.com/agriculture/agritech/) and based on the LGC oKtopure™ automated high-throughput 'sbeadex™' DNA extraction and purification system (https://www.biosearchtech.com/). Magnetic separation is used in the 'sbeadex™' technique to prepare nucleic acids. The first stage in this process is to homogenize leaf tissue samples in 96-deep-well plates using steel bead grinding. The ground tissue is treated with a DNA extraction buffer using LGC's 'sbeadex™' kit for plant DNA preparation (https://www.biosearchtech.com/). Finally, super-paramagnetic particles coated with 'sbeadexTM' surface chemistry that catches nucleic acids from a sample are used to purify extracted DNA. Purified DNA is eluted and used in downstream procedures.

The GBS analysis was carried out using the approach described by Lu et al. (2013). Purified genomic DNA was first digested with the restriction enzyme PstI and then, T4 ligase was used to ligate tailored adapters (barcodes). Following that, sequencing was performed using flow-cell attachment site tagged primers. Illumina HiSeq2000 was used for single-end sequencing. Each sequencing result's reads and tags were aligned to the *D. rotundata* reference genome v2 (https://drive.google.com/folders/1H5T4xjKAEl9LliR-4qK IR6TypCDe8nj) using Hisat2 (Kim et al., 2015). KDcompute (https://kdcompute.seqart.net/kdcompute, accessed on 30 November 2021) was used to convert the raw HapMap file to a Variant Call Format (VCF). Using the software PLINK 1.9 and VCFtools, SNP-derived markers were filtered to remove unnecessary SNP markers for quality

control. Markers and 29 accessions with more than 20% missing data were removed. Rare SNPs with 5% minor allele frequencies and low coverage read depth (5) were also eliminated. In the end, only 4,525 informative SNP markers and 133 *D. praehensilis* accessions were used for the subsequent analysis.

Data analysis

VCFtools (Danecek et al., 2011) and PLINK 1.9 were used to estimate summary statistics, such as observed and expected heterozygosity, minor allele frequency (MAF) and the polymorphic information content (PIC) (Purcell et al., 2007). SniPlay web base was used to determine mutation transversion and transition (Purcell et al., 2007). SNP Dosage format (0, 1, 2) was generated in Plink using the recodeA function, where 0 is the homozygote reference, 1 is the heterozygote, and 2 is the homozygote alternative. Dosage format was then subjected to Jaccard dissimilarity matrix using philentropy R package (Drost, 2018). Using the vegan library, the Jaccard dissimilarity matrix was then used to estimate genetic diversity indices such as Shannon–Wiener Index (H′), Inverse Simpson's (HB), Simpson's Diversity Index (D) and Pilou evenness (J) to quantify the level of genetic diversity within and among surveyed regions of Ghana. The binary file generated from the VCF file was then subjected to admixture analysis using the R package 'adegenet' (Jombart et al., 2010). After varying the number of clusters from 2 to 40, the optimal number of clusters was determined using k-means analysis. Accessions with membership proportions (Q-value) of ≤ 50% were assigned to groups using admixture analysis. Those with membership probabilities of > 50% were classified as admixtures (Salazar et al., 2017). The

generated Jaccard dissimilarity matrix was used for the hierarchical cluster (HC) and principal component analysis (PCA). The HC was plotted using the Ward.D2 method. An identity by state (IBS) dissimilarity matrix was generated in TASSEL 5.0 (Bradbury et al., 2007). The Jaccard dissimilarity matrix was then subjected to AMOVA using GenAlEx v. 6.503 (Peakall & Smouse, 2006) to partition genetic variance components among and within regions of collection.

Results

Characteristics of 4,525 SNPs retained for assessing diversity in *D. praehensilis* germplasm collection

A total of 4,525 SNPs were retained and unequally distributed across 20 *D. praehensilis* chromosomes. The number of SNPs per chromosome ranged from 94 SNPs (chromosome 13) to 487 SNPs (chromosome 5) (Table 7.1; Figure 7.1). The transition accounted for the largest part of mutations (2,757; 60.93%), while transversion accounted for 1,768 SNPs (39.03%) of the *D. praehensilis* genome (Figure 7.2). In the transition mutations, A/G had the highest rate of occurrence (1,575), while in the transversion mutations, A/T had the highest rate of occurrence (668). The average PIC of the SNPs across the 20 chromosomes was 0.1, while minor allelic frequency (MAF) ranged from 0.071 to 0.116, with a mean of 0.093. The observed heterozygosity (Ho) varied from 0.096 to 0.187 with an average of 0.127, while the expected heterozygosity (He) ranged from 0.098 to 0.175, with an average of 0.124.

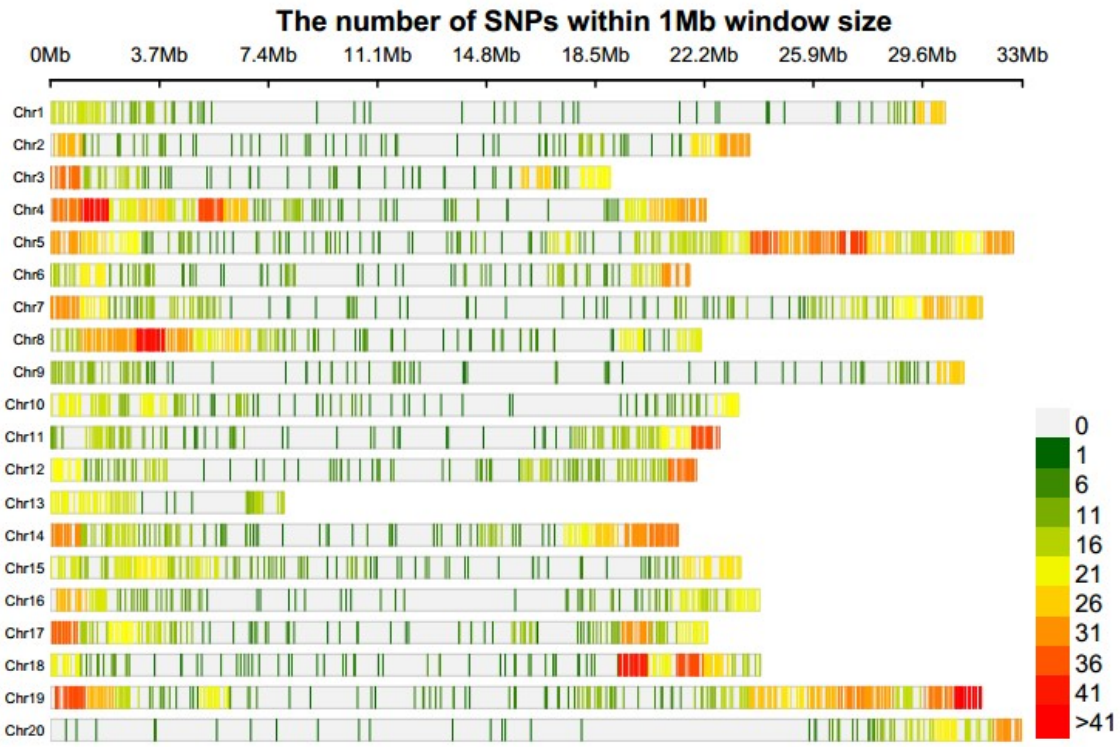


Figure 7. 1. Distribution and density of filtered SNPs across 20 yam pseudo chromosomes. The horizontal axis displays the chromosome length. The SNP density is indicated at the bottom right.

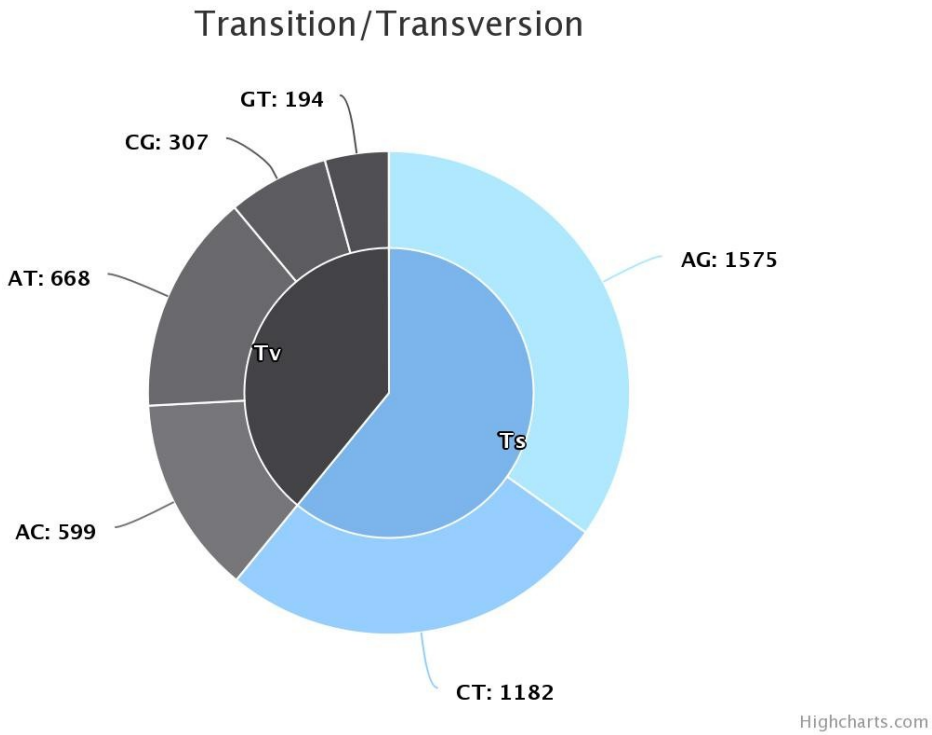


Figure 7. 2. Transition and transversion based on bi-allelic SNP markers. Tv: Transversions; Ts: Transitions; A: Adenine; T: Thymine; G: Guanine; C: Cytosine. Chart developed using SNIPLAY software.

Table 7. 1. Characteristics of the 4,525 SNP markers retained for analyses across the 20 chromosomes

Chromosome	Number of SNPs	Ho	He	MAF	PIC
1	136	0.147	0.123	0.096	0.098
2	165	0.124	0.126	0.093	0.103
3	159	0.097	0.121	0.084	0.100
4	340	0.096	0.098	0.071	0.080
5	487	0.128	0.125	0.093	0.100

6	179	0.098	0.112	0.080	0.092
7	281	0.106	0.118	0.087	0.096
8	303	0.112	0.118	0.087	0.095
9	139	0.137	0.131	0.099	0.105
10	171	0.166	0.150	0.113	0.121
11	181	0.100	0.101	0.071	0.083
12	189	0.187	0.175	0.134	0.138
13	94	0.133	0.130	0.095	0.105
14	256	0.117	0.112	0.083	0.091
15	232	0.120	0.115	0.086	0.092
16	176	0.100	0.109	0.081	0.089
17	229	0.143	0.128	0.101	0.102
18	223	0.160	0.147	0.116	0.116
		0.119	0.122	0.090	0.099
20	138	0.142	0.124	0.095	0.100
Total/	4525	0.12	0.124	0.093	0.100

Average	7

Ho: Observed heterozygosity; He: Expected heterozygosity; MAF: Minor allelic frequency; PIC: Polymorphism information content

Population structure and principal component analysis among *D. praehensilis* accessions

Population structure analysis, at a minimum value of $k = 5$ based on cross-validation error (CV error), discriminated *D. praehensilis* accessions into five clusters as the maximum number of genetic groups (Figure 7.3). Accessions with membership coefficients ≤ 0.50 were assigned to the corresponding pure groups, while those with coefficients > 0.50 were assigned to the admixture group. Among 133 *D. praehensilis* accessions, 117 (~ 88%) were assigned to genetic groups by the admixture analysis, while the remaining 16 (~12%) were considered as admixtures (Figure 7.3). The admixture accessions were EDpr2, Odonorbig, EDpr4, EDpr3, CDpr49, CDpr37, WNDpr66, WNDpr77, CDpr35, WNDpr69, WNDpr68, CDpr75, CDpr24, WNDpr8, WNDpr82 and WNDpr22.

The first and second principal components explained a total molecular variation of 69%, with PC1 and PC2 accounting for 48 and 21 % of the genetic variation, respectively (Figure 7.4). The 133 accessions were grouped into five clusters regardless of their collection regions. The principal component (PCA) was used to establish the stability of the potential population structure. The PCA resulted in five major clusters among the evaluated *D. praehensilis accessions* (Figure 7.4). The PCA also revealed some level of admixtures across the collection regions (Figure 7.4).

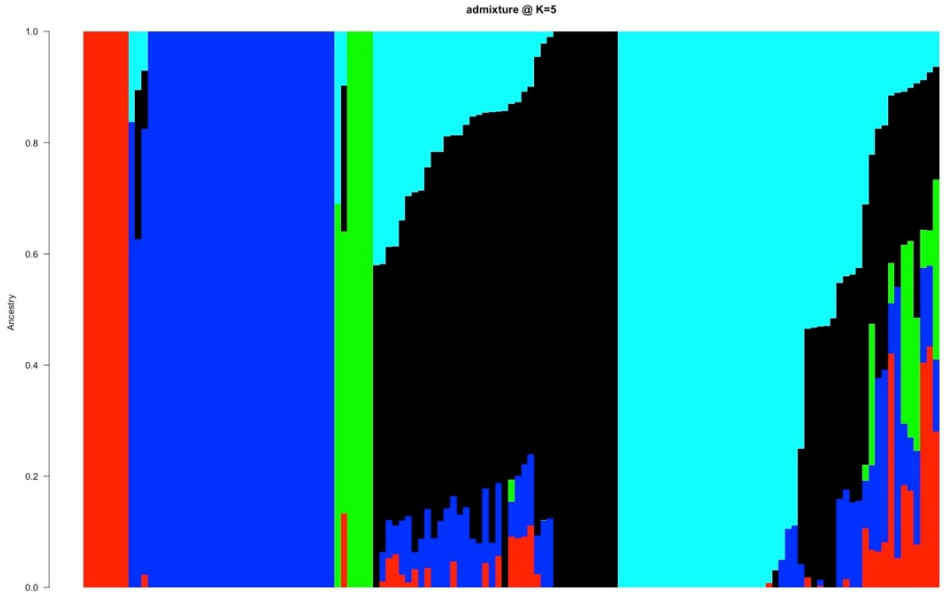


Figure 7. 3. Graphical representation of the 133 bush yam accessions' population structure based on admixture analysis. Subpopulations were set at $k = 5$.

The colours represent the five clusters: Cluster 1 (red), cluster 2 (green), cluster 3 (blue), cluster 4 (black) and cluster 5 (mint green) based on a membership coefficient of ≥50%.

Figure 7. 4. Principal component analysis plot showing clustering of the 133 *D. praehensilis* accessions into five clusters.

Each colour represents a cluster: cluster 1 (red), cluster 2 (green), cluster 3 (blue), cluster 4 (black), and cluster 5 (yellow).

Genetic diversity and differentiation based on 4,525 SNPs across regions of collection

The diversity across collection regions using 4,525 SNP markers is presented in Table 7.2. The observed Simpson's diversity index (λ) (0.99) was recorded across the three Regions where *D. praehensilis* germplasm was collected. The Shannon diversity index (H′) ranged between 4.82 for the Western North Region and 4.87 for the Eastern Region. The inverse Simpson's index (HB) ranged from 121.16 for the Western North Region to 128.52 for the Eastern Region. The Pilou evenness (J) varied from 0.53 to 0.54 across the regions.

On the basis of the identity by state (IBS) matrix, the genetic distance, based on differences at marker loci between pairs of individual accessions, ranged from 0.011 to 0.202. The highest genetic distance (0.202) was observed between CDpr45 and CDpr18; both accessions were collected from the Central Region. The lowest genetic distance (0.011) was observed between WNDpr98 and WNDpr13, and WNDpr9 and WNDpr13, all the three were collected from the Western North Region. The highest genetic distance within Eastern Region accessions was between EDpr22 and EDpr7 (0.099), while the highest genetic distance within Western North accessions was observed between WNDpr63 and WNDpr94 (0.178).

The analysis of molecular variance (AMOVA) revealed high significant genetic variability within regions (3.76; 87%), while only 13% of the variability was among regions of collection (Table 7.3). Pairwise fixation index (Fst) and Nei genetic distances among regions were below 0.5 (Table 7.4). The Fst ranged from

0.03 to 0.06, while Nei genetic distance varied from 0.12 to 0.44. The highest Nei genetic distance was observed between Central and Eastern regions, while the least was observed between Eastern and Western North. The highest Fst value in this study was between Eastern and Western regions, while the least was between Central and Eastern regions.

Table 7. 2. Diversity indices among evaluated regions based on SNP markers

Diversity indices	Across (133)	Central (58)	Eastern (17)	Western (58)	North
Λ	0.99	0.99	0.99	0.99	
H′	4.84	4.84	4.87	4.82	
HB	123.54	124.46	128.52	121.16	
J	0.53	0.53	0.54	0.53	

λ: Simpson's diversity index; H′: Shannon diversity index; HB: Inverse Simpson index; J: Pilou evenness

Table 7. 3. Analysis of molecular variance among 133 accessions of *D. praehensilis* using 4,525 SNP markers

Sources of variation	Df	SS	MS	Estimated variance	% Variation
Among regions	2	52.310	26.155	0.558	13%

Within regions	130	489.006	3.762	3.762	87%
Total	132	541.316		4.320	100%
PhiPT				0.129	
P(rand >= data)				0.001	

df: degree of freedom; SS: Sum of square; MS: Mean square

Table 7. 4. Pairwise fixation index and Nei genetic distances between the collection regions

Regions	Nei genetic distance	Pairwise fixation index (Fst)
1-2	0.44	0.03
1-3	0.34	0.05
2-3	0.12	0.06

1: Central; 2: Eastern; 3: Western North

Hierarchical clustering of *D. praehensilis* accessions based on SNP markers

The phylogenetic dendrogram based on 4,525 SNPs grouped the 133 *D. praehensilis* accessions into five distinct clusters, with each cluster containing

accessions from different regions (Fig. 7.5). The number of accessions per cluster ranged from 52 accessions for cluster 2 to five accessions for cluster 5. Cluster 1 had 32 members, of which 30 were from the Western North Region and two from the Central Region. Cluster 2 comprised 51 accessions, with 27 originating from the Central region, 15 from the Eastern Region, and nine (9) from the Western North Region. Cluster 3 had 16 accessions, 11 collected from the Western North Region and five from the Central Region. Cluster 4 contained 29 accessions with 23 accessions from the Central Region, four (4) from the Western North Region and two (2) from the Eastern Region. Cluster 5 consisted of five (5) accessions, with four (4) originating from the Western North and one from the Central regions.

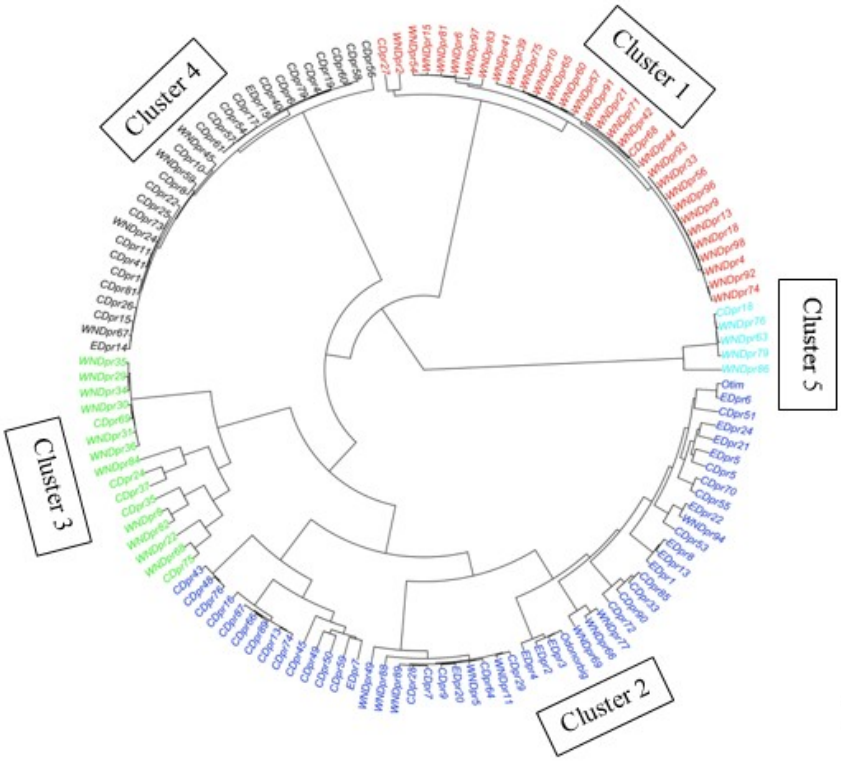


Figure 7. 5. Hierarchical clustering dendrogram of *D. praehensilis* accessions based on 4,525 SNPs generated using the UPGMA method and Jaccard's dissimilarity matrix.

Different colors refer to different groups: Cluster 1 (red), Cluster 2 (blue), Cluster 3 (green), Cluster 4 (black), and cluster 5 (mint green).

Discussion

Understanding the genetic basis of a crop's wild relatives is paramount for their conservation, management and usage for crop improvement through hybridization. The present study assessed the genetic diversity among *D. praehensilis* accessions collected from three major producing regions of Ghana to enhance their utilization as sources of genes of interest for crop improvement.

Low allelic diversity observed in evaluated genetic parameters, such as PIC, MAF, Ho and Ho, using 4,525 SNPs was contrary to allelic variability reported by Adewumi et al. (2020) using SSR markers in estimating genetic diversity in bush yam collection from the Central Region of Ghana. The low allelic variability resulted from the fact that SSR markers are co-dominant, while SNP markers are bi-allelic. Lower allelic diversity in SNPs compared to SSRs has been reported in rutabaga accessions (Yu et al., 2021), rice (Singh et al., 2013), and barley (Varshney et al., 2008). Low allelic variability in genetic characteristics has also been reported in other yam species such as *D. rotundata* (Bhattacharjee et al., 2020; Agre et al., 2021b), *D. alata* (Agre et al., 2019; Bakayoko et al., 2021) and *D. dumetorum* (Siadjeu et al., 2018) using SNP markers.

High diversity indices such as Shannon, Simpson, inverse Simpson and Pilou evenness were recorded across regions, indicating high inter-region bush

yam propagule exchange and social network in Ghana. Farmers, for example, frequently trade landrace seeds with other farmers in their areas or engage in defining localities.

The structural analysis revealed five clusters with a large proportion of admixtures among the evaluated accessions of *D. praehensilis*. All the collection regions consisted of two or more admixtures suggesting high genetic exchange and high flow of genetic materials across the collection regions of *D. praehensilis* accessions. These high admixtures could also result from *D. praehensilis* being termed as the progenitor and source of domestication of white Guinea yam (Scarcelli et al., 2006, 2017, 2019). A high proportion of admixtures has been reported in other yam species such as *D. alata* (Agre et al., 2019).

The grouping of *D. praehensilis* accessions into five clusters agrees with admixture analysis. The PCA showed a higher association among accessions within a cluster. Our finding through PCA suggests that the accessions within a cluster are similar and could be considered to improve these yam species through a hybridization process to broaden the genetic base of the yam breeding programme (Darkwa et al., 2020).

Hierarchical clusters grouped the accessions into five clusters irrespective of their collection regions. Higher number of clusters observed in this study indicates that SNP markers are capable of revealing valuable information about genetic divergence among *D. praehensilis* accessions. This encourages the detection of superior accessions that can improve the *D. praehensilis* genetic base. The results from this study suggests the ability of SNP markers in revealing the

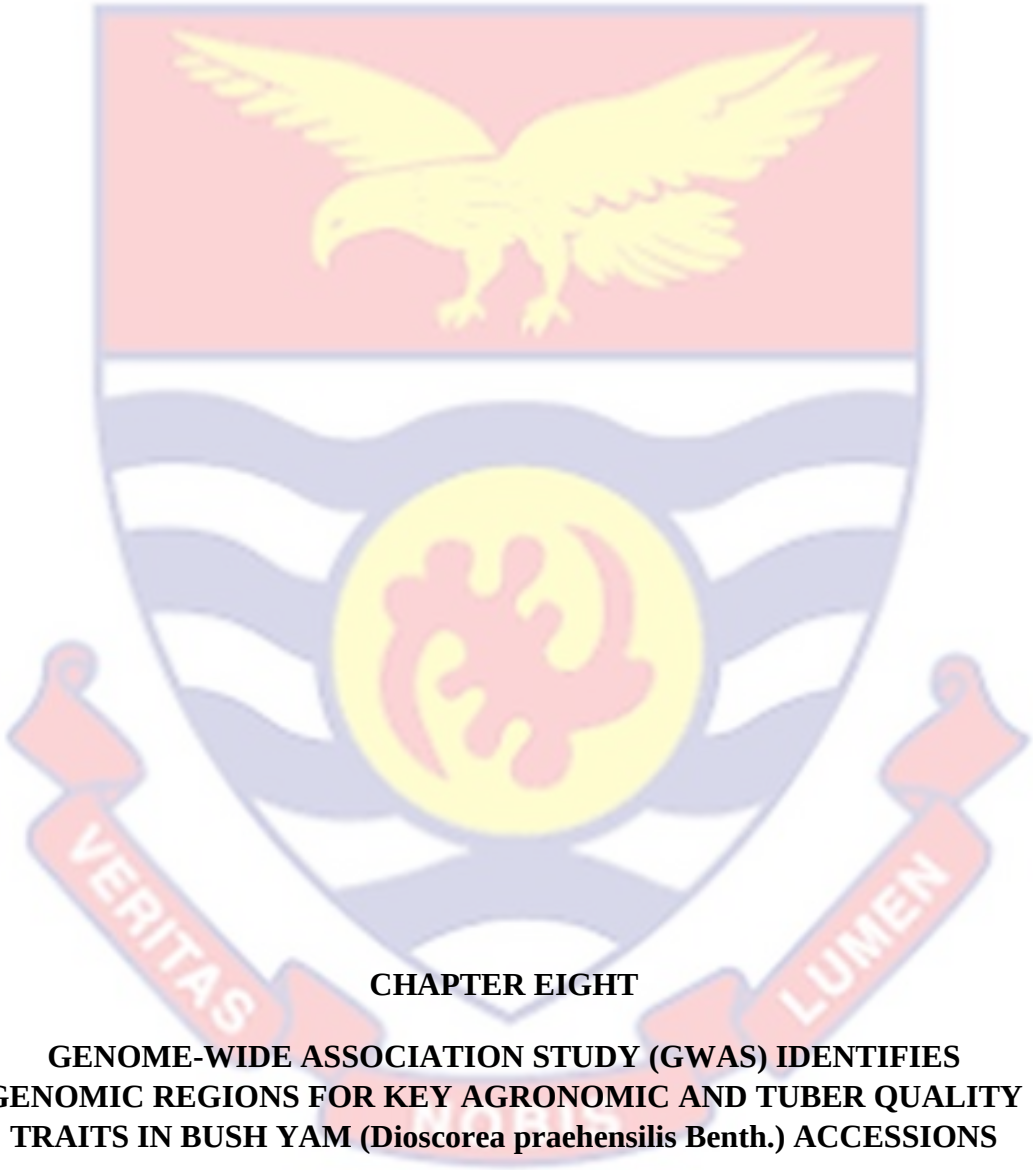
level of genetic diversity among accessions of *D. praehensilis*. These findings have also been reported in other species of yam such as white yam (Darkwa et al., 2020), greater yam (Agre et al., 2019) and trifoliate yam (Siadjeu et al., 2018). Grouping of *D. praehensilis* accessions into five clusters by structural analysis, principal component analysis and hierarchical clustering is an indication that SNP markers are highly discriminating the genetic diversity among clonally cultivated crops.

