ASSESSMENT OF GENETIC DIVERSITY OF SORGHUM (*Sorghum bicolor*) ACCESSIONS FROM TANZANIA USING SSR MARKERS: IMPLICATIONS FOR CONSERVATION

PAMELA WAMBUI KUNYUGA (Bsc)
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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other university

Pamela Wambui Kunyuga _____ _____

Signature **Date**

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Dr John Muriuki
Environmental Education Department
Kenyatta University
P.O Box 43844-00100
Nairobi

_____ _____

Signature **Date**

Dr Najma Dharani
Environmental Science Department
Kenyatta University
P.O Box 43844-00100
Nairobi

_____ _____

Signature **Date**

Dr Dionysious Kiambi
Research Scientist
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
P.O Box 30709-00100
Nairobi-Kenya

_____ _____

Signature **Date**

DEDICATION

To my mum Anne, Sister Carol and my fiancé Anthony, for their support, understanding, love and patience, I thank God for them.

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ABBREVIATIONS AND ACRONYMS

DNA:	Deoxyribonucleic acid.
RNA:	Ribonucleic acid
ICRISAT:	International Crop Research Institute for Semi-Arid Tropics.
PCR:	Polymerase Chain Reaction.
FAOSTAT:	Food and Agriculture Organization of the United Nations.
IBGR:	International Bill of Gender Rights.
EDTA:	Ethylene Diamine Tetra acetic Acid
SSR:	Simple sequence repeats.
TFPGA:	Tools for Population Genetic Analyses.
UPGMA:	Un-weighted Pair Group Method using Arithmetic Averages.
GIS:	Geographic Information Systems.
CTAB	Cetyl Trimethyl-Ammonium Bromide

ABSTRACT

Plant genetic resources are a key component of global biodiversity from which humanity derives most of its food and other essential goods and services. An understanding of the extent, distribution, and patterns of genetic variation is useful for several purposes, including selection for conservation, estimation of any possible loss of genetic diversity prior to conservation programs, understanding the genetic variability available and its potential use in breeding programs, and estimating the relative strengths of the evolutionary forces shaping natural populations. Sorghum is a subsistent and staple crop for many people living across Africa especially those in marginal arid and semi-arid tropics. The study aimed to assess the genetic diversity within as well as among 96 accessions of sorghum germplasm collected from diverse regions of Tanzania using microsatellite markers. This entailed DNA extraction whereby the CTAB method was employed, DNA quality and purity check was done and then the DNA was normalized to ensure uniformity in the amount of DNA samples used and then polymerase chain reaction was done using the 12 microsatellites. Data analysis was then carried out and different softwares were used in the data analysis and this included the genemapper version 3.7 software, allelobin software, powermarker version 3.25 software and Darwin version 5.0 software, all these were used for different stages of the analysis. The area of study included Dodoma, Morogoro, Babati, Kilimanjaro and Mtwara regions. The molecular markers revealed that there was considerable amount of genetic diversity among the Tanzania sorghum and this is indicated by the high number of alleles and the clusters generated. There are so many sub clusters dividing from the main clusters and this suggests that there is high level of diversity in these Tanzanians accessions. However this diversity was not divided according to the geographical regions and there was no appreciable diversity within the sorghum accessions but the sorghum accessions clustered according to their genotype i.e. the sorghum landraces clustered together and the varieties also clustered together. Geographical Information Systems was also used. GIS was used in this study whereby maps showing the collecting localities and the environmental conditions for those localities were generated using a combination of different GIS and computer software.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Sorghum *bicolor* is the fifth most important grain and the 2nd highly produced cereal in Africa after maize (Gerda & Christ 2007). It is one of the longest cultivated plants in many arid and semi-arid regions of sub Saharan Africa because its genetically suited to hot and dry agroecological zones where it is difficult to grow most of the food grains such as maize (Mamoudou et al., 2006, Markus and Gurling 2006). It is a staple food grain for millions of people in the semi-arid areas of the world especially in Africa and India (Taylor & Emmanbux, 2008).

In most African countries, farming depends entirely on the quality of the rainy season, a situation that makes Africa particularly vulnerable to climate change and Tanzania is not an exception. Tanzania's economic base is dependent on natural resources, rain fed agriculture and biomass for household energy and thus the economy is highly vulnerable to the adverse impacts of climate change. Tanzania has 7 agro ecological zones as shown in table 1. In Tanzania sorghum is one of the major cereal crop and most of it is grown in the semi-arid areas in Tanzania namely Singida, Shinyanga, Dodoma, Mtwara and parts of Iringa and Morogoro regions.

Ethiopia is considered the centre of origin of sorghum (Dogget and Prasada Rao, 1995). It has spread to other parts of Africa, India, south East Asia, Australia and USA, (Tawanda 2004, ICRISAT 2005). Most of the sorghum in Africa is produced in areas where rainfall is less than 500mm due to its resistance to drought. This include the northern part of

Africa, drier parts of western and central African countries, the semi arid parts of east Africa and the drier western parts of south Africa, (Taylor and Emmmanbux, 2008).

In Kenya sorghum is mainly grown in the drought prone areas of northern, eastern, western, coast and parts of rift valley. This is attributed to its tolerance nature to drought, infertile soils and high temperatures (EPZ, 2005).

Biodiversity is the variation or differences in living organisms, including all species and genetic variants within species and all ecosystems that contain and sustain these diverse forms of life. The study of genetic diversity is the process by which variation among individuals or groups of individual populations are analyzed by a specific method or a combination of methods. The data generated is very important and can be employed in mining germplasm from regions associated with adaptive or agronomically important traits.

