CHARACTERIZATION OF NUTRIENT USE-EFFICIENT GENOTYPES OF *DIOSCOREA ALATA* AND *DIOSCOREA ROTUNDATA* USING PHENOTYPIC TRAITS, SSR MARKERS AND FARMER PARTICIPATORY APPROACH.

By

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MASTERS OF PHILOSOPHY

IN

AGRONOMY (PLANT BREEDING)

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KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

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MASTERS OF PHILOSOPHY

IN

AGRONOMY (PLANT BREEDING)

JUNE 2016

DECLARATION

I hereby certify that this thesis is an exact outcome of my field and laboratory research findings and supported by references of related studies carried out and therefore has never being presented anywhere for a degree. All references cited have been duly acknowledged.

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ABSTRACT

The overall objective of this study was to identify nutrient use-efficient varieties of yam genotypes from the IITA and farmer local varieties from the Northern Region of Ghana. Both laboratory analysis and farmer description indicated low soil fertility in the study area, hence the need for nutrient use-efficient yam genotypes. In all, 45 genotypes made up of 20 *D. alata* and 21 *D. rotundata* from IITA – Ibadan with four local genotypes from farmers were evaluated in Randomized Complete Block Design (RCBD) across the three districts of the Northern Region of Ghana. A total of 20 quantitative traits and 29 qualitative traits of the farmer were used to evaluate the genotypes. The data taken ranged from sprouting to tuber maturity. Quantitative data were subjected to descriptive statistics, principal component analysis, correlation matrix and Hierachial cluster analysis using Euclidean distance coefficients. The analysis revealed that, the 20 quantitative variables and 29 qualitative farmer traits were grouped into several components in which the first six axes explained 82.70% in

D. alata and 82.42 in *D. rotundata* of the total variation. In all, six quantitative traits (Leaf area, Leaf surface area, high chlorophyll content at the lower to the middle leaves, number and weight of seed yam, ware yam and total yam) revealed the highest variability and therefore, were considered as very significant to be used to characterize nutrient use-efficient genotypes for both yam genotypes. However, qualitative information on insect pest and disease score indicated no to very low symptoms. Farmer criteria used for characterization include; medium to large leaves, medium to large canopies, smooth to slightly rough leaves with no infestation of insect pest and disease infection. Besides, high yielding, big/long tubers, smooth with few to no hairy tuber surface, no insect pest infestation and disease infection, long shelf life with high proportion of ware and seed yam productions determined farmer preferred nutrient use-efficient genotypes. The selection of the high performing genotypes was as a result of the significant variation among genotypes using the phenotypic and farmer criteria. The

use of the cluster analysis separated these genotypes into numerous clusters with similar traits. The genetic diversity conducted using seven SSRs markers to determine high performance genotypes in both species revealed a total allele number of 23 and 27 with 3.28 and 3.85 allele per loci in *D. alata* and *D. rotundata*, respectively. Mean genetic diversity recorded was 0.53 and 0.42 with polymorphic information content (PIC) found to have a mean value of 0.37 and 0.49 in *D. alata* and *D. rotundata*, respectively, showing a low level of polymorphism detected by the primers. A greater amount of dissimilarities of 0.62 to

1.00 in both species was revealed using the molecular cluster analysis based on Unweighted Pair-Group Mean Average (UPGMA). At a dissimilarity coefficient of 0.175 (17.5%) and 0.25 or (25%), the dendrogram identified two main clusters with six sub-clusters for *D. alata* and six main clusters for *D. rotundata* respectively. The study has revealed that, the use of morphological characters and farmer perception identified TDr 00/00951, TDr 09/00001, TDr 95/18988, TDr 95/19177 and Larbako as well as TDa 98/01168, TDa 09/00228, TDa 02/00012, TDa 98/01174, TDa, 92-2 and Seidubile as *D. rotundata* and *D. alata* nutrient use-efficient genotypes respectively. Besides, results from marker assisted selection, revealed that, diversity among genotypes are very high. This enhances selection of high yielding genotypes in low fertility soil for advance breeding and hybridization programmes.

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LIST OF ABBREVIATIONS

AEAs - Agricultural Extension Agents

AFLP - Amplified Fragment Length Polymorphism

AP-PCR - Arbitrarily Primed Polymerase Chain Reaction

CAPS - Cleaved Amplified Polymorphism Sequence

DAF - DNA Amplification Fingerprinting

DNA – Deoxyribonucleic Acid

dNTPs – di nucleotide tri phosphates

EDTA – Ethylene diamine tetra acetic acid

EST - Express Sequence Tags

FAMPAR - Farmer Managed Participatory Research

FAO – Food and Agricultural Organization

FAOSTAT - Food and Agricultural Organization Statistics

HCA - Hierarchical Clustering Analysis

HYV – High Yielding Varieties

IITA – International Institute of Tropical Agriculture

MoFA – Ministry of Food and Agriculture

NaCl – Sodium Chloride

NUE - Nutrient Use Efficiency

PCA - Principal Component Analysis

PCR - Polymerase Chain Reaction

PEG – Polyvinyl ethylene glycol

PPB – Participatory Plant Breeding

PRA - Participatory rural appraisal

PVP – Polyvinylpyrrolidone

QTL - Quantitative Trait Loci

RAPD - Random Amplified Polymorphic DNA

RFLP - Restriction Fragment Length Polymorphism

SDA - Stranded Displacement Amplification

SDS - Sodium Dodecyl Sulphate

SNPs - Single Nucleotide Polymorphisms

SSRs - Simple Sequence Repeats

TBE – Tris base Boric acid Extract

TDa – Tropical *Dioscorea alata*

TDr – Tropical *Dioscorea rotundata*

UPGMA - Unweighted Pair-Group Mean Average

WAP – Weeks after Planting

YMT – Yam Minisett Technology

DEDICATION

I dedicate this work to my parents and family.

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Lastly to my wife, Ms Rahinatu Mohammed Sayibu and all the children, I say may The Almighty Allah richly blessed you.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Yams (*Dioscorea* spp.) are annual or perennial herbaceous climbing plants cultivated for their starchy tubers. About 90% of world yam production takes place in the yam belt of West and Central Africa with Nigeria alone producing about 68% of the world's total (FAOSTAT, 2013). As an important crop for food security in West Africa, it also serves as a staple carbohydrate source for hundreds of millions of people (Mignouna *et al*., 2003). The tubers are processed and consumed in several forms and these includes pounded yam (fufu), boiled yam, roasted or grilled yam, fried yam slices, yam balls, mashed yams, yam chips, and yam flakes. Fresh tubers are also processed into yam flour and this is used to prepare dough called "*amala*" and "*telibowo*" in Nigeria (Mahalakshmi and Atalobhor, 2007), and "*wasawasa*" and "*tuubaani*" in Northern Ghana. Besides, the crude protein, crude fat, crude fibre and ash contents of yams are in the range of 6.7 – 7.9%, 1.0 – 1.2%, 1.2 – 1.8%, and 2.8 – 3.8%, respectively. Yam can also be processed into industrial starch (Sebio and Chang, 2000). Yam tuber is believed to constitute some pharmacologically active substances including dioscorine, saponin and sapogenin. Dioscorine is a major alkaloid in yam, medicinally, a heart stimulant (Lin and Yang, 2008). Other Native Americans used a decoction of the wild yam root to relieve labour pain and later physicians prescribed wild yam to patients with colic, morning sickness, asthma, hicough, rheumatism and gastritis related to alcoholism (Lin and Yang, 2008). Yam is produced both as sustenance and cash crop across the West African Sub-region (Baco *et al*., 2007). It provides cash income for a wide range of smallholders, including many women as producers, processors and traders (Asiedu, 2003; Hgaza *et al*., 2010). Hence, improving yam productivity

can increase food production and farmers' income in the producing areas, particularly in West Africa. In addition to yam as an important food, medicine, and cash crop, it also plays a substantial role in the socio-cultural life of people in some producing regions. Considerable amount of ritualism has been developed around the production and utilization of yam such as the celebration of Annual New Yam Festival in West Africa (Osunde and Orhevba, 2009) and wedding ceremonies in Oceania (O'Sullivan, 2008).

In the Northern Region of Ghana, yam is produced, mainly by male smallholder with little or no formal education and production has so many constraints leading to several losses of yield to the farmer. Farm labour is very expensive and has been estimated that, about 90% of the total labour costs is incurred during yam production (Ezedimma, 2000). Asante *et al.*, (2008), in his diagnostic survey, reported that, most farmers get yam planting materials from their own farm, and others from the market and from friends. These planting materials are often of a low quality and infested with fungal or bacterial diseases, viruses, and nematodes. Besides, the traditional method for obtaining seed yams involves "milking" or harvesting yam tubers after the first six months of planting. The root is gently pushed back after removing the tuber, and covered with soil for it to regenerate seed yam in three to four months. This process results in physiologically immature yams with a shorter shelf life under storage. Demand for stakes has contributed to deforestation and biodiversity loss, which reduce soil fertility while increasing production costs and the length of time spent finding stakes (Peprah and Boateng, 2010). The long growth cycle of yams also constrains production to a single season in every year (Otoo *et al.*, 2009). The gestation period of yam (8 – 12 months) on the field exposes the crop to excess of insect pests and diseases among

which anthracnose caused by *C. colletotrichum* and Yam mosaic; viral disease can be particularly damaging (Thouvenel and Dumont, 1990; Mignouna *et al*., 2003).

Northern Region is the largest region among the 10 regions and occupies an area of 70,384 square kilometers or 31% of Ghana area. It has a population of 2,479,461 million of which over 70% are in agricultural production (MoFA, 1998). It is second after Brong Ahafo Region in yam production. Yam farmers in this region cultivate yam in smallholdings, majority of whom have no formal education and are made up of mixed ethnic groups including; Dagomba, Nanumba, Gonja, Basare, Safaliba, Dagati, Mo, Bono, Kokomba, and Chokosi (Asante *et al*., 2008). Soils of Northern Region are low in organic matter (less than 2% in the topsoil), loosed structured and made up of sand silt and gravel exhibiting low fertility status. In the past, fertility regeneration by yam farmers was achieved through long fallow periods, but the recent increase in population and pressure on land used has made fallow system to disappear (MoFA, 1998). The dominance level of subsistent farming, the slow economic growth and high poverty level do not permit farmers to assess fertilizer for their yam fields.

Previous researches carried out in Northern region were application of fertilizer to yam (Sowley and Tiesaa, 2007; Carsky *et al*., 2007), morphological characterization (Dansi *et al*., 1998; 1999; 2000; Otoo *et al*., 2009), isozyme patterns (Dansi *et al*., 2000; Mignouna *et al*., 2003), diversity and ethno botany studies (Otoo *et al*., 2009), molecular characterization using SSR (Otoo *et al*., 2009) and yam value chain in Ghana (Kathryn *et al*., 2012).

No work has been done on assessment of yams in low soil fertility to select nutrient use-efficient genotypes; therefore, research on possible methods to enhance yam productivity using nutrient use-efficient genotypes is desirable to increase the production of this socio-economic and viable crop. In order to mitigate these constraints

and increase yam production, this study sought to evaluate and select with farmers and consumers yam clones from IITA that could give considerable yield under low soil nutrient conditions. In the current study, a genotype is considered nutrient use-efficient if it produces satisfactorily under low resource availability.

Besides, such genotypes should be able to adapt to the food and farming systems in Guinea Savanna Zone of Ghana where yam is extensively cultivated in low fertile soils.

The main objective of this study was to characterize nutrient use-efficient yam genotypes in low fertile soils in Northern Region of Ghana.

The specific objectives were to:

(i) Characterize accessions of *D. alata* and *D. rotundata* using phenotypic data,

(ii) Characterize accessions of *D. alata* and *D. rotundata* using SSRs markers, and

(iii) Characterize and select high yielding *D. alata* and *D. rotundata* genotypes using farmer participatory approach.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of yam

Yam is an important tropical tuber crop that belongs to the genus *Dioscorea* and family *Dioscoreaceae*. It consists of over 600 species of which only 10 of them are widely cultivated (Sesay *et al*., 2013), and also used for human consumption (Lebot,

2009). These are: *D. alata, D. esculenta* Lour, *D. batatas* Decne or D. *opposita* Thumb. originating from Asia, *D. bulbifera* L., *D. cayenensis* - *D. rotundata* complex, *D. dumetorum* Kenth originating from Africa, *D. trifida* L. originating from America, *D. nummularia* Lam. and *D. pentaphylla* L. originating from both Asia and Oceania (Maliki *et al*., 2011). Of all these species, *D. alata, D. rotundata* and *D. cayenensis* are the most widely cultivated and contribute significantly to the total economic and food growth of Africa. In West Africa, guinea yam (*D. cayenensis – D. rotundata* complex) is highly significant and symbolizes more than 95% of the total yam produced (Sesay *et al*., 2013) with considerable varietal and genetic diversity due to the continuous process of domestication from related wild species that are *Dioscorea abyssinica* Hochst, *Dioscorea praehensilis* Benth and *Dioscorea burkilliana* (Mignouna and Dansi, 2003; Dumont and Vernier, 2000; and Dansi *et al*., 2013).

The differences between *D. cayenensis and D. rotundata* is uncertain, hence, some scientists describe them as *D. cayenensis–rotundata* complex though other scientist maintained that, *D. cayenensis* and *D. rotundata* should be described as different taxa (Mignouna and Dansi, 2003). In West Africa, guinea yam (*D. cayenensis – D. rotundata* complex) is very important and constitute more than 95% of the total yam produced (Sesay *et al*., 2013) with considerable varietal and genetic diversity due to the continuous process of domestication from related wild species such as *D. abyssinica* Hochst, *D. Praehensilis* Benth and *D.burkilliana*, (Dumont and Vernier, 2000; Mignouna and Dansi, 2003; Dansi *et al*., 2013).

2.1 Taxonomy

Yam is a monocotyledonous angiosperm, which belongs to the order *Dioscoreales/ Liliflorae*, family *Dioscoreaceae,* and genus *Dioscorea*. It is considered to be among the most primitive of the angiosperms and contains over 600 species, of which only 10 of them are widely cultivated (Sesay *et al*., 2013), used for human consumption (Lebot, 2009) and for food and trade (IITA, 2009). The genus is subdivided into sections, under which the various species are classified. The section *Enantiophyllum* is the principal in terms of number of species, and includes significant species such as *D. alata*, *D. rotundata* and *D. cayenensis*. Other members of this section are *D. japonica* and *D. transversa* (Asiedu *et al*., 1998). Yam vines that twine in clockwise direction or to the right, when looked at starting from the ground upwards, have distinct characteristics of members of the section *Enantiophyllum.* These varieties include members of the species; *D. rotundata*, *D. cayenensis* and *D. alata*. On the other hand, yam vines that twine in anticlockwise direction or to the left characterize species in the sections *Lasiophyton* (*D. dumetorum* and *D. hispida*), *Opsophyton* (*D. bulbifera*), *Combilium* (*D. esculenta*) and *Macrogynodium* (*D. trifida*). *D. alata* are never found in the wild and may have resulted from crosses and domestication from *D. hamiltoni* or *D. persimilis* (Dansi *et al*., 2013). On the other hand, *D. bulbifera* is common in the wild, especially in Asia and Africa and therefore, named as separate species by some authors. Morphological characters however, do not allow clear difference between species such as *D. cayenensis* and *D. rotundata*, and also between cultivars. *D. rotundata* may in fact be a subspecies of *D. cayenensis* or alternatively and may have originated from *D. praehensilis* (Dansi *et al*., 2013).

2.2 Morphology

Yam is a multi-species, polyploidy and vegetatively propagated tuber crop that is widely cultivated in the tropics and subtropics. The adventitious roots that arose from the base of the stem absorb mineral nutrients, and water and eventually form the tuber (O'Sullivan and Ernest, 2008). The shape and size of the tuber can vary greatly due to variety, genetic and environmental factors. However, cultivated forms of yam generally produce tubers that weigh 3-5 kg or more. The number and shape of yam tubers vary greatly between species. For instance, *D. rotundata* tubers are generally larger at the head region and cylindrical in shape with white flesh whilst *D. alata* tubers have variable shapes, with majority being cylindrical (Otoo, 2009). The yam tuber grows from a corm-like structure located at the base of the vine. Sometimes, this corm remains attached to the tuber after harvest and sprouts will develop from it. In other times, when the corm is separated from the tuber, sprouting occurs from the tuber near to the point at which the corm was attached. The yam stem is usually a thin twining vine allowing the plants to climb. It is frequently winged and commonly spiny. Several species have deep striations in their stem, some contain anthocyanin, and others have large thorns. The direction of the twining is used as a taxonomic feature. Besides, the leaves of *D. rotundata*, *D. cayenensis* and *D. alata* are petiolate (except for *D. dumetorum*, *D. hispida* and D. *Pentaphylla*, which have trifoliate leaves, and hairs on their stems), and have an arrangement either opposite or alternate with axillary buds (Degras, 1993).

The reproductive system consists of male and female sexual components (Degras, 1993). The genus, *Dioscorea* is a dioeceous with an extremely irregular production of male and female flowers pollinated by insects (Edwards *et al*., 2007). The seed is flat, has a wing-like structure, and usually goes through a dormancy period of three to four

months before germination can occur. As flowering is rare, yams are vegetatively propagated using the basal nodal region of the tuber and the bulbils (Degras, 1993).

2.3 Importance of yam

About 90% of world yam productions takes place in the yam belt of West and Central Africa. The yam production zone in West Africa takes its boundaries from Cote d'Ivoire to Cameroon and said to be significant in the coastal zone of West Africa where it serves over 60 million of people their daily, dietary calories (IITA, 2009). It provides cash income for a wide range of smallholders, including many women as producers, processors and traders (Asiedu, 2003; Hgaza *et al*., 2010). Therefore, improving yam productivity can increase food production and farmers' income in the producing areas, particularly in West Africa. In addition to yam as an important food, medicine, and cash crop, it also plays a substantial role in the socio-cultural lives of people in some producing regions. Nigeria alone producing about 68% of the world's total (FAO, 2013). As an important crop for food security in West Africa, it also serves as a staple carbohydrate source for over hundreds of millions of people (Mignouna *et al*., 2003). Yam can also be processed into industrial starch for many purposes. Starch constitutes the major ingredient of yam and supplies greater percentage of the daily caloric intake. Dioscorine is the major alkaloid in yam and is medicinally a heart stimulant. Yam peels serve as a good source of carbohydrate feedstuff for rabbits, small and large ruminants. The dry vines after harvest are used to make storage structure or part of storage structure that is cool and ventilated enough for tuber storage up to six months.

2.4 Commonly cultivated yam in Ghana

White Yam (*D. rotundata*): As an African species, it was domesticated by yam farmers from forest areas in Ghana where their wild species (*D. praehensilis*) still exit. In the

Southern area (Forest and Transitional zones), major *D. rotundata* varieties grown for food and trade across the zone as early and late maturing varieties include Early maturing varieties are: Kpuno, Larbako, Asobayere, Lele, Mogninyuya, Fusheinbila, Ama Serwaa, Muchumudu, Tele, Dobre, Teacher Takyie, Lobi bayere and Nnokoben. Late maturing varieties include: Dente, Lilee, Yesu mogya, Tempi, Ama Serwaa and Nananto (Otoo, *et al*., 2012). In Northern Region (Guinea Savanna zone), popular *D. rotundata* varieties grown for food and trade include: Kpuno, Larbako, Mogninyuwa, Fusheinbila, Lelee, Chenchito. Late maturing *D. rotundata* varieties include; Yoruba, Limo, Ziglangbo and Kprinsi/Kprindjo. Other minor varieties grown includes: Kiki and Zong (Asante *et al*., 2008). Larbako and Kpuno are early maturing varieties and mature in five to six months. These varieties are planted first and harvested (milked) for food and income when food is scarce (June, July and August) and for regeneration of seed yam. They turn to give high income and serve as food security crop. Limo, Fusheinbila, Mogninyuli and Chenchito are medium maturing varieties that take seven to eight months to mature. They are harvested at a time when early maturing varieties milked might have exhausted. Ziglangbo, Yoruba and Kprinsi/Kprinjo are late maturing and harvested after eight months. The minor varieties; Kiki and zong (late maturing) are harvested at the end of the season and keep as germplasm, (Personnel communication, Puriya chief-Yakubu Abukari, 2014).