Based on Wright's F-statistics (Wright, 1984), Fst assesses the amount of genetic diversity that can be explained by population structure. A Fst number of 0 means there is no differentiation between subpopulations, whereas a value of 1 means there is complete differentiation. In differentiated populations, an Fst value greater than 0.15 can be regarded as significant (Frankham, Ballou, Briscoe, & Ballou, 2002). In this present study, no significant divergence was observed across the three collection regions. This might be due to the movement of genetic materials or the exchange of planting materials among the farmers in the three regions. In addition, analysis of molecular variance (AMOVA) revealed the genetic differentiation within and among collection regions was significant. The genetic differentiation was higher within the regions but lower among the regions. The considerable genetic variance found across accessions within regions in AMOVA suggested that human pressure was exerted on the spread of planting propagules between regions. This result confirms the large proportion of admixtures that was observed within the regions of collection. High genetic variability within the collection regions is an indication of high gene flow

between accessions across the regions of collection. This is an indication that farmers perceived the need to adopt accessions with superior and preferred traits from other regions into their regions. The current study confirmed the findings of Agre et al. (2021b) in Benin, where high diversity was identified within groups and areas as a reflection of farmers' diverse trait preferences, which could not be met in a single cultivar.

Conclusions

This study clustered 133 *D. praehensilis* accessions from Ghana into five distinct groups using admixture, principal component and hierarchical cluster analyses. The genetic diversity within regions was higher than among regions. Clustering of *D. praehensilis* into several clusters indicates the development of heterotic groups that can develop superior cultivars with preferred farmers, consumers and end-users quality traits. The identified clusters could be used to facilitate future bush yam germplasm conservation, management and breeding.



CHAPTER EIGHT

GENOME-WIDE ASSOCIATION STUDY (GWAS) IDENTIFIES GENOMIC REGIONS FOR KEY AGRONOMIC AND TUBER QUALITY TRAITS IN BUSH YAM (Dioscorea praehensilis Benth.) ACCESSIONS

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Abstract

Bush yam (*Dioscorea praehensilis* Benth.) has emerged as a food highly valued by local people for alleviating hunger during times of food scarcity. Limited information on yield potentials and resistance to yam mosaic virus and poor post-harvest tuber quality traits have significantly hindered its potential to support rural development and meet consumers' needs as an affordable tuber crop. Agronomic and tuber quality traits significantly determine the acceptability of this yam

species by farmers and end-users. Hence, conducting a genome-wide association study to identify quantitative trait nucleotides controlling agronomic and tuber quality traits and mining relevant candidate genes will expedite the improvement of bush yam through marker-assisted breeding. Using the mixed linear model (MLM), marker-trait association analysis identified twenty-one SNPs associated with the evaluated traits. The identified SNPs accounted for approximately 16% of the total phenotypic variation. Gene annotation of significant SNPs identified candidate genes with functions related to growth and development of tubers, reduction of tuber flesh oxidation and defense mechanisms against yam mosaic virus. This study provides the first valuable insight for understanding the genetic basis of agronomic and tuber quality traits in bush yam. Following validation, the markers and candidate genes identified in this novel study could provide valuable genomic resources for use in marker-assisted selection (MAS) and genomic selection (GS) to speed up the genetic improvement of agronomic and tuber quality traits in West African bush yam breeding programmes.

Keywords: SNP markers, GWAS, putative genes, post-harvest traits, bush yam

Introduction

Dioscorea spp is a significant root crop for alleviating poverty and food insecurity among the larger populations in the tropic and sub-tropic regions of the world (Cormier et al., 2019a; Wu et al., 2019). The average annual global production of yam in 2019 was 74.3 million tons, and West Africa accounted for the highest average yearly production of 69.8 million tons (FAOSTAT, 2021).

Dioscorea spp comprises more than 650 species, including the wild relative or semi-cultivated species, such as *D. praehensilis* and *D. abyssinica* and cultivated species, such as *D. rotundata* and *D. cayenensis* and *D. alata* (Scarcelli et al., 2019). Bush yam (*Dioscorea praehensilis* Benth.) is one of the relative wild species of yam that is widely distributed in rainforest zones of sub-Saharan African countries like Ghana, Nigeria, Benin, Togo and Cameroon (Scarcelli et al., 2019). *D. praehensilis* develops huge tubers with a good amount of starch content (Alexis, 2013), making it an ideal choice for combating hunger. This wild yam has obviously appeared as a food greatly valued by local people for alleviating hunger during food scarcity in many rural areas across sub-Saharan Africa (Pitalounani et al., 2017). *D. praehensilis* shares many morphological, physiological, genetic and sensory resemblances with the most recognized African Guinea yam (*D. cayenensis - D. rotundata* complex) (Dansi et al., 1999).

Despite these economic potentials of *D. praehensilis*, limited information is available on the yield potential and response to yam mosaic virus (YMV) severity. Farmers and end-users preferences of *D. praehensilis* have also been reported to be hindered by poor post-harvest tuber quality attributes, including spontaneous change in tuber flesh colour (oxidative enzymatic browning) and postharvest hardening phenomenon characterized by the inability of tuber flesh to remain soft a few days after harvesting (Adewumi et al., 2021). The quality characteristics of yam cultivars are critical for the acceptability of their cultivation and consumption. Breeding programmes routinely measure traits, such as starch and sugar content, tuber flesh colour and oxidation because they impact the

suitability of improved cultivars (Arnau, Maledon, Nudol, & Gravillon, 2016). The spontaneous change in colour of harvested crops, either vegetables, fruits or roots and tubers from white or yellow to brown, black or purple is a result of polyphenol oxidation, which influences the unacceptable changes in organoleptic characteristics and culinary qualities of agricultural produce of significant importance (Chi et al., 2014; Graham-Acquaah, Ayernor, Bediako-Amoa, Saalia, & Afoakwa, 2014; González et al., 2020). Polyphenol oxidase acts on phenols and converts them to quinines, resulting in dark-brown precipitates in plant produce (González et al., 2020). This oxidative browning has also been attributed to changes in the taste and texture of agricultural produce (Jukanti, 2017). More than 50% of the economically significant crops have been reported lost due to oxidative browning in the tropics and sub-tropics (Jiang, Duan, Qu, & Zheng, 2015).

Lignification and cell wall thickening have been identified as the major factors resulting in the tuber flesh hardening after cooking a few days after harvesting (Afoakwa & Sefa-Dedeh, 2002). The post-harvest hardening of *Dioscorea* spp is divided into a forward and backward reaction linked with phytate decrease and an irrevocable reaction linked with total phenol increase (Medoua & Mbofung, 2006). The post-harvest hardening mechanism is thought to begin with phytate enzymatic hydrolysis and then migrate to the released divalent cations to the cell wall, where they cross-react with demethoxylated pectins in the middle lamella. This initiates the lignification process, in which aromatic

compounds accumulate on the surface of the cellular wall and react as lignification precursors (Medoua & Mbofung, 2006).

However, breeding efforts have not been fully implemented due to lack of understanding of the genetic basis of agronomic and tuber quality attributes to enhance the development of improved cultivars. The agronomic and tuber quality traits are under the control of quantitatively inherited genes (i.e., these traits are polygenic), thus making the improvement of these traits difficult using conventional breeding approaches (Darkwa et al., 2020). Understanding the genetic basis of variation in bush yam's agronomic and tuber quality attributes is crucial for enhancing their selection efficiency, shortening the breeding cycle and the rate of genetic gain.

QTL mapping and genome-wide association studies (GWAS) are popular methods for identifying genetic loci that control complex traits (Stanley et al., 2021). However, the QTL mapping approach has limitations, including limited allelic diversity and mapping resolution due to a lack of recombination events (Kraakman, Niks, Van Den Berg, Stam, & Van Eeuwijk, 2004). In contrast, GWAS investigates ancestral recombination in naturally genetically diverse populations to dissect complex traits (Gómez, Álvarez, & Mosquera, 2011). GWAS is superior to QTL mapping in that it improves the resolution of QTLs due to accumulated meiotic events and shortens the time required to develop mapping populations (Darvishzadeh, 2016). GWAS is an effective method for detecting genomic regions associated with important complex quantitative traits and predicting or identifying causative genes (Brachi, Morris, & Borevitz, 2011). The

application of GWAS has been reported in other yam species. Candidate genes linked to tuber yield and YMV severity have been reported in white yam (Agre et al., 2021). Mondo et al. (2021) also used GWAS to detect genomic regions linked to sex determination and compatibility traits in *D. alata*. GWAS has also been employed to identify candidate genes associated with oxidative browning and dry matter contents in greater yam (Gataraira et al., 2020). GWAS has also been used in other root and tuber crops, such as cassava, for key tuber quality traits of root and tuber crops: waxy starch in cassava (do Carmo, e Sousa, Brito, & de Oliveira, 2020), provitamin A carotenoid content in cassava (Esuma et al., 2016), dry matter content and total carotenoid in cassava (Rabbi et al., 2017). Using GWAS, no information has been reported in *D. praehensilis* to reveal the genetic mechanisms controlling agronomic and tuber quality attributes.

The current study aimed to identify genomic regions and single-nucleotide polymorphisms (SNPs) associated with agronomic and tuber quality attributes in *D. praehensilis* accessions. These findings will serve as a basis to efficiently initiate strategies for selecting and breeding bush yam cultivars with superior agronomic and desirable postharvest tuber quality traits to enhance yam improvement programmes.

Materials and Methods

Genetic materials and experimental site

The GWAS panel comprised 162 *D. praehensilis* accessions with 71 accessions collected from the Central Region, 25 from the Eastern Region, and 66 from the Western North Region with varied agronomic and tuber quality traits as

established in (Table 6.1, Chapter 6).

The accessions were established in two growing seasons, 2020 and 2021, at Teaching and Research Farm, School of Agriculture, University of Cape Coast, Ghana (5°07´7.6´´N, 1°17´18.9´´W; 15 m above sea level) located in the Central Region with semi-deciduous forest and coastal savannah ecological zones. The annual rainfall for the experiment period was 1246.2 mm for the 2020 season and 1,170.2 mm for the 2021 season; the average maximum and minimum temperatures for the 2020 season were 27.9 and 26.9°C, while the 2021 season was 28.6 and 25°C, respectively. The average relative humidity for the 2020 season was 75.7%, while the 2021 season was 81.2%. The soil on this experimental site is sandy loam with pH (6.72), organic carbon was (1.31%), available phosphorus was (754.6 ug/g), and potassium was (0.081 cmol/kg).

Phenotyping

Phenotypic data were collected on yam mosaic virus (YMV) severity scores, the number of tubers per plot, tuber yield (tons per hectare), dry matter content, tuber flesh hardness and tuber flesh oxidation using yam standard operating protocols (Asfaw, 2016).

From 2 to 6 months after planting, the YMV severity score was established at 30 days intervals by visually analysing the relative area of plant leaf surfaces damaged by mosaic virus disease on an ordinal scale of 1–5. A score of 1 indicates no visible symptoms of virus infection; a score of 2 indicates mild mosaic, vein banding, green spotting or flecking, curling and mottling on a few

leaves but no leaf distortion; a score of 3 indicates a low incidence of the mosaic virus on the entire plant (25–50%); a score of 4 indicates severe mosaic on most leaves and leaf distortion, and a score of 5 indicates severe mosaic and bleaching with severe leaf distortion and stunting. Following the trapezoidal approach according to Campbell & Madden (1990), the virus severity scores were used to determine the area under the disease progression curve (AUDPC) as follows:

$$AUDPC = \sum_{i=1}^{N} \left(\frac{y_{i+yi+1}}{2} \right) \left(t_{i+1} - t_i \right) \quad (8.1)$$

Where: N is the number of observations, y_i is the disease severity at i^{th} observation, t_i is the time at i^{th} observation.

The number of tubers harvested per plot was assessed through hand-counted and recorded at harvesting.

The plot yield was extrapolated to the yield in tons per hectare using the following formula:

$$TTYH = \frac{TTWP \times 10}{PLS} \quad (8.2)$$

Dry matter content was estimated from each accession by sampling pest and disease-free tubers from the replications. Tubers from each accession were washed with running water to remove debris and soil particles. The tuber skin was peeled off and tuber flesh grated to smaller sizes to encourage even drying. A mass of 100g of grated tuber flesh from each accession was collected into rectangular-shaped aluminium foil bags and dried in an oven at 105°C for 24

hours. Percentage dry matter content was estimated for each genotype as follows:

$$\% \text{ dry matter content} = \frac{Dry\,tuber\,weight\,(g)}{Fresh\,tuber\,weight\,(g)} * 100 \quad (8.3)$$

Tuber flesh oxidative browning was evaluated by sampling disease and insect-free tubers from each genotype per replicate. These tubers were also washed under running water, air-dried and the skin peeled off. The peeled flesh tuber was cut into three portions (head, middle, and tail). The middle portion was chopped to obtain small tuber flesh of 5 cm diameter and 0.5 mm thickness (Gatarira et al., 2020). Hunter parameters (L*, a*, b*) were used to measure the colour of small tuber flesh using a portable chromometer or colourimeter (CHN Spec, CS-10, Baoshishan, China) immediately the surface was cut and exposed to air (0 minutes) and 60 minutes after the cut surface was exposed to air. The brightness coordinate L* is used to measure the whiteness of sample ranging from black (0) and white (100), a* coordinate is a redness (positive value) or greenness (negative value), and b* coordinate represents the yellowness (positive value) or blueness (negative value) (Abano, Ma, & Qu, 2012). White and black tiles were used to calibrate the colourimeter before each measurement. The colour change¿¿) was estimated using the formula:

$$\Delta E^{¿} = \sqrt{\Delta L^{¿2} + \Delta a^{¿2} + \Delta b^{¿2}} \quad (8.4)$$

where $\Delta E^{¿}$ is total colour change, $\Delta L^{¿}$ is the change between white and black, $\Delta a^{¿}$ is the change between red and green, while $\Delta b^{¿}$ is the change between yellow and blue.

Oxidative browning was calculated using the formula:

Oxidative browning = $F\Delta E^{¿} - I\Delta E^{¿}$ (8.5)

Where $I\Delta E^{¿}$ is the initial colour change, while $F\Delta E^{¿}$ is the final colour change

The procedure employed by Siadjeu et al. (2016) with slight modification was used in assessing the postharvest hardening of bush yam (*D. praehensilis*) accessions. Tuber flesh samples of sizes 5-cm diameter and 1-cm thickness from each accession in each replicate were assessed for tuber flesh hardening using a digital penetrometer at a 6.00 mm probe. Three measurements were taken from each accession in each replicate and the averages calculated and expressed in Newtons.

Genotyping

DNA samples were extracted for each accession using LGC oKtopure™ automated high-throughput 'sbeadex™' DNA extraction and purification system (https://www.biosearchtech.com/), which is frequently used at Intertek-AgriTech (http://www.intertek.com/agriculture/agritech/). The 'sbeadex™' technology prepares nucleic acids using magnetic separation. The first stage in this process is to homogenize leaf tissue samples in 96-deep-well plates using steel bead grinding. LGC's plant DNA preparation 'sbeadex™' kit (https://www.biosearchtech.com/) was used to incubate the ground tissue with a DNA extraction buffer. Finally, super-paramagnetic particles coated with 'sbeadexTM' surface chemistry absorb nucleic acids from a sample and are used

to purify extracted DNA. Purified DNA is eluted and used in downstream operations.

The GBS analysis was performed following the method of Lu et al. (2013). In brief, purified genomic DNA was first digested with the restriction enzyme PstI, and then customized adapters (barcodes) were ligated with T4 ligase. Following that, sequencing was performed using flow-cell attachment site tagged primers. Illumina HiSeq2000 was used for single-end sequencing. Reads and tags found in each sequencing result were aligned to the *D. rotundata* reference genome v2 (https://drive.google.com/drive/folders/1H5T4xjKAEl9LliR-4qK_IR6TypCDe8nj) with Hisat2 (Kim, Langmead, & Salzberg1, 2015). The raw HapMap file generated was first converted to a Variant Call Format (VCF) using KDcompute (https://kdcompute.seqart.net/kdcompute, accessed on 30 November 2021). SNP-derived markers were filtered to remove unwanted SNP markers for quality control using the software PLINK 1.9 and VCFtools. Markers and (twenty-nine) 29 accessions with more than 20% missing data were removed. Rare SNPs with 5% minor allele frequencies and low coverage read depth (5) were also eliminated. In the end, only 4,525 informative SNP markers and 133 *D. praehensilis* accessions were used for the subsequent association analysis.

Data Analyses

Phenotypic data analysis

Analysis of variance combined across the two growing seasons using lme4 package in the R package (R Development Core Team, 2019) was computed for agronomic and tuber quality traits measured based on a linear mixed model (LMM) analysis with restricted maximum likelihood procedure in R package. The linear model used was as follows:

$$Y_{ijk} = \mu + G_h + S_i + (G_h * S_i) + Ri_j + B_k + \varepsilon_{hijk,} \quad (8.6)$$

Where $Yijk$ = value of the observed quantitative trait; μ = population mean; G_h = effect of the $_h$th accessions; S_i = effect of the $_i$th growing seasons; (G_h * S_i) is the accessions x season interaction associated with accession h and season I; R_{ij} = effect of the jth replicate (superblock) in seasons i_{th}; Bk = effect of the kth incomplete block within the jth replicate; and $\varepsilon hijk$ = experimental error. In this analysis, accessions were considered fixed while all other factors were random. The linear mixed model analysis generated the best linear unbiased prediction (BLUP), the variance components and broad-sense heritability estimates. The accessions' BLUP values for the agronomic and tuber quality derived from the best fit model were used as input for the GWAS model.

GWAS analysis

A mixed linear model (MLM) implemented in the GAPIT (Genome Association and Prediction Integration Tools) R package (Lipka et al., 2012) was used to compute association analysis. False-positive associations were controlled by fitting population structure (Q), kinship (K) matrix and other hidden

confounding factors. The VanRaden method was used to compute the variance-covariance kinship or relatedness (K) matrix (VanRaden, 2008). Stepwise regression implemented in the lme4 R package was used to determine the phenotypic variation explained by the model for a trait and a specific SNP. The SNP loci that had a significant association with the traits were identified using an adjusted p-value and the Bonferroni correction (Benjamini & Hochberg, 1995).

The negative logarithms (-log10) of the p-values were plotted against their expected p-values to generate Quantile–quantile (QQ) plots, which fit the appropriateness of the GWAS model with the null hypothesis of no association and to determine how well the model accounted for population structure. CMplot in R package was used to display the Manhattan and Q-Q plots for GWAS.

Identification of putative genes

The Yam Generic File Format (GFF3) file was searched for probable candidate genes within the relevant genomic domain (downstream and upstream) at a specific range window of 1 MB. Using the SNPReff, the significant genes in the intergenic region were identified using the reference genome's Yam Generic File Format (GFF3). The European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) public database Interpro was utilised to determine the functions of the genes associated with the discovered SNPs (Hunter et al., 2012).

Results

Genetic and non-genetic effects on agronomic and tuber quality traits of *D. praehensilis* accessions

Estimate of variance components, mean, coefficients of variation and broad-sense heritability for agronomic and tuber quality attributes among the bush yam accessions are presented in Table 8.1. The variance estimates for genotypic effect were significant ($p \leq 0.05$) for all the evaluated traits. In contrast, the seasonal effect was significant ($p \leq 0.05$) for tuber flesh hardness, tuber yield, and yam mosaic virus severity response and genotype x season interaction was significant for tuber yield and yam mosaic virus severity response (Table 8.1).

Table 8. 1. Variance component estimates for agronomic and tuber quality traits of *D. praehensilis* accessions

Variance component	DMC (%)	NTP	TBHard (N)	TBOxi	Yield (t/ha)	YMV (AUDPC)
Genotype	9.15*	0.45*	1.37*	57.98*	97.23*	916.00*
Season	2.19^{ns}	0.00^{ns}	0.045*	0.00^{ns}	42.70*	1.30×10^{-14}*
Genotype x Season	0.00^{ns}	0.00^{ns}	0.017^{ns}	15.55^{ns}	130.75*	13.67*
Residual	5.29	0.67	0.077	33.11	125.49	26.46

DMC: Dry matter content; NTP: Number of tubers per plot; TBHard: Tuber flesh hardness; TBOxi: Tuber flesh oxidation; Yield: Tuber yield; YMV: Yam mosaic virus; *: ($p \leq 0.05$); ns: not significant

Variation, coefficients of variation and broad-sense heritability of agronomic and tuber quality traits across 2020 and 2021 seasons

The mean, minimum and maximum values, coefficients of variation and broad-sense heritability of the *D. praehensilis* accessions assessed across 2020 and 2021 seasons are presented in Table 8.2. Dry matter content ranged from 25.73 to 43.44%, with an average of 34.28%. The tubers per plot varied from ~1 to 4 with a mean value of ~2. Mean tuber flesh hardness was (50.86N) and ranged from 48.64 to 53.49 N. Mean tuber flesh oxidation was -13.01 and varied from -32.88 to 1.53. Tuber yield per hectare varied from 6.57 to 57.79 tha^{-1} with a mean value of 61.61 tha^{-1}. Response to yam mosaic virus severity (YMV) ranged

from 137.09 to 315.80 with average value 149.55. The coefficients of variation varied from 0.55% for tuber flesh hardness to 67.45% for tuber yield per hectare. High broad-sense heritability (≥60%) was recorded for all the evaluated traits, with a range of 64.84% for tuber yield per hectare to 99.00% for yam mosaic virus severity.

Table 8. 2. Mean, minimum and maximum values, coefficients of variation and broad-sense heritability of agronomic and tuber quality traits

Traits	Min	Max	Mean	CV (%)	H^2 (%)
DMC (%)	25.73	43.44	34.28	6.71	88.07
NTP	1.24	4.16	1.91	42.94	73.04
TBHard (N)	48.64	53.49	50.86	0.55	97.02
TBOxi	-32.88	1.53	-13.01	44.2	87.5
Yield (t/ha)	6.57	57.79	61.61	67.45	64.84
YMV (AUDPC)	137.09	315.8	149.55	3.44	99.00

DMC: Dry matter content; NTP: Number of tubers per plot; TBHard: Tuber flesh hardness; TBOxi: Tuber flesh oxidation; Yield: Tuber yield; YMV: Yam mosaic virus; Min: Minimum; Max: Maximum; CV: Coefficient of variation; H^2: Broad-sense heritability

Genome-wide scan for agronomic and tuber quality traits

A total of 21 significant SNPs associated with agronomic and tuber quality traits were detected at GWAS thresholds $-\log_{10}$ (P) = 3 for the number of tubers per plot, $-\log_{10}$ (P) = 4 for dry matter content, tuber yield per hectare, tuber flesh oxidation and tuber flesh hardness and $-\log_{10}$ (P) = 5 for yam mosaic virus severity response (Table 8.3). GWAS analysis using MLM model identified 1, 7, 2, 3, 7, and 1 significant SNP markers associated with dry matter content, the number of tubers per plot, tuber flesh hardness, tuber flesh oxidation, yield, and yam mosaic virus (YMV) severity, respectively.

Genome-wide scan for dry matter content

GWAS scan detected only one significant SNP marker (chrom_05_22963634) with (p = 0.00083), located on chromosome 5 at 22.96 mega-base pair (mbp) linked with variation in dry matter content (Table 8.3; Figure 8.1). This SNP marker explained 19.3% of the total phenotypic variation with quantitative trait nucleotide (QTN) effects of 0.029 and -3.05.

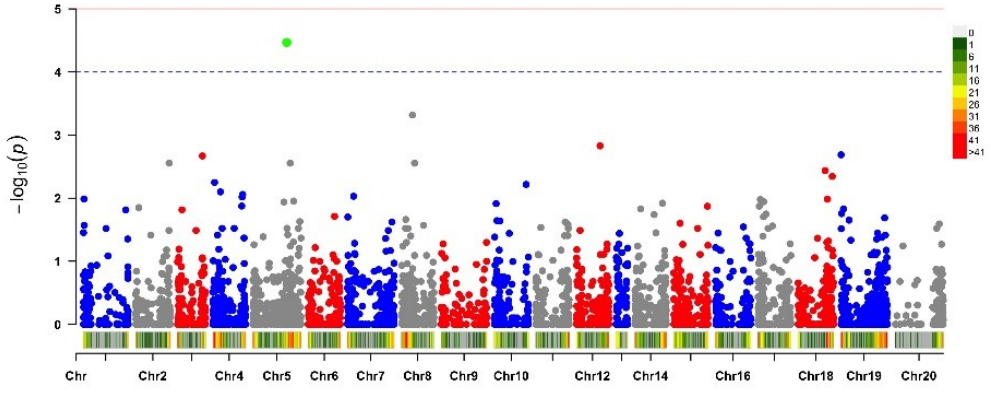


Figure 8. 1. Manhattan plot indicating SNPs linked with dry matter content (DMC).