Genetic diversity plays a very important role in survival and adaptability of a species because when a species environment changes, slight gene variations are necessary to produce changes in the organisms' anatomy that enables it to adapt and survive. A species that has a large degree of genetic diversity among its population will have more variations from which to choose the fit alleles. Increase in genetic diversity is also important for an organism to evolve. Species that have very little genetic variation are at a very great risk. This is referred to as genetic uniformity and this is dangerous because it enhances vulnerability. A good example is the 1970 epidemic of southern leaf blight (*Helminthosporium maydis*) in the corn belt of USA. The adoption of hybrid maize cultivars produced using T-cytoplasmic male sterility in the 1960s led to the loss of about 15%of the US maize crop in the early 1970s.T-cytoplasm was a man-made change in

corn plants used to foster the quick and profitable production of high-yielding, hybrid corn seed. It was a change accomplished and advanced by science and commerce without full knowledge of the potential consequences. The new strain of corn blight fungus, *Helminthosporium maydis*, was a mutation perfectly keyed to a gene in that cytoplasm. The cultivars were therefore susceptible to the new race of H. maydis, At least 80 percent of the hybrid corn in America in 1970 contained T-cytoplasm, which is why "race T" of *Helminthosporium maydis* laid waste to 15 percent of the nation's corn crop.

Table 1: Agro ecological zones

World Bank, 1994. Tanzania Agriculture, World Bank, Washington DC

Zone	Sub-Zone and Areas	Soils and Topography	Altitude	Rainfall (mm/yr)	Growing Season
I. COAST	North:Tanga (except Lushoto), Coast and Dar es Salaam) South: Eastern Lindi and Mtwara, (except Makonde Plateau)	Infertile sands on gently rolling uplands Alluvial soils in Rufigi Sand and infertile soils Fertile clays on uplands and river flood plains	under 300m	North: bimodal, 750-1200mm South: unimodal, 800-1200mm	North: October-December and March-June South: Dec-April
II. ARID LANDS	North: Serengeti, Ngorongoro Parks, part of Masailand Masai Steppe Tarangine Park, Mkomazi Reserve, Pangani and Eastern Dodoma	North: Volcanic ash and sediments. Soils variable in texture and very susceptible to water erosion South:Rolling plains of reddish sandy clays of low fertility. Susceptible to water erosion. Pangani River flood plain w/ saline, alkaline soil.	North: 1300-1800m South: 500-1500m	North: Unimodal, unreliable, 500-600mm South: Unimodal and unreliable, 400-600mm	March - May
III. SEMI-ARID LANDS	Central Dodoma, Singida, N. Iringa, some of Arusha, Shinyanga Southeastern: Morogoro (except Kilombero & Wami Basins and Uluguru Mts). Also Lindi and SW Mtwara	Central: Undulating plains, w/ rocky hills and low scarps. Well drained soils w/ low fertility. Alluvial hardpan and saline soils in Eastern Rift Valley and Lake Eyasi. Black cracking soils in Shinyanga. Southeastern: Flat, or undulating plains w/rocky hills. Moderately fertile loams and clays in South (Morogoro), infertile sands in center.	Central: 1000-1500m Southeastern: 200-600m	Central: Unimodal and unreliable:500-800mm Southeastern: Unimodal: 600-800mm	December - March
IV PLATEAUX	Western: Tabora, Rukwa (North and Center), Mbeya (North), Kigoma, part of Mara Southern: Ruvuma, and Southern Morogoro	Western: Wide sandy plains and Rift Valley scarps. Flooded swamps of Malagarasi & Ugalla rivers have clay soil with high fertility Southern: Upland plains w/ rock hills. Clay soils of low to moderate fertility in South, infertile sands in North.	800-1500m	Western: Unimodal, 800-1000mm Southern: Unimodal, very reliable, 900-1300mm	November-April
V. SOUTHERN & WESTERN HIGH-LANDS	Southern: A broad ridge from N. Morogoro to N. Lake Nyasa, covering part of Iringa, Mbeya Southwestern: Ufipa plateau in Sumbawanga. Western: Along the shore of L. Tanganyika in Kigoma and Kagera.	Southern: Undulating plains to disected hills and mountains. Moderately fertile clay soils, with volcanic soils in Mbeya. Southwestern: Undulating plateaux above Rift Valley(s). Sandy soils of low fertility Western: North-South ridges separated by swampy valleys. Loams and clay soils of low fertility in hills, with alluvium and ponded clays in valleys.	Southern: 1200-1500m Southwestern: 1400-2300m Western: 1000-1800m	Southern: Unimodal, reliable, local rainshadows, 800-1400mm Southwestern: Unimodal, reliable, 800-1000mm Western: Bimodal, 1000-2000+mm	Northern: December - April Southwestern: November - April Western: October-December and February-May.
VI. NORTHERN HIGH-LANDS	Northern: foot of Mt Kilimanjaro and Mt Meru, Eastern Rift to L. Eyasi. Granitic Mts: Uluguru Mts in Morogoro, Pare Mts in Kilimanjaro, and Usumbara Mts in Tanga, Tarime Highlands in Mara.	Northern: Volcanic uplands. Volcanic soils from lavas and ash. Deep fertile loams and clays. Soils in dry areas prone to water erosion. Granitic Mts: Steep mountain sides to highland plateaux. Soils are deep, friable and moderately fertile on upper slopes; shallow and stony on steep slopes.	Northern: 1000-2500m Granitic Mts.:1000-2000m	Northern: Bimodal, varies widely: 1000-2000mm Granitic Mts: Bimodal and very reliable 1000-2000mm	Northern: November-January and March-June Granitic Mts. October-December and March-June
VII. ALLUVIAL PLAINS	K- Kilombero (Morogoro) R- Rufiji (Coast) U- Usangu (Mbeya) W-Wami (Morogoro)	K- Central clay plain, with alluvial fans East and West R- Wide mangrove swamp delta. Alluvial soils, sandy upstream, loamy downstream in floodplain. U- Seasonally flooded clay soils in North, alluvial fans in South W- Moderately alkaline black soils in East, and alluvial fans with well drained black loam in West.		K- Unimodal, very reliable, 900-1300mm R- Unimodal, often inadequate 800-1200mm U- Unimodal, 500-800mm W- Unimodal, 600-1800mm	K-November - April R-December-April U-December-March W-December-March

1.2 Problem statement

The global climate is changing and this is already impacting on food supply and security. Climate change has a big impact on Africa's food availability and security. A report by the IPCC indicates that over the next 100years, the average temperature in Africa will rise by 3ºC to 4ºC resulting in the continent becoming generally drier than it is currently (Africa Harvest, 2007). This compounded by the fact that the African population is growing by about 24million people a year (Xinhua, 2009), and since Africa food availability and food security is tied up with its staple cereals such as sorghum and millet, there lays an emphasis on the need for crop scientists to develop improved varieties of crops that use water more efficiently such as sorghum.