Water yam: Is the next common cultivated species after *D. rotundata* and was domesticated from colleague farmers from north western part of the Guinea Savanna zone of Ghana. Its widely distribution was due to its unique characteristics of ease of propagation. In the southern area (Forest and Transitional zones), common varieties grown are late maturing and include: Matches, Akaba, Seidubile, Asamoah, Dansi, Afase pona, Apoka/Nkontina, Entrentre, Esom ne hyen, Gonglogon, Enoti and Guawa

(Otoo *et al*., 2012). In Northern Region (Guinea Savanna zone), varieties grown are: Nyuwotugu/Nyuwopeli, Nyuwozhei, Matches, Akaba and Seidubile. These species are late maturing, and can store for long with minimum to no insect and disease pest attack and use when all *rotundata-cayenensis* complex species have being used up.

The man believed to have introduced the species to Ghana is Seidu, hence the name Seidubile (means small Seidu). A small size (Match box size) of cut tuber, can be used as minisett, for propagation, hence the name Matches (Personnel communication, Tingoli farmer-Zakaria Nantogma, 2014).

Yellow yam: The name yellow originates from its yellow flesh, which is caused by the presence of carotenoids. It is native to West Africa with few wild species found in forest areas. In the Southern part (Forest and Transitional zones), *D. cayenensis* is late maturing and varieties grown are Afun, Afun/Kamba and Karangba (Otoo *et al*., 2012). It is not common in the Guinea Savanna Zone.

Aerial yam: As part of its origin as an African species, its wild types can still be found in forest of Africa. Is a perennial vine with broad leaves which length is 6 cm or more. It produces tubers generally known as bulbils at the axle of the leaves. In the Southern area (Forest and Transitional zones), *D. bulbifera* variety cultivated is Soaba (Otoo, *et al*., 2012). In Northern Region (Guinea Savanna zone), the variety cultivated is known as Furigima. It was a minor yam, but currently gaining popularity because it can be found in every yam field planted as a neglect crop. It can be stored for long, with minimum to no storage pest attack (Personnel communication, Puriya chief-Yakubu Abukari, 2014).

2.5 Morphological Characterization

It is the simplest application of morphological descriptors in a logical and repeatable measurement of genetic diversity of a crop (Hoogendyk and Williams, 2001). The conventional breeding and selection of crops and specifically yam cultivars with new or better characteristics currently suffer from the fact that, traditional cultivars have not been effectively characterize (Asiedu *et al.*, 1998). Several societies are involved in the classification of yam cultivars, with each section having its own unique series of names for the diverse cultivars (Asemota *et al*., 1996; Dansi *et al*., 1999). This extremely hinders the reliable identification of cultivars for germplasm organization and management for future use. Systems of classification and identification based on morphological characters as done in previous studies include (Dansi *et al*., 1998, 1999, 2000, Otoo, 2009 and Otoo *et al*., 2012). Major challenges inducing morphological characterization comprise low polymorphism, low repeatability, late expression and erratic environmental differences (Smith and Smith, 1992).

2.6 Principal Component Analysis (PCA)

The (PCA) is statistical software used for both quantitative and qualitative data analyses .It revealed the pattern of character variation in accessions within species and between species. The procedure group data into meaningful components that determine the amount of variation contributed from individuals within a population.

Nonetheless, it is not convenient for molecular data.

2.7 Varietal selection using farmers' perception

Participatory varietal selection (PVS) is a bottom-up approach where there is fully participation of farmers and other end-users to develop a variety and involve an intensive system of Farmer-Managed Participatory Research (FAMPAR) (Joshi and Witcombe, 2002). The actors of PVS are; researchers, farmers, consumers, extension

agents, vendors, processors and other industry stakeholders as well as farmers' and community-based organizations and non-government organizations (NGOs). The classical Mendelian breeding method is a top-bottom approach where research is conducted at research centers and findings are packaged and sent to farmers. It uses inputs and high yielding varieties (HYV) which cannot be assessed by the subsistent farmer. Farmer participation in the breeding of crop varieties for low-resource farmers is necessary to help ensure acceptance and ultimate adoption. Besides, local varieties are eco- and site-specific; the ethnic groups possess high Indigenous Technical Knowledge (ITK) on them. In addition, landraces that were going out of cultivation were revived and used efficiently to meet the food needs of various ethnic groups (Joshi *et al*., 1997) or the finished varieties (Rice *et al*., 1998). This system has been successfully tested in rice (Joshi and Witcombe, 2002; Dorward *et al*., 2007) and sorghum (Mulatu and Belete, 2001) has led to increased adoption rates by farmers.

Participation of farmers ensure acceptance and eventual adoption (Maurya *et al*., 1988; Sperling *et al*., 1993; Franzel *et al*., 1995; Gyawali *et al*., 2007;).

2.8 Soil fertility

The capacity of the soil to provide plants with enough nutrients must have the potential to contain the basic plant nutrients, sufficient minerals (trace elements), reasonable level of soil organic matter, soil pH, soil structure, microorganisms and large amounts of top soil for plant nutrition (Brady and Weil, 2002). Many agricultural activities such as ploughing and hoeing disturb the ecological balance of a given environment. As a result, organic matter loss is increased through erosion, leaching, export of harvest products and burning of crop residues. Traditionally,

African farmers restore soil fertility with cropping systems through long fallow period (Carsky *et al*., 2007). In recent times, pressure on arable land is shortening the fallow

periods required for used lands to restore their fertility status. Therefore, the need for nutrient uses-efficient genotypes to ensure sustainable yam production in low soil fertility areas.

2.9 Nutrient use efficient cultivars

The development of nutrient use efficient cultivars would enable farmers to produce at a lower fertility level of the soil. This may be one strategy towards achieving ecologically sound production of crops. Plant breeders develop lines that produce high yields through selection of genotypes that use nutrients more efficiently (Batten, 1993). Genotypes that are able to develop grow and reproduce under stress environments are described as efficiency of nutrient use (Zeng *et al*., 2009). To be able to tolerate and become more efficient, the plant uses physiological and occasionally anatomical mechanisms to circumvent stress or improve rapidly from its effects.

2.10 Molecular characterization of yam

Molecular breeding, composes genotypic and phenotypic information, and has arisen as a powerful approach and proposed new perceptions to help address these encounters (Tester and Langridge, 2010). Basic genomic resources needed for genetic studies and molecular breeding approaches are molecular markers, genetic maps and sequence information, which were not available for several less-studied crop species such as yam until recently.

Several methods such as isozyme patterns (Dansi *et al*., 2000; Mignouna *et al*., 2002) have been used to characterize yam germplasm. A major disadvantage of soluble tuber protein profiles is that the profiles may change with the physiological state of the tuber and profiles are usually dependent on tuber storage conditions (Asemota *et al*., 1992).

While isozymes have proven to be valuable tools for genetic discrimination of yam cultivars (Dansi *et al*., 2000; Mignouna *et al*., 2003), polymorphic isozyme systems are often inadequate for the unequivocal identification of individual cultivars within broad cultivar groups or morphotypes in yam (Dansi *et al*., 2000). Over the years, classification and identification methods used to characterize yam germplasm consist of morphological traits (Dansi *et al*., 1999, Otoo, 2009; Otoo *et al*,. 2012) and isozyme patterns (Dansi *et al*., 2000). Variation in profiles from soluble tuber protein profiles due to declining physiological state and storage condition of tubers also affect characterization (Asemota *et al*., 1992). Inadequate polymorphic isozyme systems to identify genotypes proposes backup from molecular markers (Asemota *et al*., 1996). Selection of any particular DNA marker in crop genetic diversity study or germplasm characterization depends largely on the objective of the research, available resources and skill (Otoo *et al*., 2009).

2.11 Molecular markers

The recent developments in molecular genetics technology have generated a range of marker types, many of which depend on the Polymerase Chain Reaction (PCR). The applications of molecular genetic markers in plants are numerous and include the following; identification of cultivars and species, identification of hybrids, paternity and kinship analyses, investigation of genetic diversity and relatedness, seed lot purity testing, gene mapping and linkage analyses in association with quantitative trait loci (QTL) in marker assisted selection. There are many and different marker systems. These markers are; restriction fragment length polymorphisms (RFLPs; Botstein *et al*., 1980), random amplified polymorphic Deoxyribonucleic acid (DNA) (RAPDs; Williams *et al*., 1991), amplified fragment length polymorphisms (AFLPs; Zabeau and Vos, 1992), diversity array technologies

(DArTs; Jones, 2009) and simple sequence repeats (SSRs, microsatellites; Tautz, 1989). Nonetheless, these and other molecular markers have technical dissimilarities in terms of cost involved, speed, quantity of DNA required, labour, levels of polymorphism, accuracy of genetic distance estimates and the statistical power of tests.

2.12 Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA was developed in 1990 (Williams *et al*., 1991) and was the first molecular marker method based on Polymerase Chain Reaction to be used in molecular genetic variation analyses (Welsh and McClelland, 1990; Williams *et al*., 1991). RAPD is different from conventional PCR as it needs one primer for amplification. Markers are generated through the random amplification of genomic DNA using short primers (10 nucleotides) and therefore, less specific. The use of RAPD requires no preceding knowledge of the genome analyzed, it can be employed across species using universal primers. DNA polymorphisms are then produced by rearrangements or deletions at or between oligonucleotide primer binding sites in the genome (Williams *et al*., 1991). Additionally, as several discrete loci are amplified by each primer, profiles are not able to distinguish heterozygous from homozygous individuals (Bardakci, 2001). Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) and DNA Amplification Fingerprinting (DAF) are self-sufficiently established methodologies, which are variants of RAPD (Welsh and McClelland, 1990). RAPD also has some limitations, such as low reproducibility and incapability to detect allelic differences in heterozygotes.

2.13 Amplified Fragment Length Polymorphism (AFLP)

These markers have a very high diversity index, resulting in a limited number of primer combinations required to screen a whole genome and has been applied to develop a

system for the fingerprinting of an organism (Faccioli *et al*., 1999) and for map expansion (Castiglioni *et al*., 1998). Some research was carried out on yam using the AFLP markers for mapping population (Mignouna *et al*., 2003), DNA fingerprinting (Powell *et al*., 1996), and parentage analysis (Gerber *et al*., 2000; Lima *et al*., 2002). Nonetheless, AFLP assays have some disadvantages. For example, polymorphic information content for bi-allelic markers are small, the maximum being 0.5 (Zabeau and Vos, 1992; Vos *et al*., 1995)

2.14 Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism markers were among the first generation of DNA markers and an essential tool to identify the genetic diversity within and between species (Old and Primrose, 1998). Most RFLP markers are co-dominant and locus-specific. They are, therefore, powerful markers or tools for comparative and synteny mapping. RFLP genotyping is extremely reproducible, and the procedure is simple and no distinct apparatus is needed. It is difficult to interpret complex RFLP allelic systems in the absence of sequence information. RFLP analysis involves huge amounts of high-quality DNA, which has low genotyping throughput, and hence highly difficult to automate.

2.15 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphism is a DNA sequence variation arising when a single nucleotide (-A, T, C, or G-) in the genome varies among members of a species or between paired chromosomes of an individual. Typically, SNPs frequencies are in a range of one SNP in every 100-300 bp in plants (Edwards *et al*., 2007; Xu, 2010). The appropriate technique for detecting SNPs is RFLP (SNPRFLP) or by using Cleaved Amplified Polymorphism Sequence (CAPS) marker technique. CAPS is less

polymorphic (the expected heterozygosity is lower). However, this limitation is made up by the comparative abundance of SNPs (Oraguzie *et al*., 2007). SNPs are codominant markers and are useful in a variety of applications, including the construction of high resolution genetic maps, mapping traits, genetic diagnostics, analysis of the genetic structure of populations and phylogenetic analysis (Rafalski, 2002). However, high costs for start-up or marker development, high-quality DNA required and high technical/equipment demands limit, to some extent, the application of SNPs in some laboratories and practical breeding programmes.

2.16 Simple Sequence Repeats (SSRs)

Simple Sequence Repeats, also called microsatellites, short tandem repeats (STRs) (Edwards *et al*., 1991), sequence-tagged microsatellite sites (STMS), simple sequence length polymorphisms (SSLP), are PCR-based markers. They are sets of repetitive sequences found inside eukaryotic genomes (Gerber, 2000). SSRs are highly variable and can vary even among individuals within a species, thus increasing their discriminative power. The flanking sequences of the repeat units are highly conserved, enabling the design of primers for their amplification in polymerase chain reactions. Research carried out showed that, SSR markers can be used to assess genetic diversity, (Otoo *et al*., 2009; Scarcelli *et al*., 2011; Norman *et al*., 2012). SSRs markers were used by Tostain *et al*., (2007) for genetic diversity study of *D. alata, D. abyssinica* and *D. Praehensilis*. The SSR-enriched clones can then be engaged by direct sequencing (Green *et al.*, 2000; Lee *et al*., 2008) or by incorporating one more step of colony hybridization with end-labelled SSR probes (Iwata *et al*., 2000)

2.17 Advantages of SSRs Markers

The hyper-variability of SSR marker yields very high allelic differences even in the midst of very closely associated species. Previous work done showed that, the quantity

of alleles differed from 1 to 37 with diversity indices of 0.29–0.95 in major crop species (Powell *et al*., 1996). The level of genetic difference discovered by SSRs analysis was almost double as detected by RFLPs, with 61 soybean lines (Morgante *et al*., 1994). In a comparative study of the utility of RFLP, RAPD, AFLP, and SSR marker systems for germplasm analysis, SSRs presented the utmost anticipated heterozygosity, while AFLPs ensured the maximum effective multiplex ratio (Powell *et al*., 1996). The co-dominant nature of SSRs is appropriate for genetical analysis in segregating F2 generations or pedigree investigation in hybrids (Scott *et al*., 2000; Slavov *et al*., 2005). Since extra and additional genomic sequences have been known in numerous eukaryotic species, it is becoming obvious that, SSRs are really plentiful in nearly all species, and are well distributed all over their genomes (Wang *et al*., 1994; Tóth *et al*., 2000). Genetic analysis is frequently hindered by the fact that, large numbers of unidentified RAPD or AFLP markers are gathered in specific sites of chromosomes or linkage maps (Vuylsteke *et al*., 1999; Kwon *et al*., 2006). In additional survey of SSRs in diverse eukaryotic genomes, Tóth *et al*. (2000) recounted that coding and non-coding regions varied significantly in SSR distribution, and characteristic variations also occurred among inter-genic regions and introns in eukaryotes from yeasts, in certain bacteria to mammals to plants.

2.18 Disadvantages of SSRs

Simple Sequence Repeats sequences can bring about replication, slippage *in vitro*, and SSR polymorphisms (Hauge and Litt, 1993; Ellegren, 2004). Slippage through PCR yields 'stutter bands' that vary in magnitude from the core product by multiples of the length of repeated unit (Hauge and Litt, 1993). The stuttering yields several ladder bands in polyacrylamide gel separation, and leads to quasi-scoring, since there are no conspicuous bands between the ladders. Stuttering similarly produces uncertainty in

SSRs with long sections of a short repeat unit (1-2 bp) as Taq polymerase slippage rises with the number of repeat units, and is inversely correlated with the length of the repeat unit (Shinde *et al*., 2003). Occurrence of homoplasy can lead to an underestimation of the real deviation among populations (Estoup *et al*., 1995).

2.19 SSRs as the marker of choice in the current study

Simple sequence repeats (SSRs) or microsatellites, is made up of a mono-, di-, and tri- or tetra nucleotide repeat, and very useful marker for a number of plant species. The main reasons for choosing SSRs are that they are abundant, reproducible, codominant, widely distributed in crop genomes and require a small quantity of DNA for PCR to detect polymorphisms. Based on these properties, SSRs are appropriate markers for use in breeding programmes, which essentially require high throughput markers as breeders need to screen a large number of breeding lines and segregating progenies.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Field characterization of nutrient use-efficient yam genotypes

3.2 Study area

The experiment was conducted in three districts of the Northern Region of Ghana. The districts and villages were: Tolon (Tingoli and Cheyohi), Mion (D C Kurah and Puriya) and East Gonja (Masaka and Kpalbe). The Northern Ghana is within the Guinea Savannah zone where the annual precipitation range from 760 to 1,140 mm and occurs from April to October in a single season, followed by a period of intense dry and warm weather (November – March). Annual temperatures vary from 23 to 35.5^{o}C with 46.6 and 76.8% as minimum and maximum relative humidity,

respectively. Yam farmers in this region cultivate yam in smallholdings, majority of whom have no formal education and are made up of mixed ethnic groups (Asante *et al*., 2008). Common yams cultivated by these farmers are: *D. rotundata* and *D. alata*.

The maturity period of *D. rotundata* varieties are; early maturing (Larbako and Kpuno), medium maturing (limo, Fushein-bila, Mogninyuli) and late maturing (Ziglangbo, Chenchito, Olodo and Kprinsi). The *D. alata* are late maturing and include; Nyuwotugu, Nyuwozhei and Seidubile/Matches.

3.3 Yam genotypes used

In total, 45 yam genotypes comprising 21 *D. rotundata*, 20 *D. alata*) genotypes from IITA Ibadan-Nigeria and four local varieties (Table 3.1) were evaluated for their nutrient use efficiency during the season.

Table 3.1 Yam genotypes, spp. and sources used for the characterization

S/N	Genotypes	spp.	Source
1	TDa 02/00064	*D. alata*	IITA-Ibadan
2	TDa 09/00314	*D. alata*	IITA-Ibadan
3	TDa 09/00364	*D. alata*	IITA-Ibadan
4	TDa 92 – 2	*D. alata*	IITA-Ibadan
5	TDa 3743	*D. alata*	IITA-Ibadan
6	TDa 02/00012	*D. alata*	IITA-Ibadan
7	TDa 00/00060	*D. alata*	IITA-Ibadan
8	TDa 01/00114	*D. alata*	IITA-Ibadan
9	TDa 09/00228	*D. alata*	IITA-Ibadan
10	TDa 93 – 36,	*D. alata*	IITA-Ibadan
11	TDa 09/00366	*D. alata*	IITA-Ibadan
12	TDa 09/00357	*D. alata*	IITA-Ibadan
13	TDa 01/00039	*D. alata*	IITA-Ibadan
14	TDa 02/00246	*D. alata*	IITA-Ibadan
15	TDa 291	*D. alata*	IITA-Ibadan
16	TDa 98/01174	*D. alata*	IITA-Ibadan
17	TDa 99/00240	*D. alata*	IITA-Ibadan
18	TDa 09/00271	*D. alata*	IITA-Ibadan
19	TDa 98/01166	*D. alata*	IITA-Ibadan
20	TDa 98/01168	*D. alata*	IITA-Ibadan
21	Seidubile	*D. alata*	Local variety - Tingoli

22	Nyuwotugu	*D. alata*	Local variety - Tingoli
23	Amula	*D. rotundata*	IITA-Ibadan
24	TDr 09/00001	*D. rotundata*	IITA-Ibadan
25	TDr 08/00917	*D. rotundata*	IITA-Ibadan
26	TDr 09/00542	*D. rotundata*	IITA-Ibadan
27	TDr 08/00444	*D. rotundata*	IITA-Ibadan
28	TDr 08/00951	*D. rotundata*	IITA-Ibadan
29	TDr 95/01932	*D. rotundata*	IITA-Ibadan
30	TDr 08/00133	*D. rotundata*	IITA-Ibadan
31	TDr 08/00990	*D. rotundata*	IITA-Ibadan
32	TDr 95/01132	*D. rotundata*	IITA-Ibadan
33	TDr 08/00921	*D. rotundata*	IITA-Ibadan
34	TDr 95/18544	*D. rotundata*	IITA-Ibadan
35	TDr 95/19177	*D. rotundata*	IITA-Ibadan
36	TDr 08/00792	*D. rotundata*	IITA-Ibadan
37	TDr 09/00123	*D. rotundata*	IITA-Ibadan
38	TDr 08/00845	*D. rotundata*	IITA-Ibadan
39	TDr 08/00944	*D. rotundata*	IITA-Ibadan
40	TDr 08/00789	*D. rotundata*	IITA-Ibadan
41	TDr 08/00845	*D. rotundata*	IITA-Ibadan
42	TDr 08/00944	*D. rotundata*	IITA-Ibadan
43	TDr 08/00789	*D. rotundata*	IITA-Ibadan
44	Larbako	*D. rotundata*	Local variety - Kpalsogu
45	Chenchito	*D. rotundata*	Local variety - Kpalsogu

3.4 Land preparation, field design and planting

The land was initially prepared by ploughing and harrowing. Soil samples were taken before ploughing to test the nutrient status (P^H, % organic carbon and nitrogen as well as Mg/kg, P and K) and percent sand, silt and clay) to determine the base fertility status prior to planting the yam genotypes. A Randomized complete block design (RCBD) with three replication, using each district as a replication for both *D. rotundata* and *D. alata*. Lining and pegging were done with the area to be occupied by a mound clearly marked and subsequently mounds were raised. One type of spacing (1.2m x 1.2m) was adopted. The size of a sub plot for a genotype was 6m x

1.2m representing five mounds. Seed yams of the genotypes acquired from International Institute of Tropical Agriculture (IITA-Nigeria) and local the local varieties collected

from farmers in Kpalsogu and Tingoli, Ghana were used. To prevent further infection of diseases and insect pest damage during sprouting, 150 –

200 sett (depending on seed size) were treated for 10 min with 100ml insecticide (*Chloropyriphose* + *Alphamethrin* 16 +1%, Cymetox super at 1 litre/ha – Modern Insecticide Limited, Ludhiana, Punjab, India), 24g of fungicide (*Mancozeb* + *Metalaxyl* 64+8% WP/W. topsin M at 70g/ha – Hunan Arshin Biotechnology Company Limited, China) and 53g of wood ash were mixed in 15 l of water (1 knapsack). Broad leaves of shrubs were used as a mulch material for each mound to prevent rottening of seed yam from sun scotch.