Vertical bars relate to 20 yam chromosomes; the green dots indicate chromosomes influencing the target trait, the dotted line indicates genome-wide Bonferroni significant threshold

Table 8. 3. Associated SNP markers identified by GWAS analyses for agronomic and tuber quality traits in *D. praehensilis*

Traits	SNP markers	Chr	Position (bp)	Allele 1	Allel e 2	PIC	QTN effect	R^2	P-value
DMC	chrom_05_22963634	5	22963634	G	A	0.06	-3.05	0.193	0.00083
NTP	chrom_03_673949	3	673949	A	G	0.13	-1.80	0.184	6.94E-05
	chrom_15_5299699	15	5299699	C	T	0.15	-1.71	0.156	0.00045
	chrom_15_7655487	15	7655487	A	G	0.05	-1.46	0.155	0.00048
	chrom_18_18469483	18	18469483	G	A	0.06	1.61	0.155	0.00049
	chrom_02_8806875	2	8806875	T	C	0.02	1.78	0.153	0.00056
	chrom_19_27703108	19	27703108	T	C	0.07	1.24	0.148	0.00081
	chrom_19_24611247	19	24611247	C	T	0.09	-1.13	0.146	0.00093
TBHard	chrom_19_29680098	19	29680098	G	A	0.37	0.84	0.095	0.00214
	chrom_14_3890023	14	3890023	T	C	0.37	0.51	0.094	0.00236
TBOxi	chrom_17_21447981	17	21447981	G	A	0.12	-27.93	0.109	0.00027
	chrom_19_22128435	19	22128435	T	C	0.20	-27.93	0.109	0.00027
	chrom_13_7087751	13	7087751	G	A	0.12	11.69	0.106	0.00033
Yield	chrom_09_30821070	9	30821070	A	T	0.02	70.78	0.250	5.77E-06
	chrom_01_28748414	1	28748414	C	A	0.02	82.74	0.230	2.17E-05
	chrom_03_2959246	3	2959246	T	C	0.02	82.74	0.033	2.17E-05
	chrom_16_22139272	16	22139272	T	C	0.25	58.72	0.217	5.57E-05
	chrom_15_4157964	15	4157964	C	A	0.15	40.90	0.216	5.81E-05
	chrom_18_22155727	18	22155727	C	G	0.15	-37.36	0.215	6.27E-05
	chrom_03_17110542	3	17110542	C	A	0.20	27.61	0.209	9.70E-05
YMV	chrom_04_5342176	4	5342176	C	T	0.13	175.40	0.226	1.91E-07

DMC: Dry matter content; NTP: Number of tubers per plot; TBHard: Tuber flesh hardness; TBOxi: Tuber flesh oxidation; Yield: Tuber yield; YMV: Yam mosaic virus; Chr: Chromosome; PIC: Polymorphism information content; R^2: R-square: QTN: Quantitative trait nucleotide; bp (base pair)

Genome-wide scan for number of tubers per plot

GWAS scan detected seven SNP markers associated with the number of tubers per plot; one SNP marker each was located on chromosome 2, 3 and 18 at 8.81, 0.67 and 18.47 mbp, respectively, while two SNP markers each were located of chromosomes 15 and 19 at 5.30, 7.66, 27.70 and 26.40 mbp , respectively (Table 8.3; Figure 8.2). The SNP markers accounted for between 14.6% (chrom_19_24611247) to 18.4% (chrom_03_673949) of the total phenotypic variance (Table 8.3). The QTN effects ranged from -1.13 (chrom_19_24611247) to -1.80 (chrom_03_673949).

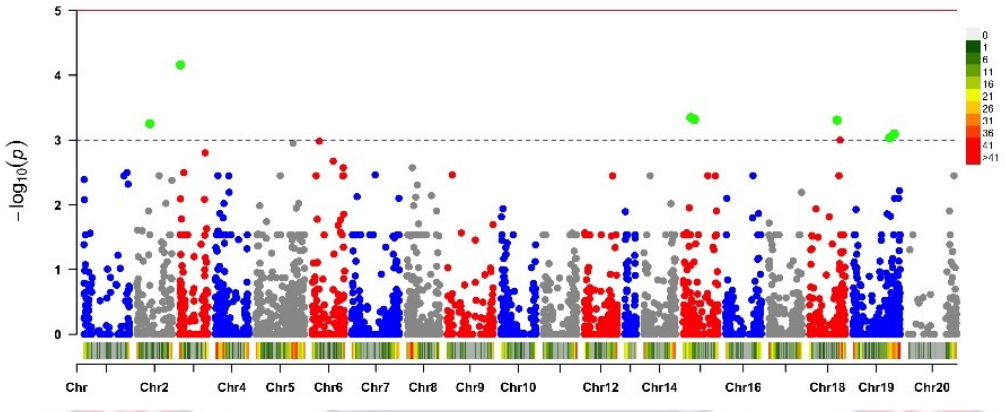


Figure 8. 2. Manhattan plot indicating SNPs linked with the number of tubers per plot (NTP).

Vertical bars relate to 20 yam chromosomes; the green dots indicate chromosomes influencing the target trait, the dotted line indicates genome-wide Bonferroni significant threshold

Genome-wide scan for tuber flesh hardness

Two significant SNP markers distributed on two chromosomes were linked to tuber flesh hardness in *D. praehensilis*. SNP chrom_19_29680098 (p = 0.00214) is located at 29.68 mbp on chromosome 19, while SNP

chrom_14_3890023 (p = 0.00236) is located at 3.89 mbp on chromosome 14 (Table 8.3; Figure 8.3). The two SNP markers explained 9.5 and 9.4% of the total phenotypic variance, with QTN effects of 0.84 and 0.51 (Table 8.3).

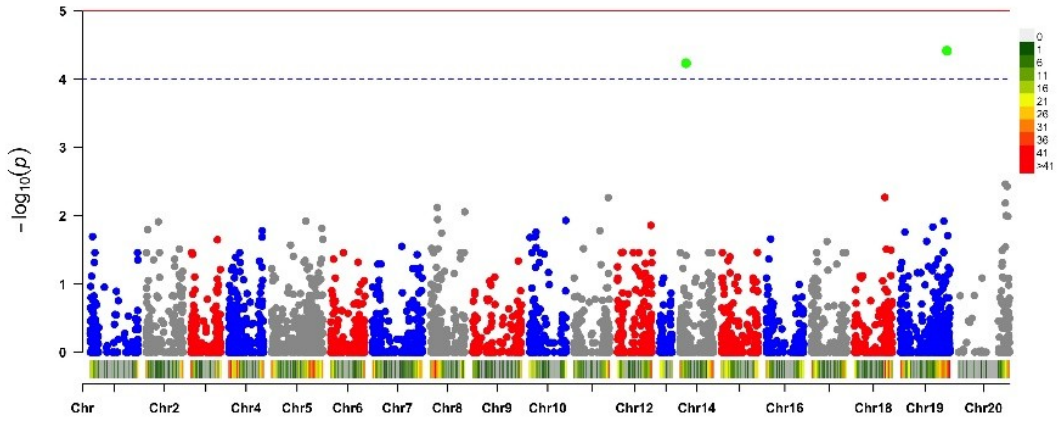


Figure 8. 3. Manhattan plot indicating SNPs linked with tuber flesh oxidation (TBHard).

Vertical bars relate to 20 yam chromosomes; the green dots indicate chromosomes influencing the target trait, the dotted line indicates genome-wide Bonferroni significant threshold

Genome-wide scan for tuber flesh oxidation

The variation in tuber flesh oxidation was found to be associated with three SNP markers located on chromosomes 17, 19 and 13 (Table 8.3; Figure 8.4). SNP chrom_17_21447981 (p = 0.00027) located on chromosome 17 at 21.44 mbp is the most significant of the three markers, followed by SNP chrom_19_22128435 (p = 0.00027) identified on chromosome 17 at 22.13 mbp and SNP chrom_13_7087751 (p = 0.00033) evolved on chromosome 13 at 7.09 mbp. The QTN effect ranged from -27.93 for chrom_17_21447981 and chrom_19_22128435 to 11.69 (chrom_13_7087751) (Table 8.3). SNP markers

chrom_17_21447981 and chrom_19_22128435 explained 10.9% of the total phenotypic variation, while chrom_13_7087751 explained 10.6% of the total phenotypic variation.

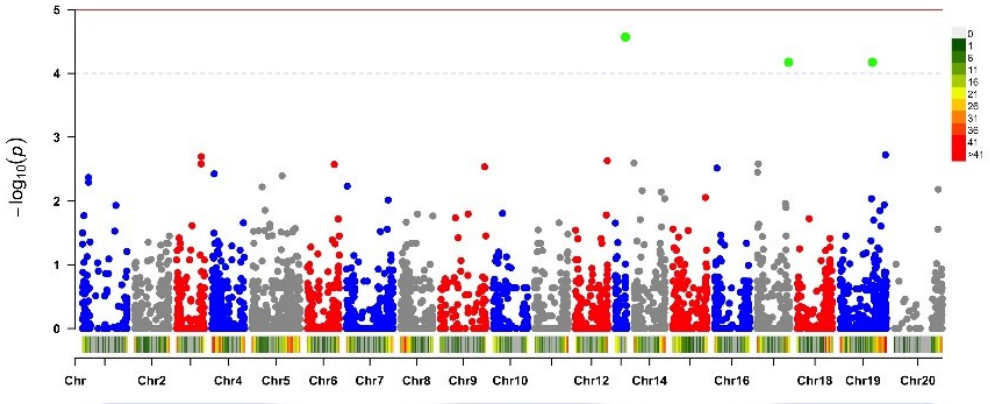


Figure 8. 4. Manhattan plot indicating SNPs linked with tuber flesh oxidation (TBOxi).

Vertical bars relate to 20 yam chromosomes; the green dots indicate chromosomes influencing the target trait, the dotted line indicates genome-wide Bonferroni significant threshold

Genome-wide scan for tuber yield

Seven SNP markers distributed on six (6) chromosomes were identified to be significantly associated with tuber yield (tha^{-1}) (Table 8.3; Figure 8.5). Of the seven detected SNP markers, two were mapped on chromosome 3 with positions of 2.96 mpb (chrom_03_2959246) and 17.11 mpb (chrom_03_17110542), respectively. One SNP marker each were located on chromosomes 1, 4, 9, 15, 16 and 18. The most significant of these markers was chrom_09_30821070, mapped on chromosome 9 at the position of 30.82 mbp and accounted for the total phenotypic variance of 25% (Table 8.3). The QTN effect ranged from -37.36 (chrom_18_22155727) and 70.78 (chrom_09_30821070) (Table 8.3).

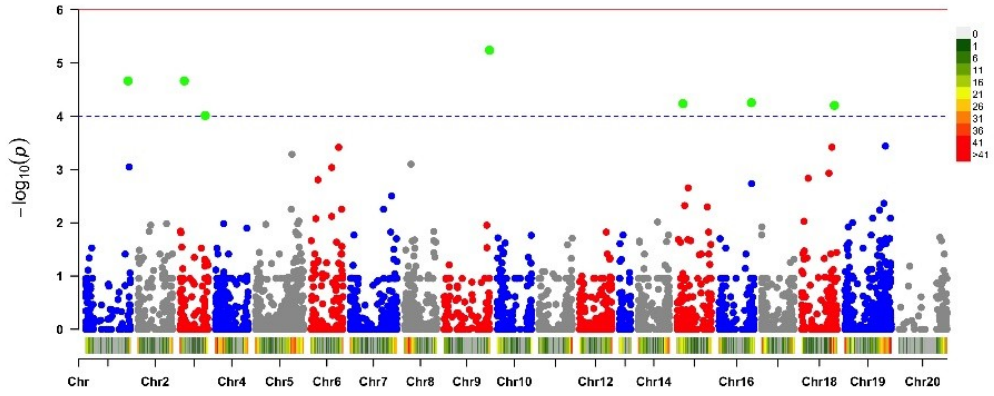


Figure 8. 5. Manhattan plot indicating SNPs linked with tuber yield (tha^{-1}).

Vertical bars relate to 20 yam chromosomes; the green dots indicate chromosomes influencing the target trait, the dotted line indicates genome-wide Bonferroni significant threshold

Genome-wide scan for yam mosaic virus (YMV) severity

One SNP locus was found to have significant association with response to yam mosaic virus (YMV) severity significantly (Table 8.3; Figure 8.6). The SNP marker is located on Chromosome 4 at 5.34 mbp and explains 22.6% of total phenotypic variance. The QTN effect observed was 175.40.

The quantile-quantile (QQ) plots of agronomic and tuber quality traits

The quantile-quantile (QQ) plots produced by plotting the negative logarithm (-log10) of the p-values against their expected p-values demonstrated that the GWAS model was appropriate for all six traits. There were difference between observed and expected values for the target traits, indicating a link between the phenotype and the markers (Figure 8.7a, b, c, d, e and f).

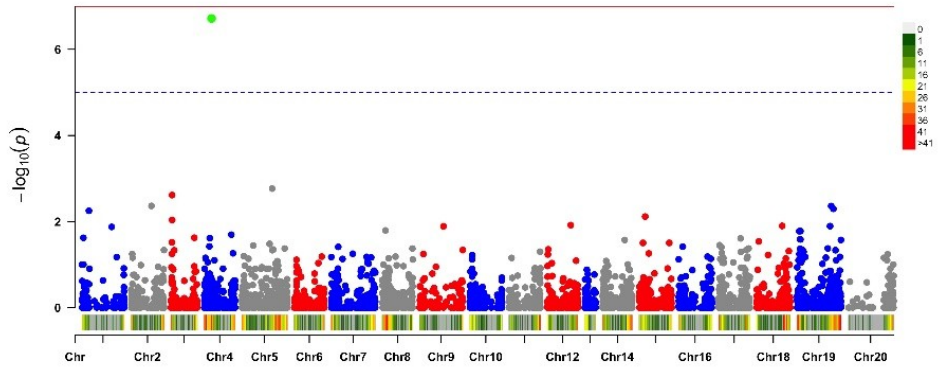


Figure 8. 6. Manhattan plot indicating SNPs linked with yam mosaic virus severity.

Vertical bars relate to 20 yam chromosomes; the green dots indicate chromosomes influencing the target trait, the dotted line indicates genome-wide Bonferroni significant threshold

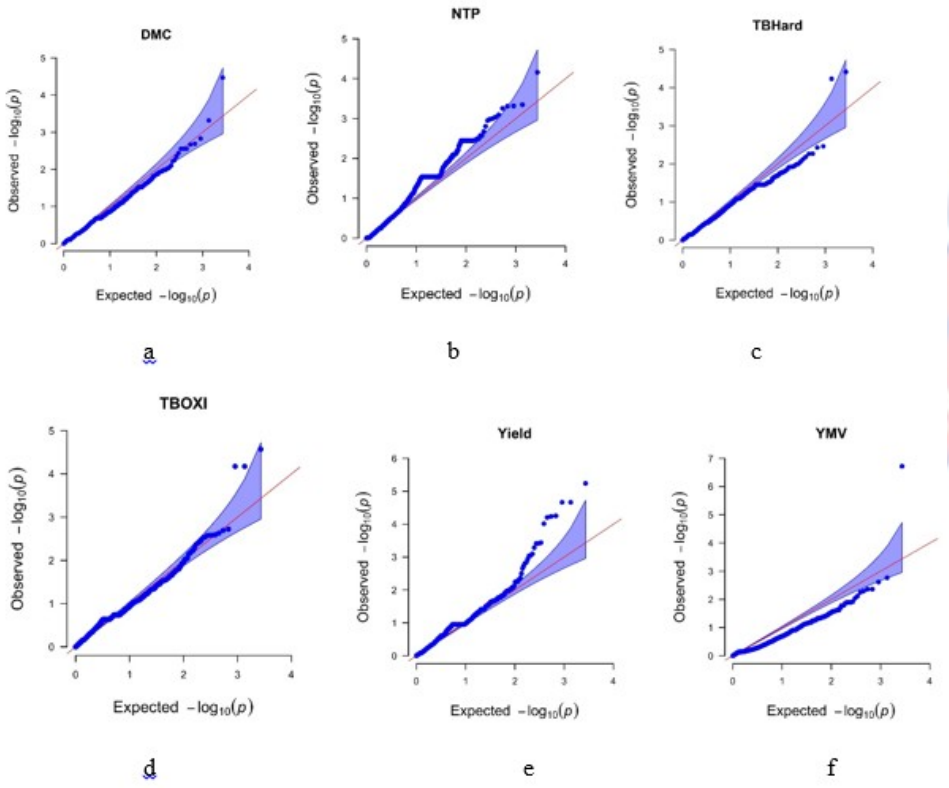


Figure 8. 7. Quantile-quantile (QQ) plots for agronomic and tuber quality traits: a: DMC; b: NTP; c: TBHard; d: THOxi; e: Yield; f: YMV

Annotation of putative genes associated with agronomic and tuber quality traits in *D. praehensilis*

Candidate genes or protein families associated with dry matter content, number of tubers per plot, tuber flesh hardness, tuber flesh oxidation tuber yield and yam mosaic virus severity in *D. praehensilis* were explored using white yam genome reference because of its close resemblance with bush yam. Thirty-six (36) putative candidate genes were discovered to be associated with the evaluated agronomic and tuber quality traits, except tuber flesh hardness.

Dry matter content

On chromosome five, the significant SNP (chrom_05_22963634) for dry matter content was located on genomic regions harbouring five putative genes (Table 8.4). The candidate genes were IPR000313 encoded as methyl-lysine recognition protein, IPR001214 encoded as protein lysine methyltransferase enzymes, IPR001810 encoded as polyubiquitination protein, transcription elongation protein, centromere binding protein and translational repression protein, IPR001810 encoded as galactose oxidase, and IPR011124 annotated as chromatin methylation protein (Table 8.4).

Number of tubers per plot

Four potential candidate genes were found in the genomic regions linked with the number of tubers per plot on chromosomes 2, 3, 5, 15 and 18. These genes include: IPR006091, which encodes Acetyl-CoA interaction enzymes, IPR006501 encoded as Invertase/pectin methylesterase inhibitor domain

superfamily, IPR006045, encoded as plant seed storage proteins, and IPR007125 encoded as gene regulation and DNA replication proteins (Table 8.4).

Tuber flesh oxidation

For tuber flesh oxidation, four putative genes, including IPR002579 (Peptide methionine sulphoxide reductase MsrA) was found on chromosome 17, IPR028324 (Serine/threonine-protein kinase CTR1/EDR1), IPR001245 (Serine-threonine/tyrosine-protein kinase catalytic domain) and IPR011009 (Protein kinase-like domain) were found on chromosome 13 (Table 8.4).

Table 8. 4. Significant SNPs associated with agronomic and tuber quality traits and candidate genes identified for 133 *D. praehensilis* accessions

Traits	SNP markers	Chr	Position	Candidate gene	Putative gene annotation
DMC	chrom_05_22963634	5	22963634	IPR000313	Methyl-lysine recognition protein
				IPR001214	Protein lysine methyltransferase enzymes
					Polyubiquitination protein, transcription elongation protein, centromere binding protein and translational repression protein
				IPR001810	
				IPR006652	Galactose Oxidase
				IPR011124	Chromatin methylation protein
	chrom_03_673949	3	673949	IPR006091	Acetyl-CoA interaction enzymes
	chrom_15_5299699	15	5299699	IPR006501	Invertase/pectin methylesterase inhibitor domain superfamily
NTP	chrom_15_7655487	15	7655487	-	-
	chrom_18_18469483	18	18469483	IPR006045	Plant seed storage proteins
	chrom_02_8806875	2	8806875	IPR007125	Gene regulation and DNA replication proteins
TBHard	chrom_19_27703108	19	27703108	-	-
	chrom_19_24611247	19	24611247	-	-
	chrom_19_29680098	19	29680098	-	-
	chrom_14_3890023	14	3890023	-	-
TBOxi	chrom_17_21447981	17	21447981	IPR002579	Peptide methionine sulphoxide reductase MsrA

Table 8. 4. Continued

	[illegible]	19	22128435	-	-
				IPR028324	Serine/threonine-protein kinase
	chrom_13_7087751	13	7087751	IPR001245	Serine-threonine/tyrosine-protein kinase catalytic domain
				IPR011009	Protein kinase-like domain
	chrom_09_30821070	9	30821070	-	-
	chrom_01_28748414	1	28748414	IPR011545	
				IPR001650	DEAD and DEAH box helicases
	chrom_03_2959246	3	2959246	IPR004813	Oligopeptide transporters
				IPR005150	Cellulose synthase
	chrom_16_22139272	16	22139272	IPR003439	ABC transporters
				IPR027417	Nucleoside triphosphate hydrolase
				IPR015655	Protein phosphatase 2C
Yield				IPR001932	Protein phosphatase 2C (PP2C)-like domain
	chrom_15_4157964	15	4157964	IPR000907	Lipoxygenase
				IPR013819	Lipoxygenase, C-terminal
				IPR008976	Lipoxygenase, plant
				IPR027433	Lipooxygenase, PLAT/LH2
				IPR001246	Lipoxygenase, domain 3
	chrom_18_22155727	18	22155727	IPR00604	
				IPR014710	11S and 7S plant seed storage proteins, and germins.
				IPR001929	
	chrom_03_17110542	3	17110542	IPR010420	Plant proteins
				IPR006702	Plant proteins
				IPR002659	Galactosyltransferases UDP-galactose, 2-acetamido-2-deoxy-D-glucose3beta-galactosyltransferase
				IPR012946	Glycosyl hydrolases
YMV	chrom_04_5342176	4	5342176		Ribosome associated membrane protein RAMP4 (or SERP1) sequences
				IPR010580	
				IPR010713	Plant xyloglucan endo-transglycosylase (XET)
				IPR025610	N-terminal region of a family of MYB and MYC transcription factors

DMC: Dry matter content; NTP: Number of tubers per plot; TBHard: Tuber flesh hardness; TBOxi: Tuber flesh oxidation; Yield: Tuber yield; YMV: Yam mosaic virus; Chr: Chromosome

Tuber yield

Two putative genes identified on chromosome 1 (IPR011545 and IPR001650) are linked to tuber yield per hectare, and these genes encode DEAD, and DEAH box helicases (Table 8.4). On Chromosome 3, three candidate genes (IPR004813, IPR005150 and IPR010420) were found encoding oligopeptide transporters, cellulose synthase and plant proteins, respectively (Table 8.4). The putative genes IPR000907, IPR013819, IPR008976, IPR027433 and IPR001246, encoding lipoxygenase, lipoxygenase, and C-terminal, lipooxygenase and PLAT/LH2 and lipoxygenase and domain 3 were identified on chromosome 15 (Table 8.4). Four putative genes were identified on chromosome 16 which encode ABC transporters, nucleoside triphosphate hydrolase, protein phosphatase 2C and protein phosphatase 2C (PP2C)-like domain, respectively. On chromosome 18, three putative genes (IPR00604, IPR014710 and IPR001929) were identified, which encode 11S and 7S plant seed storage proteins and germins (Table 8.4).

Yam mosaic virus severity

Six putative genes close to significant SNP chrom_04_5342176 on chromosome 4 were linked to yam mosaic virus severity in *D. praehensilis* (7.4). These putative genes such as IPR006702 encodes plant proteins, IPR002659 encodes galactosyltransferases UDP-galactose and 2-acetamido-2-deoxy-D-glucose3beta-galactosyltransferase, IPR012946 encodes glycosyl hydrolases, IPR010580 encodes ribosome-associated membrane protein RAMP4 (or SERP1) sequences, IPR010713 encodes Plant xyloglucan endo-transglycosylase (XET) and IPR025610 encodes N-terminal region of a family of MYB and MYC

transcription factors (Table 8.4).

Discussion

The germplasm of *D. praehensilis* used in this present study demonstrated considerable phenotypic variation for the agronomic and tuber quality traits. This is consistent with other studies on yam species, such as *D. rotundata* (Agre, Norman, Asiedu, & Asfaw, 2021) and *D. alata* (Gatarira et al., 2020). The significant difference observed for genotype, season and genotype x season interaction effects in tuber yield per hectare and yam mosaic virus severity response can be linked to varying seasonal and environmental factors and soil nutrients, which is a reflection of variations between genotypes within a season and between two seasons. This finding suggests a high genetic diversity, enabling plant breeders to take full use of genetic and environmental diversity, while facilitating genotype selection (Otoo et al., 2017).

Heritability estimates were high (≥60%) for all the evaluated agronomic and tuber quality traits, explaining the predominant role of genetic factors in these traits. High heritability traits improve the sensitivity of detecting SNPs in an association panel, allowing the identification of a true association between a marker and a putative gene (Brachi et al., 2011). The high heritability estimates reported for tuber yield and yam mosaic virus (YMV) severity in this present study are consistent with the results (Agre et al., 2021). The high heritability observed in this study suggests that selection of these traits could be useful for breeding programmes.

Genome-wide association studies have been frequently employed to

identify the genetic basis of complex characteristics (Morris et al., 2013; Sukumaran, Reynolds, & Sansaloni, 2018). However, complex genetic structures and structured features might cause erroneous signals and indirect correlations in genome-wide association studies. The quantile-quantile (Q-Q) plot, which compares observed against anticipated p-values under the null hypothesis that there is no relationship between SNP markers and the phenotypes, was used to determine model fitness for the association analysis. This finding revealed that bulk of the points in the Q-Q plots for all of the variables tested was aligned on the diagonal line, showing that erroneous allelic connections related to population structure and relative kinship were greatly reduced. These findings support the idea that attributes without p-value inflation indicate that the structural association is sufficient for GWAS (Mondo et al., 2021; Uchendu et al., 2021).

Identifying QTL and genes that influence bush yam's agronomic and tuber quality characteristics is critical for its improvement and marker-assisted breeding. The GWAS study revealed the genetic basis of some agronomic and tuber quality traits in *D. praehensilis* for the first time. A whole-genome scan for phenotypic and allelic variation in agronomic and tuber quality traits discovered genomic regions with significant -log10 values on 13 chromosomes (chromosomes 1, 2, 3, 4, 5, 9, 13, 14, 15, 16, 17, 18, and 19). Many agronomic attributes have been studied using genome-wide association mapping in *Dioscorea* spp. These traits include tuber dry matter and oxidative browning in water yam (Gatarira et al., 2020), sex determination cross-compatibility in water yam (Mondo et al., 2021), and tuber yield and yam mosaic virus resistance in

white yam (Agre et al., 2021a). The phenotypic effect values of the favourable alleles of agronomic and tuber quality traits were analyzed in this study, indicated that they positively and adversely affected the individual attributes.