Eradication of poverty and hunger is one of the millennium development goal still to be met by many African countries there is therefore a need to adapt the plants that can thrive under this changing climatic conditions. Sorghum is one example of these plants and is extremely resistant to aridity and hot conditions. The productivity of this sorghum per unit area has been rather low due to a number of production constraints, presence of insects and pests damage and diseases and inadequate supply of improved varieties. In Tanzania the main constrain is the striga weed. Yield gains have been realized by utilization of genetic variability for yield components and adaptation traits (Rai et al., 1999). Improved sorghum varieties adapted to semi-arid and tropic environments and released every year by sorghum breeders. Selection of varieties meeting specific local food and industrial requirements from this great biodiversity is of high importance to food security.

Climate change and global warming is bringing about genetic erosion this is the process whereby an already limited gene pool of an endangered species of plant or animal diminishes even more when individuals from the surviving population die off without getting a chance to meet and breed with others in their endangered low population. There is therefore a need to study the genetic diversity of plants for the efficient management and the conservation of races and their optimum utilization in plant breeding.

1.3 Research Questions

1. Are the sorghum landraces from Tanzania genetically distinct?
2. Does genetic diversity exist within the Tanzania landraces?
3. What is the importance of conserving *Sorghum bicolor* genetic resources?

1.4 Objectives

In this study the main objective was to characterize sorghum bicolor landraces and varieties from the highlands of Tanzania using molecular markers.

1.4.1 Specific Objectives

1. To assess the level of genetic diversity among and within Sorghum landraces from Tanzania and determine their genetic structure.
2. To enumerate importance of conservation of S*orghum bicolor* genetic resources.

1.5 Hypotheses

1. Sorghum landraces from Tanzania highlands are genetically distinct.
2. The genetic variability is partitioned across the geographical regions.

1.6 Significance of the study

Conservation of biodiversity is the first attempt to alleviate the pending extinction of the biosphere by humans. Diversity of sorghum in Tanzania is not well understood and a comprehensive knowledge of the diversity and genetic relationship among the cultivated sorghum will be of aid in the crop improvement strategies and development of sorghum varieties that can escape drought, tolerate low soil fertility, resist pests and diseases and in the long run enhance agricultural productivity under low input conditions. Diversity data generated from this analysis can also be used as a tool for mining germplasm collections for regions associated with adaptive or agronomically important traits. Study of the association between genes and environmental variables may also guide germplasm conservationists and breeders to target specific environments where traits of interest may be found. The aim of this study is therefore to unlock the genetic potential of Tanzania's sorghum and the results will provide a sorghum germplasm database. Germplasm assessment will provide a foundation for making informed decisions regarding the management and utilization of genetic resources.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sorghum description

Sorghum bicolor includes all cultivated sorghums as well as a group of semi wild plants often regarded as weeds. It is a grass species cultivated for its edible grain. *S. bicolor* is typically an annual, but some cultivars are perennial. It grows in clumps that may reach over 4 meters high. The grain is small, ranging from 3 to 4 mm in diameter. The species can grow in arid soils and withstand prolonged droughts. It has four features which make it one of the most drought resistant crops of all. It has a very large root-to-leaf surface area, in times of drought it will roll its leaves to lessen water-loss by transpiration, if drought continues it will go into dormancy rather than dying and its leaves are protected by a waxy cuticle. Sorghum is a c4 plant and hence it's able to reduce evapotranspiration thus conserving water under conditions of drought and high temperatures.

Figure 1: Sorghum plant.