3.5 Data taken and cultural practices

In total, 28 plant morphological data were taken, among these eight were qualitative and 20 were quantitative. Each datum was taken using four plants and averaged to obtain the final value for each parameter. Tables (3.2 and 3.3) below show data, time taken and scores (Agre *et al*., 2015) obtained.

Table 3.2 Qualitative data used, data recording time, and scores *in D. alata and D. rotundata*

Data recording Time	Type of variable	Scoring
16 Weeks After Planting (WAP)	Virus	Observation of all plants per genotype and score for severity infection level. Score (1 – 5)
	Leaf spot	Observation of all plants and score for severity infection level. Score (1 – 5)
	Anthracnose	Observation of all plants and score for severity infection level. Score (1 – 5)
	Leaf blight	Observation of all plants and score for severity infection level. Score (1 – 5)
28 WAP	Scale insect	Observation of all harvested tubers per genotype and score for severity level. Score (1 – 5)

	Yam beetle tuber	Observation of all harvested tubers per genotype and score for severity level. Score (1 – 5)
	Mealybug	Observation of all harvested tubers per genotype and score for severity level. Score (1 – 5)
	Nematode	Observation of all harvested tubers per genotype and score for severity level. level Score (1 – 5)

Diseases and pests score level. Score (1 – 5): *Where 1 – No disease symptoms. 2 – Few diseases symptoms (1-25%). 3 – Moderate diseases symptoms (25-50%). 4 – High diseases symptoms (50-75%). 5 – very severe diseases symptoms (over 80%).*

Table 3.3. Quantitative data used, recording time and data taken procedure.

Data recording Time	Data type	Data taken procedure
2-8 WAP	Plant establishment	Counting of the number of sprouted plants at 2nd, 4th, 6th and 8th weeks after planting
16 WAP	Stem height (cm)	Three plants were measured with a calibrated measuring stick from the base to the growing tip
16 WAP	Stem number	Counting of number of stems/vine(s) germinates from seed yam
16 WAP	Stem diameter	Stem girth taken at 0.5m above ground, before the diameter was estimated
16 WAP	Internode length	Immediate internode measured 1m above ground.
16 WAP	SPAD reading	SPAD values of mature leaves at the uppermost, part of the plant
16 WAP	SPAD reading	SPAD values of mature leaves at the middle portion of the plant
20 WAP	SPAD reading	SPAD content of mature leaves at the lower portion of the plant
20 WAP	Leaf Area	Estimation was done using the formular stated below
20 WAP	Leaf Area Index	Estimation was done using the formular stated below
26 WAP	Leaf Surface Area	Measuring length and breadth of the widest portion of the lower, middle and upper leaves of a plant.
26 WAP	Plants harvested	Counting number of plants harvested
26 WAP	Seed yam number	Counting number of seed yam
26 WAP	Ware yam number	Counting number of ware yam

26 WAP	Total yam number	Counting number of seed and ware yam number
26 WAP	Seed yam weight	Taking the weight of seed yam
26 WAP	Ware yam weight	Taking the weight of ware yam
26 WAP	Total yam weight	Taking total weight of seed and ware yam
26 WAP	Tuber diameter	Measuring the girth of the tuber and later find the diameter
26 WAP	Tuber length	Measuring tuber length from the center of the head to the distal end of the tail

The formulae for leaf area and leaf area index proposed by Andres (2004) below were used.

Leaf Area (LA) = $\frac{M \times \pi r^2 \times ND}{m}$

Where;

M = Weight of imperforated leaves *m* = Weight of disc leaves *ND* = number of disc leaves r^2 = Square of radius of the disc and π = 3.142

Leaf Area Index (LAI) = $\frac{\text{Leaf Area } (m^2)}{\text{Ground area } (m^2)}$

Where, ground area = Area occupied by plant stand and it is 1.44m^2

3.6 Statistical Analysis

Statistical analysis was performed using quantitative data. The severerity data on insect and disease infestation/infection ranged between one and two, meaning that, insect pest

infestation and diseases infection on leaves and tubers were far below threshold to be considered as significant. For quantitative data, Statistica software (version 12.0) was used to compute descriptive statistics (Mean, minimum, maximum, variance, standard deviation and coefficient of variation, correlation and coefficients analysis). In other to determine variables that offered the utmost contribution, Multiple Component Analysis was done using quantitative data. Statistica analytical software (version 9.3) was used for farmer correspondence analysis and farmer preference of the genotypes was selected and ranked. Mean tuber yield in t/ha was determined using Statistix (version 9.2). Bands were scored as present (1) and absent (0) for each genotype and Unweighted Pair Group Method with an Arithmetic Mean was used to show genetic relationships among species. Special code was used for missing data.

3.7 Participatory evaluation with farmers to determine nutrient-use efficient yam genotypes

3.7.1 Selection of farmers

The farmers were nominated based on their indigenous knowledge in yam cultivation. Careful selection was carried out to target persons (both men and women) in the villages adjudged to have indigenous knowledge about yam, and also to ensure gender and age representation.. Selection of participants was done with the help of Agricultural Extension Agents (AEAs) of the Ministry of Food and Agriculture, village chiefs, opinion leaders and Assembly men who were more familiar with farmers in their communities within the study sites.

3.7.2 Farmer participatory varietal selection

At 16 weeks after planting (WAP), the selected yam farmers made up of males and females evaluated the vegetative phase of the yam crop on the field based on the morphological characters of the various genotypes. Names of genotypes were not given

to farmers so as to avoid bias, but genotypes were coded. Also a checklist was designed to give farmers free mind in evaluating the general performance of each variety according to their own criteria, and also to allow researchers to gather precise and enough information. Among the various groups formed, a group leader was appointed for each group. Preference analysis was used and farmers perception as to whether they like the plants of a treatment or not and the reasons behind their perception were obtained. Yam characteristics considered by farmers as reasons for preference or not, during this phase in each genotype were; sprouting %, plant vigour, number of stems per stand, magnitude of canopy cover, leaf texture, leaf size, leaf coloure, leaf shape, presence or absence of thorns, flowering ability, insect damage and disease infection. Farmers also concluded by further evaluating each genotype as good, very good, fairly good and not good. The use of farmer's opinion to evaluate tubers of yam genotypes in the field was done using preference analysis by groups of male and female farmers separately. At seven to eight months after planting, yam genotypes were harvested and farmer evaluation on the tuber harvested was carried out. Yam tubers harvested were arranged on the field in an open place. Each genotype was arranged as ware and seed yam. Following the above arrangement the performance of each genotype according to farmers criteria was evaluated in two forms: Farmer characterization of the tubers and preference ranking analysis for the best five genotypes. Farmers in their various groups were taken round the various treatments of yams to describe them. Farmer characterization was done based on the tuber physical features which included the following: yield in terms of quantity, tuber size (small, medium or big), tuber shape (round or straight), tuber surface (smooth or rough), and proportion of ware to seed yam, rotten tubers (presence, number and weight.), insect infestation (damage caused), hairiness and nematode damaged. Besides, ranking based on preference analysis carried

out by the farmer groups was based on gender (male and female elders and youth) and age (below and above 50 years) to select the best five genotypes and reason(s) for their selection. The process involved the use of coloured cardboard to represent the various groups on gender and age. Four colours representing (Pink – elder women, yellow – young women, blue – elder men, and white – young men) were used. Each colour cardboard was cut into five sets. Each colour set was marked 1st, 2nd, 3rd, 4th, and 5th positions. The first step involved allowing a group of farmers (gender and age category), with a set of cardboard marked 1st position to go round the arranged tubers to select their best treatment by placing cards on their desired treatment. When all the groups have gone round to exhaust the first position cards, each treatment was attended and counted the cards placed or voted for and the number was recorded based on the various sex and age groups. A treatment with high number of votes indicated farmer preference and therefore was ranked first. Finally reasons for selection were then attributed to the best genotype. This step was repeated until the best five (5) genotypes were chosen.

3.7.3 Data analysis

Statistical analytical software package (version SAS 9.3) was used to perform correspondence analysis and associate genotypes with respective preference parameters as described by the farmers at the vegetative and tuber characterization phases. This was presented as a scatter diagram using the first two factors of the Principal component analysis (PCA). In addition, best genotypes chosen and ranked by farmers were presented with reasons associated to their choice.

Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) is a multivariate tool in statistical software's that is use for both quantitative and qualitative data analysis .It revealed the pattern of character variation in accessions within specie and between species. The procedure

group data into meaningful components that determined the amount of variation contributed from individuals within a population. Nonetheless, it is not convenient for molecular data. (Cornell University, 2003).

3.8 Molecular characterization of yam genotypes

3.8.1. The use of molecular markers, to identify the performance of high nutrient use-efficient genotypes

3.8.2 Experimental location

The study was carried out at the Bioscience Center of International Institute of Tropical Agriculture (IITA) - Ibadan, Nigeria.

3.8.3 Yam genotypes used

Yam genotypes used were 45 tubers made up of 20 *D. alata* genotypes and 21 *D. Rotundata* genotypes, collected from IITA Yam Breeding Programme and four local farmer varieties, provided by the farmers from their previous harvest. These genotypes were grown in three districts (Tolon, Mion and East Gonja) of Northern Region of Ghana and characterized using the IPGRI yam descriptor and farmer preference criteria to select high yielding genotypes grown under low soil fertility and under no staking condition as nutrient use-efficiency evaluation.

3.8.4 DNA Extraction

Genomic DNA was extracted from freshly harvested tubers of the 45 yam genotypes planted in the Northern Region of Ghana as described above using SDS (Sodium Dodecyl Sulphate) procedure. Tuber tissue of each genotype was sampled at the middle portion of the tuber and ground gently separately without much destruction, into marshy fine paste using hand held pestle and mortar. About 2g of ground tissue was put in 2ml eppendorf tubes. The sample was first washed with Hepes. An amount of 450µl of Hepes was added to the sampled tissue, mixed, shoke with hand and centrifuged at 10000rpm for five minutes. Secondary metabolite such as starch was removed by decanting. After the Hepes wash, it was further mixed with 450µl of 1 x

SDS extraction buffer made up of 1% SDS (w/v), 100 mM Tris HCl (pH 8.0) 10ml, 10mM EDTA (pH 8.0) 2ml, 2M NaCl 40ml, 2% PVP, 4% PEG, 1% B-Mecarpthoethanol and 0.05ng/ml Protenase K. Samples were then incubated in a 65^{o}C water bath with intermittent inversions for 30 min. The samples were removed from the water bath and shoke to mix thoroughly. An amount of 450µl mixture of chloroform isoamyl alcohol (24:1) was added to the sample, well covered and mixed thoroughly before centrifugation at 13000rpm for 10min. The supernatant obtained after centrifugation was transferred through gentle pippeting into a newly labelled 1.5ml eppendorf tube. The DNA was precipitated by adding 300µl of ice- cold Isopropanol and incubated at -20^{o}C for 1h. Samples were centrifuged at 13000rpm for 10min and the ice-cold isopropanol was decanted to obtain pellets. The DNA pellet was washed with 200µl of 70% ethanol and dried for 30 min at normal room temperature. The resulting DNA pellet was resuspended into 200µl of Tris EDTA low (salty water) (Sigma) with 3µl of RNase.

3.8.5 DNA purification

A volume of 450µl of Chlorofoam Isoamyl alcohol (24:1) was added to each of the dissolved DNA sample. It was covered and shaken well for proper mixing and centrifuged at 13000rpm for 15min. The supernatant was removed with care into a newly labelled eppendorf tube. DNA was precipitated by adding 315µl ice-cold Ethanol sodium acetic acid with PEG to the supernatant, inverted for proper mixing, incubated at -20^{o}C for 1h and was centrifuged at 13000rpm for 20min. Afterwards, the supernatant was decanted and pellets were washed with 200µl of 70% ethanol and dried on a laboratory working bench for 30min. The pellets were resuspended in 100µl ultrapure water and stored in fridge.

3.8 .6 DNA quality and quantity determination

The quality of DNA of each of the yam genotypes was checked by electrophoresis on 1% agarose gel stained with ethidium bromide and visualized under UV light using gel documentation. Quantification was done using a DNA Lambda 50 bp/molecular weight marker. The quality of DNA were determined by recording the absorbance reading at 260 and 280 nm (A 260 and A 280), respectively, using a spectrophotometer (Beckman Coulter DU530 - Labtronics company, Punchkula – India). The milli-Q water was used as a standard reference to set the spectrophotometer at 260 nm wavelength (blanking). For each genotype sample, 2µl of DNA was taken and the quality was measured at 280/260. A value of 1.8 was taken for high quality DNA. After DNA quantification, DNA was set to 25ng/µl as working solution for the PCR.

Table 3.4. List of SSR markers used for optimization of *D. rotundata* and *D. alata* genotypes

No.	Marker	Forward primer (5' to 3')	Reverse primer (3' to 5')	Annealing temperature
1	YSR2	TAGATTTCGCTTTTCCACTAGC	CCTAATCATCATCATCGTCATC	55ºC
2	YSR3	TCACTCAAACAATGAGCGTAG	GATGGCTGCTGCATGACTG	55ºC
3	YSR5	AGGATTATCACTGAAAGGGCT	CCTTCCAATTACTCTCCAAGA	55ºC
4	YSR6	ACAGAGCTGTTGACACAAACA	CCTCAAATGAACCTTTGGTCTA	55ºC
5	YSR9	AGGAACATTCCCACTCAGTTATG	ATTGGGCAAGTGTGGTGTG	55ºC
6	YSR10	ACCCAAAATATTCTCCCCATTATAC	TTGACACTCAATCTTATATTGCTCC	55ºC
7	YSR 36	CCTTACCACCGGACTCCTC	TGCAGCAATACACCGGAAC	55ºC
8	YSR 53	CTCATAAGCAGAGCCTTCTCTC	TACAGTCCCTGTTTGAGCATAG	55ºC
9	YSR11	GGATGGCGTAGAGGAAGAGG	GGATAAGACCACGAGTGTTGC	55ºC
10	YSR12	TGAGCATTCTTGTTTTGCCG	CTTTCAGGGCGTGCATGG	55ºC
11	YSR13	CCAATCACATCACGTCTAGTCT	GACAATAGAAACTTCGAGACCC	55ºC
12	YSR 32	GAGGTCTGCGACGGATTTG	TCGCATTCTTCATCCTTCAC	55ºC
13	YSR 33	ACCATGGGATGAAGGGAAGG	GCATATGGTGCATGGGAGC	55ºC
14	YSR 66	ATATTGACTGACCACCAGATCA	GAAGAGTCTTGGATTTCTACCA	55ºC
15	YSR 75	TCGCTCAACCTAATCCTCTATT	TCAAACCAGCCAAAACATC	55ºC
16	YSR21	AATGATGCATCTGAGGATAGTG	GATGCTATTACGACAACCTTGA	55ºC
17	YSR23	TTAAGACTTGCAGGGTTAAAGG	GTGGCTAGTTTTTGTAGCTGGT	55ºC
18	YSR24	GGTGTTGTTGGGTTTCATTGTC	TCCCTCTTCTCATTTCACTCCC	55ºC
19	YSR78	ATGACTACTGCAAGGACAACAG	GGTGATATGCATGATTCAACCT	55ºC
20	YSR 74	TGGTGTTTGAGAATGGAGGATTG	ACTTGATCTTTGTCTTGATGGC	55ºC

3.8 .7 Polymerase chain reaction (PCR) and genotyping with agarose

Polymerase chain reaction was carried out in a PCR system. In all, 20 SSR primers listed in Table 3.4 were screened to choose the best SSR primer. About 10µl of PCR reaction mix include in a 2µl of 25 ng/µl of DNA; 1µl of 10X PCR buffer, 0.4µl dNTPs (5mM), 0.4µl $MgCl_2$ (50mM); 1µl (5mM) of each of forward and reverse primers, 0.06µl Taq polymerase and adjusted to 10µl by adding double distilled water. The PCR reactions were carried out in a Themolyne Amplitron 11 Thermocycler – (Labtronics company, Punchkula – India). Pre denaturation at 94ºC for 5min following annealing at 55ºC for 20s and an extension for 30s at 72ºC for 32 cycles and a final extension of 5min at 72ºC.

The amplified PCR products were analyzed using 2.5% agarose gel electrophoresis in 0.5 × TAE buffer (pH 8.0) at 100 Volts for 2h, stained with Ethidium bromide and visualised on Gel documentation system (Zhou *et al*., 2007). To determine the size of the SSR fragments, a 500 bp DNA Ladder was used. During the initial screening, a total of 20 primers were used and only 12 that could amplify visible bands were selected for further examination. Different annealing temperatures were used in trials to optimize the amplification condition for the 12 selected primers. Finally, a total of seven SSR primers as shown in (Table 3.5) produced clearer and reproducible bands and therefore, were selected for the amplification of all the samples. The gel was captured and photographed with a computer system coupled with Ultra Violet (UV) lighting system.

Table 3.5 SSR markers selected for genotyping *D. alata* and *D. rotundata*

No	Primer	Right (forward) primer (5 to 3)	Left (Reverse) primer (3 to 5)	Annealing
1	YSR 32	GAGGTCTGCGACGGATTTG	TCGCATTCTTCATCCTTCAC	55ºC
2	YSR 33	ACCATGGGATGAAGGGAAGG	GCATATGGTGCATGGGAGC	55ºC

3	YSR 36	CCTTACCACCGGACTCCTC	TGCAGCAATACACCGGAAC	55^{o}C
4	YSR 53	CTCATAAGCAGAGCCTTCTCTC	TACAGTCCCTGTTTGAGCATAG	55^{o}C
5	YSR 66	ATATTGACTGACCACCAGATCA	GAAGAGTCTTGGATTTCTACCA	55^{o}C
6	YSR 74	TGGTGTTTGAGAATGGAGGATTG	ACTTGATCTTTGTCTTGATGGC	55^{o}C
7	YSR 75	TCGCTCAACCTAATCCTCTATT	TCAAACCAGCCAAAACATC	55^{o}C

3.8.8 Data analysis

The genomic SSR markers that, resulted in complex patterns (that is, more than 2 alleles) were excluded to maintain a strict di-allelic model of inheritance as reported by Fregene *et al*. (2003). Seven markers (Table 3.5) that gave distinct di-allelic patterns were chosen for gene diversity analysis. To determine the relationship among genotypes based on a hierarchical cluster analysis, the alleles were recorded as bands and scored as 1 or 0 for presence and absence, respectively. The data in this form were used to calculate genetic distances between pairs (simple matching coefficient) of the yam genotypes from comparisons of the band scores. Then using the unweighted pair-group mean average (UPGMA) cluster method of Nei's genetic distances (Rohlf, (1998), a dendrogram of genetic similarity was generated. The genetic distances and dendrogram were computed with the NTSYS-PC computer programme, version. 2.02 (Rohlf, 1998). Genetic diversity was estimated using three statistics averaged over loci, the percentage of polymorphic loci (P), the mean number of alleles per locus or allelic richness (A); the average gene diversity (He); was computed, according to Rohlf (1998).