Significant marker-trait associations were detected using the strict threshold of -log10 in all the assessed, number of tubers (6.94×10^{-5} to 9.30×10^{-4}), tuber yield (2.17×10^{-5} to 9.70×10^{-5}), tuber flesh oxidation (2.70×10^{-4} to 3.30×10^{-4}), tuber flesh hardness (2.14×10^{-3} to 2.36×10^{-3}), dry matter content (8.30×10^{-4}) and yam mosaic virus severity response (1.91×10^{-7}) (Table 8.3). The significant markers co-located with candidate genes for the evaluated traits could be essential for developing functional markers that would be useful for marker-assisted selection to enhance the development of *D. praehensilis* for agronomic and tuber quality traits. Detection of significant marker-trait associations using GWAS has been reported in some yam species such as *D. rotundata* (Agre et al., 2021a) and *D. alata* (Gatarira et al., 2020; Mondo et al., 2021) and other root and tuber crops such as cassava (Uchendu et al., 2021).

This study identified putative candidate genes that are located within the genomic regions of the target traits in *D. praehensilis*. The annotation analysis identified potential involvement in tuber yield, post-harvest tuber qualities and resistance to YMV. The SNP in chromosome 5 is close to putative genes encode Methyl-lysine recognition, protein-lysine Methyltransferase enzymes, Galactose oxidase, Polyubiquitination protein and chromatin methylation protein. Mininno et al. (2012) reported that methyl-lysine recognition protein has been known to be involved in the diverse cellular process such as maintaining genome stability, and

regulating cell cycle progression. Protein-lysine methyltransferase enzymes are majorly involved in carbon metabolism and methylating chloroplast fructose in plants (Mininno et al., 2012). Galactose oxidase is a member of a protein family called alpha amylase involved in cell to cell adhesion between seed coat epidermal cells (do Carmo et al., 2020). This protein has been reported to be involved in initiation of degradation in many starchy crops such as cassava (do Carmo et al., 2020).

SNPs in chromosomes 2, 3, 15, 18 and 19 are close to candidate genes for dry matter content, which encode Acetyl-CoA interaction enzymes, Plant seed storage protein, Invertase/pectin methylesterase inhibitor domain superfamily and Gene regulation and DNA replication proteins. Acetyl-CoA interaction enzymes, a small alpha-helical domain from type I and II citrate synthase enzymes, and a homologous domain found in the related enzyme ATP citrate synthase, has been implicated in fruit development, carbohydrate metabolism, and cell wall extension (Camardella et al., 2000). Plant seed storage comprises plant proteins that have inhibitory activity against serine proteinases from the trypsin and subtilisin families, thiol proteinases and aspartic proteinases, and some proteins that are probably involved in seed storage. This putative gene has been reported in sweet potato as sproramin and proteinase inhibitor, the major tuberous root protein (Hattori, Yoshida, & Nakamura, 1989).

SNPs in chromosomes 13, 17 and 19 are close to putative genes for tuber flesh oxidation, which encode Peptide methionine sulphoxide reductase MsrA, Serine/threonine-protein kinase (STKs), Tetratricopeptide-like helical domain

superfamily and Protein kinase. Peptide methionine sulphoxide reductase (Msr) reverses the inactivation of many proteins due to the oxidation of critical methionine residues by reducing methionine sulphoxide, (MetO), to methionine (Lowther, Brot, Weissbach, Honek, & Matthews, 2000). Serine/threonine-protein kinase (STKs) catalyze the transfer of the gamma-phosphoryl group from ATP to serine/threonine residues on protein substrates. In potatoes, serine/threonine-protein kinase (STK) was found to be involved in starch and sugar production and to induce glucose pyrophosphorylase (Geigenberger, 2003; Tiessen et al., 2003). STK has been shown to stimulate several enzymes in the starch biosynthesis pathways in potato (*Solanum tuberosum*) and wheat (*Triticum aestivum*) (Purcell, Smith, & Halford, 1998). Tetratricopeptide-like helical domain superfamily genes have been found to mediate protein-protein interactions and be involved in creating protein and starch, which are plants' primary storage carbohydrates (Kurtz., 2001). Protein kinase, which involves developmental processes in plants and endosperm that were first isolated in maize (Cao, Li, Suh, Guo, & Becraft, 2005; Demko, Ako, Perroud, Quatrano, & Olsen, 2016). Mutations in this protein affect cell wall thickness and structure, cuticle formation, vesicle trafficking, and tumour-like outgrowths, with similar effects seen in rice (Cao et al., 2005; Demko et al., 2016; Gaudioso-Pedraza & Benitez-Alfonso, 2014).

The SNPs in chromosomes 1, 3, 9, 15, 16 and 18 are near to candidate genes for tuber yield, which encode The DEAD-box helicases, Cellulose synthase (CESA), ABC transporters, Protein phosphatase 2C, Lipoxygenases and Plant

protein family. The DEAD-box helicases have been reported to be involved in various aspects of RNA metabolism, including nuclear transcription, pre mRNA splicing, ribosome biogenesis, nucleocytoplasmic transport, translation, RNA decay and organellar gene expression (Aubourg, Kreis, & Lecharny, 1999). The cellulose synthase (CESA) superfamily includes a wide variety of glycosyltransferase family 2 enzymes that share the common characteristic of catalyzing the elongation of polysaccharide chains. Cellulose synthase has been reported in sweet potato for playing a significant role in primary cell wall biosynthesis (Oomen et al., 2004). The plant cell wall seems to play an important role in stress perception by facilitating activation of signalling pathways and remodelling growth strategies in response to stresses (Kesten, Menna, & Sánchez-Rodríguez, 2017). ABC transporters belong to the ATP-Binding Cassette (ABC) superfamily, which uses the hydrolysis of ATP to energise diverse biological systems. ABC transporters minimally consist of two conserved regions: a highly conserved ATP binding cassette (ABC) and a less conserved transmembrane domain (TMD) (Fernandez-lopez et al., 1996). ABC transporters play an important role in organ growth, plant nutrition, plant development, response to abiotic stress, and the interaction of the plant with its environment (Kang et al., 2011). Protein phosphatase 2C (PP2C, also known as Protein phosphatase 1) is involved in regulating cellular responses to stress in various eukaryotes. It consists of two domains: an N-terminal catalytic domain and a C-terminal domain characteristic of mammalian PP2Cs (Das, Helps, Cohen, & Barford, 1996). Protein phosphatase 2C has been reported to significant role in plant growth and

development in *Arabidopsis thaliana* (Singh et al., 2018). Lipoxygenases are a class of iron-containing dioxygenases that catalyses lipids' hydroperoxidation, containing a cis, cis-1, 4-pentadiene structure. They are common in plants where they are involved in several diverse aspects of plant physiology, including growth and development, pest resistance, and senescence or responses to wounding (Kolomiets, Hannapel, Chen, Tymeson, & Gladon, 2001). Lipoxygenases has been reported to be involved in tuber development in sweet potato (Kolomiets et al., 2001) Plant seed storage proteins provide the major nitrogen source for the developing plant (Kesari et al., 2017).

The SNP in chromosome 4 is near to putative genes for yam mosaic virus, which encode Glycosyl hydrolase, Ribosome-associated membrane protein RAMP4 (or SERP1) sequences and Plant protease inhibitor. The plant proteins such as plant protease inhibitor play an important role in natural plant defence. They are also known to possess potent antibiotic activity against bacteria, fungi, and even certain viruses (Kim et al., 2009). Glycosyl hydrolases (GHs) are a group of enzymes that hydrolyze the glycosidic link between carbohydrate and noncarbohydrate molecules. GHs are classified into different families based on their amino acid sequences. Both plants and pathogens use these proteins for a variety of purposes (Chatterjee, Mazumder, & Basu, 2013). Although some recent investigations have suggested that these enzymes are involved in plant defensive responses, little is known about their role during host-microbe interactions (Chatterjee et al., 2013). The Ribosome associated membrane protein RAMP4 (or SERP1) sequences consist of several ribosomes associated membrane protein

RAMP4 (or SERP1) sequences. Stabilisation of membrane proteins in response to stress involves the concerted action of a rescue unit in the ER membrane comprised of SERP1/RAMP4, other components of the translocon, and molecular chaperones in the ER (Yamaguchi et al., 1999).

The found putative candidate genes and SNPs connected to these essential agronomic and tuber quality traits could aid in the development of new breeding strategies in future bush yam improvement programmes. At the early seedling stages of *D. praehensilis*, promising agronomic and tuber quality trait SNP markers might be transformed into inexpensive Kompetitive Allele- Specific PCR (KASP-PCR) markers for validation, verification and used for phenotype prediction. Marker-assisted selection and genomic prediction might also be used to enhance conventional yam breeding programs, which would speed up the selection of bush yam materials and cut down on the time and expense required to create new bush yam varieties.

Conclusions

The present study offers the first genome-wide association mapping strategy in bush yam to decode the genetic architecture of agronomic and tuber quality variables. The study discovered 21 significant SNPs linked to variation in assessed characteristics. The linked SNP markers could be used and investigated in MAS of the evaluated traits to improve their selection effectiveness and rate of genetic gain. Except for tuber flesh hardness, this study discovered 36 significant candidate genes for most of the traits evaluated. In bush yam population improvement, the genetic loci controlling these studied features could be

exploited for selection and effective pyramiding of beneficial alleles. Further genetic studies involving transcript/transcriptome analysis, fine mapping, joint linkage mapping, and mapping using different mapping populations will be required to validate associations and candidate genes identified in this study so that marker-assisted breeding approaches can be used as tools to accelerate genetic improvement of agronomic and tuber quality traits in bush yam germplasm, especially in Ghana, where yam is a major staple food.

CHAPTER NINE

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

Bush yam is a high-yielding and disease-tolerant species of yam that is used to fill hunger gaps among farmers living in the rural areas of West, East, and Central Africa. Despite the enormous advantages, its production and utilisation have been constrained by several factors, including limited research on its genetic diversity, conservation, management, yield potentials, framers and end-users preference criteria, and poor post-harvest tuber quality traits (flesh tuber oxidation and tuber flesh hardness). Limited understanding of the genetic structure of the traits that contribute to enhanced productivity and tuber quality under prevailing production challenges and the inadequate breeding enabling technologies that can expedite yam improvement further aggravate the situation. This study was carried out to elucidate the genetic diversity and genome-wide association of agronomic and tuber quality traits of bush yam. More specifically, this study sought to (1) estimate the perception of farmers on the diversity, distribution, and management of bush yam, (2) assess genetic diversity and population structure of bush yam using SSR markers, (3) explore *D. praehensilis* as new sources for yield and YMV genes for improving *D. rotundata*, (4) evaluate the extent of genetic variability among the accessions of bush yam (5) assess the diversity among an association panel of yam using SNP markers, (6) identify SNP markers and putative candidate genes for important agronomic and tuber quality traits in *D. praehensilis.*

A participatory rural appraisal survey was conducted to assess the utilization, diversity knowledge, distribution, management and farmers' varietal preferences *of D. praehensilis* in 23 villages from three regions of Ghana.

Forty-three (43) accessions of *D. praehensilis* collected from a farmer's field in the Central Region, were genotyped for diversity studies using simple sequence repeat (SSR) markers as a preliminary study for this research work.

One hundred and sixty-two (162) of *D. praehensilis* accessions collected during regional germplasm collection from Ghana's three regions (Central, Eastern, and Western North). Ten major grown landraces were collected from local markets in Ghana were utilised for diversity studies and genome-wide association studies. These accessions were evaluated at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast, for two seasons (2020 and 2021) and genotyped using genotyping-by-sequencing protocol. The results of these studies are summarised below:

1. Forty-two (42) *D. praehensilis* accessions were inventoried across the three regions surveyed and grouped into seven classes based on colour of the tuber flesh identified by farmers.
2. Shannon diversity index, equitability, and Margalef species richness revealed the presence of moderate diversity and distribution in the surveyed regions.
3. Farmers' trait preferences mainly included early maturity, smooth tuber texture, tuber flesh colour stability, good storage aptitude and high tuber productivity.

4. *D. praehensilis* production and utilization rates had declined mainly due to poor culinary quality and agronomic traits of most accessions.
5. Simple sequence repeat (SSR) markers revealed low genetic diversity among the three genetic populations from the Central Region in a preliminary investigation conducted.
6. *D. praehensilis* accessions out-performed the best *D. rotundata* landraces for tuber yield, yam mosaic virus (YMV) resistance, plant vigour and tuber size.
7. The qualitative traits revealed a high level of diversity among the evaluated *D. praehensilis* accessions.
8. The quantitative traits revealed significant variation among the evaluated *D. praehensilis* accessions.
9. Clustering analysis using quantitative traits identified three clusters, with cluster 3 containing the best performing accessions for tuber yield-related traits. In contrast, cluster 1 had the best performing resistance to yam mosaic virus (YMV) severity.
10. All the identified three clusters had good post-harvest tuber quality attributes and dry matter content.
11. A total of 4,525 single nucleotide polymorphic (SNP) markers were generated using the genotyping-by-sequencing (GBS) platform.
12. A high level of genetic diversity was revealed among the accessions of *D. praehensilis* by the single nucleotide polymorphic (SNP) markers.

13. The SNP markers grouped the accessions irrespective of region of the collection into five clusters when compared with the morphological traits.
14. Using a mixed linear model, genome-wide association analysis identified 21 SNPs associated with six agronomic and tuber quality traits in *D. praehensilis*.
15. The identified SNPs accounted for approximately 16% of the total phenotypic variation.
16. Thirty-six (36) putative candidate genes were found to be associated with the evaluated agronomic and tuber quality traits, except tuber flesh hardness.
17. The identified candidate genes have functions related to the growth and development of tubers, reduction of tuber flesh oxidation, and defence mechanisms against the yam mosaic virus.

Conclusions

This study provided insight into *D. praehensilis* diversity, distribution, and farmers' varietal preferences in Ghana, guiding its genetic resource conservation and plant breeding interventions.

This study identified some promising *D. praehensilis* accessions for traits such as high yield potential (WNDpr76, CDpr28, CDPr7, EDpr14 and WNDpr63), resistance to YMV (WNDpr76, CDpr7, EDpr14, CDpr28 and EDpr13), high dry matter content (WNDpr76, CDpr28 and WNDpr24), low tuber flesh oxidation (WNDpr76, CDpr5, WNDpr31, CDpr40 and WNDpr94) and the high number of tubers per plant (WNDpr76, CDpr7, CDpr68, CDpr29 and

CDpr58). These accessions could be employed in the yam breeding scheme to improve bush yam as well as improve the white Guinea, which is the major cultivated yam species in West Africa.

This study also revealed the significance of single nucleotide polymorphic (SNP) markers as a robust tool for unravelling the level of genetic diversity among bush yam accessions when compared with the simple sequence repeat (SSR) markers.

This study also provides the first valuable insight for understanding the genetic basis of agronomic and tuber quality traits in bush yam.

Recommendations

1. The germplasm collection of *D. praehensilis* holds significant genetic diversity for species not yet fully harnessed for breeding. Thus, nationwide germplasm collection is recommended to enhance the management and conservation of this yam species.
2. There is the need to include some of the superior accessions in breeding and improvement programmes to broaden the genetic base of *D. praehensilis* in Ghana.
3. The identified superior bush yam accessions should be evaluated for organoleptic properties and sensory analysis to determine the acceptable accessions by the consumers.
4. Further studies are recommended to validate the candidate genes identified in this study and the development of diagnostic SNP markers for marker-assisted selection.

REFERENCES

Abano, E. E., Ma, H., & Qu, W. (2012). Influence of combined microwave-vacuum drying on drying kinetics and quality of dried tomato slices. *Journal of Food Quality*, 35(3), 159–168. https://doi.org/10.1111/j.1745-4557.2012.0 0446.x

Abdel-Shafy, H., Bortfeldt, R.H., Tetens, J., & Brockmann, G.A. (2014). Single nucleotide polymorphism and haplotype effects associated with somatic cell score in German Holstein cattle. *Genetics Selection Evolution*, 46(1):35. DOI: 10.1186/1297-9686-46-35. PMID: 24898131; PMCID: PMC407 8941.

Abdin, M.Z., Kiran, U., Kamaluddin, & Ali, A. (2017). Plant Biotechnology: Principles and applications. https://doi.org/10.1007/978-981-10-2961-5

Abebe, W., Demissew, S., Fay, M.F., Smith R.J., Nordal, I., & Wilkin, P. (2013). Genetic diversity and population structure of Guinea yams (*Dioscorea* spp.) and their wild relatives in South and Southwest Ethiopia as revealed by microsatellite markers. *Genetic Resources and Crop Evolution***,** 60: 529–541.

Aboagye, L.M., Nyadanu, D., Opoku-Agyeman, M.O., Owusu, S.K., & Asiedu-Darko, E. (2015). Survey of diversity and production of yams in four communities in Southern Ghana. *African Journal Agricultural Research*, 10, 2453–2459. https://doi.org/10.5897/ajar2014.9468

Acinas, S. G., Sarma-Rupavtarm, R., Klepac-Ceraj, V., & Polz, M. F. (2005). PCR-induced sequence artifacts and bias: insights from comparison of two 16s rRNA clone libraries constructed from the same sample. *Applied and Environmental Microbiology*, 71(12), 8966–8969. https://doi.org/10.1128/AEM.71.12.8966-8969.2005

Adaramola, T.F., Sonibare, M.A., Sartie, A., Lopez-Montes, A., Franco, J., & Albach, D.C. (2016). Integration of ploidy level, secondary metabolite profile, and morphological traits analyses to define a breeding strategy for

trifoliate yam (*Dioscorea dumetorum* (Kunth) Pax). *Plant Genetic Resources: Characterisation and Utilisation*, 14(1), 1–10. https://doi.org/10.1017/S1479262114000975

Adeigbe, O.O., Ilori, C.O., & Adewale, B.D. (2015). Phenotypic Diversity and Ploidy Level of Some *Dioscorea dumetorum* Genotypes. *IOSR Journal of Agriculture and Veterinary Science*, Ver. II. 8, 47–52. https://doi.org/10.9790/2380-08324752

Adeniji, M., Shoyinka, S., Ikotun. T., Asiedu, R., Hughes, J.D., & Odu, B. (2012). Yield loss in guinea yam (*Dioscorea rotundata* Poir.) due to infection by yam mosaic virus (YMV) genus potyvirus. Ife Journal of Science. 14, 237–244.

Adewumi, A.S., Asare, P.A., Adu, M.O., Taah, K.J., Akaba, S., Mondo, J.M., & Agre, P.A. (2021). Farmers' perceptions on varietal diversity, trait preferences and diversity management of bush yam (*Dioscorea praehensilis* Benth.) in Ghana. *Scientific African*, 12, e00808. https://doi.org/10.1016/j .sciaf.2021.e00808

Adewumi, A.S., Taah, K.J., Asare, P.A., Agre, P.A., & Adu, M.O. (2020). Assessment of Genetic Diversity of *Dioscorea Praehensilis* (Berth.) Collected from Central Region, Ghana Using Simple Sequence Repeat. *ES Journal of Agriculture and Current Research*, 1(1), 1–7.

Adigoun-Akotegnon, F.A., Adoukonou-Sagbadja, H., Fadinan, C., Tchougourou, A., Agassounon-Tchibozo, M., & Ahanhanzo, C. (2019). Diversity, distribution, and ethnobotanical importance of cultivated and wild African trifoliate yam [Dioscorea dumetorum (Kunth) Pax] in Benin. *Genetic Resources and Crop Evolution*, 66(3), 659–683. https://doi.org/10.1007/s10722-019-00739-z.

Adoukonou-Sagbadja, H., Wagner, C., Dansi, A., Ahlemeyer, J., Daïnou, O., Akpagana, K., Ordon, F., & Friedt, W. (2007). Genetic diversity and population differentiation of traditional fonio millet (*Digitaria* spp.)

landraces from different agro-ecological zones of West Africa. *Theoretical and Applied Genetics*, 115(7), 917–931. https://doi.org/10.1007/s00122-007-0618-x

Afoakwa, E.O., & Sefa-Dedeh, S. (2002). Textural and microstructural changes associated with post-harvest hardening of trifoliate yam (Dioscorea dumetorum) pax tubers. *Food Chemistry*, 77(3), 279–284. https://doi.org/10.1016/S0308-8146 (01)00343-0

Agre, P., Asibe, F., Darkwa, K., Edemodu, A., Bauchet, G., Asiedu, R., Adebola, P., & Asfaw, A. (2019). Phenotypic and molecular assessment of genetic structure and diversity in a panel of winged yam (*Dioscorea alata*) clones and cultivars. *Scientific Reports*, 9(1). https://doi.org/10.1038/s41598-019-54761-3

Agre, P.A, Norman, P. E., Asiedu, R., & Asfaw, A. (2021a). Identification of Quantitative Trait Nucleotides and Candidate Genes for Tuber Yield and Mosaic Virus Tolerance in an Elite Population of White Guinea Yam (*Dioscorea rotundata*) Using Genome-Wide Association Scan. *BMC Plant Biology*, 1–17. https://doi.org/10.1186/s12870-021-03314-w

Agre, P.A., Dassou, A.G., Loko, L.E.Y., Idossou, R., Dadonougbo, E., Gbaguidi, A., Mondo, J.M., Muyideen, Y., Adebola, P.O., Asiedu, R., Dansi, A.A., & Asfaw, A. (2021b). Diversity of white Guinea yam (*Dioscorea rotundata* Poir.) cultivars from Benin as revealed by agro-morphological traits and SNP markers. *Plant Genetic Resources*: Characterization and Utilization, October, 1–10. https://doi.org/10.1017/s1479262121000526

Aighewi, B.A., Asiedu, R., Maroya, N., & Balogun, M. (2015). Improved propagation methods to raise the productivity of yam (*Dioscorea rotundata* Poir.). Food Security, 7(4), 823–834. https://doi.org/10.1007/s12571-015-0481-6

Ajala, S.O., Olayiwola, M.O., Ilesanmi, O.J., Gedil, M., Job, A.O., & Olaniyan, A.B. (2019). Assessment of genetic diversity among low-nitrogen-tolerant

early generation maize inbred lines using SNP markers. *South African Journal of Plant and Soil*, 36(3), 181–188. https://doi.org/10.1080/02571862.2018.1537010

Al Salameen, F., Habibi, N., Kumar, V., Al Amad, S., Dashti, J., & Talebi, L. (2018). Genetic diversity and population structure of *Haloxylon salicornicum* (moq.) in Kuwait by ISSR markers. *PLoS ONE*, 13(11): e0207369. https://doi.org/ 10.1371/journal.pone.0207369.

Alabi, T.R., Adebola, P.O., Asfaw, A., De Koeyer, D., Lopez-Montes, A., & Asiedu, R. (2019). Spatial multivariate cluster analysis for defining target population of environments in West Africa for yam breeding. *International Journal of Applied Geospatial Research*, 10, 1–30. https://doi.org/10.4018 /IJAGR.2019070104

Alamu, O.E., Adesokan, M., & Maziya-Dixon, B. (2019). Calibration development for nutritional evaluation of Yam (*Dioscorea* spp) using Near-Infrared Reflectance Spectrophotometry (NIRS). *Cogent Chemistry*, 5(1), 1–13. https://doi.org/10.1080/23312009.2019.1565623

Alamu, E.O., Adesokan, M., Asfaw, A., & Maziya-Dixon, B. (2020). Effect of Sample Preparation Methods on the Prediction Performances of Near Infrared Reflectance Spectroscopy for Quality Traits of Fresh Yam (*Dioscorea* spp.). *Applied Science*, 10, 6035; DOI: 10.3390/app10176035

Alexis, S.D. (2013). Technical Sheet of Some Wild Yam (*Dioscorea*) Species Starch Functional Properties. *IOSR Journal of Pharmacy* (IOSRPHR), 03(06), 66–72. https://doi.org/10.9790/3013-0361066-72

Andriamparany, J.N., Brinkmann, K., Jeannoda, V., & Buerkert, A. (2014). Effects of socio-economic household characteristics on traditional knowledge and usage of wild yams and medicinal plants in the Mahafaly region of south-western Madagascar. *Journal of Ethnobiology and Ethnomedicine*, 10, 1-21. https://doi.org/10.1186/1746-4269-10-82

Anokye, M., Tetteh, J.P., & Otoo, E. (2014). Morphological Characterization of Some Water Yam (*Dioscorea alata* L.) Germplasm in Ghana. *Journal of Agricultural Science and Technology B*, 518–532.

Arnau, G., Abraham, K., Sheela, M.N., Sartie, A., & Asiedu, R. (2010). Yams. In Root and Tuber Crops (pp. 127-148). *Springer*, New York, NY.

Arnau, G., Bhattacharjee, R., Sheela, M.N., Chair, H., Malapa, R., Lebot, V., Abraham, K., Perrier, X., Petro, D., Penet, L., & Pavis, C. (2017). Understanding the genetic diversity and population structure of yam (*Dioscorea alata* L.) using microsatellite markers. *PLoS ONE*, 12(3), 1–17. https://doi.org/10.1371/journal.pone.0174150

Arnau, G., Maledon, E., Nudol, E., & Gravillon, M.C. (2016). Progress and challenges in gene/crop improvement of yam (*Dioscorea alata* L.). In: *Electronic Proceedings of World Congress on Root and Tuber Crops*. Nanning: World Congress on Root and Tuber Crops. 1, Nanning (Chine).