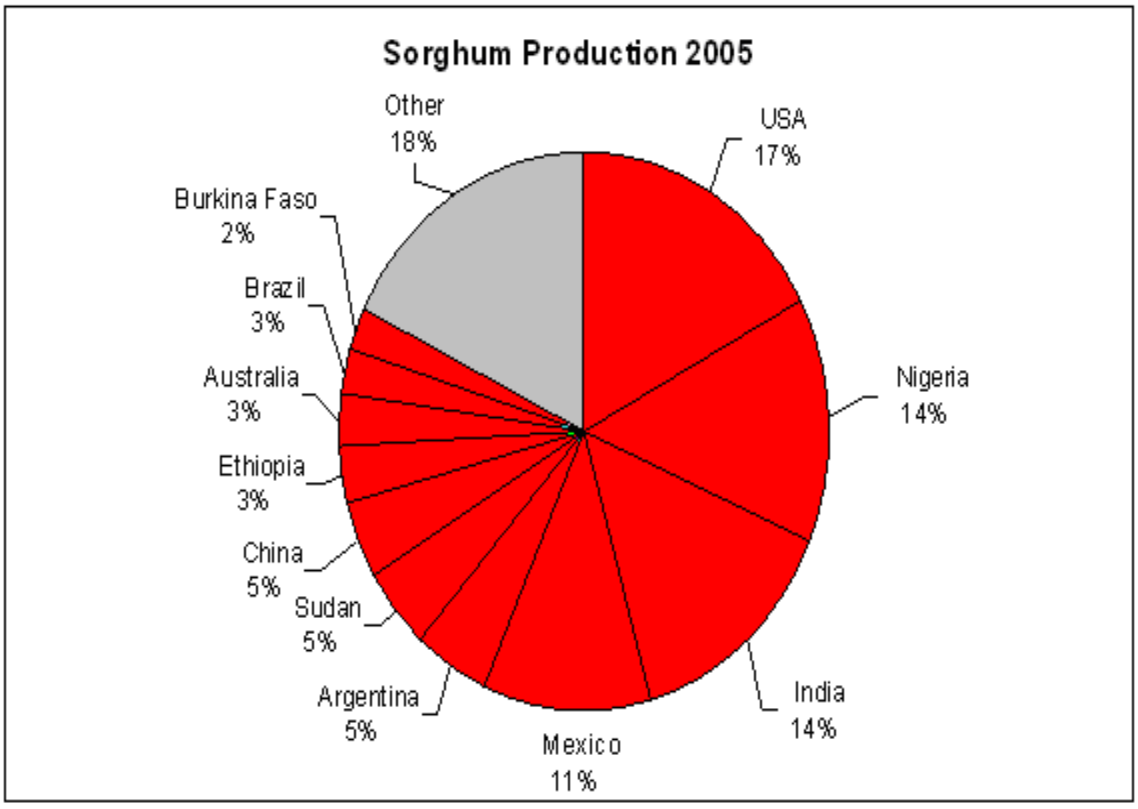
2.2 Sorghum production and distribution

Sorghum has its origin in Ethiopia and from here it spread to other parts of Africa, India, Asia, Australia and the US. (Tawanda, 2004; ICRISAT, 2005). It's the fifth most important cereal crop in the world after wheat, rice, maize and barley. Its one of the longest cultivated plants in many arid and semi arid regions of sub-Saharan Africa and other areas where other crops such as maize would fail (Mamoudou et al., 2006; Markus and Gurling, 2006). Together with pearl millet [*Pennisetum americanum* (L.) and finger millet [*Eleusine coracana* (L.) Gaertn] it represents Africa's main contribution to the world food supply (De Vries and Toenniessen 2001).

Major producers are the USA, India, Nigeria, China, Mexico, Sudan and Argentina. The crop occupies 25% or more of arable land in Mauritania, Gambia, Mali, Burkina Faso, Ghana, Niger, Somalia and Yemen, and >10% of this area in Nigeria, Chad, Sudan, Tanzania and Mozambique. Eighty percent of the area devoted to sorghum is located within Africa and Asia, with average yields of 810 and 1150 kilograms per hectare, respectively. The bulk of African sorghum production is centered in the savanna zone of east, west and central Africa, where the grain of this crop is a major component of the daily menu for millions of people. Twelve out of the twenty largest sorghum producing countries in the world are in Africa, with Nigeria as one of the leading world producers of the crop.

The area under sorghum in Africa is 10%, 36% in Asia, 21% in central and South America in USA 20 %, (International starch institute, 2001). Sorghum and millet are important food crops in Tanzania and are mainly grown in three of the country's 7

agroecological zones. In Kenya the area under sorghum cultivation is 0.12 ha (FAO, 1995).



Source – FAO 2005; http://www.gramene.org/species/sorghum/sorghum maps and stats html.

Figure 1: Top sorghum producers 2005

2.3 Significance of the crop

Sorghum bicolor is one of the most important staple cereals in the semi-arid regions of the world (Dogett, 1988; Rohrbach et al., 2002) and represents Africa's main contribution to the world's food supply (Purseglove, 1987). It's a staple food for millions of people in India and Africa. Its popularity as a cereal crop globally is due to its productivity and tolerance to drought and stress (Dogett, 1988). Sorghum bicolor is also used as a feed for livestock and this is popular in the USA where it's commonly used as a maize substitute for livestock feed because their nutritional values are very similar.

It's also being exploited as a major alcohol producer, for example Africa Harvest is partnering with East Africa Breweries Ltd to link sorghum farmers to this major alcohol producer and this will improve the livelihoods of farmers and therefore contribute to poverty alleviation and contribute to sustainable development while improving the environment. In the United States, sweet sorghum has been grown mainly for its syrup and used as a sugar substitute. Comparing crops as sources of ethanol, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Food and Agriculture Organization (FAO) underscored the following: Sweet sorghum can grow like no crop has grown in dry-lands, acidic or basic soils, and waterlogged fields; Sweet sorghum grows faster than sugarcane, 200 days (two crops) against 365 days; Sweet sorghum needs 4.5 times less water than sugarcane, 8,000 (two crops) against 36,000 cubic meters; Cost of cultivation of sweet sorghum is three times less than that of sugarcane; Sweet sorghum is easily planted, five kilograms of seeds to a hectare. Sugarcane requires the handling of 5,000 cuttings. Ethanol production process from sweet sorghum is eco-friendly while that from sugarcane is not. Ethanol from sweet

sorghum is better than from sugarcane for two reasons: it has lower sulphur content (is less polluting) and higher octane (yields more power).

The straw of traditional tall sorghums is used to make palisades in villages or around homesteads. The plant bases are an important source of fuel for cooking and the stems of wild varieties are used to make baskets or fish traps. Dye extracted from sorghum is used in West Africa to color leather red. In western Africa, Nigeria has emerged as a pioneer in the industrial utilization of sorghum.

2.4 Genetic diversity and its conservation

Conservation of biodiversity has become important during the last decade attempting to alleviate the pending extinction of the biosphere by humans (Soule, 1986, 1987; Wilson & Petta, 1988; Wilson 1989, 1992; Ehrlich & Wilson, 1991). Genetic diversity is a level of biodiversity that refers to the total number of genetic characteristics in the genetic makeup of a species. It's the variation in living organisms within a given ecosystem (Chandra et al., 2001). It's recognized as one of the three fundamentals level of biodiversity the others being ecological biodiversity and species biodiversity.

The sustainable use of genetic resources for food and agriculture will be the foundation for many of the adaptation strategies required in food and agriculture .In order to adapt to climate change and global warming, plants and animals important for food security for example sorghum will need to adapt to abiotic changes which include heat, drought, floods and salinity among others. New resistance will be required for crop breeds, animal breeds, fish breeds and forest varieties, therefore genetic diversity which is currently underutilized will become more attractive for breeders and farmers in crop improvement strategies in this era of climate change and global warming.

Genetic conservation is the management, preservation, and use of genetic resources so that they may yield the greatest suitable benefit to the present generation, while maintaining their potential needs and aspirations of generations to come (IUCN 1980). It encompasses the collection, maintenance and preservation of intra and interspecific variation. The genetic diversity within a species is one of the biological systems allowing for further development and adaptation to changing environmental conditions.