CHAPTER FOUR

4.0 RESULTS

4.1 Field evaluation of yam genotypes using agromophological characters

4.2 The major constraint

During the inception of the study, Participatory rural appraisal (PRA) was conducted to know the yam cultivation status, constraints and how the farmers on their own were addressing these constraints based on their indigenous knowledge, experiences and resources in the study area. Soil fertility was prioritized as the major constraint. This was because; land is fixed and subjected to so many uses. The pressure on land has limited the traditional bush fallow systems where exhausted lands were kept under fallow to rejuvenate in fertility. Farmers could not afford fertilizing their yam fields with chemical fertilizer. Some of the farmers used farm yard manure from their livestock and mulching to improve soil fertility and texture. The description of the soil fertility and texture by the farmer as summarized in Table 4.1 revealed that, soils used in yam cultivation are sandy and gravel soils, and of low water holding capacity. Nutrient losses through erosion and leaching are high on these soils. Hence, the soil fertility was described to be low resulting in low tuber yields. Similar soil fertility status was obtained when soil was sampled and analyzed as in table 4.2. The results showed that, pH values ranged from 4.94 to 6.12 indicating largely acidic soil condition. Organic matter content varied between 0.2 to 0.9%, thus, less than 1%. Percent N ranged from 2.2 to 8.6%, thus, less than 10%. Available P and K (mg/kg) were found to vary from 3.4 to 30.2 and 33 to 103, respectively. Since the amount of soil organic matter content in a soil influences the availability of other nutrients, percent Nitrogen content, available P and K in mg/kg were low, giving a general conclusion that the soils' fertility status was low. The soil fractions were found to be sandy, with very low silt and clay fractions. This type of texture has very poor water and nutrient holding capacity for plant growth. Hence, the need to use nutrient useefficient yam genotypes

which could give considerable yield in low soil fertility conditions as described for sustainable yam production in Northern Region.

Table 4.1. Farmer descriptions of soil fertility status and texture across the three districts before planting *D.alata* and *D. rotundata* genotypes

S/N	Districts	Community	Soil fertility status	Yield	Soil texture
1	East Gonja	Masaka	Low soil fertility	Low yam yield	Sandy and gravel soils
2	East Gonja	Kpalbe	Low soil fertility	Low yam yield	Sandy and gravel soils
3	Tolon	Tingoli	Low soil fertility	Low yam yield	Sandy and gravel soils
4	Tolon	Cheyohi	Low soil fertility	Low yam yield	Sandy and gravel soils
5	Mion	Puriya	Low soil fertility	Low yam yield	Sandy and gravel soils
6	Mion	D C Kurah	Low soil fertility	Low yam yield	Sandy and gravel soils

Table 4.2. Soil fertility status on the soil of the three districts before planting of *D. alata* and *D. rotundata* genotypes

S/N	District	Community	Experimental type	pH value	Organic Carbon and Nutrient Content				Percent Soil Texture		
					% OC	% N	Mg/kg P	Mg/kg K	% Sand	% Silt	% Clay
1	East Gonja	Masaka	*D. rotundata*	5.77	0.507	0.048	30.187	33	75.16	23.96	0.88
2	East Gonja	Kpalbe	*D. alata*	6.12	0.819	0.078	6.489	90	69.16	29.96	0.88
3	Tolon	Tingoli	*D. rotundata*	5.73	0.507	0.047	3.437	90	55.16	43.96	0.88
4	Tolon	Cheyohi	*D. alata*	4.94	0.234	0.022	3.612	58	69.76	29.8	0.44
5	Mion	Puriya	*D. rotundata*	5.69	0.97	0.086	15.442	75	33.76	57.8	8.44
6	Mion	D C Kurah	*D. alata*	4.99	0.819	0.079	4.035	103	53.76	41.8	4.44

Source: Laboratory of CSIR – SARI, 2015

4.3 Statistical descriptive analysis on *D. alata* and *D. rotundata*

The quantitative variables study as summarized in the Table 4.3 was performed to estimate the variability of each trait among the *D.alata* and *D. rotundata* genotypes. The study revealed that, the plant stand (PtS) varied from 1.00 to 10.00 with the mean of 5.12. The mean value of stem diameter (StD) was 0.55 and it varied from 0.35 to 0.76. Value of the leaf area (LA) varied from 0.42 to 8.81 with the mean as 2.55. For the low SPAD values, (SLo), 48.36 cm^2 was found as general mean and varied from 38.12 to 61.20 cm^2. Total yam number (TYN) varied from 5555.20 to 34720.00 and 2840.54 was recorded as mean. The total yam weight (TYW) varied from 2876.80 to 15566.13kg where the mean value was 12073.22kg. The standard deviation observed was highest in seed yam number 6935.71 and lowest in stem diameter 0.10. Some parameters such as leaf area (LA), Leaf area index (LAI) and Leaf surface area (LSA) were found to present high value of the coefficient while some such as low SPAD values (SLo), SPAD middle (SMi) and SPAD up (SUp) showed low coefficient values.

Table 4.3. Statistic descriptive of the quantitative parameters of *D. alata and* genotypes

Parameters	Mean	Minimum	Maximum	Variance	Std.Dev.	CV(%)
PtS	5.12	1.00	10.00	5.75	2.40	46.82
PHt (cm)	3.25	1.44	8.29	2.41	1.55	47.76
StN	2.12	1.00	5.00	1.16	1.08	50.77
StD (cm)	0.55	0.35	0.76	0.01	0.10	17.60
ILn (cm)	12.46	5.80	23.00	16.32	4.04	32.41
LSA (cm^2)	24.24	0.53	72.95	583.16	24.15	99.62
LA (cm)	2.55	0.42	8.81	4.98	2.23	87.64
LAI	30.22	1.94	77.89	741.97	27.24	90.15
SUp	31.84	21.67	41.87	25.91	5.09	15.99

SMd	40.61	29.57	47.20	18.31	4.28	10.54
SLo	48.36	38.12	61.20	23.31	4.83	9.98
PtH	4471.77	992.00	6944.00	3873866.00	1968.21	44.01
SYN	9115.18	0.00	32694.67	48104102.90	6935.71	76.09
WYN	2840.54	0.00	6944.00	4266342.88	2065.51	72.72
TYN	12073.2	5555.20	34720.00	39243802.10	6264.49	51.89
SYW (kg)	3885.86	0.00	14379.87	6916223.84	2629.87	67.68
WYW (kg)	3787.73	0.00	9721.60	7904520.63	2811.50	74.23
TYW (kg)	7673.58	2876.80	15566.13	7712542.75	2777.15	36.19
TDi (cm)	7.66	4.14	10.18	1.53	1.24	16.15
Tbl (cm)	26.38	14.50	38.00	32.19	5.67	21.51

Key: PSt = Plant Stand, PtH = Plant Height, StN = stem number, StD = Stem diameter, ILn = Internode length, LSA = leaf surface area, LA = leaf area, LAI = leaf area index, SUP = SPAD reading (top leaves of plant), SLM = SPAD reading (middle leaves of plant), SLO = SPAD reading (lower leaves of plant), PtH = Plant harvested, SYN = seed yam Number, WYN = ware yam number, TYN = total yam number, SYW = seed yam weight, WYW = ware yam weight, TYW = total yam weight, TDi = Tuber diameter, Tbl = tuber length

4.4 Factor and principal component analysis (PCA) of *D. alata* and *D. rotundata*

The use of the principal component analysis revealed that, the 20 quantitative variables were grouped into several components in which the first six axes explained 82.70% of the total variation obtained (Table 4.4 and Figure 4.1). As recommended in the Statistica software, variables with values more than 50% have an association with the axe. Therefore, any value that was 50% and above, positive or negative was considered to be associated to the axes. The score of principal component 1 (PC1) was positively associated with plant establishment (PSt), plant height (PHt), leaf area index (LAI), stem number (StN), leaf surface area (LSA), seed yam number (SYN), Total yam

number (TYN) and seed yam weight (SYW), but presented a negative association with tuber length (TLn) and leaf area (LfA) and accounted for 30.23% total variability. Principal component 2 (PC2) was found to have positive associate with SPAD middle (SMd) and SPAD low (Slo), but found to be have negative association with ware yam number (WYN) and ware yam weight (WYW) accounting for 17.68% variability. Total yam numbers (TYN) and total yam weight (TYW) were positively associated with PC3 and contributed 12.25%. Internode length (ILn) and plant harvested (PtH) were positively associated with the PC4 while plant establishment (PSt) and plant height (PHt) were negatively associated with the same factor and contributed 9.06% Three parameters (PSt, StD and PtH) were found to be associated with the PC 5 accounting for 7.18% as their contribution. The PC6 contributed 6.30% as its variability, but had no association with any of the variables. The subsequent PCs were considered to be less significant since their correlation values were less than 50%. In total, 18 out of the 20 quantitative parameters presented high variability and SPAD up (SUp) and tuber diameter (Tdi) was not associated with any of the given six axes. In all, six quantitative traits (Leaf Area, Leaf surface area, high chlorophyll content at the lower to the middle leaves, seed yam, ware yam and total yam) out of the 20 studied revealed the highest variability and therefore, were considered as very significant to be used in characterizing the nutrient use-efficient genotypes of *D. alata* and *D. rotundata* species.

Table 4.4. Principal components analysis (PCA) of *D. alata* and *D. rotundata* parameters on the factor axis

S/N	Variables	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
1	PSt	**0.5865**	-0.2377			**-0.5149**	-0.1016
		0.11917	**0.51659**				

2	PHt	**0.51566**	-0.247	0.04035	**-0.5532**	-0.1345	0.18941	3	StN	**0.5885**	-0.1382
	-0.2523	0.37604	0.28237	-0.1718							
4	StD	0.2337	-0.2491	0.25368	-0.4666	**-0.5909**	0.21808				
5	ILn	-0.001	-0.4477	-0.1796	**-0.5816**	-0.3024	-0.2517				
6	LfA	**-0.8277**	0.03514	0.19249	-0.1836	-0.0412	-0.354				
7	LAI	**0.77523**	-0.2314	-0.036	-0.0518	0.13884	0.37146	8	LSA	**0.82118**	
-0.2125	-0.1455	0.03847	0.04196	0.41299							
9	Sup	-0.4505	0.44376	0.41082	0.14914	-0.0075	0.17571				
10	SMd	-0.3587	**0.65539**	0.44703	0.12039	-0.2878	0.22909				
11	SLo	-0.2035	**0.70325**	0.24172	0.03632	-0.2189	0.35751				
12	PtH	0.40508	-0.3259	0.13233	**0.59035**	**-0.5214**	-0.2037				
13	SYN	**0.78706**	0.32784	0.38519	-0.0846	0.04545	-0.2868				
14	WYN	-0.3576	**-0.7735**	0.33153	0.03919	0.07527	0.02355				
15	TYN	**0.73669**	0.12569	**0.53348**	-0.0683	0.06197	-0.3094				
16	SYW	**0.67554**	0.4192	0.43375	-0.2234	0.19665	-0.2128				
17	WYW	-0.4364	**-0.7082**	0.42913	0.14152	0.10647	0.1949				
18	TYW	0.19789	-0.32	**0.84518**	-0.0683	0.29401	-0.0042				
19	Tdi	-0.088	-0.3668	0.28815	0.17009	0.23223	0.30973				
20	TLn	**-0.7439**	-0.4174	0.24089	0.03119	-0.0648	-0.171				
Proportion		**30.23**	**17.68**	**12.25**	**9.06**	**7.18**	**6.30**	**Eigen value**		**6.05**	**3.54**
2.45	**1.81**	**1.81**	**1.26**	**% Cumulative**		**30.23**	**47.91**	**60.16**	**69.23**	**76.4**	**82.7**

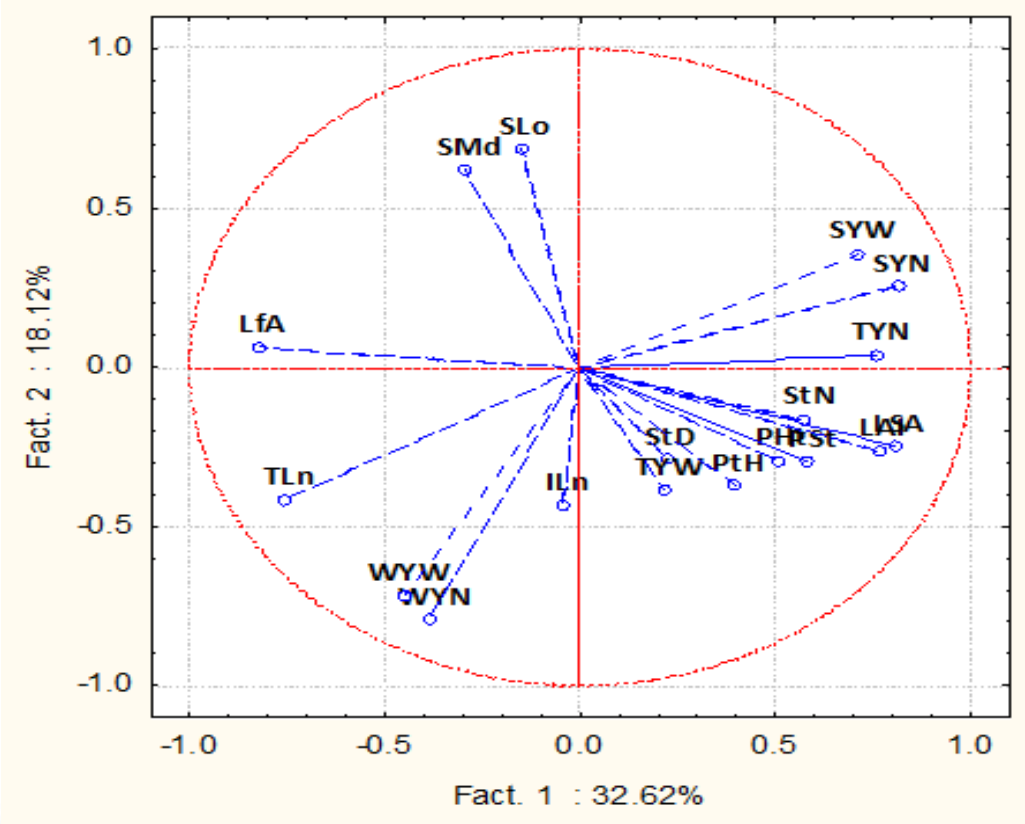

Figure 4.1. Principal component analysis (PCA) of *D. alata* and *D. rotundata* based on quantitative data

4.5 Observed correlation among quantitative data of *D. alata*

Summary of the correlation is presented in Table 4.5. Each value bolded per column was significantly-correlated. Plant establishment was significant and correlated positively (r=0.38, p<0.05) with stem number (StN), (r = 0.37, p < 0.05) leaf area index (LAI), (r = 0.46, p < 0.05) leaf surface area (LSA), (r = 0.92, p < 0.05) plant harvested (PtH), (r = 0.39, p < 0.05) seed yam number (SYN) and (r = 0.43, p < 0.05) total yam number (TYN). However, it correlated negatively (r= -0.49, p<0.05) with leaf area (LfA). Plant height correlated positively (r = 0.43, p < 0.05) with stem diameter (StD), (r = 0.40, p < 0.05) internode length (ILn), (r = 0.48, p < 0.05) leaf area index (LAI), (r = 0.52, p < 0.05) leaf surface area (LSA) and (r = 0.34, p < 0.05) seed yam weight

(SYW). Nonetheless, it had a negative correlation (-0.33, $p < 0.05$) with leaf area (LA) and ($r = -0.33$, $p < 0.05$) SPAD middle (SMd). Stem number was observed to have positive correlation ($r = 0.42$, $p < 0.05$) with leaf area index (LAI), ($r = 0.52$, $p < 0.05$) leaf surface area (LSA) and ($r = 0.34$, $p < 0.05$) plant harvested (PtH). In contrast, it correlated negatively with ($r = -0.55$, $p < 0.05$), leaf area (LfA), ($r = -0.18$, $p < 0.05$) SPAD up (Sup) and ($r = -0.30$, $p < 0.05$) SPAD middle (SMd). Positive correlation ($r = 0.35$, $p < 0.05$) was found between Stem diameter (StD) and internode length (ILn). However, negative correlation ($r = -0.31$, $p < 0.05$) was recorded between internode length (ILn), ($r = -0.31$, $p < 0.05$) SPAD up (Sup), ($r = 34$, $p < 0.05$), SPAD middle (SMd) and ($r = -0.33$, $p < 0.05$) SPAD low (Slo). Leaf area (LfA) was found to have significant and positive correlation with ($r = 0.37$, $p < 0.05$) SPAD up (Sup), ($r = -0.30$, $p < 0.05$) ware yam weight (WYW) and ($r = 0.67$, $p < 0.05$) tuber length (TLn), but recorded negative correlation ($r = -0.75$, $p < 0.05$) with leaf area index (LAI), ($r = -0.88$, $p < 0.05$) leaf surface area (LSA), ($r = -0.47$, $p < 0.05$) seed yam number (SYN), ($r = -0.41$, $p < 0.05$) total yam number (TYN) and ($r = -0.33$, $p < 0.05$) seed yam number (SYW). Total yam number (TYN) were positively correlated ($r = 0.45$, $p < 0.05$) with leaf area index (LAI), ($r = 0.31$, $p < 0.05$) leaf surface area (LSA) and ($r = 0.90$, $p < 0.05$) seed yam number (SYN). Seed yam number (SYN) had a significant positive correlation ($r = 0.96$, $p < 0.05$) with total yam number (TYN), ($r = 0.90$, $p < 0.05$) seed yam weight (SYW) and ($r = 0.37$, $p < 0.05$) total yam weight (TYW), but had a negative correlation ($r = -0.40$, $p < 0.05$) with ware yam number (WYN), ($r = -0.47$, $p < 0.05$) ware yam weight (WYW) and ($r = -0.57$, $p < 0.05$) tuber length (TLn). Ware yam number (WYN) recorded positive significant correlation ($r = 0.88$, $p < 0.05$) with ware yam weight (WYW), ($r = 0.46$, $p < 0.05$) total yam weight

(TYW) and ($r = 0.67$, $p < 0.05$) tuber length (TLn). It however had negative correlation ($r = -0.45$, $p < 0.05$) with seed yam weight (SYW).

Total yam number (TYN) emerged significant and positively correlated ($r = 0.84$, $p < 0.05$) with seed yam weight (SYW), ($r = 0.54$, $p < 0.05$) total yam weight (TYW), but negatively correlated ($r = -0.41$, $p < 0.05$) with tuber length. The weight of seed yam (SYW) significantly correlated negatively ($r = -0.48$, $p < 0.05$) with weight of ware yam (WYW) and ($r = -0.57$, $p < 0.05$) tuber length (TLn), but positively correlated ($r = 0.46$, $p < 0.05$) with total yam number (TYN).

Total yam weight (TYW) was found to be positively correlated ($r = 0.37$, $p < 0.05$) with seed yam number (SYN), ($r = 0.46$, $p < 0.05$) ware yam number (WYN), ($r = 0.54$, $p < 0.05$) total yam number (TYN), ($r = 0.46$, $p < 0.05$) seed yam weight (SYW) and ($r = 0.56$, $p < 0.05$) ware yam weight (WYW).