Asfaw, A. (2016). Standard operating protocol for yam variety performance evaluation trial, (eds.); IITA, Ibadan, Nigeria, pp. 27 (Issue April). https://doi.org/10.13140/RG.2.1.3656.4887

Asfaw, A., Aderonmu, D.S., Darkwa, K., De Koeyer, D., Agre, P., Abe, A., Olasanmi, B., Adebola, P., & Asiedu, R. (2021). Genetic parameters, prediction, and selection in a white Guinea yam early-generation breeding population using pedigree information. *Crop Science*, 61(2), 1038–1051. https://doi.org/10.1002/csc2.20382

Asiedu, R., & Sartie, A. (2010). Crops that feed the World 1. Yams. *Food Security*, 2(4), 305–315. https://doi.org/10.1007/s12571-010-0085-0

Atwell, S., Huang, Y.S., Vilhjálmsson, B.J., Willems, G., Horton, M., Li, Y., Meng, D., Platt, A., Tarone, A.M., Hu, T.T., Jiang, R., Muliyati, N.W., Zhang, X., Amer, M.A., Baxter, I., Brachi, B., Chory, J., Dean, C., Debieu, M., … & Nordborg, M. (2010). Genome-wide association study

of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*, 465(7298), 627–631. https://doi.org/10.1038/nature08800

Aubourg, S., Kreis, M., & Lecharny, A. (1999). The DEAD-box RNA helicase family in *Arabidopsis thaliana*. *Nucleic Acids Research*, 27(2), 628–636. https://doi.org/10.1093/nar/27.2.628

Avouhou, H.T., Vodouhe, R.S., Dansi, A., Bellon, M., & Kpeki, B. (2012). Ethnobotanical factors influencing the use and management of wild edible plants in agricultural environments in Benin. *Ethnobotany Research and Applications*, 10, 571–592. https://doi.org/10.17348/era.10.0.571-592

Ayisah, K.D., Mawussi, G., Tchaniley, L., & Aziadekey, M.K. (2020). Potentials of Cultivated Varieties and Wild Yam Seeds as Efficient Alternative Plant Genetic Resources for Resistant Genotypes against Yam Mosaic Virus (YMV) in Togo. *Journal of Experimental Agriculture International*, 42, 82–93. https://doi.org/10.9734/jeai/2020/v42i730557

Bakayoko, L., Da, N., Kouassi, A.B., Agre, P.A., Akintayo, O.T., Asfaw, A., Simon, A., & Guetta, P.N. (2021). Diversity of Water Yam (*Dioscorea alata* L.) Accessions from Côte d'Ivoire Based on SNP Markers and Agronomic Traits. Plant, https://doi.org/10.3390/plants10122562

Bassey, E., & Akpan, U. (2015). Evaluation of Guinea White Yam (*Dioscorea rotundata* Poir.) for Yield and Yield Components in Nigeria. *Journal of Experimental Agriculture International*, 8, 216–223. https://doi.org/10.9734/ajea/2015/16496

Bekele, A., & Bekele, E. (2018). Proximate and Mineral Composition Variability in Ethiopian Yam (Dioscorea spp). *Journal of Food and Nutrition Sciences*, 6(1), 12. https://doi.org/10.11648/j.jfns.20180601.12

Bekele, A. (2014). *Studies on Molecular Genetic Diversity and Useful Genomic Traits of Yam (Dioscorea spp) Germplasm Collections from Ethiopia*.

Ph.D. A thesis submitted to the School of Graduate Studies, Addis Ababa University, Ethiopia.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate : A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society*, 57(1), 289–300.

Beyene, T.M., Mekbib, F., & Shimelis, H. (2021). Genetic Diversity of Yam (Dioscorea spp.) Landraces from Ethiopia Assessed by Morphological and Microsatellite Markers Genetic Diversity of Yam (*Dioscorea* spp.) Landraces from Ethiopia Assessed by Morphological and Microsatellite Markers. *Ethiopia Journal of Crop Science*, 30–47.

Beyerlein, P., & Pereira, H.D.S. (2018). Morphological diversity and identification key for landraces of the Amerindian yam in central Amazon. *Pesquisa Agropecuaria Brasileira*, 53(4), 405–418. https://doi.org/10.1590/S0100-204X2018000400002

Bhandari, H., Bhanu, N.A., Srivastava, K., Singh, M., & Shreya, H.A. (2017). Assessment of Genetic Diversity in Crop Plants - An Overview. *Advance Plants and Agricultural Research*, 7, 279–286. https://doi.org/10.15406/apar.2017.07.00255

Bhattacharjee, R., Agre, P., Bauchet, G., de Koeyer, D., Lopez-Montes, A., Lava Kumar, P., Abberton, M., Adebola, P., Asfaw, A., & Asiedu, R. (2020). Genotyping-by-sequencing to unlock genetic diversity and population structure in white yam (*Dioscorea rotundata* Poir.). *Agronomy*, 10(9). https://doi.org/10.3390/AGRONOMY10091437

Bhattacharjee, R., Lava, Kumar P., Gedil, M., Sartie, A., Dumet, D., Otoo, E., & Asiedu, R. (2011). Yams (*Dioscorea* spp.). In C. Kole, (Ed.), *Wild Crop Relatives: Genomic and Breeding Resources*, (Vol. 8).

Bhattacharjee, R., Nwadili, C.O., Saski, C.A., Agre, P., Scheffler, B.E., Augusto, J., Lopez-Montes, A., Onyeka, J.T., Lava-Kumar, P., & Bandyopadhyay, R. (2018). An EST-SSR-based genetic linkage map and identification of QTLs for anthracnose disease resistance in water yam (*Dioscorea alata* L.). *PLoS ONE*, 13(10), 1–16. https://doi.org/10.1371/journal.pone.0197717.

Brachi, B., Morris, G.P., & Borevitz, J.O. (2011). Genome-wide association studies in plants: the missing heritability is in the field. *Genome Biology*, 12:232, http://genomebiology.com/2011/12/10/232

Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y., & Buckler, E.S. (2007). TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics*, 23(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Braimah, H., Anchirinah, V.M., & Adu-Mensah, J. (2008). Yam pests in the Ashanti and Brong Ahafo regions of Ghana: A study of farmers' indigenous technical knowledge and control practices. *Ghana Journal of Agricultural Science*, 40(1), 33–42. https://doi.org/10.4314/gjas.v40i1.2152

Brakenhoff, R.H., Schoenmakers, J.G.G., & Lubsen, N.H. (1991). Chimeric cDNA clones: A novel PCR artifact. *Nucleic Acids Research*, 19(8), 1949. https://doi.org/10.1093/nar/19.8.1949

Bressan, E.A., Neto, T.B., Zucchi, M.I., Rabello, R.J., & Veasey, E.A. (2011). Morphological variation and isozyme diversity in *Dioscorea alata* L. landraces from Vale do Ribeira, Brazil. *Scientia Agricola*, 68(4), 494–502. https://doi.org/10.1590/S0103-90162011000400016

Bressan, E.A., Neto, T.B., Zucchi, M.I., Rabello, R.J., & Veasey, E.A. (2014). Genetic structure and diversity in the *Dioscorea cayenensis*/*D. rotundata* complex revealed by morphological and isozyme markers. *Genetics and*

Molecular Research, 13(1), 425–437. https://doi.org/10.4238/2014. January.21.10

Brillouet, J.M., Treche, S., & Sealy, L. (1981). Alterations in cell wall constituents of yams *Dioscorea dumetorum* and *Dioscorea rotundata* with maturation and storage conditions in relation to post-harvest hardening of *D. dumetorum* yam tubers. *Journal of Food Science* 46: 1954-1965.

Burkill, I.H. (1960). The organography and the evolution of Dioscoreaceae, the family of the Yams. *Journal of the Linnean Society of London, Botany*, 56(367), 319–412. https://doi.org/10.1111/j.1095-8339.1960.tb02508.x

Camardella, L., Carratore, V., Ciardiello, M.A., Servillo, L., Balestrieri, C., & Giovane, A. (2000). Kiwi protein inhibitor of pectin methylesterase: Amino-acid sequence and structural importance of two disulfide bridges. *European Journal of Biochemistry*, 267(14), 4561–4565. https://doi.org/10.1046/j.1432-1327.2000.01510.x

Campbell, C.L., & Madden, L.V. (1990). Introduction to plant disease epidemiology. John Wiley & Sons. 1990.

Cao, X., Li, K., Suh, S. G., Guo, T., & Becraft, P.W. (2005). Molecular analysis of the CRINKLY4 gene family in *Arabidopsis thaliana*. *Planta*, 220(5), 645–657. https://doi.org/10.1007/s00425-004-1378-3

Cao, T., Sun, J., Shan, N., Chen, X., Wang, P., Zhu, Q., Xiao, Y., Zhang, H., Zhou, Q., & Huang, Y. (2021). Uncovering the genetic diversity of yams (*Dioscorea* spp.) in China by combining phenotypic trait and molecular marker analyses. *Ecology and Evolution*, 11(15), 9970–9986. https://doi.org/10.1002/ece3.7727

Cen, H., & He, Y. (2007). Theory and application of near-infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science and Technology*, 18(2), 72–83. https://doi.org/10.1016/j.tifs.2006.09.003

Chaïr, H., Perrier, X., Agbangla, C., Marchand, J.L., Dainou, O., & Noyer, J.L. (2005). Use of cpSSRs for the characterisation of yam phylogeny in Benin. *Genome*, 48(4), 674–684. https://doi.org/10.1139/G05-018

Chatterjee, M., Mazumder, M., & Basu, D. (2013). Functional analysis of the promoter of a glycosyl hydrolase gene induced in resistant *Sinapis alba* by *Alternaria brassicicola*. *Phytopathology*, 103(8), 841–850. https://doi.org/1 0.1094/PHYTO-11-12-0303-R

Chen, W., Hou, L., Zhang, Z., Pang, X., & Li, Y. (2017). Genetic diversity, population structure, and linkage disequilibrium of a core collection of *Ziziphus jujuba* assessed with genome-wide SNPs developed by genotyping-by-sequencing and SSR Markers. *Frontiers in Plant Science*, 8(April), 1–14. https://doi.org/10.3389/fpls.2017.00575

Chi, M., Bhagwat, B., Lane, W.D., Tang, G., Su, Y., Sun, R. … & Xiang, Y. (2014). Reduced polyphenol oxidase gene expression and enzymatic browning in potato (*Solanum tuberosum* L.) with artificial microRNAs. *BMC Plant Biology*, 14(1), 1–18. https://doi.org/10.1186/1471-2229-14-62

Clarke, W.E., Higgins, E.E., Plieske, J., Wieseke, R., Sidebottom, C., Khedikar, Y., Batley, J., Edwards, D., Meng, J., Li, R., Lawley, C.T., Pauquet, J., Laga, B., Cheung, W., Iniguez-Luy, F., Dyrszka, E., Rae, S., Stich, B., Snowdon, R.J., … & Parkin, I.A.P. (2016). A high-density SNP genotyping array for Brassica napus and its ancestral diploid species based on optimised selection of single-locus markers in the allotetraploid genome. *Theoretical and Applied Genetics*, 129(10), 1887–1899. https://doi.org/10.1007/s001 22-016-2746-7

Cline, J., Braman, J.C., & Hogrefe, H.H. (1996). PCR fidelity of Pfu DNA polymerase and other thermostable DNA polymerases. *Nucleic Acids Research*, 24(18), 3546–3551. https://doi.org/10.1093/nar/24.18.3546

Contreras-Soto, R. I., Mora, F., De Oliveira, M. A. R., Higashi, W., Scapim, C. A., & Schuster, I. (2017). A genome-wide association study for agronomic traits in soybean using SNP markers and SNP-Based haplotype analysis. *PLoS ONE*, 12(2), 1–22. https://doi.org/10.1371/journal.pone.0171105

Cormier, F., Mournet, P., Causse, S., Arnau, G., Maledon, E., Gomez, R.M. … & Chair, H. (2019a). Development of a cost-effective single nucleotide polymorphism genotyping array for management of greater yam germplasm collections. *Ecology and Evolution*, 9(10), 5617–5636. https://doi.org/ 10.1002/ece3.5141

Cormier, F., Lawac, F., Maledon, E., Gravillon, M.C., Nudol, E., Mournet, P., Vignes, H., Chaïr, H., & Arnau, G. (2019b). A reference high-density genetic map of greater yam (*Dioscorea alata* L.). *Theoretical and Applied Genetics*, 132(6), 1733–1744. https://doi.org/10.1007/s00122-019-03311-6

Coursey, D. G. (1976). Yams: *Dioscorea* spp. (Dioscoreaceae). *Evolution of Crop Plants*. NW Simmonds, Ed.

Couto, R.S., Martins, A.C., Bolson, M., Lopes, R.C., Smidt, E.C., & Braga, J.M.A. (2018). Time calibrated tree of *Dioscorea* (Dioscoreaceae) indicates four origins of yams in the Neotropics since the Eocene, *Botanical Journal of the Linnean Society*. Volume 188, Issue 2, 144–160, https://doi.org/ 10.1093/botlinnean/boy052.

Craufurd, P.Q., Battley, N.H., Ile, E.L., & Asiedu, R. (2006). Phase of dormancy in yam tubers (*Dioscorea rotundata*). *Annals of Botany*, 97: 497-504.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dansi, A., Dantsey-Barry, H., Dossou-Aminon, I., Kpenu, E.K., Agré, A.P., Sunu, Y.D., Kombaté, K., Loko, Y.L., Dansi, M., Assogba, P., & Vodouhè, R. (2013). Varietal diversity and genetic erosion of cultivated yams (*Dioscorea cayenensis* Poir - *D. rotundata* Lam complex and *D. alata* L.) in Togo. *International Journal of Biodiversity Conservation*, 5(2), 223–239. https://doi.org/10.5897/IJBC12.131

Dansi, A., Mignouna, H.D., Zoundjihékpon, J., Sangare, A., Asiedu, R., & Quin, F.M. (1999). Morphological diversity, cultivar groups and possible descent in the cultivated yams (*Dioscorea cayenensis/D. rotundata*) complex in Benin Republic. *Genetic Resources and Crop Evolution*, 46(4), 371–388. https://doi.org/10.1023/A:1008698123887

Dansi, A., Orobiyi, A., Dansi, M., Assogba, P., Sanni, A., & Akpagana, K. (2013). Sélection de sites pour la conservation in situ des ignames sauvages apparentées aux ignames cultivées : cas de *Dioscorea praehensilis* au Bénin. *International Journal of Biological and Chemical Sciences*, 7(1), 60. https://doi.org/10.4314/ijbcs.v7i1.6

Darkwa, K., Agre, P., Olasanmi, B., Iseki, K., Matsumoto, R., Powell, A., Bauchet, G., De Koeyer, D., Muranaka, S., Adebola, P., Asiedu, R., Terauchi, R., & Asfaw, A. (2020). Comparative assessment of genetic diversity matrices and clustering methods in white Guinea yam (*Dioscorea rotundata*) based on morphological and molecular markers. *Scientific Reports*, 10(1). https://doi.org/10.1038/s41598-020-69925-9

Darkwa, K., Olasanmi, B., Asiedu, R., & Asfaw, A. (2019). Review of empirical and emerging breeding methods and tools for yam (*Dioscorea* spp.) improvement: Status and prospects. *Plant Breeding*, https://doi.org /10.1111/pbr.12783

Darvishzadeh, R. (2016). Population structure, linkage disequilibrium, and association mapping for morphological traits in sunflower (*Helianthus*

annuus L.). *Biotechnology and Biotechnological Equipment*, 30(2), 236–246. https://doi.org/10.1080/13102818.2015.1136568

Das, A. K., Helps, N. R., Cohen, P. T. W., & Barford, D. (1996). Crystal structure of the protein serine/threonine phosphatase 2C at 2.0 Å resolution. *EMBO Journal*, 15(24), 6798–6809. https://doi.org/10.1002/j.1460-2075.1996.tb01071.x

Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., & Blaxter, M. L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *In Nature Reviews Genetics,* (Vol. 12, Issue 7, pp. 499–510). https://doi.org/10.1038/nrg3012

de Mendiburu, F. (2021). Agricolae tutorial (Version 1.3-5). Universidad Nacional Agraria, La Molina, PERU, 88 p.

Demko, V., Ako, E., Perroud, P. F., Quatrano, R., & Olsen, O. A. (2016). The phenotype of the CRINKLY4 deletion mutant of *Physcomitrella patens* suggests a broad role in developmental regulation in early land plants. *Planta*, 244(1), 275–284. https://doi.org/10.1007/s00425-016-2526-2

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., & Guarino, L. (2017). Past and future use of wild relatives in crop breeding. *Crop Science*. 57, 1070–1082. https://doi.org/10.2135/cropsci2016.10.0885

Deshmukh, S.N., Basu, M., & Reddy, P.S. (1986). Genetic variability, character association, and path coefficient analysis of quantitative traits in Virginia bunch varieties of groundnut. *Indian Journal of Agricultural Science*, 56, 515–518.

Di Giusto, B., Dounias, E., & McKey, D.B. (2017). Facing herbivory on the climb up Lost opportunities as the main cost of herbivory in the wild yam *Dioscorea praehensilis*. *Ecology and Evolution*, 7(16), 6493–6506. https://doi.org/10.1002/ece3.3066

Djedatin, G., Adeoti, K., Ewedje, E., & Agbangla, C. (2017). Does the Domestication of Yam Wild Type *Dioscorea praehensilis* and *Dioscorea abyssinica* has a Genetic Basis? *International Journal of Sciences*, 3(11), 18–28. https://doi.org/10.18483/ijsci.1431

do Carmo, C.D., e Sousa, M.B., Brito, A.C., & de Oliveira, E.J. (2020). Genome-wide association studies for waxy starch in cassava. *Euphytica*, 216(5). https://doi.org/10.1007/s10681-020-02615-9

Doyle, J.J., & Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus***,** v.12, p.13-15.

Drost, H.G. (2018). Philentropy: information theory and distance quantification with R. *Journal of Open Source Software*, *3*(26), 765.

Dumont, R., Dansi, A., Vernier, P.H., & Zoundjihékpon, J. (2005). Biodiversity and domestication of yams in West Africa. Traditional practices leading to *Dioscorea rotundata* Poir. Edité par Collection Repères, *CIRAD*, 119 pages.

Dumont, R., Dansi, A., Vernier, P., & Zoundihèkpon, J. (2006) Biodiversity and Domestication of Yams in West Africa Traditional Practices leading to *Dioscorea rotundata* Poir. *CIRAD and IPGRI, Montpellier and Rome*, pp. 95. https://doi.org/10.1017/S0014479707005649

Duran, C., Appleby, N., Edwards, D., & Batley, J. (2009). Molecular Genetic Markers: Discovery, Applications, Data Storage and Visualisation. *Current Bioinformatics*, 4(1), 16–27. https://doi.org/10.2174/157489309787158198

Dutta, B. (2015). Food and medicinal values of certain species of Dioscorea with special reference to Assam. *Journal of Pharmacognosy and Phytochemistry*, 3(4), 15–18.

Efisue, A. (2016). Genetic diversity study of *Dioscorea* spp using Morphological Traits and Isozyme Markers Analyses. *Nigerian Journal of Biotechnology*, 30(1), 7. https://doi.org/10.4314/njb.v30i1.2

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., & Mitchell, S.E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, 6(5), 1–10. https://doi.org/10.1371/journal.pone.0019379

Emshwiller, E. Calberto-Sánchez, G., Girma, G., Jansky, S., Sardos, J., Staver, C., Stoddard, L.F., & Rou, N. (2015). Unavailability of wild relatives. In: Redden, R. Yadav, S.S., Maxted, N., Ehsan M.E., Dulloo, M.E., Guarino, L., and Smith, P. (Eds), Crop wild relatives and climate change. Wiley-Blackwell, pp. 224-249.

Esuma, W., Herselman, L., Labuschagne, M.T., Ramu, P., Lu, F., Baguma, Y., Buckler, E.S., & Kawuki, R.S. (2016). Genome-wide association mapping of provitamin A carotenoid content in cassava. *Euphytica*. https://doi.org/10.1007/s10681-016-1772-5

Etikan, I., Alkassim, R., & Abubakar, S. (2016). Comparison of snowball sampling and sequential sampling technique. *Biom. Biostat. Int. J.*, 3(1), 55.

FAOSTAT (2017). Food and Agriculture Organization Cooperate Statistical Database, http:/www/faostat.fao.org/2017.

FAOSTAT (2020). Food and Agriculture Organization Cooperate Statistical Database, Available online: http://www.fao.org/faostat/en/#data/QL (accessed on 8th December 2020).

FAOSTAT. (2021). Food and Agriculture Organization Cooperate Statistical Database, Available online: http://www.fao.org/faostat/en/#data/QL (accessed on 10th August 2021).

Farris, S., Schaich, K.M., Liu, L.S., Cooke, P.H., Piergiovanni, L., & Yam, K.L. (2011). Gelatin-pectin composite films from polyion-complex hydrogels. *Food Hydrocolloids*, 25(1), 61–70. https://doi.org/10.1016/j.foodhyd.2010.05.006

Fernandez-lopez, M., Haeze, W.D., Mergaert, P., Verplancke, C., Prome, J., Van Montagu, M., & Holsters, M. (1996). Role of nodI and nodJ in lipo-oligosaccharide secretion in Azorhizobium caulinodans and Escherichia coli. *Molecular Microbiology*, 20, 993–1000.

Frankham, R., Ballou, S.E.J.D., Briscoe, D.A., & Ballou, J.D. (2002). *Introduction to conservation genetics*. Cambridge University Press.

Frossard, E., Aighewi, B.A., Aké, S., Barjolle, D., Baumann, P., Bernet, T., Dao, D., Diby, L.N., Floquet, A., Hgaza, V.K., & Ilboudo, L.J. (2017). The challenge of improving soil fertility in yam cropping systems of West Africa. *Frontiers in Plant Science*, 8, 1953.

Fu, Y.B., Cheng, B., & Peterson, G.W. (2014). Genetic diversity analysis of yellow mustard (*Sinapis alba* L.) germplasm based on genotyping by sequencing. *Genetic Resources and Crop Evolution*, 61(3), 579–594. https://doi.org/ 10.1007/s10722-013-0058-1

Gatarira, C., Agre, P., Matsumoto, R., Edemodu, A., Adetimirin, V., Bhattacharjee, R., Asiedu, R., & Asfaw, A. (2020). Genome-Wide Association Analysis for Tuber Dry Matter and Oxidative Browning in Water Yam (*Dioscorea alata* L.). *Plants*, 9(8), 969. https://doi.org/10.3390/plants9080969

Gaudioso-Pedraza, R., & Benitez-Alfonso, Y. (2014). A phylogenetic approach to study the origin and evolution of plasmodesmata-localized glycosyl hydrolases family 17. *Frontiers in Plant Science*, 5(MAY), 1–14. https://doi.org/10.3389/fpls.2014.00212

Gbadamosi, I.T., Abberton, M., Igwe, A.C., Soyode, F., & Olawuyi, O.J. (2020). Phenotypic Characterization of *Dioscorea burkilliana* J. Miège (Dioscoreaceae) Genetic Resources. *Journal of Applied Life Sciences International*, 23(1), 41–57. https://doi.org/10.9734/jalsi/2020/v23i130141

Geigenberger, P. (2003). Regulation of sucrose to starch conversion in growing potato tubers. *Journal of Experimental Botany*, 54(382), 457–465. https://doi.org/10.1093/jxb/erg074

Girma, G., Bhattacharjee, R., Lopez-Montes, A., Gueye, B., Ofodile, S., Franco, J., & Abberton, M. (2018). Re-defining the yam (*Dioscorea* spp.) core collection using morphological traits. *Plant Genetic Resources: Characterisation and Utilisation*, 16(3), 193–200. https://doi.org/10.1017/S1479262117000144

Girma, G., Gedil, M., & Spillane, C. (2017). Morphological, SSR and ploidy analysis of water yam (*Dioscorea alata* L.) accessions for utilization of aerial tubers as planting materials. *Genetic Resources and Crop Evolution*, 64(2), 291–305. https://doi.org/10.1007/s10722-015-0351-2

Girma, G., Hyma, K.E., Asiedu, R., Mitchell, S.E., Gedil, M., & Spillane, C. (2014). Next-generation sequencing based genotyping, cytometry and phenotyping for understanding diversity and evolution of guinea yams. *Theoretical and Applied Genetics*, 127(8), 1783–1794. https://doi.org/10.1007/s00122-014-2339-2

Gnamkoulamba, A., Tchala, W., Tostain, S., & Soumana, I. (2002). Le bayere, une forme domestiquée de *Dioscorea praehensilis* dans la prefecture de Wawa (Togo). *Annales des Sciences Agronomiques*, 3(2), 45–53. https://doi.org/ 10.4314/asab.v3i2.43382

Gómez, G., Álvarez, M.F., & Mosquera, T. (2011). Association mapping, a method to detect quantitative trait loci: statistical bases. *Agronomía Colombiana*, 29(3), 367–376.

González, M. N., Massa, G. A., Andersson, M., Turesson, H., Olsson, N., Fält, A. S., … & Feingold, S. E. (2020). Reduced Enzymatic Browning in Potato Tubers by Specific Editing of a Polyphenol Oxidase Gene via Ribonucleoprotein Complexes Delivery of the CRISPR/Cas9 System.

Frontiers in Plant Science, 10, 1–12. https://doi.org/10.3389/fpls.2019.01649

Govindaraj, M., Vetriventhan, M., & Srinivasan, M. (2015). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genetics Research International*, 2015, 14. https://doi.org/10.1155/2015/431487

Graham-Acquaah, S., Ayernor, G.S., Bediako-Amoa, B., Saalia, F.K., & Afoakwa, E.O. (2014). Spatial distribution of total phenolic content, enzymatic activities, and browning in white yam (*Dioscorea rotundata*) tubers. *Journal of Food Science and Technology*, 51(10), 2833–2838. https://doi.org/10.1007/s13197-012-0760-6

Hajjar, R., & Hodgkin, T. (2007). The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica*, 156(1–2), 1–13. https://doi.org/10.1007/s10681-007-9363-0

Haleegoah, J.A.S. (2018). Changing gender roles in seed yam production among smallholder farmers in Ghana: Implications for food security. *3rd Global Food Security, Food Safety & Sustainability Conference*: New York, USA, 21–22 May 2018.

Hammer, Ø., Harper, D.A., & Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia electronica*, 4(1), 9.

Hattori, T., Yoshida, N., & Nakamura, K. (1989). Structural relationship among the members of a multigene family coding for the sweet potato tuberous root storage protein. *Plant Molecular Biology*, 13(5), 563–572. https://doi.org/ 10.1007/BF00027316

Hildebrand, E., Demissew, S., & Wilkin, P. (2000). Local and regional landrace disappearance in species of *Dioscorea* L. (Yams) in Southwest Ethiopia: causes of agrobiodiversity loss and strategies for conservation. In: Stepp,

J. R., Wyndham, F.S., Zarger, R.K. (eds), Ethnobiology and biocultural diversity, Proceedings of the 7th International Congress of Ethnobiology and Biocultural Diversity: Athens, Georgia, USA, October 2000, pp. 678–695.