The sources of tremendous variation in plants support all other forms of life on land. It's the basis for food and other human needs for the millennium. It includes the resources that contribute to people's livelihoods by provision of food, medicine, food for domestic animals, fiber, clothing, shelter, energy, and a multiple of other products and services. This is an asset and also a tool to promote national, regional and international economies all over the world. Therefore the conservation and sustainable use of plant genetic resources are essential to the sustainable development of agricultural production and hence the knowledge of crop genetic diversity in the cultivated crops is very paramount.

2.5 Sorghum and environment.

Diversity is currently facing eminent threat and heavy pressures from both the environment and socio economic factors. (Kiambi et al., 2008). In the recent years it has been evident on the implications that global warming might have for climate patterns and the resulting changes is not only in the comparison of ecosystem but also the patterns of genetic diversity, resilience and distribution of species. (Parmeson and Yohe., 2003; Jutro., 1991). Climate change is now a global issue and this raises some serious conservation concerns as this has led to numerous shifts in the distribution, abundance and extinction of species. (Thomas et al., 2004; Williams et al., 2003; Pounds et al., 1999).

2.6 Production constraint

Despite the threat of climate change and global warming leading to variable and drier climate there is still no clear policy and government commitment for the development of sorghum which can prove to be of use in this era of changing climate. Sorghum could as well be termed as an orphaned crop with little and uncoordinated support to farmers compared to other cereal crops and does not receive the attention it deserves and thus produces just a fraction of what it could. Sorghum also suffers from lack of status and it is mostly referred to as a coarse grain, animal feed and food for peasant classes and people need to be educated about its values. Another production constraint in sorghum is losses due to biotic stresses which include diseases, insect pests and striga and also the abiotic stresses such as drought, high temperatures and low soil fertility (Ngugi et al., 2002). Sorghum also has a lot of undeveloped genetic potential and that's partly what this study aims to address, genetic studies need to be carried out so as to unlock this genetic potential of sorghum and therefore used to address the issue of food security and to mitigate and adapt to the global warming and climate change.

2.7 Methods of assessing the genetic diversity

Information about the genetic diversity and genetic relatedness is a very important element in plant breeding and crop improvement strategies, (Siddiqui and Naz, 2009). Information from genetic diversity assessment can be of great use in diverse areas including analysis of genetic variability in cultivars, identifying diverse parental combination to create segregating progenies with maximum genetic variability for further selection and introgressing desirable genes from diverse germplasm into the available genetic base.

Study of genetic diversity is the process by which variation among individuals of populations is analyzed by a specific method or a combination of methods. This characterization is the observation, measurement and documentation of highly heritable traits. The characterization aims at describing and understanding the genetic diversity of an organism under study. Genetic diversity has been developed based mostly on morphological descriptors, molecular marker technology (Mace et al., 2005) among others.

Morphological descriptors are based on the morphological characteristics which are obtained in the field or from field specimen (Gaines et al., 1999). They discriminate individuals based on physical characteristics for example maturity cycle, growth habit, leaf shape, hairiness, nature of corolla and panicle/pod/fruit size (Van der Maesen., 1990, Gaines et al., 1999). These morphological traits are reliable, easy to study and relatively low cost to study. However they have their shortcoming and these include limited polymorphism, also these characters may not be significantly distinct and hence require that plants grow to full maturity prior to identification (Ratnaparkle et al., 1995). Another shortcoming is that the characters are often influenced by environmental factors resulting in differences in expression and therefore one should be very careful with false positives due to influences by the environment.

DNA based markers on the other hand have been used successfully in DNA fingerprinting of plant genome and in genetic diversity studies (Agranama and Tuinstra, 2004). These markers system have several advantages over the other marker types and therefore have been used in recent studies successfully. Unlike the morphological

markers they are not affected by the environment. They can also be detected in all tissues at all stages of development (Soriano et al., 2005).

There are many types of molecular markers which have been developed and applied. They include restriction fragment polymorphism, amplified fragment length polymorphism, random amplified polymorphic DNA, sequence tagged sites, microsatellites also known as simple sequence repeats and single nucleotide polymorphism (Tawanda, 2004; Chandra et al., 2001). These marker systems are available for assessing the genetic diversity though they differ in principle, application, amount of polymorphism detected and also the time required. In this study microsatellite markers were used in the assessment of the genetic diversity of sorghum.

2.8 SSR markers

Microsatellite are short repeated DNA sequences in the genome 2 to 4 nucleotides in length for example, … (GCC) 17…) (Tawanda, 2004; Reisch, 1998) they are distributed throughout the genome of eukaryotes and abundant in genomes of plants where they are thought to be a source of genetic variation (Mahalakshmi et al., 2002). They tend to occur in non-coding regions of the DNA, although a few human genetic disorders are caused by (trinucleotide) microsatellite regions in coding regions. The SSR markers have proved to be a variable asset for breeding programs and have been used for a wide range of application and this include measuring of genetic diversity (Xiao et al.,1996) and in assigning lines to heterotic groups (Senior et al., 1998) SSR markers have been used in the genetic analysis of breeding schemes, genetic distance analysis (Chen et al., 1997)and in population genetic fingerprinting for legal protection of cultivars and parental lines and in establishing genome relationship in species with putative interspecific parents.

SSR markers have been developed in pigeon pea and have been used to assess the degree and distribution of genetic diversity in pigeon peas landraces from Andhara Pradesh in India (Newbury et al., 2004; Buhariwalla and Crouch, 2004; Bramel et al., 2004). Polymorphisms have been observed with this kind of marker in loquat (Sorriano et al., 2005), peanut (Krishna et al., 2004), perennial ryegrass (Kubik et al., 2001), rice (Liu et al., 2000), maize (Senior et al., 1998; Senior and Heun, 1993) and in barley (Shaghai et al., 1994). SSRs have also been found to occur in other plant genomes including soyabean (*Glycine max* L.) (Akkaya et al., 1992), rice (*Oryza sativa* L.) (Wu et al., 1993) and barley (*Hordeumn vulgare* L.). The results from Agranama and Tuinstra (2004) in the analysis of genetic diversity among sorghum lines indicated that the genetic distances calculated from SSR data were highly correlated with the distances based on the geographic origin and race classifications. SSR markers are therefore useful in the estimation of genetic similarities among diverse genotypes of sorghum because they display high levels of polymorphism.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Area of study