Table 4.5 Correlation between quantitative parameters of *Dioscorea alata*

Variables	PSt	PHt	StN	StD	ILn	LfA	LAI	LSA	SUp	SMd	SLo	PtH	SYN	WYN	TYN	SYW	WYW	TYW	TDi	TLn
PSt	1.00																			
PHt	0.18	1.00																		
StN	0.38	0.13	1.00																	
StD	0.21	0.43	-0.25	1.00																
ILn	-0.01	0.40	-0.04	0.35	1.00															
LfA	-0.49	-0.33	-0.55	-0.16	0.16	1.00														
LAI	0.37	0.48	0.42	0.22	0.03	-0.75	1.00													
LSA	0.46	0.52	0.52	0.23	0.00	-0.88	0.82	1.00												
SUp	-0.23	-0.24	-0.30	-0.18	-0.31	0.37	-0.40	-0.42	1.00											
SMd	-0.13	-0.33	-0.43	0.01	-0.34	0.29	-0.42	-0.40	0.66	1.00										
SLo	-0.17	-0.18	-0.28	-0.04	-0.33	0.16	-0.18	-0.19	0.47	0.79	1.00									
PtH	0.92	0.05	0.34	0.17	-0.01	-0.29	0.20	0.29	-0.22	-0.14	-0.21	1.00								
SYN	0.39	0.28	0.31	0.16	-0.12	-0.47	0.44	0.39	-0.13	0.01	0.04	0.22	1.00							
WYN	-0.03	-0.06	-0.11	0.16	0.21	0.29	-0.06	-0.13	-0.03	-0.24	-0.34	0.09	-0.40	1.00						
TYN	0.43	0.28	0.29	0.22	-0.07	-0.41	0.45	0.38	-0.14	-0.04	-0.06	0.28	0.96	-0.14	1.00					
SYW	0.16	0.34	0.24	0.10	-0.13	-0.33	0.35	0.31	-0.02	0.09	0.14	0.03	0.90	-0.45	0.84	1.00				

WYW	-0.05	-0.09	-0.20	0.09	0.07	0.30	-0.12	-0.15	0.09	-0.07	-0.23	0.09	-0.47	0.88	-0.24	-0.48	1.00			
TYW	0.09	0.23	0.03	0.19	-0.05	-0.01	0.21	0.14	0.07	0.02	-0.11	0.12	0.37	0.46	0.54	0.46	0.56	1.00		
TDi	0.06	-0.02	-0.07	-0.01	0.00	0.04	0.09	-0.01	0.03	-0.06	-0.16	0.09	-0.16	0.25	-0.08	-0.08	0.41	0.34	1.00	
TLn	-0.24	-0.26	-0.38	-0.06	0.17	0.67	-0.50	-0.60	0.25	0.09	-0.08	-0.07	-0.57	0.67	-0.41	-0.57	0.68	0.15	0.12	1.00

Marked (red) correlations are significant at p < 0.05

4.6 Cluster analysis of *D. alata*

Cluster analysis was performed using the dendrogram of the hierarchical cluster analysis (HCA). The analysis separated 22 nutrient use-efficient *D. alata* genotypes into numerous clusters with Euclidean distance dissimilarities ranging from 0 to 16000 (Fig. 4.2). With linkage distance of 4200, the dendrogram identified 11 main clusters, named 1 to 11 in (Fig. 4.2 and Table 4.6). Besides, cluster 1 was subdivided at a linkage distance of 3750 into three sub clusters and these were; 1a, 1b and 1c (Table 4.6). The main Cluster 1 had eight genotypes and these were highly characterized in plant establishment (PtS) and plants harvested (PtH), but was found low in leaf area index (LAI) and plant height (PHt). Cluster 2 with three genotypes was characterized to have high number of stem (StN), leaf area (LA) and ware yam number (WYN), but was characterized low in plant height (PHt) and SPAD readings (SPAD). Clusters 3, 4, 6, 7 and 9 contained one genotype each, and were characterized low in plant establishment (PtS), leaf area (LA), plant harvested (PtH) and total yam weight (TYW). Cluster 5 with three genotypes was highly characterized in plant establishment (PtS) and seed yam number (SYN). Cluster 8 recorded one genotype and this was highly characterized in leaf area index (LAI) and ware yam number (WYN). Cluster 10 recorded 1 genotype and was characterized high in SPAD reading (SPAD), leaf surface area (LSA), tuber diameter (TbD) and tuber length (TLn). In contrast, it was characterized low in seed yam weight (SYW). The last cluster (11) had one genotype and was characterized high in plant height (PHt), stem diameter (StD), internode length (ILn), seed yam number (SYN), total yam number (TYN), seed yam weight (SYW) and total yam weight (TYW). It was however, characterized low in tuber length (TLn).

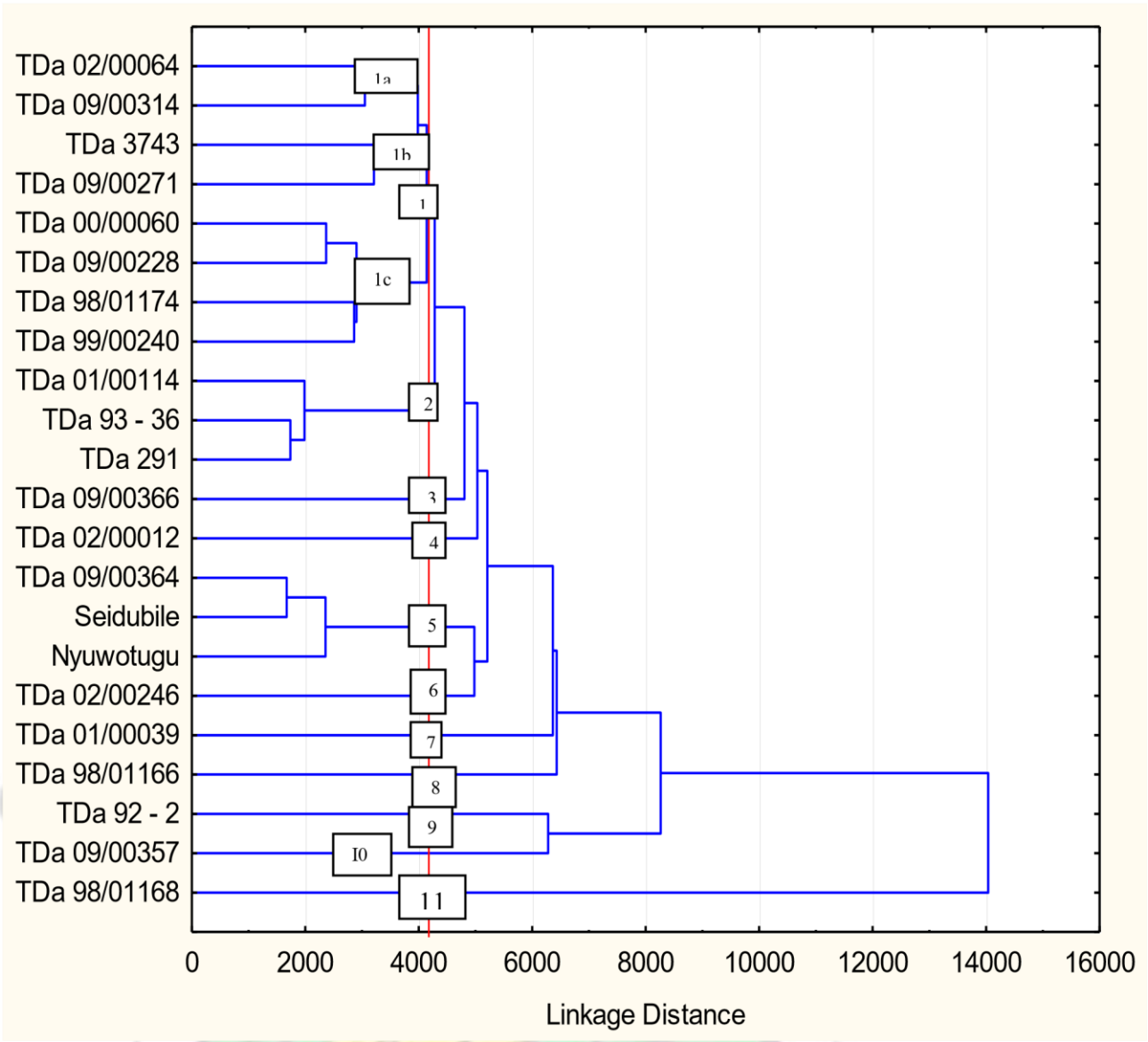

Figure 4.2. Dendrogram of 22 yam genotypes of *D. alata*, based on quantitative data and Hierarchical clustering analysis (HCA) using single linkage that were evaluated for nutrient use-efficiency

Table 4.6. Cluster, number and name of varieties of *D. alata* and their characteristics

Cluster	Genotype number	Genotype names	Characteristics
1a	2	TDa 02/00064 and TDa 09/00314	Plant establishment (PtS) and
1b	2	TDa 3743 and TDa 09/00271	Plant establishment (PtS) and Plant harvested (PtH),

1c	4	TDa 00/00060, TDa 09/00228, TDa 98/01174 and TDa 99/00240	Plant harvested (PtH),
2	3	TDa 01/00114, TDa 93 – 36 and TDa 291	number of the stem (StN), leaf area (LA) and ware yam number (WYNo),
3	1	TDa 09/00366	Leaf area (LA), plant harvested (PtH) and total yam weight (TYW).
4	1	TDa 02/00012	Leaf area (LA), plant harvested (PtH) and total yam weight (TYW).
5	3	TDa 09/00364, Seidubile and Nyuwotugu	Plant establishment (PtS) and seed yam number (SYN).
6	1	TDa 02/00246	Leaf area (LA), plant harvested (PtH) and total yam weight (TYW).
7	1	TDa 01/00039	Leaf area (LA), plant harvested (PtH) and total yam weight (TYW).
8	1	TDa 98/01166	Leaf area index (LAI) and ware yam number (WYN).
9	1	TDa 09/00357	Leaf area (LA), plant harvested (PtH) and total yam weight (TYW).
10	1	TDa 92 – 2	SPAD reading (SPAD), leaf surface area (LSA), tuber diameter (TbD) and tuber length (TLn).
11	1	TDa 98/01168	Seed yam number (SYN), total yam number (TYN), seed yam weight (SYW) and total yam weight (TYW).

4.7 Cluster analysis of *D. rotundata*

The analysis separated 23 *D. rotundata* nutrient used-efficient genotypes into numerous clusters with Euclidean distance dissimilarities ranging from 0 to 60000 (Figure 4.3). At linkage distance of 1750, the dendrogram identified four main clusters: Cluster 1, 2, 3, and 4. Clusters 1 recorded the highest number of genotypes nine as in (Figure 4.3 and Table 4.7). The varieties of this cluster are characterized by high plant height (PHt), leaf surface area (LSA) and plant harvested (PtH). However, they have the lowest tuber length (TLn),

ware yam weight (WYW) and ware yam number (WYN). Cluster 2 composed of five genotypes and are found to have high stem diameter (StD), ware yam

Weight (WYW), total yam weight (TYW), Tuber length (TLn) and tuber Diameter (TbD). In contrast, varieties of this cluster were also characterized by low seed yam weight (SYW), leaf area index (LAI), leaf area (LA) and stem number (StN). Cluster 3 had seven genotypes and these were characterized by stem number (StN), total yam number (TYN) and seed yam weight (SYW) with lowest characteristics found for plant harvested (PtH), seed yam number (SYN) and tuber diameter (TbD). The 4th cluster had the lowest number of genotypes two and was characterized by high leaf surface area (LSA), leaf area (LA), leaf area index (LAI), SPAD reading (SPAD) and seed yam number (SYN) but was found to have lower characteristics in plant height (PHt).

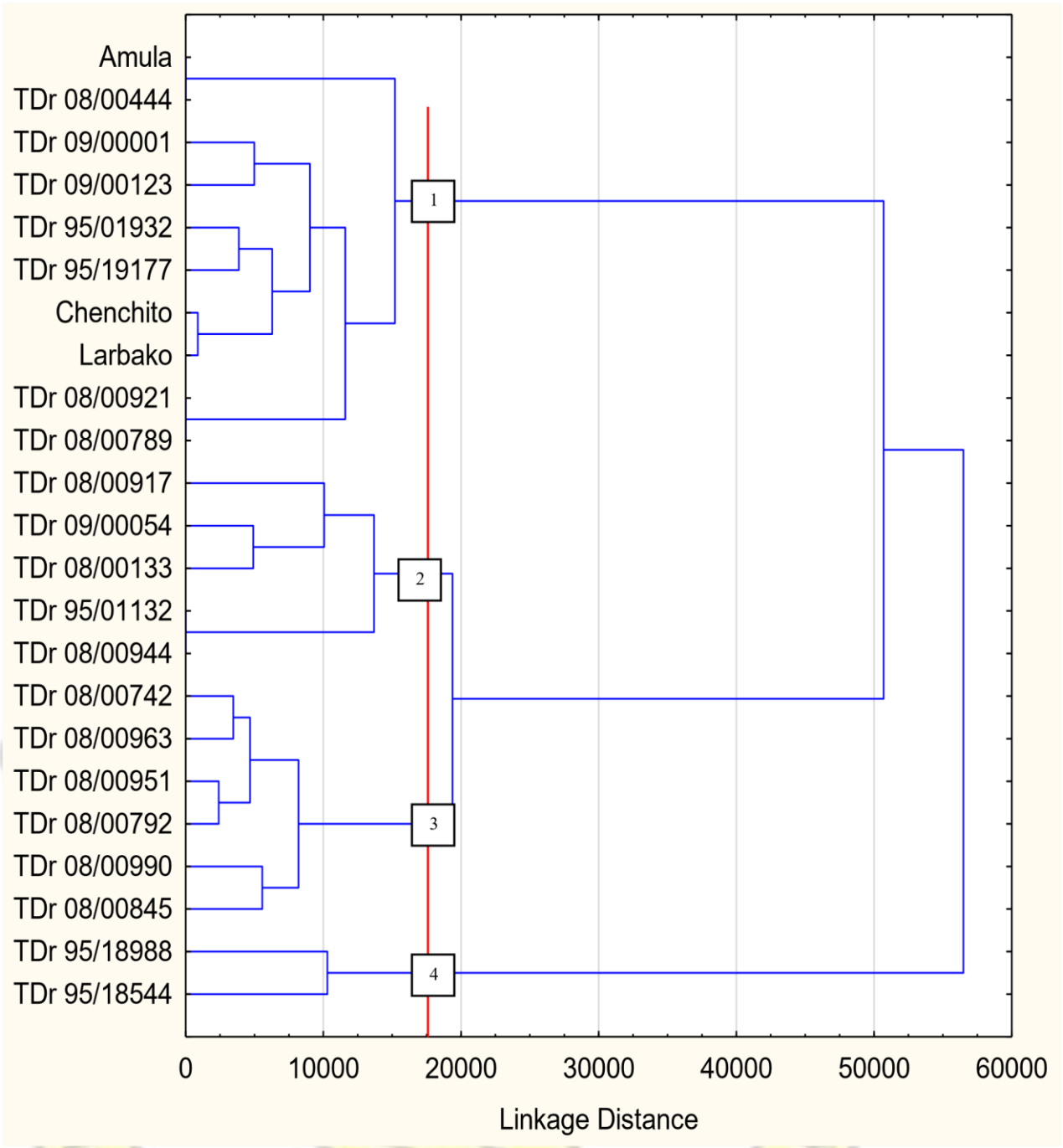

Figure 4.3. Dendrogram of 23 yam genotypes of *D. rotundata* based on quantitative data and Hierarchical clustering analysis (HCA) using single linkage that were evaluated for nutrient use-efficiency

Table 4.7. Cluster, number and name of accessions of *D. rotundata* and their characteristics

Cluster	Genotype number	Genotype names	Characteristics

1	9	Amula, TDr 08/00444, TDr 09/00001, TDr 09/00123, TDr 95/19177, TDr 95/01932, Chenchito, Larbako and TDr 08/00921.	Plant height (PHt), leaf surface area (LSA) and plant harvested (PtH)
2	5	TDr 08/00789, TDr 08/00917, TDr 09/00054, TDr 08/00133 and TDr 95/01132	Ware yam Weight (WYW), total yam weight (TYW), Tuber length (TLn) and tuber Diameter (TbD)
3	7	TDr 08/00742, TDr 08/00963, TDr 08/00951, TDr 08/00792, TDr 08/00990, TDr 08/00845 and TDr 08/00944	Stem number (StN), total yam number (TYN) and seed yam weight (SYW)
4	2	TDr 95/18544 and TDr 95/18988	Leaf surface area (LSA), leaf area (LA), leaf area index (LAI), SPAD reading (SPAD) and seed yam number (SYN)

4.8 Identification of nutrient use-efficient yam varieties using farmer participatory approach

4.8.1 Vegetative phase characterization of *D. alata* by farmers

Farmers, with their local knowledge and experience, described yam plants grown in low fertile soils with no stakes and having high potential for yield to have four distinct categories of characters. Vigorous sprout, good plant establishments, insect and disease free plants and plant growth. In fig. 4.4, farmers observed several associations between the variables and genotypes for characterization. According to the farmers, good plant establishment characteristics had high sprouting percent, high vigorous growth and with 1 to 2 stems per stand. Also, Good growth characters involved big basal stem, medium to large size of leaves, green to dark green leaves, medium to large plant canopies. Besides, healthy plants with no signs of viral, other mosaic diseases and any insect pest damage on the yam plant. Therefore, any genotype that had good and close association with these characters, had high chances of yielding high in low fertile soil, and therefore, could be classified as a nutrient use-efficient variety.

The genotypes TDa 08/01174 and TDa 01/00114 were highly associated with no insect infestation and presence of male flowers, but poorly associated with severe disease

symptoms and low vigour. There were genotypes that were highly associated with very good sprouting and dark green leaves such as TDa 09/00314 and TDa 09/00228. However, they were poorly associated with multi stem and small canopy size. TDa 9336 and TDa 92-2 were found to be closely associated with no thorns on leaves, medium leaf size and average sprouting, but poorly associated with small canopy size. TDa 09/00271, TDa 09/00357, TDa 98/01168 and TDa 09/00240 recorded high association with good sprout, large canopy size and narrow leaves size. In contrast, these genotypes were poorly associated with small leaf size. Genotypes TDa 09/00364, TDa 02/00064,

TDa 291 and TDa 3743 were highly associated with round and large leaves. TDa 00/00060, TDa 02/00248, Nyuwotugu and TDa 98/01166 were found to have recorded good association with single stem per stand, light green leaves, few insect infestations, but poorly associated with rough leaf surface and yellow green leaf colour. The genotypes TDa 02/00012, TDa 09/00366 and TDa 01/00039 were found to have recorded high association with more vigour, no disease, presence of flowers and medium canopy, but poorly associated with poor sprouting. Besides, more insect infestation and thorns on leaves were found to have no association with any genotype.

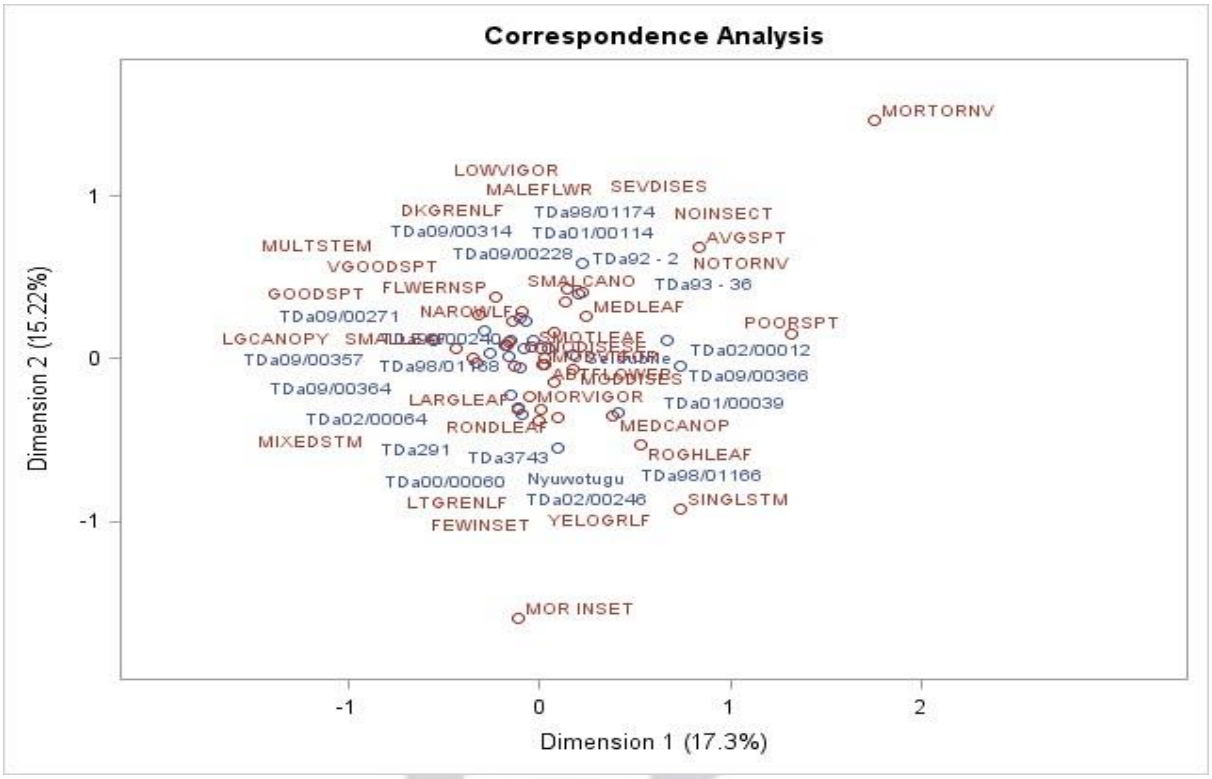

Figure 4.4. Correspondence analysis of vegetative Phase variables in association with *D. alata* genotypes

4.8.2 Tuber characterization of *D. alata* by farmer

Farmer's knowledge and experience for yam tuber characterization was categorized into four groups. Farmer selection for high yielding genotypes, genotypes for both ware and seed yam production: genotypes with big, long and smooth skin tubers were good for food, seed yam, socio-cultural purposes, gift and high marketability. Besides, genotypes with minimal or no infestation on of field and store insect pest and diseases and genotypes that can store for long without much losses.