Hildebrand, E.A. (2003). Motives and opportunities for domestication: An ethnoarchaeological study in southwest Ethiopia. *Journal of Anthropological Archaeology*, 22(4), 358–375. https://doi.org/10. 1016/S0278-4165 (03)00031-X

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C., Zhu, C., Lu, T., Zhang, Z., Li, M., Fan, D., Guo, Y., Wang, A., Wang, L., Deng, L., Li, W., Lu, Y., Weng, Q., … & Han, B. (2010). Genome-wide association studies of 14 agronomic traits in rice landraces. *Nature Genetics*, 42(11), 961–967. https://doi.org/10.1038/ng.695

Huang, X., Zhao, Y., Wei, X., Li, C., Wang, A., Zhao, Q., Li, W., Guo, Y., Deng, L., Zhu, C., Fan, D., Lu, Y., Weng, Q., Liu, K., Zhou, T., Jing, Y., Si, L., Dong, G., Huang, T., … & Han, B. (2012). Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nature Genetics*, 44(1), 32–39. https://doi.org/10.1038/ng. 1018

Hunter, S., Jones, P., Mitchell, A., Apweiler, R., Attwood, T.K., Bateman, A. … & Yong, S.Y. (2012). InterPro in 2011: New developments in the family and domain prediction database. *Nucleic Acids Research*, 40(D1), 306–312. https://doi.org/10.1093/nar/gkr948

Ile, E.I., Crauford, P.Q., Battey, N.H., & Asiedu, R. (2006). Phases of dormancy in yam tubers (*Dioscorea rotundata*). *Annals of Botany*, 97(4), 497–504. https://doi.org/10.1093/aob/mcl002

Jia, G., Huang, X., Zhi, H., Zhao, Y., Zhao, Q., Li, W., Chai, Y., Yang, L., Liu, K., Lu, H., Zhu, C., Lu, Y., Zhou, C., Fan, D., Weng, Q., Guo, Y., Huang, T., Zhang, L., Lu, T., … Han, B. (2013). A haplotype map of genomic

variations and genome-wide association studies of agronomic traits in foxtail millet (*Setaria italica*). *Nature Genetics*, 45(8), 957–961. https://doi.org/10.1038/ng.2673

Jiang, Y., Duan, X., Qu, H., & Zheng, S. (2015). Browning: Enzymatic Browning. *Encyclopedia of Food and Health*, 508–514. https://doi.org/10.1016/B978-0-12-384947-2.00090-8

Johnson, M.W., Robinson, H.F., & Comstock, R.E. (1955). Genotypic and phenotypic correlations in soybeans and their implication in selection. *Agronomy Journal*, 47, 477–483.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, 11(94), 1–15. http://www.biomedcentral.com

Jukanti, A. (2017). Polyphenol Oxidases (PPOs) in Plants, (2012), 1–131. https://doi.org/10.1007/978-981-10-5747-2

Kadiri, M., Ojewumi, A.W., Abiola, T,O., & Hussain, A.M. (2014). Ethno-botanical survey of plants commonly used for ceremonial activities among Yoruba tribe of South West, Nigeria. *Direct Research Journal of Health and Pharmacology*, 2(February), 1–5.

Kang, J., Park, J., Choi, H., Burla, B., Kretzschmar, T., Lee, Y., Martinoia, E. (2011). Plant ABC Transporters. Arabidopsis Book; 9:e0153. doi: 10.1199/tab.0153. *Epub*. PMID: 22303277; PMCID: PMC3268509.

Kemausuor, F., Akowuah, J.O., & Ofori, E. (2013). Assessment of Feedstock Options for Biofuels Production in Ghana. *Journal of Sustainability and Bioenergy System*, 3(02), 119–128. https://doi.org/10.4236/jsbs.2013.32017

Kesari, P., Neetu, B.S.P., Sharma, A., Katiki, M., Kumar, P., Gurjar, B. … & Kumar, P. (2017). Structural, Functional, and Evolutionary Aspects of

Seed Globulins. *Protein & Peptide Letters*, 24(3), 267–277. https://doi.org/10 .2174/092986652366616122011264l

Kesten, C., Menna, A., & Sánchez-Rodríguez, C. (2017). Regulation of cellulose synthesis in response to stress. *Current Opinion in Plant Biology*, 40, 106–113. https://doi.org/10.1016/j.pbi.2017.08.010

Kim, D., Langmead, B., & Salzberg1, S.L. (2015). HISAT: a fast spliced aligner with low memory requirements Daehwan HHS Public Access. *Nature Methods*, 12(4), 357–360. https://doi.org/10.1038/nmeth.3317.HISAT

Kim, J.Y., Park, S.C., Hwang, I., Cheong, H., Nah, J.W., Hahm, K.S., & Park, Y. (2009). Protease inhibitors from plants with antimicrobial activity. *International Journal of Molecular Sciences*, 10(6), 2860–2872. https://doi.org/10.3390/ijms10062860

Kim, W., & Tabita, F.R. (2006). Both subunits of ATP-citrate lyase from Chlorobium tepidum contribute to catalytic activity. *Journal of Bacteriology*, 188(18), 6544–6552. https://doi.org/10.1128/JB.00523-06

Kolomiets, M.V., Hannapel, D.J., Hao, C., Tymeson, M., & Gladon, R.J. (2001). Lipoxygenase is involved in the control of potato tuber development. *The Plant Cell*, Vol. 13, 613-626.

Koradaa, R.R., Naskara, S.K., & Edison, S. (2010). Insect pests and their management in yam production and storage: A world review. *International Journal of Pest Management*, 56(4), 337–349. https://doi.org/10. 1080/09670874.2010.500406

Kouam, E.B., Avana-Tientcheu, M.L., Lekeumo, V.D., Akitio, H.M., Khasa, D.P., & Pasquet, R.S. (2018). Agro-ecological distribution of the phenotypic diversity of aerial yam (*Dioscorea bulbifera* L.) in Cameroon using multivariate analysis: Prospect for germplasm conservation and improvement. *Open Agriculture*, 3(1), 190–206. https://doi.org/10. 1515/opag-2018-0020

Kraakman, A.T.W., Niks, R.E., Van Den Berg, P.M.M.M., Stam, P., & Van Eeuwijk, F.A. (2004). Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. *Genetics*, 168(1), 435–446. https://doi.org/10.1534/genetics.104.026831

Kulibaba, R.A., & Liashenko, Y.V. (2016). Influence of the PCR artifacts on the genotyping efficiency by the microsatellite loci using native polyacrylamide gel electrophoresis. *Cytology and Genetics,* 50(3), 162–167. https://doi.org/ 10.3103/S0095452716030087

Kumar, J., Mir, R., Kumar, N., Kumar, A., Mohan, A., Prabhu, K.V., Balyan, H.S., & Gupta, P.K. (2010). Marker-assisted selection for pre-harvest sprouting tolerance and leaf rust resistance in bread wheat. *Plant Breeding*, 129(6), 617–621. https://doi.org/10.1111/j.1439-0523.2009.01758.x

Kumar, S., Ambreen, H., Variath, M.T., Rao, A.R., Agarwal, M., Kumar, A., Goel, S., & Jagannath, A. (2016). Utilization of molecular, phenotypic, and geographical diversity to develop the compact composite core collection in the oilseed crop, safflower (*Carthamus tinctorius* L.) through maximization strategy. *Frontiers in Plant Science*, (7): 1–14. https://doi.org/10.3389 /fpls.2016.01554

Kumar, S., Das, G., Shin, H.S., & Patra, J.K. (2017a). Dioscorea spp. (A Wild Edible Tuber): A study on its ethnopharmacological potential and traditional use by the local people of Similipal biosphere reserve, India. *Frontiers in Pharmacology*, 8(FEB), 1–17. https://doi.org/10.3 389/fphar .2017.00052

Kumar, R., Bohra, A., Pandey, A.K., Pandey, M.K., & Kumar, A. (2017b). Metabolomics for plant improvement: Status and prospects. *Frontiers in Plant Science*, 8(August), 1–27. https://doi.org/10.3389/fpls.2017.01302

Kump, K.L., Bradbury, P.J., Wisser, R.J., Buckler, E.S., Belcher, A.R., Oropeza-Rosas, M.A., Zwonitzer, J. C., Kresovich, S., McMullen, M.D., Ware, D.,

Balint-Kurti, P.J., & Holland, J.B. (2011). Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nature Genetics*, 43(2), 163–168. https://doi.org/10.1038/ng.747

Kurtz D.M.J. (2001). Germin is a manganese containing homohexamer with oxalate oxidase and superoxide dismutase activities. *Chemtracts*, 14(8), 461–465.

Laly, J., Gbemavo, D.S.J.C., Gbaguidi, A.A., Dossou-Aminon, I., & Dansi, A. (2019). *Dioscorea dumetorum* (Kunth) Pax, a neglected and underutilized yam species in Benin: folk classification and post-harvest conservation. *Genetic Resources and Crop Evolution*, 66(4), 965–979. https://doi.org/10.1007/s10722-019-00762-0

Lebot, V., & Malapa, R. (2013). Application of near-infrared reflectance spectroscopy for the evaluation of yam (*Dioscorea alata*) germplasm and breeding lines. *Journal of the Science of Food and Agriculture*, 93(7), 1788–1797. https://doi.org/10.1002/jsfa.6002

Lebot, V., Malapa, R., & Molisalé, T. (2019a). Development of HP-TLC method for rapid quantification of sugars, catechins, phenolic acids, and saponins to assess Yam (*Dioscorea* spp.) tuber flour quality. *Plant Genetic Resources: Characterisation and Utilisation*, 17(1), 62–72. https://doi.org/10.1017/ S1479262118000333

Lebot, V., Abraham, K., Kaoh, J., Rogers, C., & Molisalé, T. (2019b). Development of Anthracnose resistant hybrids of the Greater Yam (*Dioscorea alata* L.) and interspecific hybrids with *D. nummularia* Lam. *Genetic Resources and Crop Evolution*, 66, 871–883. https://doi.org/10.1007/s10722-019-00756-y

Lev, L. S., & Shriver, A. L. (1998). A trend analysis of yam production, area, yield, and trade (1961-1996). *Colloques-CIRAD*, 11-20.

Li, H., Peng, Z., Yang, X., Wang, W., Fu, J., Wang, J., Han, Y., Chai, Y., Guo, T., Yang, N., Liu, J., Warburton, M. L., Cheng, Y., Hao, X., Zhang, P., Zhao, J., Liu, Y., Wang, G., Li, J., & Yan, J. (2013). Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. *Nature Genetics*, 45(1), 43–50. https://doi.org/10.1038/ng.2484

Lipka, A.E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P.J. … & Zhang, Z. (2012). GAPIT: Genome association and prediction integrated tool. *Bioinformatics*, 28(18), 2397–2399. https://doi.org/10.1093/ bioinformatics /bts444

Liu, H.J., & Yan, J. (2019). Crop genome-wide association study: a harvest of biological relevance. *Plant Journal*, 97(1), 8–18. https://doi.org/10 .1111/tpj.14139

Loko, Y.L., Dansi, A., Dansi, M., Vodouhe, R., & Sanni, A. (2013). Indigenous Knowledge and Management of Yam (*Dioscorea Cayenensis – Dioscorea Rotundata* Complex) Pests and Diseases in. *International Journal of Applied Biology and Pharmaceutical Technology*, 4(3), 276–279.

Loko, Y. L., Adjatin, A., Dansi, A., Vodouhè, R., & Sanni, A. (2015). Participatory evaluation of Guinea yam (*Dioscorea cayenensis* Lam.–*D. rotundata* Poir. complex) landraces from Benin and agro-morphological characterization of cultivars tolerant to drought, high soil moisture, and chips storage insects. *Genetic Resources and Crop Evolution*, 62(8), 1181–1192. https://doi.org/ 10.1007/s10722-015-0221-y

Loko, Y.L., Bhattacharjee, R., Agre, P., Dossou-Aminon, I., Orobiyi, A., Dejedatin, G.L., & Dansi, A. (2016). Genetic diversity and relationship of Guinea yam (*Dioscorea cayenensis* Lam.–*D. rotundata* Poir. complex) germplasm in Benin (West Africa) using microsatellite markers. *Genetic Resources and Crop Evolution*, DOI 10.1007/s10722-016-0430-z.

Lorenz, A.J., Hamblin, M.T., & Jannink, J.L. (2010). Performance of single nucleotide polymorphisms versus haplotypes for genome-wide association

analysis in barley. *PLoS ONE*, 5(11), 1–11. https://doi.org/10.1371 /journal.pone.0014079

Lowther, W.T., Brot, N., Weissbach, H., Honek, J.F., & Matthews, B.W. (2000). Thiol-disulfide exchange is involved in the catalytic mechanism of peptide methionine sulfoxide reductase. *Proceedings of the National Academy of Sciences of the United States of America*, 97(12), 6463–6468. https://doi.org/10.1073/pnas.97.12.6463

Lu, F., Lipka, A. E., Glaubitz, J., Elshire, R., Cherney, J.H., Casler, M.D., ... & Costich, D. E. (2013). Switchgrass genomic diversity, ploidy, and evolution: novel insights from a network-based SNP discovery protocol. *PLoS genetics*, *9*(1), e1003215.

Maechler, M., Rousseew, P., Struyf, A., Hubert, M., & Hornik, K. (2019). Cluster Analysis Basics and Extensions. R package version 2.1.0.

Maneenoon, K., Sirirugsa, P., & Sridith, K. (2008). Ethnobotany of Dioscorea L. (Dioscoreaceae), a major food plant of the Sakai tribe at Banthad Range, Peninsular Thailand. *Ethnobotany Research and Applications*, 6, 385–393. https://doi.org/10.17348/era.6.0.385-394

Marie, F.S.N., Ngwe, D.N.O., & Simon, J. (2015). Evolution and phylogenetic diversity of yam species (*Dioscorea* spp.): Implication for conservation and agricultural practices. *PLoS ONE***,** 10: 1-13.

Matsumoto, R., Ishikawa, H., Asfaw, A., & Asiedu, R. (2021). Low soil nutrient tolerance and mineral fertilizer response in White Guinea Yam (*Dioscorea rotundata*) genotypes. *Frontiers in Plant Science*, 12, 223.

Mayes, S., Massawe, F.J., Alderson, P.G., Roberts, J.A., Azam-Ali, S.N., & Hermann, M. (2012). The potential for underutilized crops to improve the security of food production. *Journal of Experimental Botany*, 63(3), 1075–1079. https://doi.org/10.1093/jxb/err396

Medoua, G.N., & Mbofung, C.M.F. (2006). Hard-to-cook defect in trifoliate yam *Dioscorea dumetorum* tubers after harvest. *Food Research International*, 39(5), 513–518. https://doi.org/10.1016/j.foodres.2005.10.005

Mengesha, W.A., Demissew, S., Fay, M.F., Smith, R.J., Nordal, I., & Wilkin, P. (2013). Genetic diversity and population structure of Guinea yams and their wild relatives in South and South West Ethiopia as revealed by microsatellite markers. *Genetic Resources and Crop Evolution*, 60(2), 529–541. https://doi.org/10.1007/s10722-012-9856-0

Mensah, C. (2013). Residents' satisfaction and behavioural intention with Asogli Yam Festival in Ghana. *International Journal of Asian Social Science*, 3(3), 682–702.

Michael, T.P., & VanBuren, R. (2015). Progress, challenges, and the future of crop genomes. *Current Opinion in Plant Biology*, 24, 71–81. https://doi.org/10.1016/j.pbi.2015.02.002

Mignouna, H.D., Abang, M.M., & Fagbemi, S.A. (2003). A comparative assessment of molecular marker assays (AFLP, RAPD, and SSR) for white yam (*Dioscorea rotundata*) germplasm characterisation. *Annals of Applied Biology*, 142(3), 269–276. https://doi.org/10.1111/j.1744-7348.2003.tb0 0250.x

Mignouna, H.D., & Dansi, A. (2003). Yam (*Dioscorea* ssp.) domestication by the Nago and Fon ethnic groups in Benin. *Genetic Resources and Crop Evolution*, 50, 519–528. https://doi.org/10.1023/A

Mininno, M., Brugière, S., Pautre, V., Gilgen, A., Ma, S., Ferro, M. … & Ravanel, S. (2012). Characterization of chloroplastic fructose 1, 6-bisphosphate aldolases as lysine-methylated proteins in plants. *Journal of Biological Chemistry*, 287(25), 21034–21044. https://doi.org/10.1074/jbc.M112. 359976

Mondo, J.M., Agre, P.A., Asiedu, R., Akoroda, M.O., & Asfaw, A. (2021). Genome-wide association studies for sex determination and cross-compatibility in water yam (Dioscorea alata L.). *Plants*, 10(7), 1–18. https://doi.org/10.3390/plants10071412

Mondo, J.M., Agre, P.A., Edemodu, A., Adebola, P., Asiedu, R., Akoroda, M.O., & Asfaw, A. (2020). Floral Biology and Pollination Efficiency in Yam (*Dioscorea* spp.). *Agriculture*, 10, 560.

Morris, G.P., Ramu, P., Deshpande, S.P., Hash, C.T., Shah, T., Upadhyaya, H.D., Riera-Lizarazu, O., Brown, P.J., Acharya, C.B., Mitchell, S.E., Harriman, J., Glaubitz, J.C., Buckler, E.S., & Kresovich, S. (2013). Population genomic and genome-wide association studies of agro-climatic traits in sorghum. *Proceedings of the National Academy of Sciences of the United States of America*, 110(2), 453–458. https://doi.org/10.1073/pnas.1215985110

Mosissa, D. (2018). Tuber Wild Plants of Shinasha People as Food and Medicine in Bullen District, Northwest of Ethiopia. *Biodiversity International Journal*, 2(1), 53–58. https://doi.org/10.15406/bij.2018.02.00043

Mueller, U.G., & Wolfenbarger, L.L.R. (1999). AFLP genotyping and fingerprinting. *Trends in Ecology and Evolution*, 14(10), 389–394. https://doi.org/10.1016/S0169-5347 (99)01659-6

Mulualem, T., Mekbib, F., Shimelis, H., Gebre, E., & Amelework, B. (2018). Genetic diversity of yam (*Dioscorea* spp.) landrace collections from Ethiopia using simple sequence repeat markers. *Australian Journal of Crop Science*, 12(8), 1222–1230. https://doi.org/10.21475/ajcs.18.12.08.PNE885

Mwirigi, P.N., Kahangi, E.M., Nyende, A.B., & Mamati, E.G. (2009). Morphological variability within the Kenyan yam (*Dioscorea* spp.). *Journal of Applied Biosciences*, 16, 894–901.

Nascimento, W.F., Rodrigues, J.F., Koehler, S., Gepts, P., & Veasey, E.A. (2013) Spatial structured genetic diversity of the endangered Amerindian yam (*Dioscorea trifida* L.) assessed by SSR and ISSR markers in Southern Brazil. *Genetic Resources and Crop Evolution***,** 60: 2405-2420. http://dx.doi.org/10.1007/s10722-013-0008-y.

Ngailo, S., Shimelis, H., Sibiya, J., Amelework, B., & Mtunda, K. (2016). Genetic diversity assessment of Tanzanian sweet potato genotypes using simple sequence repeat markers. *S. African Journal of Botany***,** 102: 40-45.

Nordborg, M., & Weigel, D. (2008). Next-generation genetics in plants. *Nature*, 456(7223), 720–723. https://doi.org/10.1038/nature07629

Norman, P.E, Tongoona, P., & Shanahan, P.E. (2011). Diversity of the morphological traits of yam (Dioscorea spp.) genotypes from Sierra Leone. *Journal of Applied Biosciences*, 45, 3045–3058.

Norman, P.E., Tongoona, P.B., Danquah, A., Danquah, E.Y., Agre, P.A., Agbona, A., Asiedu, R., & Asfaw, A. (2021). Genetic parameter estimation and selection in an advanced breeding population of white Guinea yam. *Journal of Crop Improvement*, 35(6), 790–815. https://doi.org/10.1080/15427528.2021.1881012

Oben, J.A., Egbe, A.E., Chuyong, G.B., & Tabot, P.T. (2016). Diversity of Yam (*Dioscorea* spp) Populations in the South Western Region of Cameroon. *American Journal of Life Sciences*, 4(6), 187–194. https://doi.org/10.11648/j.ajls.20160406.17

Obidiegwu, J.E., Asiedu, R., Ene-Obong, E.E., Muoneke, C.O., & Kolesnikova-Allen, M. (2009). Genetic characterization of some water yam (*Dioscorea alata* L.) accessions in West Africa with some simple sequence repeats. *Journal of Food, Agriculture and Environment***,** 7, 634-638.

Obidiegwu, J.E., & Akpabio, E.M. (2017). The geography of yam cultivation in southern Nigeria: Exploring its social meanings and cultural functions. *Journal of Ethnic Foods*, 4, 28–35.

Obidiegwu, J.E., Lyons, J.B., & Chilaka, C.A. (2020). The *Dioscorea* Genus (Yam) - An Appraissal of Nutritional and Therapeutic Potentials. *Food*, 1–45.

Okoro, G.O., Onu, O.B., Ngasoh, G.F., & Nicholas, N. (2019). The Effect of Climate Change on Abiotic Plant Stress: A Review. *Abiotic and Biotic Stress in Plants*, 1–13. https://doi.org/10.5772/intechopen.82681

Oladeji, A.E., Bussie, M.D., Roman, F., Ibironke, P., Robert, A., & Therese, G. (2016). Characterization and classification of the provitamin A carotenoids of deep yellow-fleshed bitter yam (*Dioscorea dumetorum*) varieties. *Journal of Food and Nutrition Research*. https://doi.org/10.12691/jfnr-4-10-2

Olatoye, K.K., & Arueya, G.L. (2019). Nutrient and phytochemical composition of flour made from selected cultivars of Aerial yam (*Dioscorea bulbifera*) in Nigeria. *Journal of Food Composition and Analysis*, 79(January), 23–27. https://doi.org/10.1016/j.jfca.2018.12.007

Onda, Y., & Mochida, K. (2016). Exploring Genetic Diversity in Plants Using High-Throughput Sequencing Techniques. *Current Genomics*, 17(4), 358–367. https://doi.org/10.2174/1389202917666160331202742

Oomen, R.J.F.J., Tzitzikas, E.N., Bakx, E.J., Straatman-Engelen, I., Bush, M.S., McCann, M.C., … & Vincken, J.P. (2004). Modulation of the cellulose content of tuber cell walls by antisense expression of different potato (*Solanum tuberosum* L.) CesA clones. *Phytochemistry*, 65(5), 535–546. https://doi.org/10.1016/j.phytochem.2003.12.019

Orobiyi, A., Dansi, A., Assogba, P., Loko, L.Y., Dansi, M., Vodouhè, R., Akouegninou, A., & Sanni, A. (2013). Chili (*Capsicum annuum* L.) in southern Benin: production constraints, varietal diversity, preference criteria and participatory evaluation. *International Research Journal of Agricultural Science and. Soil*, 3, 107–120.

Otoo, E., Akromah, R., Kololesnikova-Allen, M., & Asiedu, R. (2009). Ethno-botany and morphological characterisation of the yam pona complex in Ghana. *International African Crop Science Conference Proceeding*, 9, 407–414.

Otoo, E., Opoku Agyeman, M., Dansi, A., Aboagye, L.M., Acheremu, K., & Tetteh, J.P. (2015a). Increasing farmers and breeders access to yam (*Dioscorea* spp) diversity: The case of Forest-Savannah Transition Agroecology. African Journal of Agricultural Research, 10(8), 772–782. https://doi.org/10.589 7/ajar2014.8850

Otto, E., Anokye, M., Asare, P.A., & Tetteh, J.P. (2015b). Molecular Categorization of some Water Yam (*Dioscorea alata* L.) Gerplasm in Ghana using Microsatellite (SSR) Markers. *Journal of Agricultural Science***,** Vol. 7, No.10; 2015.

Otoo, E., Osei, K., Adomako, J., Agyeman, A., Amele, A., de Koeyer, D. … & Asiedu, R. (2017). GGE Biplot Analysis of 12 *Dioscorea rotundata* Genotypes in Ghana. *Journal of Agricultural Science*, 10(1), 249. https://doi.org/10.5539/jas.v10n1p249

Paál, D., Kopernický, J., Gasper, J., Vašíček, D., Vašíčková, K., Bauerová, M., & Bauer, M. (2013). Microsatellite analysis of the slovak carniolan honey bee (*Apis mellifera* Carnica).*Journal of Microbiology, Biotechnology and Food Sciences***,** 2:1517U 1525.

Padhan, B., & Panda, D. (2018). Variation of photosynthetic characteristics and yield in wild and cultivated species of yams (*Dioscorea* spp.) from Koraput, India. *Photosynthetica*, 56, 1010–1018. https://doi.org/10.1007/s11099-018-0823-7

Padhan, B., Mukherjee, A.K., Mohanty, S.K., Lenka, S.K., & Panda, D. (2019). Genetic variability and inter species relationship between wild and cultivated yams (*Dioscorea* spp.) from Koraput, India based on molecular

and morphological markers. *Physiology and Molecular Biology of Plants*, 25(5), 1225–1233. https://doi.org/10.1007/s12298-019-00691-3

Patel, A., Desai, K.D., & Ahlawat, T. (2019). Characterization of greater yam (*Dioscorea alata* L.) genotypes by using morphological markers. *International Journal of Chemical Studies*, 7, 4961–4967.

Peakall, R., & Smouse, P.E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1), 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Peakall, R., & Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics***,** Volume 28, Issue 19, Pages 2537–2539, https://doi.org/10 .1093/bioinformatics/bts460.

Pitalounani, W.E.N., Dourma, M., Wala, K., Woegan, Y., Gbogbo, A., Batawila, K., Dansi, A., Tozo, K., & Akpagana, K. (2017). Agrodiversity, peasant management, and importance of *Dioscorea praehensilis* Benth in the Subhumid Zone of Togo. *African Journal of Food, Agriculture, Nutrition and Development*, 17(3), 12455–12475. https://doi.org/10.18697/ajfand.79.15930

Plazas, M., Vilanova, S., Gramazio, P., Rodríguez-Burruezo, A., Fita, A., Herraiz, F.J., ... & Prohens, J. (2016). Interspecific hybridization between eggplant and wild relatives from different genepools. *Journal of the American Society for Horticultural Science*, *141*(1), 34-44.

Polycarp, D., Afoakwa, E.O., Budu, A.S., & Otoo, E. (2012). Characterization of chemical composition and anti-nutritional factors in seven species within the Ghanaian yam (*Dioscorea*) germplasm. *International Food Research Journal*, 19(3), 985–992.