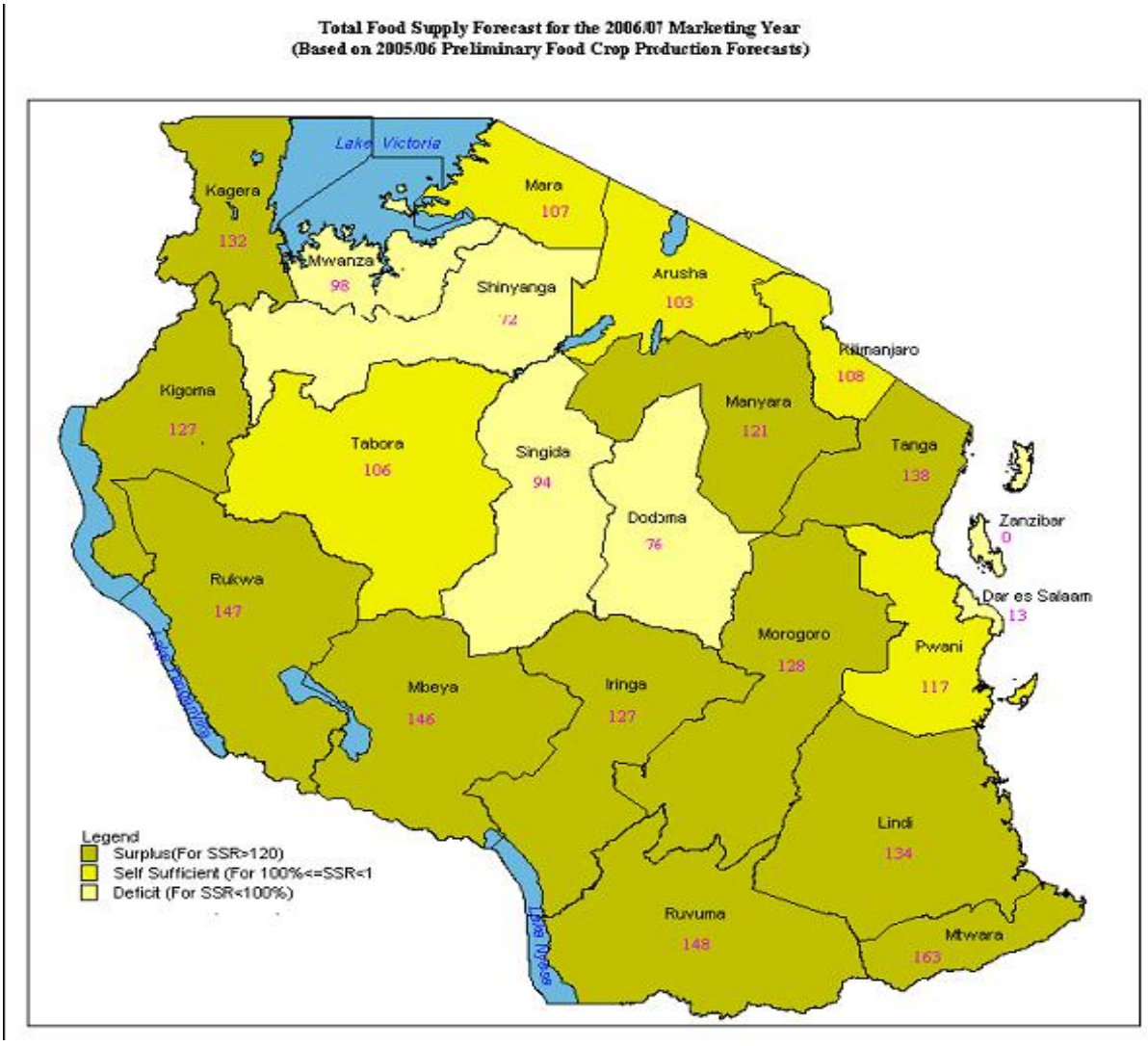


Figure 3: Map showing different regions of Tanzania and their food production

Dodoma Region has a savanna type of climate, which is characterized by a long dry season lasting between late April and early December, and a short single wet season occurring during the remaining months. In the long dry season, persistent desiccating winds and low humidity contribute to high evapo-transpiration and to soil erosion. The average rainfall of the area is 570mm and apart from the rainfall being relatively low, it is rather unpredictable in frequency and amount. In contrast, Morogoro region has an annual rainfall ranging from 600mm in low lands to 1200mm in the highland plateau The average annual temperature varies between 18ºC on the mountains to 30ºC in river valleys. In most parts of the region, the average temperatures are almost uniform at 25ºC. In general the hot season runs from July to September. Kilimanjaro on the other hand has a bi modal type of rainfall which is very reliable and the growing season is October to December, and then March to June.

3.2 Plant materials

Ninety six accessions of sorghum were obtained from diverse geographical regions of Tanzania. The materials were sown in trays and kept in the green house. The seeds were planted in bulk of about 10 seedlings for interpopulation diversity analysis and about five seedlings for intrapopulation analysis.

3.2 DNA extraction

For the interpopulation diversity sampling about 10 leaves from different plants were harvested from 2weeks old seedlings and put into separate extraction caps that contain two marble balls DNA was isolated from these leaf tissues using a mini-prep method. DNA was extracted using the CTAB protocol as developed by Mace et al. (2004). Two steel beads were put in each of the wells of a Geno Grinder 2000 (Spex CertiPrep, USA) plate together with leaf samples and then placed in a bucket with liquid nitrogen which