Yam tubers from genotypes TDa 09/00271, TDa 92-2 and Nyuwotugu were highly associated with more seed yam, smooth surface and medium size tubers. Genotypes TDa 02/00246, TDa 93-36 and Seidubile were highly associated with medium yield and medium size tubers. Tubers from genotypes TDa 09/00314, TDa 01/00114 and TDa 00/00060 were found to have high association with no insect infestation. However, the genotypes were found to be poorly associated with rough tuber surface, very hairy and small

size tubers. Genotype TDa 02/00246 tubers was highly associated with no hair on the tuber skin. Tubers from genotypes TDa 02/00246, TDa 09/00228, TDa 93-36 and TDa 02/00064 were found to be highly associated with medium to high tuber yield and no insect and nematode infestation. Genotype TDa 09/00366 was found to be associated poorly with low yield, rough surface and small size tubers. TDa 37-43, TDa 98/01174, TDa 01/00039, TDa 02/00012, TDa 09/00364 and TDA 291 tubers were highly associated with few hairs, more ware yam, long tubers, big tuber size and no rotten tubers. Genotype TDa 98/01168 was found to have hairy and thorny tuber surface. TDa 99/00240 and TDa 98/01168 were equally described to have associated with equal proportion of seed and ware yam. No genotype was associated with, branched tubers and few to more rotten tubers.

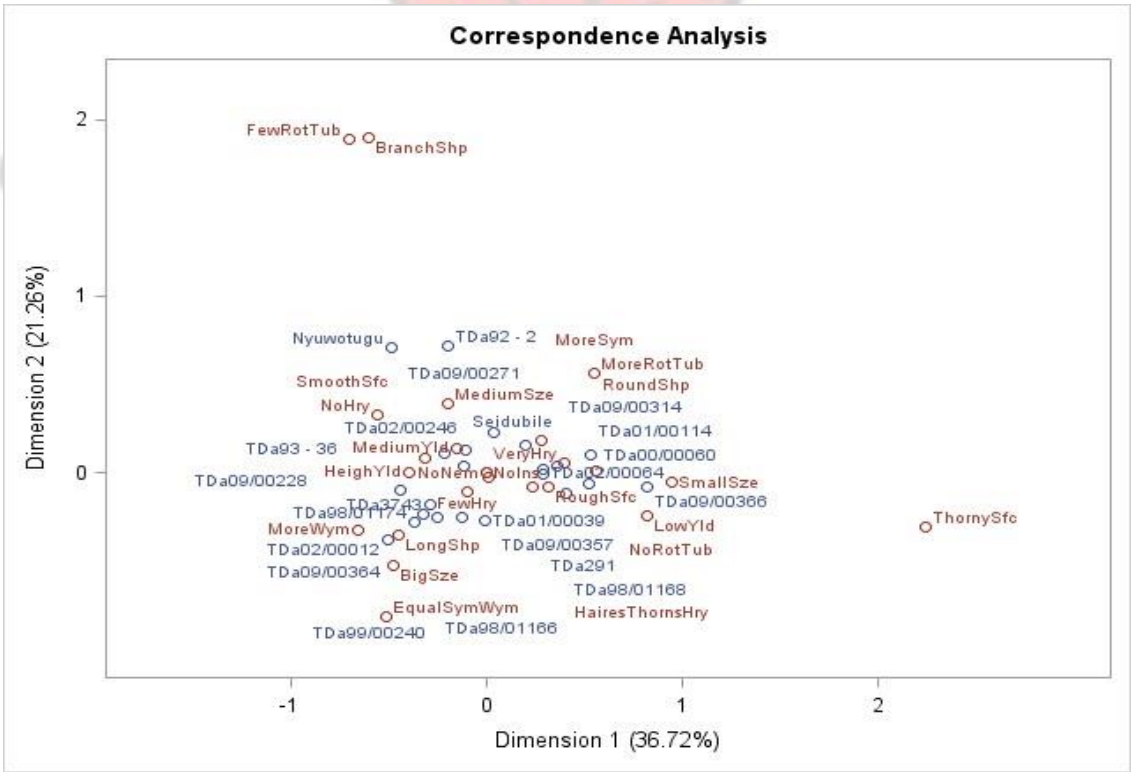

Figure 4.5. *D. alata* tuber variables in association with farmer descriptive parameters

Table 4.8. Farmer preference ranking of *D. alata* across the three districts

District	Community	Genotype	Men Elders	Women Elders	Men Youth	Women Youth	Sum total	Rank
Tolon	Cheyohi	Nyuwotugu	10	7	8	7	32	1st
Tolon	Cheyohi	TDa 09/00364	9	8	6	7	30	2nd
Tolon	Cheyohi	TDa 3743	8	6	7	6	27	3rd
Tolon	Cheyohi	TDa 98/01168	7	8	6	5	26	4th

Tolon	Cheyohi	Seidubile	6	6	5	4	21	5th
East Gonja	Kpalbe	TDa 09/00228	9	8	9	8	34	1st
East Gonja	Kpalbe	TDa 02/00012	8	9	8	8	31	2nd
East Gonja	Kpalbe	TDa 98/01174	9	7	6	7	29	3rd
East Gonja	Kpalbe	Nyuwotugu	7	6	4	3	20	4th
East Gonja	Kpalbe	Seidubile	4	3	6	2	15	5th
Mion	D.C Kurah	TDa 09/00364	7	7	7	8	29	1st
Mion	D.C Kurah	TDa 02/00064	7	8	6	7	28	2nd
Mion	D.C Kurah	TDa 98/01174	7	5	6	6	24	3rd
Mion	D.C Kurah	TDa 02/00012	6	7	5	4	22	4th
Mion	D.C Kurah	TDa 09/00228	5	4	5	4	18	5th

4.8.3 Farmer preference ranking of *D. alata* across the three districts

The results of farmers' preference and selection of the best five genotypes of *D. alata* as in Table 4.8 revealed that, almost the same variety was preferred and selected in each district. These genotypes included: TDa 98/01168, TDa 09/00228, TDa 02/00012, TDa 98/01174 and Seidubile were selected as high yielding NUE varieties. Farmers' selection was based on genotypes that were high yielding in seed and ware yam and mainly for food and income security. Genotypes that satisfied these objectives were big/long ware yam, smooth with few to no hairy tuber surface, no insect pest and disease infestation, long storage properties with high proportion of ware and seed yam production.

4.8.4. Preference criteria used by farmers for selection of *D. alata* tubers

At the vegetative phase, farmer preference on selection of genotypes was based on high sprouting percent, high vigour, one to two numbers of stems, medium to large leaves, medium to large canopies spread evenly on the mound, smooth leaves with no infestation of insect pest and disease. According to farmers, genotypes with these characters have a good potential to give high yield.

Table 4.9. Breeder and farmers' selection of *D. alata* across the three districts

S/N	*D. alata* genotypes	Mean yield t/ha	Percent ware yam	Percent seed yam	Farmer selection of
1	TDa 98/01168	11.1	8	92	TDa 98/01168
2	TDa 09/00228	10.7	53	47	TDa 09/00228
3	TDa 02/00012	10.2	71	29	TDa 02/00012
4	TDa 98/01174	10	62	38	TDa 98/01174
5	TDa 92-2	9.8	35	65	Not selected
6	Seidubile	8.6	77	33	Seidubile

4.8.5 Breeder and farmers' selection for *D. alata* genotypes

Mean yield obtained from statistical analysis was used by the breeder as a criterion to select the best six *D. alata* genotypes. Mean yield per hectare (Appendix 8) varied from 8.6 t/ha in Seidubile to 11.1 t/ha in TDa 98/01168. Both descriptive and multiple component analysis revealed; Leaf area, Leaf surface area and high chlorophyll content at the lower to the middle leaves. Besides, correlation analysis performed revealed that, total yam weight was highly and positively correlated (r= with the number and weight of seed yam, ware yam and total yam. It is important to note that, the farmer and the breeder criteria were almost the same (Table 4.9) hence; the genotypes chosen by the breeder were the same as those chosen by the farmer, confirming both evaluations to be the same in determining nutrient use-efficient genotypes in *D. alata*. However, TDa 92 – 2 was high yielding, but was not chosen by the farmers, because of thorny and hairy tuber surface.

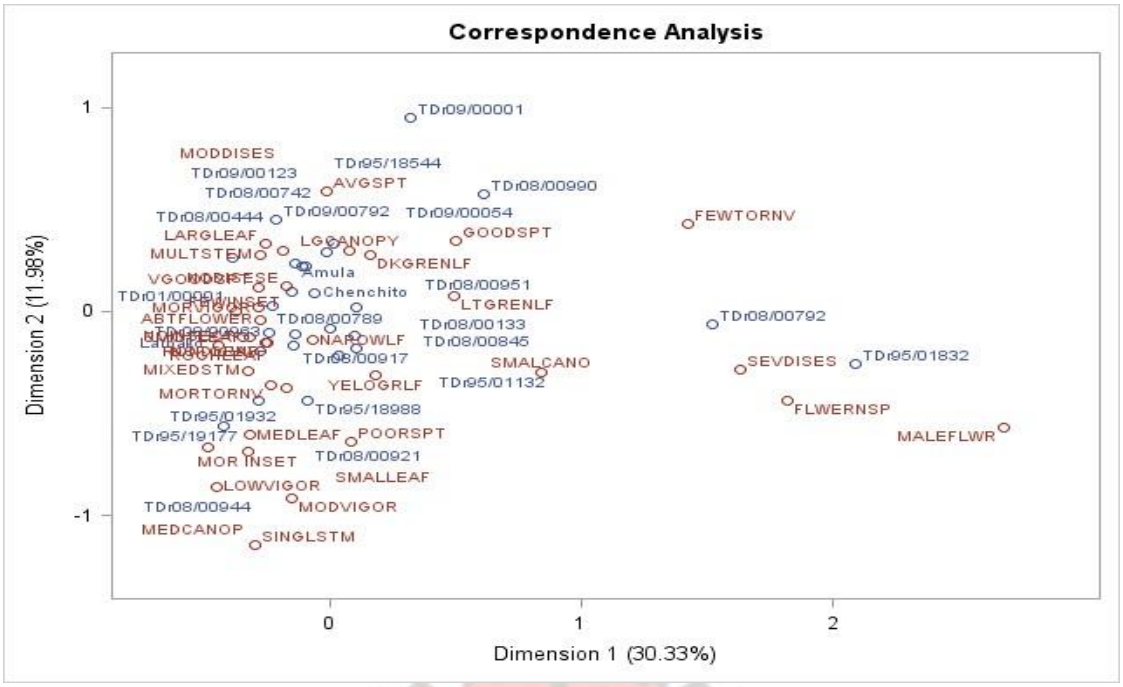

Figure 4.6. *D. rotundata* vegetative phase characterization by farmers

4.8.6 Vegetative phase characterization of *D. rotundata* by farmers

Farmers with their local knowledge and experience described *D. rotundata* species plants grown in low fertile soils with no stakes and possessing high potentials for yield to have 4 distinct categories of characters as in (Fig. 4.6). Vigorous sprout, good plant establishments, Insect and disease free plants and high plant growth. In fig. 4.4. Farmers observed several associations between the variables and genotypes for characterization. According to the farmers, good plant establishment characteristics have high sprouting percent, high vigorous growth and with 1 to 2 stems per stand. Also, Good growth characters involved big basal stem, medium to large size of leaves, green to dark green leaves, medium to large plant canopies. Besides, clean and healthy plants have no signs of viral, other mosaic diseases and any insect pest damage on the yam plant. Therefore, any genotype that has good and close association with these characters, have high chances of yielding high, and therefore be selected by farmers as a nutrient use-efficient variety. Genotypes TDa 09/00123, TDa 95/18544 and TDa 09/00742 were highly associated with average sprouting but with low association in more disease incidence. Genotypes TDa

08/00444, TDA 09/00792, TDa 09/00054 and Amula recorded highly associated with good sprouting, large leaves, large canopies, dark green leaves. There was no low association with any character. High association was found with TDa 08/00963, Larbako, Chenchito, TDa 08/00789, TDa 08/00951 and TDa 08/00133 to very good sprouting, more vigorous growth and large green leaves. However, there were low associated with narrow and rough leaves with mixed stems. TDr 95/001932, TDr 95/18988, TDr 95/01132 and TDr 95/00917 were found to be associated with yellow green leaves, medium leaf size and small canopy size. In contrast, it was found to have low association with more insect infestation and thorny stems. Genotype TDr 08/00921 and TDr 08/00944 presented high association with more vigorous growth, single stem and medium canopy. Yet it was also found to have low association with poor sprouting, low vigour, small leaf size and more insect damage. Genotype TDr 95/01832 and TDr 08/00792 were associated with severe disease and female flower. Finally, the genotype TDr 09/00001 was not found to be in association with any farmer criteria. Also the variable with few thorny stems were found not in association with any genotype.

4.8.7 Tuber characterization of *D. rotundata* by farmer

Farmer knowledge and experience for yam tuber characterization were categorized into four groups. Farmer selection for high yielding genotypes for both ware and seed yam production, genotypes with big, long and smooth skin tubers were good for food, seed yam, socio-cultural purposes, gift and high marketability. Besides, Genotypes with minimal or no infection of field and storage insect pest and diseases and genotypes that could store for long without much losses. As in (Figure 4.7), TDr 95/00054 genotype was found to have low yield with small size tubers. The genotypes TDr 08/00963, TDr 08/00944, TDr 09/00001, TDr 95/18544, TDr 08/00952 and TDr 08/00990 were observed to have high association with rough surface but no rot on tubers. Further records was found with TDr 95/01132 and TDr 08/00789 to be associated with no insect infestation but with

very hairy tuber surface. TDr 08/00917, TDr 08/00845, Amula, TDr 09/00792 and TDr 95/01932 genotypes were highly associated with high yield, big tubers, few hairy tuber surfaces and few insect. No low association was found with these genotypes. Records show that, TDr 09/00123 genotypes were associated with more ware yam and long tubers. TDr 95/19177 and TDr 08/00133 were not associated with nematode but had high insect infestation. The genotype TDr 95/18988 gave a medium yield with equal seed and ware yam produced. TDr 08/00444 was associated with medium size tubers with branched shape. TDr 08/00921, TDr 01/00001 and Chenchito were found to have no hairs with smooth surface. TDr 08/00944 and TDr 08/00963 and Larbako were found isolated with no association with any descriptive parameter. Similarly, tubers that were round with hairy and thorny surfaces were found isolated with no association with any genotype.

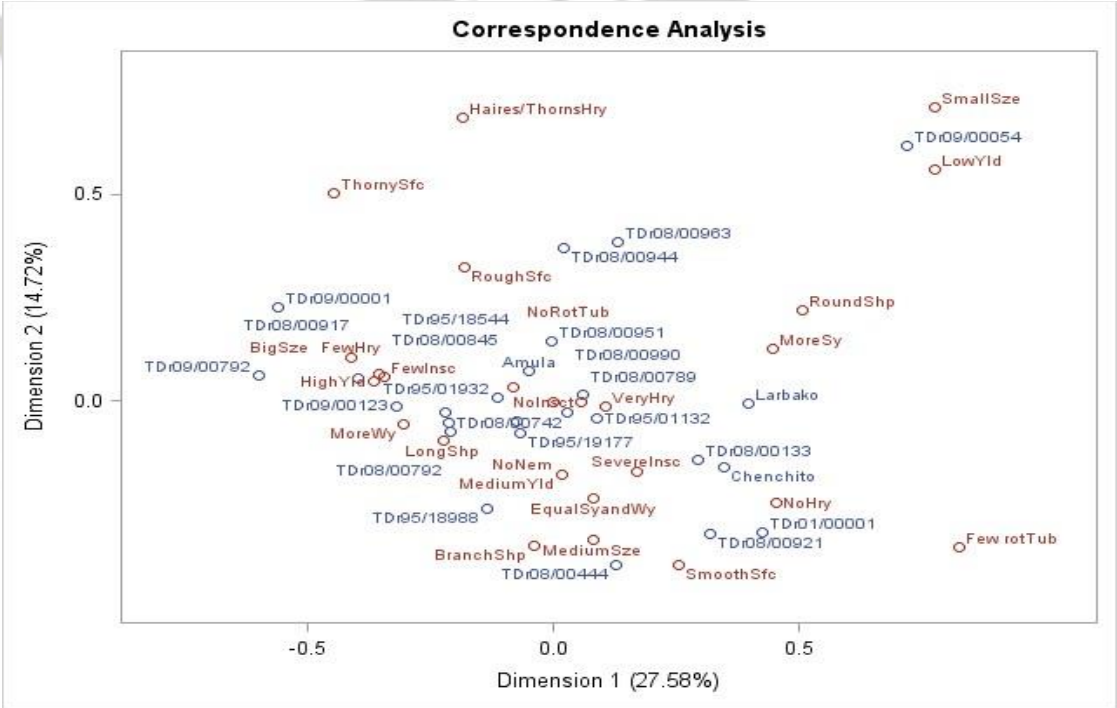

Figure 4.7. Farmer description of *D. rotundata* nutrient use-efficient genotype tubers with its associated variables

4.8.8. Farmer preference ranking of *D. rotundata* across the three districts

The results of farmer preference and selection of the best five genotypes of *D. rotundata* as in Table 4.10 revealed that, almost the same variety was preferred and selected in each district. These genotypes included: *D. rotundata* genotypes: TDr 95/19177, TDr 09/00001, TDr 09/18988, TDr 00/00951 and Larbako were selected as high yielding NUE genotypes. Farmers' selection was based on genotypes that were high yielding in seed and ware yam and mainly for food and income security. Genotypes that satisfied these objectives were big/long ware yam, smooth with few to no hairy tuber surface, no insect pest and disease infestation, long storage properties with high proportion of ware and seed yam production.

Table 4.10. Farmer preference ranking of *D. rotundata* across the three districts

S/N	District	Community	Genotype	Men Elders	Women Elders	Men Youth	Women Youth	Sum total	Rank
1	Tolon	Tingoli	TDr 09/00001	8	7	6	7	28	1st
	Tolon	Tingoli	TDr 95/19177	7	5	8	5	25	2nd
	Tolon	Tingoli	TDr 00/00951	8	4	5	4	21	3rd
	Tolon	Tingoli	Larbako	5	6	4	4	19	4th
	Tolon	Tingoli	TDr 95/18988	6	4	3	3	16	5th
2	East Gonja	Masaka	TDr 95/18988	8	8	7	6	29	1st
	East Gonja	Masaka	TDr 00/0951	7	7	6	7	27	2nd
	East Gonja	Masaka	TDr 09/00001	6	6	7	6	25	3rd
	East Gonja	Masaka	TDr 95/19177	6	6	6	6	24	4th
	East Gonja	Masaka	Larbako	6	4	5	4	19	5th
3	Mion	Puriya	TDr 09/00001	7	7	8	7	29	1st
	Mion	Puriya	Larbako	8	5	7	5	25	2nd
	Mion	Puriya	TDr 00/00951	7	4	6	4	21	3rd
	Mion	Puriya	TDr 95/19177	6	5	5	4	20	4th
	Mion	Puriya	TDr 95/18988	6	5	4	3	18	5th

4.8.9 Preference criteria used by farmers for selection of *D. rotundata* nutrient useefficient genotype tubers

At the vegetative phase, farmer selected genotypes based on high sprouting percent, high vigour, 1 to 2 stems, medium to large leaves, medium to large canopies spread evenly on the mound, smooth to slightly rough leaves with no infection of insect pest and disease. According to farmers, genotypes with these characters have potential to give high yield.