Price, E.J., Bhattacharjee, R., Lopez-Montes, A., & Fraser, P.D. (2017). Metabolite profiling of yam (*Dioscorea* spp.) accessions for use in crop

improvement programmes. *Metabolomics*, 13(11), 1–12. https://doi.org/10.1007/s11306-017-1279-7

Price, E.J., Bhattacharjee, R., Lopez-Montes, A., & Fraser, P.D. (2018). Carotenoid profiling of yams: Clarity, comparisons and diversity. *Food Chemistry*, 259(March), 130–138. https://doi.org/10.1016/j.foodchem.2018.03.066

Price, E.J.; Drapal, M.; Perez-Fons, L.; Amah, D.; Bhattacharjee, R.; Heider, B.; Rouard, M.; Swennen, R.; Becerra Lopez-Lavalle, L.A., & Fraser, P.D. (2020). Metabolite database for root, tuber, and banana crops to facilitate modern breeding in understudied crops. *The Plant Journal*, 101, 1258–1268.

Purcell, P.C., Smith, A.M., & Halford, N.G. (1998). Antisense expression of a sucrose non-fermenting-1-related protein kinase sequence in potato results in decreased expression of sucrose synthase in tubers and loss of sucrose-inducibility of sucrose synthase transcripts in leaves. *Plant Journal*, 14(2), 195–202. https://doi.org/10.1046/j.1365-313X.1998.00108.x

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J., & Sham, P.C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81(3), 559–575. https://doi.org/10.1086/519795

Purnomo, D.B.S., Rugayah, S.I., & Shiwachi, H. (2012). Phenetic analysis and intra-spesific classification of Indonesian water yam germplasm (*Dioscorea Alata* L.) based on morphological characters. *Sabrao Journal of Breeding and Genetics*.

R Development Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing: Vienna, Austria.

Rabbi, I.Y., Udoh, L.I., Wolfe, M., Parkes, E.Y., Gedil, M.A., Dixon, A., Ramu, P., Jannink, J.L., & Kulakow, P. (2017). Genome-Wide Association Mapping of Correlated Traits in Cassava: Dry Matter and Total Carotenoid Content. *The Plant Genome*, 10(3), plantgenome2016.09.0094. https://doi.org/10.3835/plantgenome2016.09.0094

Rafalski, J.A. (2010). Association genetics in crop improvement. *Current Opinion in Plant Biology*, 13(2), 174–180. https://doi.org/10.1016/j.pbi.2009.12.004

Ray, D.K., Mueller, N.D., West, P.C., & Foley, J.A. (2013). Yield Trends Are Insufficient to Double Global Crop Production by 2050. *PLoS ONE*, 8(6). https://doi.org/10.1371/journal.pone.0066428

Rife, T.W., & Poland, J.A. (2014). Field book: An open-source application for field data collection on android. *Crop Science*, 54(4), 1624–1627. https://doi.org/10.2135/cropsci2013.08.0579

Rivera-Jiménez, H.J., Álvarez-Sotol, A., Palacio-Mejía, J.D., Barrios-Leal, D.Y., & López-Álvarez, D. (2011). Intra and interspecific genetic diversity of yam (*Dioscorea* spp.) from the Colombian Caribbean region by AFLP markers. *ACTA AGRONÓMICA*, (60) 4 2011, p. 325-333.

Sahu, S.C., Dhal, N.K., & Mohanty, R.C. (2010). Potential medicinal plants used by the tribal of deogarh district, Orissa, India. *Studies on Ethno-Medicine*, 4(1), 53–61. https://doi.org/10.1080/09735070.2010.11886362

Salazar, E., González, M., Araya, C., Mejía, N., & Carrasco, B. (2017). Genetic diversity and intra-racial structure of Chilean Choclero corn (*Zea mays* L.) germplasm revealed by simple sequence repeat markers (SSRs). *Scientia Horticulturae*, 225(August), 620–629. https://doi.org/10.1016/j.scienta.2017.08.006

Sartie, A., Franco, J., & Asiedu, R. (2012). Phenotypic analysis of tuber yield- and maturity-related traits in white yam (*Dioscorea rotundata*). *African*

Journal of Biotechnology, 11(17), 3964–3975. https://doi.org/10.5897/AJB11.1292

Sato, H. (2001). The potential of edible wild yams and yam-like plants as a staple food resource in the African tropical rain forest. *African Study Monographs*, 26, 123–134.

Sato, H. (2006). A brief report on a large mountain-top community of *Dioscorea Praehensilis* in the tropical rainforest of Southeastern Cameroon. *African Study Monographs*, 33(May), 21–28.

Scarcelli, N., Tostain, S., Mariac, C., Agbangla, C., Da, O., Berthaud, J., & Pham, J.L. (2006). Genetic nature of yams (*Dioscorea* sp.) domesticated by farmers in Benin (West Africa). *Genetic Resources and Crop Evolution*, 53(1), 121–130. https://doi.org/10.1007/s10722-004-1950-5

Scarcelli, N., Couderc, M., Baco, M.N., Egah, J., & Vigouroux, Y. (2013) Clonal diversity and estimation of relative clone age: application to agrobiodiversity of yam (*Dioscorea rotundata*). *BMC Plant Biology* 13: 178. http://dx.doi.org/10.1186/1471-2229-13-178.

Scarcelli, N., Chaïr, H., Causse, S., Vesta, R., Couvreur, T.L. P., & Vigouroux, Y. (2017). Crop wild relative conservation: Wild yams are not that wild. *Biological Conservation*, 210 (May), 325–333. https://doi.org/10.1016/j.biocon.2017.05.001

Scarcelli, N., Cubry, P., Akakpo, R., Thuillet, A.C., Obidiegwu, J., Baco, M.N., Otoo, E., Sonké, B., Dansi, A., Djedatin, G., Mariac, C., Couderc, M., Causse, S., Alix, K., Chaïr, H., François, O., & Vigouroux, Y. (2019). Yam genomics supports West Africa as a major cradle of crop domestication. *Science Advances*, 5(5), 1–8. https://doi.org/10.1126/sciadv.aaw1947

Schaal, B.A., Hayworth, D.A., Olsen, K.M., Rauscher, J.T., & Smith, W.A. (1998). Phylogeographic studies in plants: Problems and prospects.

Molecular Ecology, 7(4), 465–474. https://doi.org/10.1046/j.1365-294x.1998.00318.x

Sesay, L., Norman, P.E., Massaquoi, A., Kobba, F., Allieu, A.P., Gboku, M.L., & Fomba, S.N. (2013). Assessment of farmers' indigenous knowledge and selection criteria of yam in Sierra Leone. *Sky Journal of Agricultural Research*, 2(1), 1–6.

Shabanimofrad, M., Rafii, M.Y., Megat Wahab, P.E., Biabani, A.R., & Latif, M.A. (2013). Phenotypic, genotypic and genetic divergence found in 48 newly collected Malaysian accessions of *Jatropha curcas* L. *Industrial Crops and Products*, 42(1), 543–551. https://doi.org/10.1016/j.indcrop.2012.06.023

Sharif, B.M., Burgarella, C., Cormier, F., Mournet, P., Causse, S., Van, K.N., Kaoh, J., Rajaonah, M.T., Lakshan, S.R., Waki, J., Bhattacharjee, R., Badara, G., Pachakkil, B., Arnau, G., & Chaïr, H. (2020). Genome-wide genotyping elucidates the geographical diversification and dispersal of the polyploid and clonally propagated yam (*Dioscorea alata*). *Annals of Botany*, 126(6), 1029–1038. https://doi.org/10.1093/aob/mcaa122

Sharma, L.N., & Bastakoti, R. (2010). Ethnobotany of Dioscorea L. with emphasis on food value in Chepang communities in Dhading district, central Nepal. *Botanica Orientalis: Journal of Plant Science*, 6, 12–17. https://doi.org/10.3126/botor.v6i0.2905

Sheela, M.N., Abhilash, P.V., Asha, K.I., & Arnau, G. (2016). Genetic diversity analysis in greater yam (*Dioscorea alata* L.) native to India using morphological and molecular markers. *Acta Horticulturae*. https://doi.org/10.17660/ActaHortic.2016.1118.8

Sheikh, N., & Kumar, Y. (2017). Morphological characterization of meghalayan *Dioscorea* spp. (yam), northeast India. *Journal of Agricultural Science and Technology*, 19(2), 487–497.

Siadjeu, C., Toukam, G.M.S., Bell, J.M., & Nkwate, S. (2015). Genetic diversity of sweet yam *Dioscorea dumetorum* (Kunth) Pax revealed by morphological traits in two agro-ecological zones of Cameroon. *African Journal of Biotechnology*, 14(9), 781–793. https://doi.org/10.5897/ajb2014.14067

Siadjeu, C., Akdowa Panyoo, E., Mahbou Somo Toukam, G., Bell, J.M., Nono, B., & Medoua, G.N. (2016). Influence of Cultivar on the Postharvest Hardening of Trifoliate Yam (*Dioscorea dumetorum*) Tubers. *Advances in Agriculture*, 2016(May), 1–7. https://doi.org/10.1155/2016/2658983

Siadjeu, C., Mayland-Quellhorst, E., & Albach, D.C. (2018). Genetic diversity and population structure of trifoliate yam (*Dioscorea dumetorum* Kunth) in Cameroon revealed by genotyping-by-sequencing (GBS). *BMC Plant Biology*, 18(1), 1–14. https://doi.org/10.1186/s12870-018-1593-x

Silva, L.R.G., Bajay, M.M., Monteiro, M., Mezette, T.F., Nascimento, W.F., Zucchi, M.I., Pinheiro, J.B., & Veasey, E.A. (2014). Isolation and characterization of microsatellites for the yam *Dioscorea cayenensis* (Dioscoreaceae) and cross-amplification in *D. rotundata*. *Genetics and Molecular Research*, 13: 2766-2771. http://dx.doi.org/10.4238/2014.April.14.5.

Silva, D.M., Siqueira, M.V.B.M., Carrasco, N.F., Mantello, C.C., Nascimento, W.F., & Veasey, E.A. (2016). Genetic diversity among air yam (Dioscorea bulbifera) varieties based on single sequence repeat markers. *Genetics and Molecular Research*, 15(2), 1–12. https://doi.org/10.4238/gmr.15027929

Silva, L.R.G., Mezette, T.F., Nascimento, W.F., Silva, E.F., & Veasey, E.A. (2017). Spatially structured morphological and molecular diversity among *Dioscorea cayenensis* and *D. rotundata* yam accessions. *Plant Genetic Resources: Characterisation and Utilisation*, 15(4), 296–309. https://doi.org/10.1017/S1479262115000647

Singh, N., Choudhury, D.R., Singh, A.K., Kumar, S., Srinivasan, K., Tyagi, R.K., Singh, N.K., & Singh, R. (2013). Comparison of SSR and SNP markers in the estimation of genetic diversity and population structure of Indian rice varieties. *PLoS ONE*, 8(12), 1–14. https://doi.org/10.1371 /journal.pone.0084136

Singh, A., Yadav, A.K., Kaur, K., Sanyal, S.K., Jha, S.K., Fernandes, J.L., Sharma, P., Tokas, I., Pandey, A., Luan, S., & Pandey, G.K. (2018). A protein phosphatase 2C, AP2C1, interacts with and negatively regulates the function of CIPK9 under potassium-deficient conditions in Arabidopsis, *Journal of Experimental Botany*, Volume 69, Issue 16, Pages 4003–4015, https://doi.org/10.1093/jxb/ery182

Siqueira, M.V., Dequigiovanni, G., Corazon-Guivin, M.A., Feltran, J.C., & Veasey, E.A. (2012). DNA fingerprinting of water yam (*Dioscorea alata*) cultivars in Brazil based on microsatellite markers. *Horticultura Brasileira*, 30(4), 653–659. https://doi.org/10.1590/s0102-05362012000400015

Siqueira, M.V.B.M., Bonatelli, M.L., Günther, T., Gawenda, I., Schmid, K.J., Pavinato, V.A.C., & Veasey, E.A. (2014). Water yam (*Dioscorea alata* L.) diversity pattern in Brazil: an analysis with SSR and morphological markers. *Genetic Resources and Crop Evolution***,** 61: 611-624. http://dx.doi.org/10.1007/s10722-013-0063-4

Son, I.S., Kim, J.H., Sohn, H.Y., Son, K.H., Kim, J.S., & Kwon, C.S. (2007). Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (Dioscorea spp.), on high-cholesterol fed rats. *Bioscience, Biotechnology, and Biochemistry*, 71(12), 3063–3071. https://doi.org/ 10.1271/bbb.70472

Songuimondenin, S., Kouadio, M.T., Siaka, B., Kouassi, A.K., Konan, H., & Kouadio, P.E.J. (2018). Functional Properties of Flours from Fresh and Boiled Wild Yam *Dioscorea praehensilis* Tubers. Advance Research, 17(3), 1–13. https://doi.org/10.9734/air/2018/45146

Sonibare, M.A., Asiedu, R., & Albach, D.C. (2010). Genetic diversity of *Dioscorea dumetorum* (Kunth) Pax using Amplified Fragment Length Polymorphisms (AFLP) and cpDNA. *Biochemical Systematics and Ecology*, 38(3), 320–334. https://doi.org/10.1016/j.bse.2010.01.012

Sosinski, B., Gannavarapu, M., Hager, L.D., Beck, L.E., KIng, J.J., Ryder, C.D., Rajapakse, S., Baird, W.V., Ballard, R.E., & Abbot, A.G. (2000). Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. *Theoretical and Applied Genetics*, 101(3), 421–428. https://doi.org/10.1007/s001220051499

Stanley, A.E., Menkir, A., Ifie, B., Paterne, A. A., Unachukwu, N. N., Meseka, S., … & Gedil, M. (2021). Association analysis for resistance to *Striga hermonthica* in diverse tropical maize inbred lines. *Scientific Reports*, 11(1). https://doi.org/10.1038/s41598-021-03566-4

Sugihara, Y., Darkwa, K., Yaegashi, H., Natsume, S., Shimizu, M., Abe, A., Hirabuchi, A., Ito, K., Oikawa, K., Tamiru-Oli, M., Ohta, A., Matsumoto, R., Agre, P., de Koeyer, D., Pachakkil, B., Yamanaka, S., Muranaka, S., Takagi, H., White, B., … & Terauchi, R. (2020). Genome analyses reveal the hybrid origin of the staple crop white Guinea yam (*Dioscorea rotundata*). *Proceedings of the National Academy of Sciences of the United States of America*, 117(50), 31987–31992. https://doi.org/10.1073/pnas.2015830117

Sugihara, Y., Kudoh, A., Oli, M.T., Takagi, H., Natsume, S., Shimizu, M., Abe, A., Asiedu, R., Asfaw, A., Adebola, P., & Terauchi, R. (2021). Population Genomics of Yams: Evolution and Domestication of Dioscorea Species. In: *Population Genomics. Springer, Cham*. 1–28. https://doi.org/10.1007/13836_2021_94

Sukumaran, S., Reynolds, M.P., & Sansaloni, C. (2018). Genome-wide association analyses identify QTL hotspots for yield and component traits in durum wheat grown under yield potential, drought, and heat stress

environments. *Frontiers in Plant Science*, 9(February), 1–16. https://doi.org/10. 3389/fpls.2018.00081

Swarnkar, S., & Katewa, S.S. (2008). Ethnobotanical observation on tuberous plants from tribal area of Rajasthan (India). *Ethnobotanical Leaflets*, 647666.

Takeda, S., & Matsuoka, M. (2008). Genetic approaches to crop improvement: Responding to environmental and population changes. *Nature Reviews Genetics*, 9(6), 444–457. https://doi.org/10.1038/nrg2342

Tamiru, M., Becker, H.C., & Maass, B.L. (2008). Diversity, distribution, and management of yam landraces (*Dioscorea* spp.) in Southern Ethiopia. *Genetic Resources and Crop Evolution*, 55(1), 115–131. https://doi.org/10.1007/s10722-007-9219-4

Tamiru, M., Becker, H., & Maass, B.L. (2009). Genetic diversity in yam germplasm from Ethiopia and their relatedness to the main cultivated *Dioscorea* species assessed by AFLP markers. *Crop Sci.*, 47: 1744 - 1753.

Tamiru, M., Yamanaka, S., Mitsuoka, C., Babil, P., Takagi, H., Lopez-Montes, A., Sartie, A., Asiedu, R., & Terauchi, R. (2015). Development of genomic simple sequence repeat markers for yam. *Crop Science*, 55(5), 2191–2200. https://doi.org/10.2135/cropsci2014.10.0725

Tamiru, M., Natsume, S., Takagi, H., White, B., Yaegashi, H., Shimizu, M., Yoshida, K., Uemura, A., Oikawa, K., Abe, A., Urasaki, N., Matsumura, H., Babil, P., Yamanaka, S., Matsumoto, R., Muranaka, S., Girma, G., Lopez-Montes, A., Gedil, M., … & Terauchi, R. (2017). Genome sequencing of the staple food crop white Guinea yam enables the development of a molecular marker for sex determination. *BMC Biology*, 15(1). https://doi.org/10.1186/s12915-017-0419-x

Teixeira, H., Rodrıguez-Echeverrı, A.S., & Nabais, C. (2014). Genetic Diversity and Differentiation of *Juniperus thurifera* in Spain and Morocco as

Determined by SSR. *PLoS ONE*, 9(2): e88996. doi:10.1371/journal .pone.0088996.

Terauchi, R., Chikaleke, V.A., Thottappilly, G., & Hahn, S.K. (1992). Origin and phylogeny of Guinea yams as revealed by RFLP analysis of chloroplast DNA and nuclear ribosomal DNA. *Theoretical and Applied Genetics*, 83(6–7), 743–751. https://doi.org/10.1007/BF00226693

Tetteh, J.P., & Saakwa, C. (1991). Prospects and constraints to yam production in Ghana. *In Symposium on Tropical Root Crops in a Developing Economy*, 380 (pp. 355-359).

Tewodros, M., Firew, M., Hussein, S., Endale, G., & Beyene, A. (2018). Genetic diversity of yam (*Dioscorea* spp.) landrace collections from Ethiopia using simple sequence repeat markers. *AJCS*, 12(08):1223-1230, doi: 10.21475/ajcs.18.12.08.PNE885.

Tewedros, M., Firew, M., Shimelis, H., & Tafesse, E. G. (2020). Interrelationship and Path analysis of Tuber yield and related traits in yam (Dioscorea spp.). *Research Journal of Pharmacognosy and Phytochemistry*, 12(4), 207–218.

Thouvenel, J.C., & Dumont, R. (1975). Perte de rendement de l'igname infectee par le virus de la mosaique en Côte d'Ivoire. *Agronomical Tropical*, 45, 125–129.

Tiessen, A., Prescha, K., Branscheid, A., Palacios, N., McKibbin, R., Halford, N. G., & Geigenberger, P. (2003). Evidence that SNF1-related kinase and hexokinase are involved in separate sugar-signalling pathways modulating post-translational redox activation of ADP-glucose pyrophosphorylase in potato tubers. *Plant Journal*, 35(4), 490–500. https://doi.org/10.1046/j .1365-313X.2003.01823.x

Tostain, S., Allomasso, T., & Sokpon, N. (2002). Conservation de l'igname sauvage Dioscorea praehensilis dans les forêts sacrées du département de l'atlantique (sud du Bénin) et dans les jardins de cases. *Annals Agronomical Science*, 4 (1), 55–72.

Tostain, S., Scarcelli, N., Brottier, P., Marchand, J.L., Pham, J.L., & Noyer, J.L. (2006). Development of DNA microsatellite markers in tropical yam (*Dioscorea* sp.). *Molecular Ecology Notes*, 6(1), 173–175. https://doi.org/10. 1111/j .1471-8286.2005.01182.x

Treche, S., & Guion, P. (1979). Etude des potentialities nutritionnelles de quelques tubercules tropicaux au Cameroun. *L'Agronomie Tropicale*, 34**:** 127-131.

Tsykun, T., Rellstab, C., Dutech, C., Sipos, G., & Prospero, S. (2017). Comparative assessment of SSR and SNP markers for inferring the population genetic structure of the common fungus *Armillaria cepistipes*. *Heredity*, 119(5), 371–380. https://doi.org/10.1038/hdy.2017.48

Turner, M.F., Heuberger, A.L., Kirkwood, J.S., Collins, C.C., Wolfrum, E.J., Broeckling, C.D., Prenni, J.E., & Jahn, C.E. (2016). Non-targeted metabolomics in diverse sorghum breeding lines indicates primary and secondary metabolite profiles are associated with plant biomass accumulation and photosynthesis. *Frontiers in Plant Science*, 7(JULY2016), 1–17. https://doi.org/10.3389/fpls.2016.00953

Uchendu, K., Njoku, D.N., Paterne, A., Rabbi, I.Y., Dzidzienyo, D., Tongoona, P., … & Egesi, C. (2021). Genome-Wide Association Study of Root Mealiness and Other Texture-Associated Traits in Cassava. *Frontiers in Plant Science*, 12(December). https://doi.org/10.3389/fpls.2021.770434

Umar, U.U., Ado, S.G., Aba, D.A., & Bugaje, S.M. (2014). Combining ability, gene action and heterosis in maize (Zea mays L.) under drought stress. *International Journal of Agriculture Innovations and Research*, *3*(3), 2319-1473.

Van Raden, P.M. (2008). Efficient methods to compute genomic predictions. *Journal of Dairy Science*, 91(11), 4414–4423. https://doi.org/10.3168 /jds.2007-0980

Varshney, R.K., Mahendar, T., Aggarwal, R.K., & Börner, A. (2007). Genic molecular markers in plants: Development and applications. *Genomics-Assisted Crop Improvement*, 1, 13–29. https://doi.org/10.1007/978-1-4020-6295-7_2

Varshney, R.K., Thiel, T., Sretenovic-Rajicic, T., Baum, M., Valkoun, J., Guo, P., Grando, S., Ceccarelli, S., & Graner, A. (2008). Identification and validation of a core set of informative genic SSR and SNP markers for assaying functional diversity in barley. *Molecular Breeding*, 22(1), 1–13. https://doi.org/10.1007/s11032-007-9151-5

Vaseeharan, B., Rajakamaran, P., Jayaseelan, D., & Vincent, A.Y. (2013). Molecular markers and their application in genetic diversity of penaeid shrimp. *Aquaculture International*, 21(2), 219–241. https://doi.org/10.1007/s10499-012-9582-9

Vignal, A., Milan, D., SanCristobal, M., & Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. 34, 275–305. https://doi.org/10.1051/gse

Wei, T., & Simko, V. (2017) R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). Available from https://github.com/taiyun/corrplot (accessed on 1st August 2021).

Wright, S. (1984). Evolution and the genetics of populations, volume 4: variability within and among natural populations (Vol. 4). University of Chicago Press.

Wu, W., Chen, C., Zhang, Q., Ahmed, J.Z., Xu, Y., Huang, X. … & Huang, D. (2019). A comparative assessment of diversity of greater yam (Dioscorea alata) in China. *Scientia Horticulturae*, 243, 116–124. https://doi.org/10.10 16/j.scienta.2018.08.016

Wumbei, A., Bawa, J.K.A., Akudugu, M.A., & Spanoghe, P. (2019). Absence of effects of herbicides use on yam rots: A case study in wulensi, Ghana.

Agriculture (Switzerland), 9(5), 1–15. https://doi.org/10.3390/agriculture 9050095

Yamaguchi, A., Hori, O., Stern, D.M., Hartmann, E., Ogawa, S., & Tohyama, M. (1999). Membrane Proteins during Stress and Facilitates Subsequent Glycosylation. *The Journal of Cell Biology*, 147(6), 1195–1204.

Yan, J., Yang, X., Shah, T., Sánchez-Villeda, H., Li, J., Warburton, M., Zhou, Y., Crouch, J. H., & Xu, Y. (2010). High-throughput SNP genotyping with the Goldengate assay in Maize. *Molecular Breeding*, 25(3), 441–451. https://doi.org/10.1007/s11032-009-9343-2

Yu, Z., Fredua-Agyeman, R., Hwang, S.F., & Strelkov, S.E. (2021). Molecular genetic diversity and population structure analyses of rutabaga accessions from Nordic countries as revealed by single nucleotide polymorphism markers. *BMC Genomics*, 22(1), 1–13. https://doi.org/10.1186/s12864-021-07762-4

Zalapa, J.E., Cuevas, H., Zhu, H., Steffan, S., Senalik, D., Zeldin, E., McCown, B., Harbut, R., & Simon, P. (2012). Using next-generation sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant sciences. *American Journal of Botany*, 99(2), 193–208. https://doi.org/10.3732/ajb.1100394

Zannou, A., Ahanchédé, A., Struik, P.C., Richards, P., Zoundjihékpon, J., Tossou, R., & Vodouhè, S. (2004). Yam and cowpea diversity management by farmers in the Guinea-Sudan transition zone of Benin. *NJAS – Wageningen Journal of Life Science*, 52, 393–420. https://doi.org/10.1016/S1573-5214 (04)80023-X

Zannou, A., Agbicodo, E., Zoundjihékpon, J., Struik, P.C., Ahanchédé, A., Kossou, D.K., & Sanni, A. (2009). Genetic variability in yam cultivars from Guinea- Sudan zone of Benin assessed by random amplified polymorphic DNA. *African Journal of Biotechnology*, 8(1), 026–036. https://doi.org/10. 5897/AJB2009.000-9005

Zavinon, F., Adoukonou-Sagbadja, H., Ahoton, L., Vodouhe, R.S., & Ahanhanzo, C. (2018) Quantitative Analysis, Distribution and Traditional Management of Pigeon Pea [Cajanus cajan (L.) Millsp.] Landraces' Diversity in Southern Benin. *European Science Journal*, 14(9), 184–211. https://doi.org/10. 19044/esj.2018.v14n9p184

Zhang, S., Chen, X., Lu, C., Ye, J., Zou, M., Lu, K., Feng, S., Pei, J., Liu, C., Zhou, X., Ma, P., Li, Z., Liu, C., Liao, Q., Xia, Z., & Wang, W. (2018). Genome-wide association studies of 11 agronomic traits in cassava (Manihot esculenta Crantz). *Frontiers in Plant Science*, 9(April), 1–15. https://doi.org/ 10.3389/fpls.2018.00503

Zhao, K., Tung, C.W., Eizenga, G.C., Wright, M.H., Ali, M.L., Price, A.H., Norton, G. J., Islam, M. R., Reynolds, A., Mezey, J., McClung, A. M., Bustamante, C. D., & McCouch, S. R. (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. *Nature Communications*, 2(1), 1–10. https://doi.org/10.1038/ncomms1467

Zhigang, W., Wu, J., Xiao, X., Zheng, M., Chuan, Y., Xin, C., & Nitin, M. (2014) Genetic diversity analysis of yams (*Dioscore*a spp.) cultivated in China using ISSR and SRAP markers. *Genetic Resources and Crop Evolution*, 61: 639–650.