makes the leaf material brittle making it easy to grind.450µl Preheated (65^{o}C) Extraction Buffer (EB) was added to the leaf samples and ground using the Genogrinder. The EB contains 1 M Tris-HCl for stabilizing the PH, 2% w/v Cetyltrimethyl Ammonium Bromide (CTAB) which is a cationic detergent which aids in lysis of cell membranes and form complexes with nucleic acids, 1.4M NaCl helps for the formation of CTAB and nucleic acid complexes, β-Mercapto-ethanol (0.2-2%) protects the DNA against quinones, disulphides, peroxidases and polyphenol oxidases. EDTA (20mM) in EB chelates divalent ions particularly Ca^{2+} and Mg^{2+} and prevents activity of metal-dependent nucleases. The macerated substance was transferred to fresh microfuge tubes and incubated for 15 minutes at 65^{o}C with occasional mixing. Solvent extraction was done by adding 450µl Chloroform: isoamyalcohol (24:1) to each sample and mixed thoroughly by inversion. The tubes were centrifuged at 12000 rpm for 10 minutes at 24^{o}C and the upper portion transferred into fresh tubes (about 400µl). 0.7 volumes of iso-propanol (stored at -20^{o}C) was added and inverted once to mix and the tubes centrifuged after 20-30 minutes at 12000rpm for 15 minutes. This helps to precipitate the crude DNA pellet. The supernatant was decanted and the pellet air dried for 30 minutes. 200µl Low salt TE buffer (1mM Tris and 0.1mM EDTA [PH 8]) with 3µl RNase A (10mg/ml) was added to each sample and incubated overnight at room temperature. The volume was then transferred to fresh tubes and chloroform: isoamyalcohol (24:1) was added to each tube and inverted twice to mix and centrifuged. Later the aqueous layer was transferred into fresh tubes. DNA purification was done by adding 315µl ethanol and 1/10 volume of 3M sodium acetate solution (PH 5.2) to each sample and then placed in -20^{o}C for 5 minutes. The tubes were then centrifuged at 12000rpm for 5 minutes and the supernatant decanted.

200µl of 70% ethanol was added and centrifuged at 3500 rpm for 5 minutes. This is for washing the DNA pellet. DNA pellet was air-dried for one hour. The pellet was then re-suspended in 100µl low salt TE (10mM Tris, 1mM EDTA [PH 8]) buffer and stored at 4^{o}C.

3.3 DNA Quality and Purity Check

DNA quality and quantity has to be checked before embarking on a genetic diversity project so as to ensure high quality results, DNA quality check was done using 0.8% (w/v) agarose gel electrophoresis stained with Gel red (5µl/100µl). The good quality DNA shows a clear band while a bad one shows a smear. The DNA quantity and quality was also checked using a nanodrop spectrophotometer. It assesses the purity of nucleic acids using the ratio of absorbance at 260 and 280 nm. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA.

3.4 DNA normalization

This was to ensure uniformity in the amount of DNA samples used. Using the nanodrop readings and the agarose 0.8% gel images, all DNA samples were diluted to the required concentration (5ng/ul) this was done by adding 40ul double distilled water into 5ul of DNA for each sample to make a 1:10 dilution.

For each sample 2ul of the diluted DNA were mixed with 1ul bromo-phenol blue dye and 3ul double distilled water and then they were filled in the wells of submerged gels in an electrophoresis unit containing 1XTBE buffer. The outer wells at the left edges of the gel were filled with 5 nanograms and 10 nanograms concentrations of lambda DNA standards for comparison. An electric current of 120V was applied for 30minutes

due to the ethidium bromide (1%), the DNA fragments emitted a luminous glow under UV light and were photographed using a video capture system (Flowgen IS 1000). the

concentration of the DNA fragments was estimated according to the thickness of the band in comparison with the Lambda DNA standards at the edge of the gel.

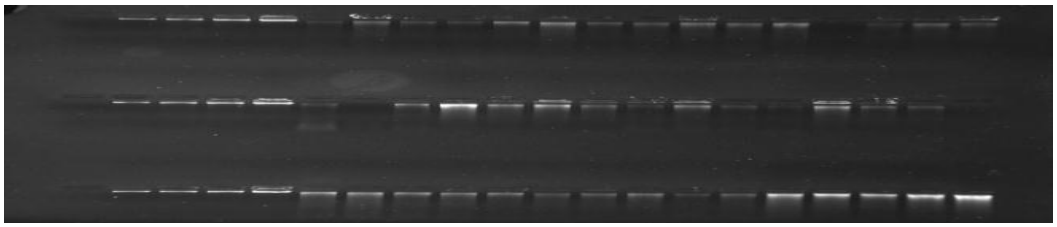


Figure 4: Example of a Normalized DNA sample.

3.5 Optimization of PCR products

A set of 12 sorghum SSR primers was used for the genotyping. The SSR markers were chosen based on genome position, repeat size (ranging from dinucleotide to hexanuceotides repeats) and the number of previously reported alleles (ranging from two to six). Optimization of the primers was necessary to avoid non amplification and stuttering a problem of unoptimization (Figure 5). The annealing temperatures of the 12 primers were optimized using the touchdown PCR amplification procedure. The PCR products were again ran on agarose and sent to the sequencer to check for amplification.

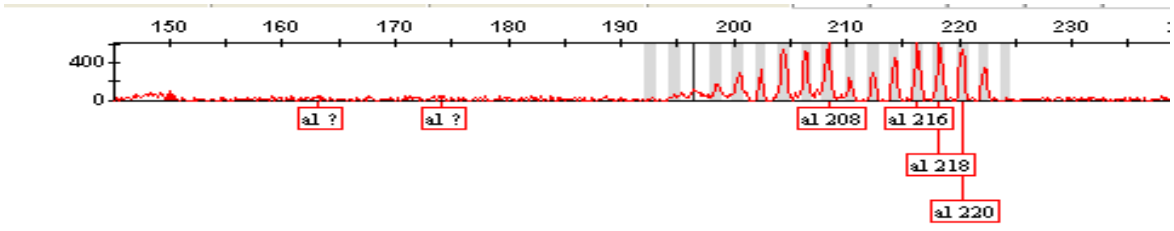


Figure 5: ABI sequencer output showing stuttering of peaks due to unoptimized PCR conditions

3.6 PCR and capillary electrophoresis

A set of 12 SSR primers was used for genotyping. SSR were suitable because of their genome position, repeat size (ranging from di-nucleotide to haxa-nucleotide repeats) and the number of previously reported alleles (ranging from two to six). Forward primers were labelled with FAM, HEX, NED, VIC, or PET (PE- Applied Biosystems). PCR conditions for each of the 12 primers were optimized and PCR reactions were set in 10µl volumes in 96-well PCR plates manually. Each PCR reaction contained 0.2pmol of primer, 1-2 m*M* Mgcl2, 0.1-0.2 m*M* dNTP, 0.2U Amplitaq Gold polymerase (PE-Applied Biosystems) and 1x PCR buffer (PE-Applied Biosystems). Temperature cycling was carried out using the GeneAmp PCR systems 9600 (PE Applied Biosystems) and touch down PCR amplification: one 15min denaturation cycle, followed by ten cycles of 94°C for 10s, 61°C for 20s (ramp of 1 per cycle) and 72°C for 30s, then by 31cycles of 94°C for 10s, 54°C for 20s and 72°C for 30s. After completion of the 31cycles, a final extension of 20min at 72°C was included.