Table 4.11. Breeder and farmers selection of *D. rotundata* nutrient use-efficient genotypes across the 3 districts

D. rotundata genotypes	Mean yield t/ha	Percent ware yam	Percent seed yam	Farmer selection
TDr 09/00001	12.1	98	2	TDr 09/00001
TDr 95/18988	10.9	40	60	TDr 95/18988
TDr 00/00951	11.3	20	80	TDr 00/00951
TDr 95/19177	10.1	97	3	TDr 95/19177
Larbako	9.7	79	21	Larbako

4.8.10. Breeder and farmer selection criteria for *D. rotundata* tubers

Mean yield obtained from statistical analysis was used by the breeder as a criterion to select the best five *D. rotundata* nutrient use-efficient genotypes. Mean yield per hectare (Appendix 7) varied from 9.7 t/ha in Larbako to 12.1 t/ha in TDr 09/00001. Both descriptive and multiple component analysis revealed; Leaf Area, Leaf surface area, high chlorophyll content at the lower to the middle leaves. Besides, correlation analysis performed revealed that, Yield (total yam weight – kg/ha was highly and positively correlated with the number and weight of seed yam, ware yam and total yam. It is important to note that, the farmer and the breeder criteria were almost the same hence; the

genotypes chosen by the breeder were the same as those chosen by the farmer, confirming both evaluations to be the same (Table 4.11) in determining nutrient useefficient genotypes in *D. rotundata*. It is important to note that, the farmer and the breeder criteria were nearly the same hence; genotypes chosen by the breeder as high yielding were the same chosen by the farmer confirming both evaluations to be the same in determining nutrient use-efficient genotypes in *D. rotundata*.

4.9 Molecular characterization

4.9.1 Genetic diversity study of *D. rotundata*

A total of 30 primers were screened to assess the genetic diversity of *D. rotundata* yam genotypes from IITA and northern Ghana. Out of these, seven genomic SSR primers which produced clear and reproducible bands were selected for amplification of 20 *D. rotundata* yam genotypes DNA and PCR samples. A total number of 27 different alleles were amplified with the seven SSR loci that were used to analyze the 20 yam genotypes. An average of 3.85 alleles was observed per locus and varied from one to seven alleles (Table 4.12). The primer SSR 53 recorded the highest number of alleles followed by SSR33 and SSR36 recording the same number. However, the lowest number of alleles was found in primers SSR74, SSR32 and SSR66 (Table 4.12). The mean allele frequency was 0.57 and varied from 0.35 to 1.00. Lower allele frequency was found in SSR33 and the highest in SSR74. Gene diversity recorded an average value of 0.53 and vary from 0.00 (SSR74) to 0.75 (SSR33). Further observation showed that, average Polymorphic information content (PIC) was 0.49 and ranged from 0.00 detected in SSR74 to 0.70 detected in SSR33.

Table 4.12. Detection of Polymorphism base on SSR primers, together with Major Allele Frequency (A), Sample Size (number), Allele number (A), Gene Diversity and polymorphism information content (PIC) during the assessment of *20 D. rotundata* genotypes.

Marker	Major Allele Frequency	Sample Size	Number of observations.	Allele number	Availability	Gene Diversity	PIC
SSR 36	0.45	20	20	5	1	0.7	0.655
SSR 32	0.85	20	20	2	1	0.255	0.222
SSR 53	0.45	20	20	7	1	0.725	0.692
SSR 33	0.35	20	20	5	1	0.75	0.708
SSR 75	0.45	20	20	4	1	0.67	0.609
SSR 74	1	20	20	1	1	0	0
SSR 66	0.45	20	20	3	1	0.635	0.559
Mean	**0.57**	**20**	**20**	**3.85**	**1**	**0.53**	**0.492**

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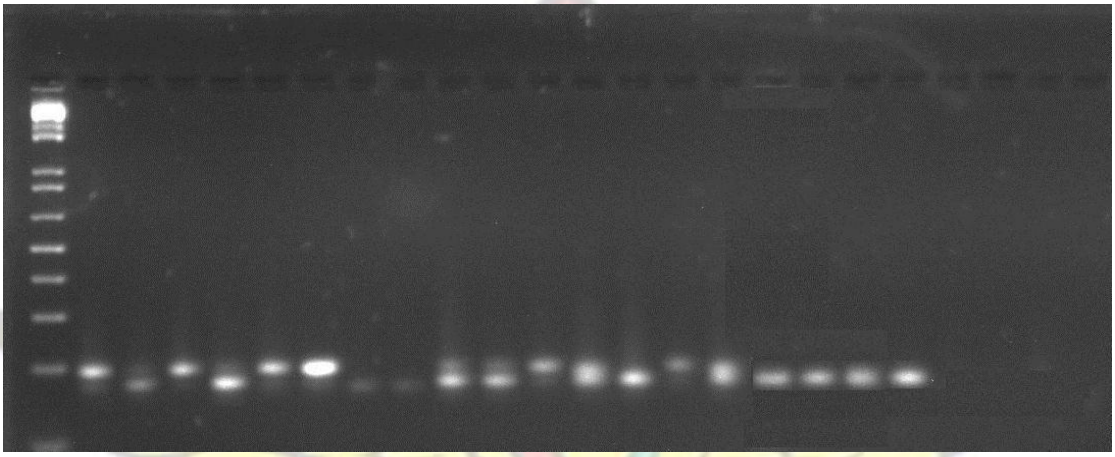

Fig. 4.8. A representation of a gel electrophoregram of *D. rotundata* using SSR marker (SSR 36)

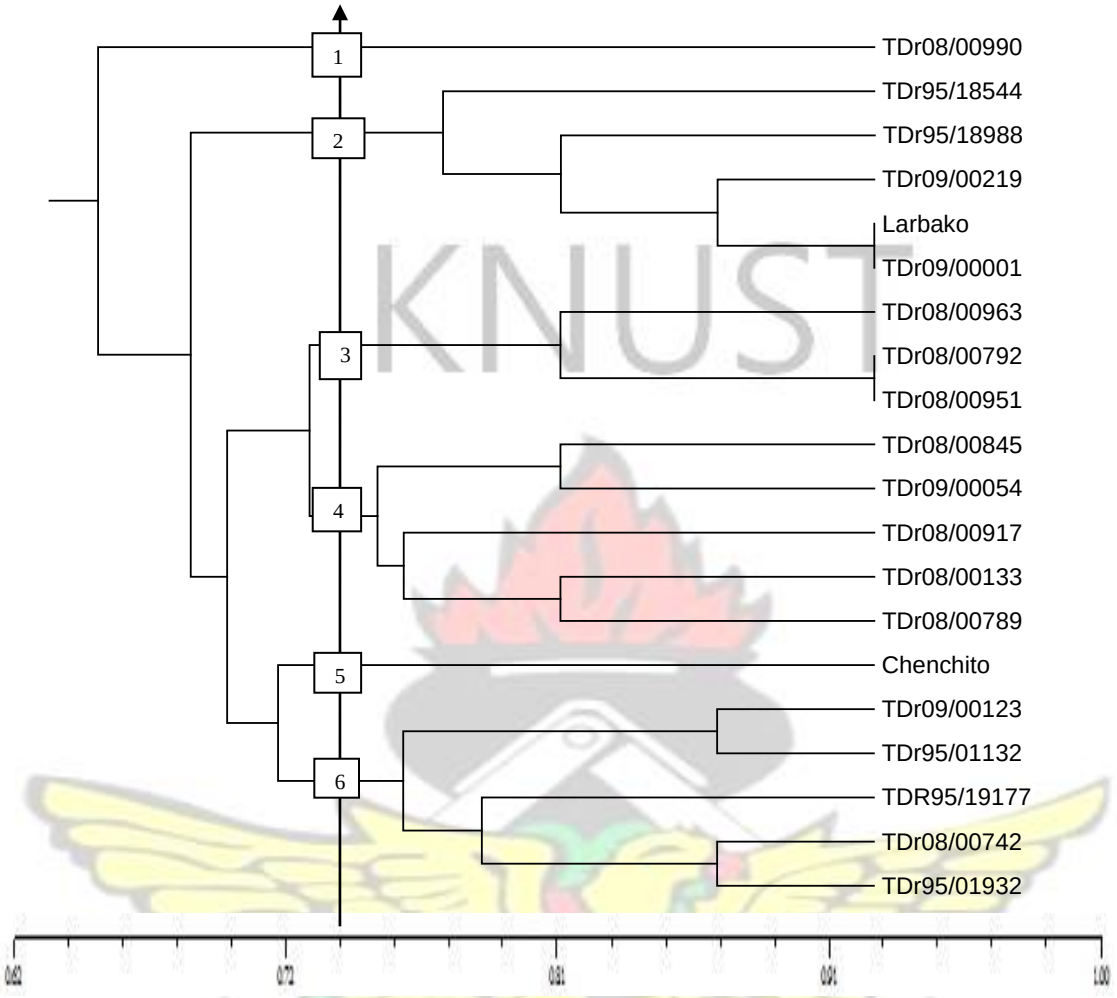

Figure 4.9 A dendrogram showing the genetic relationship among *20 D. rotundata* genotypes using the *UPGMA* (Unweighted Pair Group Method with an Arithmetic Mean) method

4.9.2 Cluster analysis of *D. rotundata*

Bands were score as in (Fig. 4.8) and special codes were used for missing data (Appendix 2). Cluster analysis was performed using the Unweighted Pair Group Method with an Arithmetic Mean (UPGMA) cluster analysis. The analysis separated 20 *D, rotundata* genotypes into numerous clusters using dissimilarity coefficient distance (Fig. 4.9). With dissimilarity coefficient distance of 0.25 (25%), the dendrogram identified six main clusters: Cluster 1, 2, 3, 4, 5 and 6. Clusters 2, 4 and 6 recorded the highest number of genotypes (5) each (Fig. 4.9 and Table 4.13), followed by cluster 3 with three accessions.

Clusters 1 and 5 had one accession each as the lowest. The clusters, variety number as well as the variety names obtained in the agro morphological evaluation were found to be different from the molecular evaluation. Cluster 2 genotypes were found to be high yielding except TDr 09/00921, and were chosen by the breeder and farmers. Clusters 1, 3 4 and 5 genotypes were found to have secondary genome, hence were described to be genetically related with low tuber yield. Cluster 6 genotypes had moderate to high yielding genotypes. Larbako and TDr 09/00001 in cluster 2 and TDr 08/00951 and TDr 08/00792 in cluster 3 were found to have secondary genome.

Table 4.13 Cluster, number, name and characteristics of *D. rotundata* accessions

Cluster number	Variety number	Variety names	Characteristics
1	1	TDr 08/00990	Low tuber yield
2	5	TDr 95/18544, TDr 09/00921, TDr 95/18988, Larbako and TDr 09/00001	High tuber yield (except TDr 09/00921) and genetical related
3	3	TDr 08/00963, TDr 08/00951 and TDr 08/00792	Genetically related and low tuber yield,
4	5	TDr 08/00854 and TDr 09/00054, TDr 08/00917 TDr 08/00133 and TDr 08/00789	Genetically related with low tuber yield
5	1	Chenchito	Moderate tuber yield
6	5	TDr 09/00123, TDr 95/01132, TDr 95/19177 , TDr 08/00742 and TDr 95/01932	Genetically related with moderate to high tuber yield

4.9.3 Genetic diversity study – *D. alata*

A total of twenty (20) primers were screened to assess the genetic diversity of *D. alata* yam genotypes from IITA and Northern Ghana. Out of these, seven genomic SSR primers which produced clear and reproducible bands were selected for amplification of 22 *D. alata* genotypes DNA and PCR samples. A total number of 23 different alleles were amplified with the seven SSR loci that were used to analyze 22 yam genotypes. An average of 3.28 numbers of alleles was observed per locus and varied from one to nine alleles (Table 4.14). The primers SSR75 recorded the highest number of alleles follow by SSR36

and SSR66. Nonetheless, the lowest number of alleles was found equally in primers SSR32 and SSR53 on one hand and SSR33 and SSR74 on the other (Table 4.14). The mean allele frequency was 0.64 and varied from 0.18 to 1.00. Lower allele frequency was found in SSR75 and the highest equally in SSR32 and SSR53. Gene diversity recorded an average value of 0.42 and varied equally from 0.00 (SSR32) and 0.00 (SSR53) to 0.87 (SSR75). Further observation showed that, average Polymorphic information content (PIC) was 0.37 and ranged equally from 0.00 detected in SSR32 and SSR53 to 0.85 detected in SSR75.

Table 4.14. Detection of Polymorphism base on SSR primers, together with Major Allele Frequency (A), Sample Size (number), Allele number (A), Gene Diversity and polymorphism information content (PIC) during the assessment of *22 D. alata* genotypes.

Marker	Major Allele Frequency	Sample Size	No. of obs.	Allele Number	Availability	Gene Diversity	PIC
SSR36	0.409	22	22	5	1	0.714	0.668
SSR 32	1	22	22	1	1	0	0
SSR 66	0.727	22	22	3	1	0.429	0.385
SSR 53	1	22	22	1	1	0	0
SSR 75	0.181	22	22	9	1	0.871	0.858
SSR 74	0.681	22	22	2	1	0.433	0.339
SSR 33	0.5	22	22	2	1	0.5	0.375
Mean	**0.642**	**22**	**22**	**3.285**	**1**	**0.421**	**0.375**

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

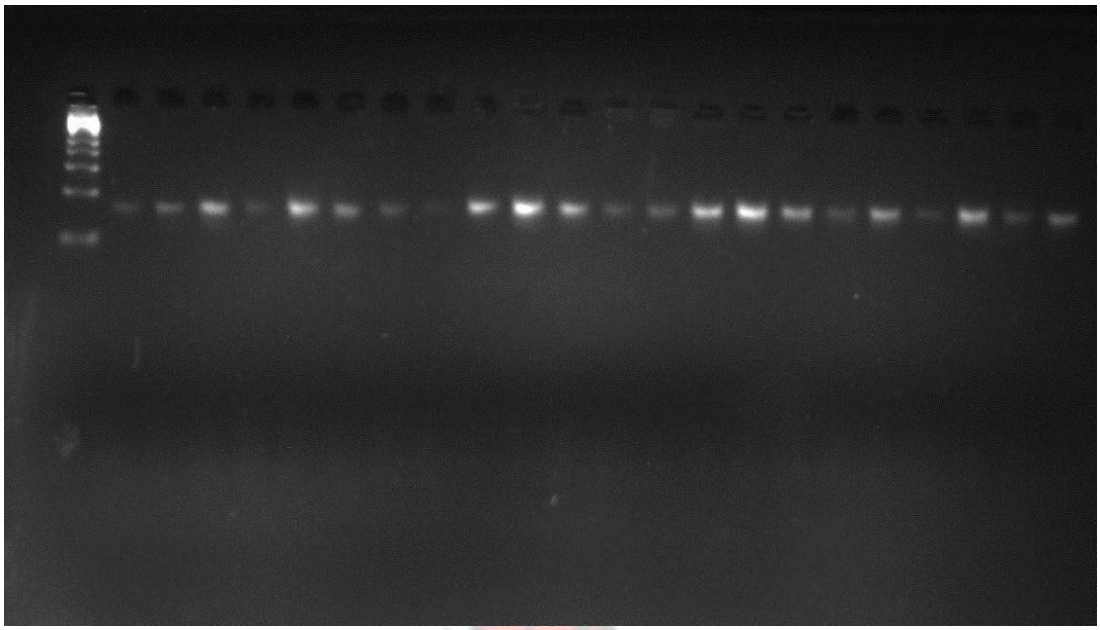

Figure 4.10 A representation of a gel electrophoregram picture of *D. alata* using SSR marker (SSR 36)

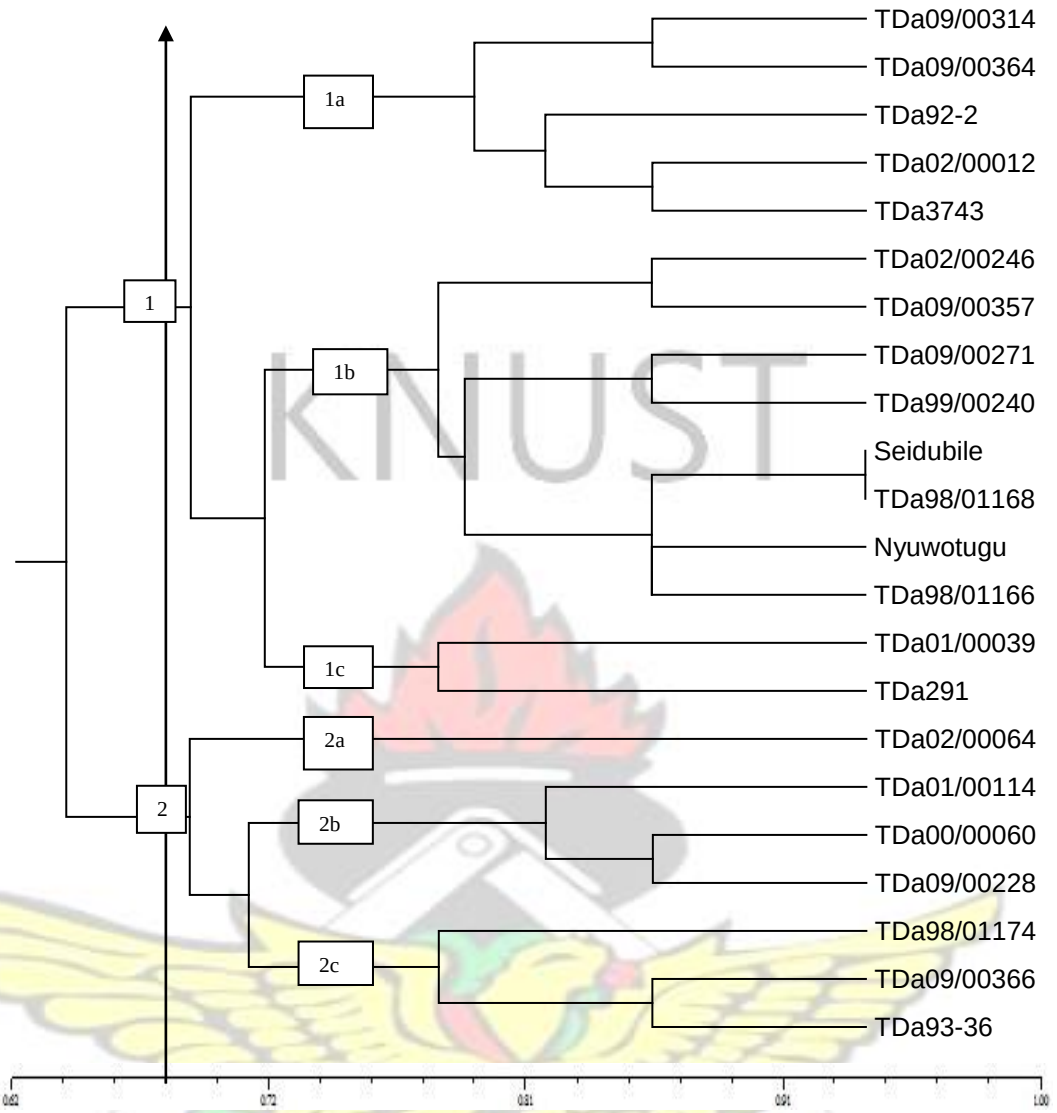

Figure 4.11. A dendrogram showing the genetic relationship among 22 *D. alata* genotypes using the *UPGMA* (Unweighted Pair Group Method with an Arithmetic Mean) method

4.9.4 Cluster analysis of *D. alata* genotypes

Bands were scored as in (Fig. 4.10) and special codes were used for missing data (Appendix 3). Cluster analysis was performed using the Unweighted Pair Group Method with an Arithmetic Mean (UPGMA) cluster analysis (Figure 4.9). The analysis separated 22 *Dioscorea alata* genotypes into numerous clusters with Euclidean distance dissimilarities. With dissimilarity coefficient distance of 0.175

or (17.5%), the dendrogram identified 2 main clusters, namely 1 and 2, with sub classes 1a, 1b and 1c and 2a, 2b and 2c at dissimilarity coefficient distance of 0.225, (Figure 4.10 and Table 4.15). Sub cluster 1b recorded the highest number (8) of genotypes, following sub class 1a with (5) genotypes. Sub class 2b and 2c recorded (3) genotypes each with sub class 1c found with 2 genotypes. The lowest genotype (1) was found with sub class 2a. Cluster 1b had 2 accessions (Seidubile and TDa 98/01168) clustered to have secondary genome. In addition, this cluster has majority of the highest yielding genotypes which was selected by both breeder and the farmer.