APPENDICES

APPENDIX A: QUESTIONNAIRE FOR FARMERS' PERCEPTIONS OF THE DIVERSITY, MANAGEMENT, CONSERVATION AND PREFERENCE CRITERIA OF BUSH YAM IN GHANA

Farmers' Indigenous Knowledge on the Diversity, Management, and Conservation of Bush Yam in Ghana

Dear participant,

I am a graduate student of the School of Agriculture, University of Cape Coast. I am conducting this research in partial fulfillment of the requirements for the award of a Ph.D. in Crop Science (PlanBreedingng and Genetics). I assure you that the responses you give will be treated with strict confidentiality. I would be grateful if you would agree to answer the questions below as objectively as you can.

Section A: Socio-economic characteristics

a. Region ----------

b. District ----------

c. Community/Village ----------

d. Sex 1. Male [ ] 2. Female [ ]

e. Age----------

f. Occupation……

g. Educational level 1. Informal [ ] 2. Primary [ ] 3. Secondary [ ] 4. Tertiary [ ]

h. Marital status 1. Single [ ] 2. Married [ ] 3. Divorced [ ] Widow [ ]

i. Family income level 1. Low [ ] 2. Medium [ ] 3. High [ ]

j. Family Size ----------

k. Total farm size for bush yam cultivation----------

Section B: Bush yam diversity

1. How many years have you been growing bush yam? ----------

2. How many types of bush yams do you grow? ----------

3. How did you get your bush yam types? 1. Market [ ] 2. Farmers' field [ ] 3. Wild [ ]

4. List bush yam types grown in your field and their tuber quality attributes

Bush yam accessions	Presence of thorns on tubers	Tuber yield (high, medium, low)	Length of Tuber storability	Tuber flesh color	Tuber color after peeling	Tuber color after cooking	Taste (Bitter or Sweet, bland)	Tuber hardening a few days after harvesting (Yes or No)	Tuber texture

5. List the agronomic attributes of the bush yam accessions named above (Question 4)

Bush yam accessions	Stem/ vine color	Presence of thorns on the stem	Time of plantin g	Time of harvesti ng	Twinning direction	Sex (Male, Female or Monoecy)	Leaf color	Flowering (High or Low)

6a. which of the above bush yam types are your favorite varieties?

6b. what attributes make them your favorites?

1. ---------- 2. ---------- 3. ----------

4. ---------- 5. ---------- 6. ----------

7. Why are you still cultivating the remaining ones?

Section C: Bush yam management and conservation

8. How do you grow the bush yam? 1. Sole crop [ ] 2. Intercrop [ ]

9. If as a sole crop,

a. Do you use stake or you allow the vines to crawl on the ground? ----------

b. If you stake, what are the staking materials you are using?

1. ---------- 2. ---------- 3. ---------- 4. ----------

10. If as intercrop,

a. Which crops do you grow along with the bush yam? List the crops

1. ---------- 2. ---------- 3. ---------- 4. ----------

5. ---------- 6. ----------

b. Do these crops serve as life stake to bush yam? 1. Yes [ ] 2. No [ ]

11. For what purpose do you grow bush yam? 1. Food [ ] 2. Medicine [ ] 3. Social value [ ] 4. For money [ ] 5. Other [ ] specify----------

12. Which labor do you use for bush yam cultivation? 1. Family labor [ ] 2. Hired labor [ ] 3. Self [ ] 4. Both family and hired labors [ ]

13. If family labor, how many are you in your family? ----------

14. How many of them assist you with farm work? ----------

15. If hired labor, how many casual laborers do you hire? ----------

16. What activities do you hire them for? 1. Planting [ ] 2. Weeding [ ] Harvesting [ ]

17. Do you have customers who willingly buy the bush yam varieties? 1. Yes [ ] 2. No. [ ]

18. What do they use it for? ----------

19. What signs do you note for maturity in bush yam? ----------

20. Do you harvest immediately after maturity? 1. Yes [ ] 2. No. [ ]

21. When do you normally harvest your yam? 1. Once in a year [ ] 2.Twice in a year [ ] others [ ] specify----------

22. How do you store the bush yam tubers? 1. In situ (without harvest) [ ] 2. Ex situ (after harvest) [ ]

23. If In situ, how long do you keep them in the mounds before harvesting?

24. If Ex situ, how do you keep them after harvesting? ----------

Section D: Which of the following are the problems you are facing in bush yam production

1. What problem do you face in marketing bush yam? 1. Low commercial value [ ] 2. Lack of organized market for sale [ ] 3. Not preferred by the consumers 4. Others----------

2. What are the challenges with the agronomic performance of bush yam? 1. Lower productivity [ ] 2. Delay in breaking of dormancy [ ] 3. Lack of seed yams [ ] 4.

3. Ecology 1. Inadaptability to dry areas [ ] 2. Decreased soil fertility [ ] 3. Bush burning [ ] 4. Others……

……
……..

4. Socio-cultural activities 1. Loss of cultural values [ ] 2. Migration [ ] 3. Introduction of new varieties [ ] 4. Schooling of young people [ ] 4. Others
……
……
……

5a. Biological challenges 1. Insect pest attack [ ] 2. Disease attack [ ]

5b. List the insect pests affecting Bush yam

1. ---------- 2. ---------- 3. ----------

5c. List the diseases affecting Bush Yam

1. ---------- 2. ---------- 3. ----------

Which of the following attributes do you think is a major challenge to the consumption of bush yam?

1. Blackening of tuber after peeling [ ] 2. Fibrous texture of tubers [ ] 3. Difficulty in chewing [ ] 4. Bitterness [ ] 5. Inability to store for a longer period

APPENDIX B: Ph.D. CANDIDATE COLLECTING INFORMATION FROM BUSH YAM FARMERS

APPENDIX C: BEST LINEAR UNBIASED PREDICTION MEANS FOR THE AGRONOMIC TUBER QUALITY TRAITS OF BUSH YAM ACCESSIONS EVALUATED IN THE 2020 AND 2021 SEASONS

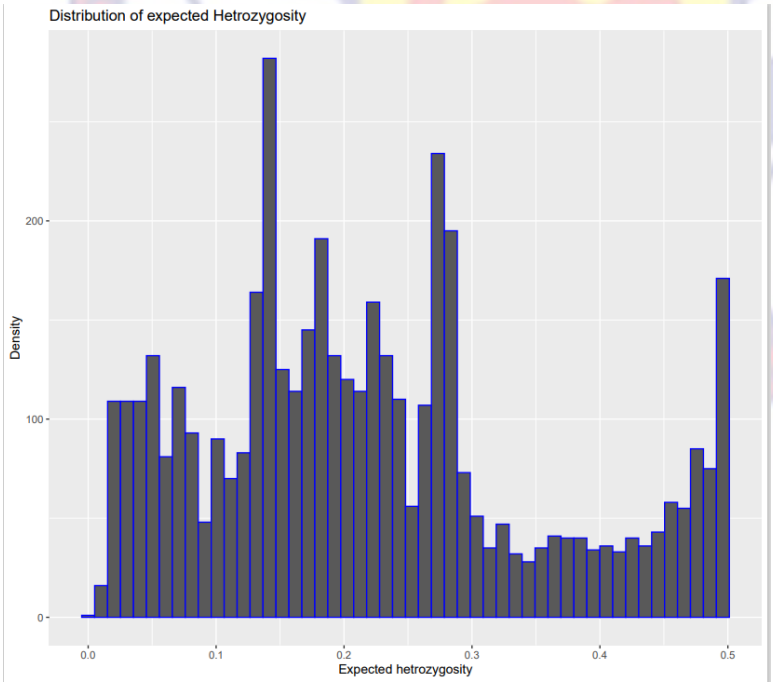
Genotype	BLUP _NTP	BLUP_ Yield	BLUP_ YMV	BLUP_ DMC	BLUP_ TBOXI	BLUP_ TBHard
CDpr1	1.61	12.42	137.09	32.65	-14.39	49.83
CDpr10	2.34	15.26	137.09	33.74	-9.05	50.02
CDpr11	2.70	29.40	137.09	34.76	-7.77	50.82
CDpr13	1.43	10.15	137.09	34.02	-5.62	49.71
CDpr15	1.97	13.27	181.77	32.52	-6.98	49.47
CDpr16	1.79	12.87	137.09	36.09	-7.87	52.17
CDpr17	2.16	15.91	271.12	34.74	-8.97	50.42
CDpr18	1.61	9.26	137.09	34.18	-17.41	51.88
CDpr19	1.61	11.51	137.09	33.90	-26.83	53.31
CDpr22	2.34	28.57	137.09	29.33	-18.82	52.63
CDpr24	2.34	27.82	137.09	33.00	-17.59	50.90
CDpr25	2.34	17.89	137.09	31.95	-5.45	52.68
CDpr26	2.34	21.71	137.09	33.94	-15.16	52.34
CDpr27	2.16	18.08	137.09	36.18	-5.13	50.05
CDpr28	2.34	27.78	137.09	37.85	-8.90	49.71
CDpr29	3.07	14.55	137.09	39.39	-6.77	50.57
CDpr33	1.79	14.54	211.55	34.17	-28.54	52.09
CDpr35	1.61	12.06	137.09	33.60	-0.97	49.52
CDpr37	1.43	18.79	196.66	36.69	-12.33	49.64
CDpr4	1.61	17.18	140.07	32.96	-2.91	50.53
CDpr40	1.43	16.14	140.07	33.40	-21.83	50.92
CDpr41	2.16	24.29	140.07	34.08	-13.01	50.14
CDpr43	1.24	6.90	140.07	30.78	-21.33	52.81
CDpr45	1.97	20.92	140.07	35.98	-7.14	49.72
CDpr48	1.43	12.46	140.07	37.54	-9.21	49.61
CDpr49	1.24	9.25	140.07	38.12	-12.11	49.91
CDpr5	1.97	21.46	140.07	35.77	-21.92	49.72
CDpr50	1.24	7.22	140.07	43.36	-3.96	52.39
CDpr51	1.43	10.89	140.07	34.10	-26.71	53.32
CDpr53	1.43	11.88	140.07	35.35	-9.51	50.51
CDpr54	1.24	6.98	162.41	33.19	-12.03	49.63
CDpr55	1.79	10.89	140.07	40.01	-11.37	50.41
CDpr56	2.34	20.07	140.07	37.70	-7.01	49.42
CDpr57	1.97	33.39	173.58	31.96	-17.75	52.81
CDpr58	2.52	33.28	140.07	35.62	-14.05	50.88
CDpr59	1.79	18.37	140.07	33.00	-4.42	50.86
CDpr6	2.16	17.88	140.07	36.97	-22.00	49.85
CDpr60	2.34	31.36	140.07	30.24	-12.98	50.71

CDpr61	1.61	12.00	140.07	31.35	-15.31	49.94
CDpr64	2.34	18.85	140.07	35.62	-9.19	50.39
CDpr66	1.24	9.92	140.07	35.58	-4.89	50.59
CDpr68	2.52	17.38	140.07	30.65	-7.51	52.22
CDpr69	2.52	19.74	140.07	40.05	-10.91	50.80
CDpr7	3.25	39.00	140.07	36.19	-12.33	51.28
CDpr70	2.52	22.39	140.07	35.18	-12.54	49.48
CDpr72	1.61	12.42	140.07	34.58	-5.84	49.32
CDpr73	2.70	20.26	137.09	31.19	-19.06	51.36
CDpr74	1.43	11.16	211.55	32.68	-6.42	49.73
CDpr75	1.24	6.98	139.57	33.37	-5.97	50.97
CDpr76	1.61	14.46	139.57	32.22	-18.97	51.94
CDpr79	1.61	15.85	139.57	32.69	-11.28	49.03
CDpr8	3.43	25.56	161.91	34.96	-24.05	50.78
CDpr81	1.24	10.87	139.57	32.12	-10.66	50.11
CDpr85	1.61	10.65	139.57	36.38	-3.41	50.58
CDpr87	1.43	10.52	139.57	35.27	-14.33	50.49
CDpr89	1.79	11.94	173.08	35.70	-5.47	49.32
CDpr9	2.16	18.23	139.57	34.53	-12.15	49.34
CDpr90	1.97	14.13	139.57	32.49	-9.09	50.49
Dp/Asamankese/ Assin/C/002	1.61	13.07	139.57	36.44	-5.09	50.50
Dp/Asamankese/ Assin/C/009	1.43	14.57	137.09	25.73	-9.18	51.94
Dp/Asesewa/UP/E/ 001	1.24	6.57	271.12	31.47	-13.59	50.93
Dp/UP/E/001	3.62	26.54	137.09	31.96	-22.08	50.59
EDpr1	2.89	25.05	211.55	35.61	-24.20	52.64
EDpr13	2.16	23.76	137.09	33.72	-9.91	50.51
EDpr14	2.70	31.72	137.09	36.13	-7.74	50.20
EDpr15	1.61	15.89	137.09	33.17	-13.62	52.86
EDpr2	1.24	9.25	137.09	32.59	-2.91	50.37
EDpr20	1.43	9.51	137.09	32.26	-10.87	49.64
EDpr21	2.34	20.01	137.09	30.43	-11.53	50.11
EDpr22	1.97	12.95	137.09	41.31	-15.47	50.48
EDpr24	1.24	7.63	157.57	31.67	-25.90	49.53
EDpr3	2.34	15.20	137.09	34.76	-15.46	49.61
EDpr4	1.43	11.01	137.09	35.56	1.53	50.19
EDpr5	1.43	15.18	211.55	31.48	-17.53	53.49
EDpr6	1.97	11.30	137.09	30.99	-19.21	49.83
EDpr7	2.34	13.66	137.09	28.39	-10.71	50.20
EDpr8	2.52	19.32	137.09	34.92	-18.61	50.97
Odonor big	1.24	9.83	137.09	32.41	-23.23	51.74
Otim	1.24	10.15	137.09	32.93	-27.31	51.53

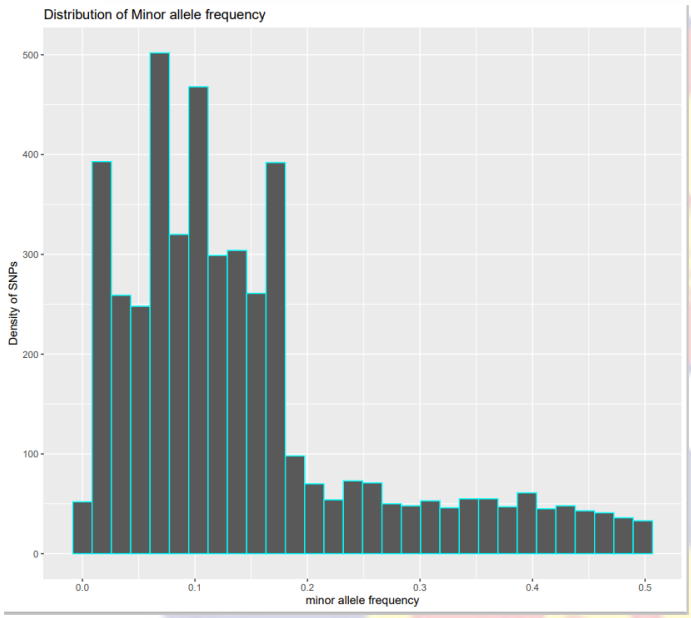
PGR/20/002	1.61	13.00	241.34	30.68	-15.92	50.67
WNDpr10	1.24	6.98	139.57	37.75	-7.53	50.07
WNDpr11	1.79	22.12	139.57	34.32	-14.28	50.22
WNDpr13	2.52	19.95	139.57	32.02	-7.64	50.54
WNDpr15	2.70	30.43	173.08	31.82	-11.96	51.03
WNDpr18	1.97	18.33	139.57	30.58	-5.82	50.90
WNDpr2	1.43	14.52	139.57	34.07	-11.50	53.41
WNDpr21	1.97	22.86	139.57	34.70	-27.94	51.90
WNDpr22	1.43	11.01	139.57	36.36	-6.84	50.94
WNDpr24	2.34	19.90	139.57	39.30	-4.18	50.04
WNDpr29	1.43	10.18	195.42	33.65	-28.75	52.42
WNDpr30	2.52	19.86	139.57	34.44	-15.13	52.64
WNDpr31	1.61	10.89	139.57	29.36	-23.19	50.48
WNDpr33	1.24	9.75	139.57	31.85	-20.15	50.40
WNDpr34	1.79	17.57	139.57	32.91	-17.88	52.83
WNDpr35	1.79	15.01	139.57	33.88	-6.61	49.80
WNDpr36	2.16	16.08	139.57	35.95	-30.00	49.69
WNDpr39	1.24	10.14	139.57	34.95	-7.75	51.26
WNDpr4	1.79	17.03	139.57	38.17	-8.67	50.59
WNDpr41	1.24	6.57	139.57	33.33	-12.72	51.07
WNDpr42	1.24	6.57	137.09	31.44	-21.39	50.81
WNDpr44	1.97	13.90	137.09	33.16	-10.42	50.01
WNDpr45	1.43	11.88	137.09	36.15	-12.80	51.45
WNDpr49	1.79	14.72	137.09	30.15	-27.01	50.10
WNDpr5	1.97	14.12	137.09	37.26	-16.05	48.64
WNDpr54	1.79	9.94	137.09	34.69	-7.81	49.14
WNDpr56	1.61	9.42	137.09	34.59	-12.34	52.06
WNDpr57	2.70	19.92	137.09	32.52	-13.89	52.85
WNDpr59	1.24	7.22	166.87	33.14	-5.49	49.62
WNDpr6	2.52	17.52	137.09	35.26	-6.90	50.48
WNDpr60	1.79	15.78	137.09	33.27	-15.02	53.01
WNDpr63	3.43	42.70	137.09	35.50	-5.16	50.00
WNDpr65	1.24	12.80	137.09	33.29	-16.05	52.44
WNDpr66	1.24	11.31	137.09	36.80	-4.32	49.13
WNDpr67	1.24	6.98	137.09	30.78	-9.28	51.66
WNDpr68	1.61	19.45	211.55	35.87	-32.88	51.97
WNDpr69	2.70	25.39	137.09	33.77	-22.58	52.64
WNDpr71	1.24	18.12	137.09	32.61	-17.26	52.17
WNDpr74	1.24	7.08	137.09	41.23	-9.13	50.87
WNDpr75	1.97	14.44	137.09	32.47	-12.26	50.57
WNDpr76	4.16	57.79	137.09	43.44	-18.11	49.73
WNDpr77	2.16	16.66	241.34	29.83	-23.44	53.23
WNDpr79	2.70	24.53	137.09	36.36	-8.54	49.92

WNDpr8	1.43	12.86	151.98	33.14	-9.44	50.66
WNDpr81	1.97	13.71	137.09	33.22	-15.43	49.60
WNDpr82	2.16	13.39	151.98	34.68	-4.76	50.43
WNDpr83	2.70	25.72	137.09	34.78	-17.72	51.02
WNDpr84	2.16	24.38	137.09	37.69	-8.33	49.73
WNDpr86	2.65	33.82	226.16	32.93	-11.23	49.64
WNDpr87	1.51	14.80	137.66	36.71	-20.15	52.79
WNDpr88	2.34	22.81	137.09	38.41	-1.44	50.40
WNDpr89	1.24	6.98	137.09	38.59	-6.71	50.42
WNDpr9	1.97	10.81	137.09	36.64	-10.03	50.20
WNDpr91	1.79	16.17	315.80	33.27	-12.02	52.79
WNDpr92	1.43	11.47	137.09	29.62	-12.73	51.40
WNDpr93	1.97	26.53	211.55	33.17	-24.22	52.05
WNDpr94	2.16	19.09	137.09	36.76	-23.37	50.97
WNDpr96	1.61	12.76	137.09	36.57	-8.71	50.03
WNDpr97	1.79	12.92	137.09	32.27	-6.40	49.32
WNDpr98	1.43	12.75	137.09	33.92	-5.64	51.95

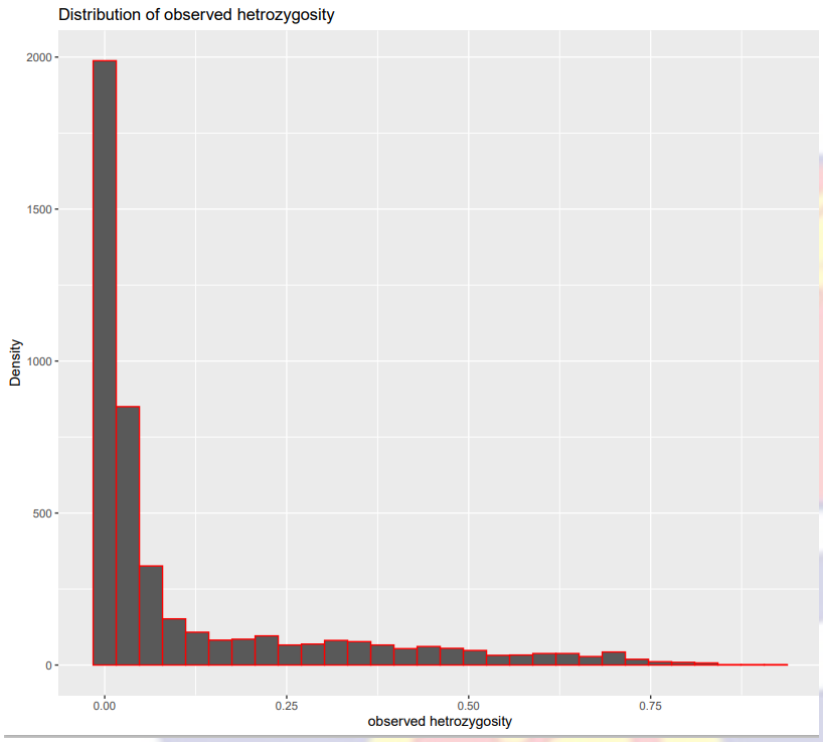
APPENDIX D: DISTRIBUTION OF EXPECTED HETEROZYGOSITY OF 4,525 SNPs GENERATED



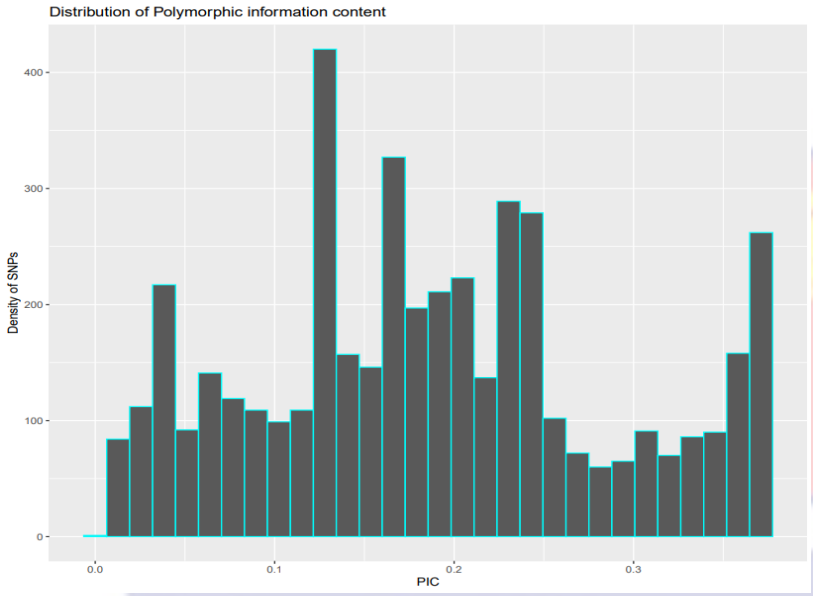
APPENDIX E: DISTRIBUTION OF MINOR ALLELE FREQUENCY OF 4,525 SNPs GENERATED



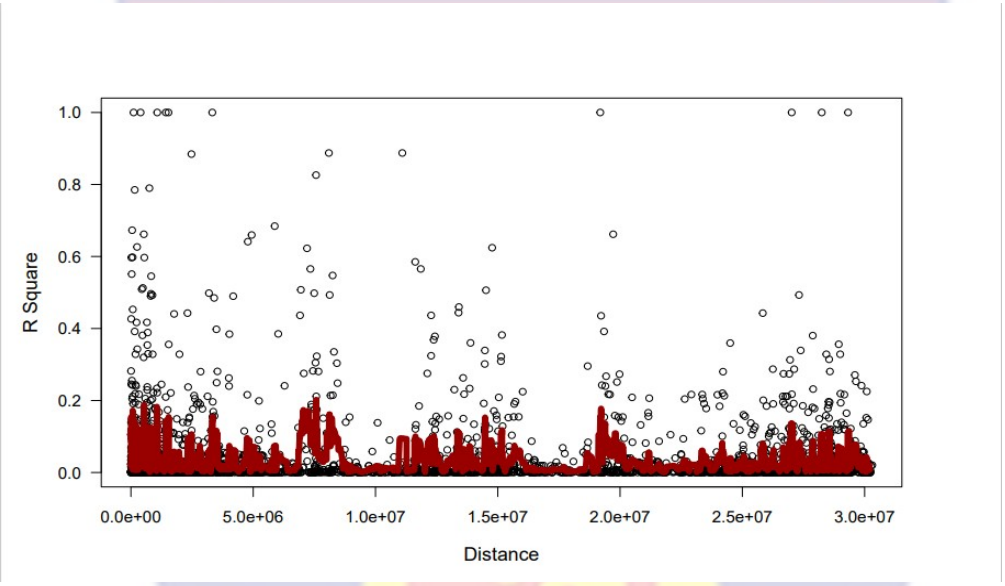
APPENDIX F: DISTRIBUTION OF OBSERVED HETEROZYGOSITY OF 4,525 SNPs GENERATED



APPENDIX G: DISTRIBUTION OF POLYMORPHIC INFORMATION CONTENT OF 4,525 SNPs GENERATED



APPENDIX H: GENOME-WIDE LINKAGE DISEQUILIBRIUM (LD) DECAY AMONG THE SNP PAIRS AS A FUNCTION OF GENETIC DISTANCE IN BASE PAIRS BASED ON THE JOINT ANALYSIS OF THE 20 CHROMOSOMES IN 133 BUSH ACCESSIONS. THE DOTS CORRESPOND TO THE OBSERVED LD (R^2) VALUES. THE REDLINE REPRESENTS THE NONLINEAR TREND OF EXPECTED LD DECAY



APPENDIX I: PICTURE OF BUSH YAM (*Dioscorea praehensilis*) AT VEGETATIVE STATE (6 MONTHS AFTER PLANTING)