Table 4.15 Cluster, number, name and characteristics of *D. alata* accessions

Cluster	Sub cluster	Variety number	Variety name	Characteristics
1	1a	5	TDa 09/00314 and TDa 09/00364, TDa 92 - 2, TDa 02/00012 and TDa 3743	Average tuber number and weight.
	1b	8	TDa 02/00246, TDa 09/00357, TDa 09/00271, TDa 99/00240, Seidubile, TDa 98/01168, Nyuwotugu and TDa 98/01166	High number and weight of ware and seed yam.
	1c	2	TDa 01/00039, TDa 291	Low number and weight of ware and seed yam
2	2a	1	TDa 02/00064	Low number and weight of ware and seed yam
	2b	3	TDa 01/00114, TDa, 00/00060 and TDa 09/00228	Moderate to high number and weight of ware and seed yam
	2c	3	TDa 98/01174, TDa 09/00366 and TDa 93 – 36	High ware and seed yam number and weight

CHAPTER FIVE

5.0 DISCUSSION

The description of the soil status in the study area revealed that, the soils are made up of sandy and silty soil with very little clay fraction. Hence, the ability for water

and nutrient holding capacity is low, hence low soil fertility resulting to low yield obtained by yam farmers. Yam is grown on mounds made by heaping the top soil which is largely sandy and deficient in major nutrients. Its water-holding capacity is also low and therefore, requires varieties that can efficiently use the low nutrient levels to achieve economic yield. Therefore, the use of nutrient use-efficient yam genotypes in low fertility soil.

5.1 Characterization using phenotypic traits

Characterization using phenotypic traits showed clearly that, the variability of each trait studied was well identified. Both *D. alata* and *D. rotundata* were evaluated on the same traits, but varied among and between different species. The descriptive and the multiple component analysis grouped the 20 quantitative variables into several components in which the first six axis explained 82.70% in *D. alata* and 82.42% in *D. rotundata* of the total variability obtained. As recommended by the Statistica version (12.0) software, variables with values 50% and above plus (+) or minus (-) have association with these six axis and therefore, significant (Agre *et al*., 2015). This was also found by Agre (2015) in his study in agromophological characterization of elite cassava (*Manihot esculenta*) cultivars collected in Benin.

In all, six quantitative traits (Leaf area, leaf surface area, high chlorophyll content of lower to middle leaves, as well as number and weight of seed yam, ware yam and total yam) were found to be significant. Such traits with highest variability were therefore considered as very significant traits to be used to characterize nutrient use-efficient genotypes. The variation in morphological traits within and between these genotypes may be due to sexual recombination and possibly mutation (Norman *et al*., 2011). Other studies revealed that, this morphological variability is the challenge resulting from evolutionary dynamics of guinea yams (*D. cayenensis – D. rotundata*

complex) found during domestication of yams (*D. cayenensis* - *D. rotundata*) within the Bariba ethnic group in Benin (Mignouna and Dansi, 2003).

Correlation matrix conducted revealed that, total yam number correlated positively ($r = 0.45$, $p < 0.05$) with leaf area and ($r = 0.38$, $p < 0.05$) leaf surface area. Besides, total yam weight (yield in kg/ha) correlated positively ($r = 0.37$, $p< 0.05$) with seed yam number, ($r = 0.46$, $p < 0.05$), ware yam number, ($r = 0.54$, $p < 0.05$), total yam number, ($r = 0.46$, $p < 0.05$), seed yam weight ($r = 0.56$, $p < 0.05$) and ware yam weight. Positive correlation with yam yield (total yam number and total yam weight) therefore, means that, when leaf area and leaf surface area as well as seed yam and ware yam number and weight increases, there will be a correspondence increase in yam yield (Agre *et al*., 2015). This also confirmed the same traits as in descriptive and multiple component analysis.

5.2 Farmer characterization for high yielding NUE genotypes

The farmer qualitative traits resulting from vegetative and tuber characterization revealed that, genotypes and variables co exit when a Biplot was conducted through correspondence analysis. The study revealed that, genotypes and variables that lies at the center of the graph shows high stability and persistence performance during its cultivation period.

The six important traits used by the farmer as very impotant include (medium to large leaves, green to dark green leaves, medium to large canopies spread evenly on the mound. According to farmers, genotypes with these characters had a high potential to give high yield.

At the tuber characterization phase, farmers selected yam tubers that were high yielding for food and income security. Therefore, for genotypes to be high yielding for food and income security, its tuber characters must include, high yielding, big

and long tubers, smooth with few to no hairy tuber surface, no insect pest damage and disease infection, long shelf life with high proportion of ware and seed yam production.

Farmers also ranked the genotypes yam tubers according to the best five preferred genotypes across the three districts of the study area. *D. alata* genotypes such as TDa 98/01168, TDa 09/00228, TDa 02/00012, TDa 98/01174 and Seidubile as well as *D. rotundata* genotypes such as: TDr 95/19177, TDr 09/00001, TDr 09/18988, TDr 00/00951 and Larbako were selected as high yielding and preferred across the three districts. Reasons for farmers choice of yam tubers was based on its physical features (high yielding, big and long tubers, smooth with few to no hairy tuber surface, no insect pest damage and disease infection).

5.3 Characterization using marker assisted selection for diversity studies The genetic diversity conducted using seven SSRs markers to determine high performance genotypes in both species revealed total allele number of 23 and 27 in *D. alata* and *D. rotundata* respectively. The allele number 27 in *D. rotundata* was similar to that reported by Otoo *et al.,* (2009) work on puna (27 allele with 13 SSRs makers). Mean genetic diversity was 0.42 and 0.53 in *D. alata* and *D. rotundata* respectively. Polymorphic information content (PIC) was found to have a mean of 0.37 and 0.49 in *D. alata* and *D. rotundata* respectively which was similar to studies carried out by Muthamia *et al*. (2013). A dendrogram was used to cluster these genotypes to identify similarities and duplicates, but the quantitative traits clustered genotypes according to its morphological characters. Marker assisted selection was done to confirm similarities that existed among genotypes of different species.

Lower percent similarities were observed in *D. alata* (17.5%) and in *D. rotundata* (17.5%). The lower percentages obtained revealed that, genotypes are not similar but have differences among them. It further means that, there is diversity among these

genotypes which enhances selection for advance breeding work. Besides, it further revealed that Seidubile and TDa 98/01168 were the same and likely to have the same secondary genome in the *D. alata* nutrient-use efficient genotypes. Also Larbako and TDr 09/00001 as well as TDr 08/00792 and TDr 08/00951 were clustered to be similar at different cluster groups and are likely to have the same genome in the *D. rotundata* genotypes. Complementing molecular tools to phenotypic evaluation for accurate findings are important.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

Laboratory analysis and farmer description of the soil status in the study area revealed that, the soils are made up of sand, silt gravel soil with very little clay fraction. Hence the ability for water and nutrient holding capacity is low resulting in low soil fertility and low yield obtained by yam farmers. The analysis of the quantitative parameters for the agromophological characterization in this study revealed that, six important parameters (Leaf area, leaf surface area, high chlorophyll content at the lower to middle leaves as well as number and weight of seed yam, ware yam and total yam) which contribution to the total variability were highly significant. High yielding and farmer preferred nutrient use-efficient *D. rotundata* accessions determined/varieties were; TDr 95/19177, TDr 09/00001, TDr 09/18988, TDr 00/00789 and Larbako as well as *D.alata* accessions/varieties such as TDa 98/01168, TDa 09/00228, TDa 02/00012, TDa 98/01174, TDa, 92-2 and Seidubile. These genotypes further revealed an appreciable yield in the low nutrient soils in the Northern Region. In addition, the use of farmer participatory varietal selection in the

study gives the farmer confidence in the accessions/varieties they participated in selecting; hence adoption of these accessions/varieties would be very high in their farming systems. Results from the marker assisted selection revealed that diversity among genotypes are very high. This enhances selection of high environmental fit and yielding genotypes for advance breeding work.

6.2 Recommendations

- The study recommends that, the six traits (Leaf area, leaf surface area, high chlorophyll content at the lower to middle leaves as well as number and weight of Seed yam, ware yam and total yam) which contributed very high to total variability should be used in advance breeding programme to improve yam productivity and strengthen the gene pool of cultivated yams.
- The participatory method used should be set as a standard protocol for participatory varietal breeding in yam.
- Marker assisted selection should always be used as complementary to agro morphological and farmer participatory breeding to find diversity among genotypes and enhance selection for advance breeding work.
- The genotypes selected by agro morphology and participatory farmer criteria should be evaluated further by conducting baby trials to scale up and out of these selected new genotypes that could be released as nutrient use-efficient varieties.
- Molecular characterization revealed that most genotypes were closely related with secondary genome and others were widely related with high diversity. Hence, crossing the widely related genotypes will produce genotypes with high with yielding.

- Results from the marker assisted selection revealed that diversity among genotypes were very high. This enhances selection of high environmental fit and yielding genotypes for advance breeding work.
- Genotypes which were high yielding would be advanced in multilocational trials and if performance persists, they would be released to farmers.

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Appendix 1. Eigenvalues of correlation matrix, and related statistics Value Active variables only

number	Eigenvalue	% Total	Cumulative	Cumulative
1	6.045588	30.22794	6.04559	30.2279
2	3.536484	17.68242	9.58207	47.9104
3	2.450717	12.25358	12.03279	60.1639
4	1.812539	9.06269	13.84533	69.2266
5	1.435166	7.17583	15.28049	76.4025
6	1.260199	6.30099	16.54069	82.7035
7	0.833957	4.16979	17.37465	86.8732
8	0.672545	3.36273	18.04719	90.2360
9	0.464278	2.32139	18.51147	92.5574
10	0.378406	1.89203	18.88988	94.4494
11	0.326980	1.63490	19.21686	96.0843
12	0.222179	1.11089	19.43904	97.1952
13	0.188443	0.94221	19.62748	98.1374
14	0.134131	0.67065	19.76161	98.8081

15	0.095562	0.47781	19.85717	99.2859
16	0.059868	0.29934	19.91704	99.5852
17	0.054375	0.27187	19.97142	99.8571
18	0.026763	0.13381	19.99818	99.9909
19	0.001822	0.00911	20.00000	100.0000

Appendix 2. Scores of bands of *D. rotundata* gel electrophoresis

Genotype	SSR 3653 33	SSR 36	SSR 36	SSR 36	SSR 3233	SSR 3233	SSR 5331	SSR 5330	SSR 5331	SSR	SSR	SSR	SSR	SSR 3310	SSR 7500	SSR 7500	SSR 7511	SSR 7411	SSR 7411	SSR 6611	SSR 6600	SSR 6611
TDr 08/00990	0	0	0	1	?	?				1	1	0	1									
TDr 08/00133	1	0	0	0	?	?	0	0	0	1	1	0	0	0	0	0	1	1	1	1	0	1
TDr 08/00789	1	0	0	0	1	1	0	0	0	1	1	0	1	0	0	0	1	1	1	1	0	1
TDr 09/00054	1	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	1	1	1	0	0	1
TDr 08/00845	1	0	0	0	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	0	0	1
TDr 08/00917	1	1	1	0	1	1	0	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1
TDr 08/00951	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1	0	1	1	1	0	1
TDr 08/00792	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1	0	1	1	1	0	1
Chenchito	1	0	0	0	?	?	0	0	1	1	0	1	0	1	0	0	1	1	1	0	1	1
TDr 95/01932	1	0	0	1	1	1	0	0	0	1	0	1	0	1	0	0	1	1	1	1	0	1
TDr 08/00742	1	0	0	1	1	1	0	0	0	1	0	1	0	1	0	0	1	1	1	0	1	1
TDr 95/01132	0	0	0	1	1	1	0	0	0	1	0	1	0	1	0	1	0	1	1	0	1	1
TDr 95/19177	1	0	0	1	1	1	0	0	1	1	0	1	0	1	1	?	?	1	1	0	1	1
TDr 08/00963	1	0	0	1	1	1	0	1	1	1	0	1	0	1	0	1	0	1	1	1	0	1
TDr 09/00123	0	0	0	1	1	1	0	0	0	1	0	1	0	1	0	1	0	1	1	0	0	1

TDr 95/18988	1	0	0	1	1	1	1	1	0	1	?	?	?	?	?	?	?	1	1	0	0	1
Larbako	1	0	0	0	1	1	0	0	0	1	?	?	?	?	?	?	?	1	1	0	0	1
TDr 09/00001	1	0	0	0	1	1	0	0	0	1	?	?	?	?	?	?	?	1	1	0	0	1
TDr 09/00219	?	?	?	?	1	1	0	0	0	1	?	?	?	?	?	?	?	1	1	0	0	1
TDr 95/18544	?	?	?	?	1	1	?	?	?	?	?	?	?	?	?	?	?	1	1	1	0	1

1 = Present,

0 = Absent

? = Special code for missing data used during analysis.

Appendix 3. Scores of bands of *D. alata* gel electrophoresis

S/N	Genotypes	SSR36	SSR36	SSR36	SSR36	SSR36	SSR32	SSR32	SSR66	SSR66	SSR53	SSR53	SSR75	SSR75	SSR75	SSR75	SSR75	SSR74	SSR74	SSR33	SSR33
1	TDa02/00064	0	0	0	1	0	1	1	?	?	1	1	1	0	0	0	0	1	1	1	0
2	TDa09/00314	1	0	0	0	0	1	1	?	?	1	1	1	0	0	0	0	0	1	1	0

3	TDa09/00364	1	0	0	0	0	1	1	1	0	1	1	1	0	0	0	0	0	1	1	0
4	TDa92 – 2	1	0	0	0	0	1	1	1	0	1	1	0	1	0	0	0	0	1	1	0
5	TDa3743	1	0	0	0	0	1	1	1	0	1	1	0	0	1	0	0	0	1	1	0
6	TDa02/00012	1	1	1	0	0	1	1	1	0	1	1	0	0	1	0	0	0	1	1	0
7	TDa00/00060	1	0	0	0	0	1	1	1	1	1	1	?	?	?	?	?		1	1	1	0
8	TDa01/00114	1	0	0	0	0	1	1	1	0	1	1	0	0	0	1	0	1	1	1	0
9	TDa09/00228	1	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	1	1	1	0
10	TDa93 – 36	1	0	0	1	0	1	1	1	0	1	1	0	0	1	0	1	1	1	1	0
11	TDa09/00366	1	0	0	1	0	1	1	1	0	1	1	0	0	0	1	1	1	1	1	0
12	TDa09/00357	1	0	0	1	0	1	1	1	0	1	1	0	0	0	1	1	0	1	0	1
13	TDa01/00039	1	0	0	1	0	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1
14	TDa02/00246	1	0	0	1	0	1	1	1	0	1	1	0	0	0	0	1	0	1	0	1
15	TDa291	0	0	0	1	0	1	1	1	1	1	1	0	0	0	0	1	0	1	0	1
16	TDa98/01174	1	0	0	1	0	1	1	1	0	1	1	?	?	?	?	?		1	1	0	1
17	TDa99/00240	1	0	0	0	0	1	1	1	0	1	1	?	?	?	?	?		0	1	0	1
18	TDa09/00271	1	0	0	0	0	1	1	1	0	1	1	0	0	0	1	0	0	1	0	1
19	TDa98/01166	?	?	?	?	?	1	1	1	0	1	1	0	0	0	1	0	0	1	0	1
20	TDa98/01168	?	?	?	?	?	1	1	1	0	1	1	1	0	0	1	0	0	1	0	1
21	Seidubile	?	?	?	?	?	1	1	1	0	1	1	1	0	0	1	0	0	1	0	1
22	Nyuwotugu	?	?	?	?	?	1	1	1	0	1	1	1	0	0	0	0	0	1	0	1

1 = Present,

0 = Absent

? = Special code for missing data used during analysis.

Appendix 4. *D. alata* and *D. rotundata* genotypes on the gel plate

S/N	*D. alata*	*D. rotundata*
1	TDa 02/00064	TDr 08/00990
2	TDa 09/00314	TDr 08/00133
3	TDa 09/00364	TDr 08/00789
4	TDa 92 – 2	TDr 09/00054
5	TDa 3743	TDr 08/00845
6	TDa 02/00012	TDr 08/00917
7	TDa 00/00060	TDr 08/00951
8	TDa 01/00114	TDr 08/00792
9	TDa 09/00228	Chenchito
10	TDa 93 – 36	TDr 95/01932
11	TDa 09/00366	TDr 08/00742
12	TDa 09/00357	TDr 95/01132
13	TDa 01/00039	TDR 95/19177
14	TDa 02/00246	TDr 08/00963
15	TDa 291	TDr 09/00123
16	TDa 98/01174	TDr 95/18988
17	TDa 99/00240	Larbako
18	TDa 09/00271	TDr 09/00001
19	TDa 98/01166	TDr 09/00219
20	TDa 98/01168	TDr 95/18544
21	Seidubile	
22	Nyuwotugu	

Appendix 5. High yielding and farmer preferred *D.alata* nutrient use-efficient

genotypes


TDa 09/00228


TDa 02/00012


Seidubile


TDa 09/00228


TDa 02/00012


Seidubile


TDa 98/01168


TDa 98/01174


TDa 98/01168


TDa 98/01174

Appendix 6. High yielding and farmer preferred *D. rotundata* Nutrient useefficient genotypes

Appendix 7. Mean Yield (kg/ha) of nutrient use-efficient *D. rotundata* genotypes

Genotypes	Seed yam weight (kg/ha)	Ware yam weight (kg/ha)	Total yam weight (kg/ha)
Amula	1037	6944	7981
TDr 09/00001	2720	9424	12144
TDr 08/00917	3425	3472	6897
TDr 09/00054	4074	1130	5204
TDr 95/18988	5410	4762	10172
TDr 08/00742	3010	2777	5787

TDr 08/00444	2297	6944	9241
TDr 08/00944	2585	2315	4900
TDr 00/00951	2001	9325	11326
TDr 95/01932	2282	6944	9226
Chenchito	2304	4629	6933
TDr 08/00133	1894	4265	6159
TDr 08/00990	3379	5902	9281
TDr 95/01132	2105	6770	8875
Larbako	3646	6076	9722
TDr 95/18544	6134	1020	7154
TDr 95/19177	1852	8332	10184
TDr 08/00792	2639	2315	4954
TDr 09/00123	3067	1722	4789
TDr 08/00845	3075	3075	6150
TDr 08/00944	2616	3472	6088
TDr 00/00789	3511	5902	9413
TDr 08/00963	4409	1091	5500
Lsd (0.05)	2775.6	3714	3878.9
C V	55.84	79.58	40.15

Appendix 8. Mean Yield (kg/ha) of nutrient use-efficient *D. alata* genotypes

Genotypes	Seed yam weight (kg/ha)	Ware yam weight (kg/ha)	Total yam weight (kg/ha)
TDa 02/00064	4149	3651	7800
TDa 09/00314	3993	3387	7380
TDa 09/00364	5381	2710	8091
TDa 92 - 2	6421	3433	9854
TDa 3743	3893	3343	7236
TDa 02/00012	2893	7330	10223
TDa 00/00060	4051	2001	6052
TDa 01/00114	3086	1901	4987
TDa 09/00228	5054	5710	10764
TDa 93 - 36	2500	1389	3889
TDa 09/00366	5073	592	5665
TDa 09/00357	5555	1505	7060
TDa 01/00039	4861	470	5331

TDa 02/00246	4722	666	5388
TDa 291	2951	1575	4526
TDa 98/01174	5266	4745	10011
TDa 99/00240	3742	4108	7850
TDa 09/00271	5787	2315	8102
TDa 98/01166	2169	3144	5313
TDa 98/01168	10050	1132	11182
Seidubile	6269	2362	8631
Nyuwotugu	4086	2577	6663
Lsd (0.05)	5079.9	3343	5545.3
C V	66.53	7433	45.64