After the PCR, a few accessions in each primer were randomly selected and their PCR products run on a 2.5% agarose gel to check for amplification. Genotyping was carried out by capillary electrophoresis using the ABI PRISM 3730 (Applied Biosystems), a fluorescent based capillary detection system that uses polymer as the separation matrix. This facilitated the accurate sizing of the microsatellite alleles to within ± 0.3 base pairs (Buhariwalla and Crouch, 2004). PCR products were co-loaded post-PCR based on dye label, fragment size and fluorescence to reduce the unit cost of high throughput genotyping. 0.5-1µl of labelled PCR products (depending on the intensity of the bands on agarose gel) were loaded mixed with formamide (PE-Applied Biosystems) and ROX-

labelled GS500LIZ-3730 size standard (PE-Applied Biosystems). DNA fragments were denatured and size-fractioned using capillary electrophoresis on an ABI 3730 automatic DNA sequencer (PE-Applied Biosystems). The peaks were sized and the alleles called using GeneMapper software and the internal ROX GS500LIZ-3730 size standard. This system has the advantages of automated filling of capillaries, automated sample loading and rapid electrophoresis (Buhariwalla and Crouch, 2004). To verify the repeatability of each PCR and each capillary electrophoresis run, a control sample (accession BTx623) was included during the PCR of each SSR marker and during each capillary electrophoresis run. Allelobin software was used for checking the quality of markers.

3.7 Data analysis

All SSR markers showed high reproducibility, with high consistency in the amplified product between the PCR and ABI runs of the control, Btx623 and therefore all the 12 markers were included in the analysis. Alleles were called and scored using the GeneMapper version 3.7 software then the data was subjected to allelobin software to check the quality of the SSR markers that were used in this study. The data generated from the allelobin was analysed using PowerMarker version 3.25 to calculate the Polymorphic Information Content (PIC), heterozygosity and number of alleles for each marker, %of polymorphic loci estimates, genetic diversity within and among accessions, genetic distances within and among accessions. PIC values give the information the measure of usefulness of each marker in distinguishing one individual from another.

TFPGA provided three estimates of heterozygosity; direct count, expected heterozygosities under Hardy-Weinburg Equilibrium (H-W) and Nei`s (1978) unbiased heterozygosity. The estimates were calculated from each locus and averages obtained

over each loci. Allele and genotype frequencies were calculated using haplotype diversity values calculated according to Nei (1978) (PowerMarker version 3.25).

Darwin Version 5.0 software was used to calculate the principle component analysis (PCA) and clustering within and among the accessions. To determine the genetic relationships and differentiation, the 96 accessions were clustered based on the matrix of genetic similarities using the Un-weighted Pair Group Method using Arithmetic Averages (UPGMA) clustering algorithm. Dissimilarity was calculated from allelic data, where dissimilarity index was calculated by simple matching. The distances were computed for microsatellite data (12 loci) and trees constructed using the neighbour- joining method using Darwin Version 5.0 software. The principle component scores were not standardized and thus had variance equal to the corresponding Eigen values. The UPGMA results were used to generate dendrograms. The robustness of the phylogenies was evaluated by bootstrapping (1000 permutations) replicates over all loci. An exact test was used to determine possible deviations from Hardy-Weinberg Equilibrium and the existence of non-random associations of genotypes across polymorphic co-dominant loci (Guo and Thompson 1992; Weir 1990). Exact tests were performed using the program TFPGA. Analysis of molecular variance (AMOVA, Excoffier et al., 1992) was used to partition SSR variation within the accessions. For this purpose, a modified version of the data was used by dividing the allele sizes for each primer into the repeat as described in the user's manual. Significance levels for variance component estimates were computed by a non-parametric permutation procedure, using 1000 permutations. AMOVA and F_{st} indices were calculated using the ARLEQUIN program, version 3.11 (http://cmpg.unibe.ch/software/arlequin3).

Geographical information use in the Data Analysis

Geographic Information System was also used whereby current climate conditions were downloaded from WorldClim (http://www.worldclim.org). Bioclimatic variables for the 92 accession records were extracted from the climatic datasets using DIVA GIS software. Each point had 19 bioclimatic climatic variables; only variables related to rainfall and temperature were used in the study. The average monthly temperature and annual mean rainfall variable were extracted from the climate dataset to produce a table showing the relationship. All base maps were obtained from DIVA GIS (http://www.diva-gis.org) with a reference scale of 1:10000 as ESRI shape files or grids. Using DIVA GIS, the average monthly temperature and annual mean rainfall climate grids were extracted from the current climate dataset as GIS files.

Maps were generated using a combination of different GIS and computer software. Accession points shape files were created using ArcGIS 9.3.1 and exported to DIVA GIS for further manipulation. The accession records used in this study was obtained from databases held in the Tanzania National Gene bank. The passport data contained 92 accession records of *Sorghum bicolor*. Each accession record consisted information on the latitude, longitude and altitude. Using ArcGIS 9.3.1 the passport data was overlaid with the average monthly temperature and annual mean rainfall climate grids to produce GIS maps showing the collecting localities and the environmental conditions of those localities.

CHAPTER FOUR

RESULTS AND DISCUSSION

The neighbor joining phenogram depicting genetic relation among the sorghum *bicolor* accessions showed 2major groups (fig 6). Each cluster consisted of 46 accessions. The two main clusters mainly subdivided according to their genetic background. Most varieties are found in the upper cluster while most landraces are in the lower cluster and therefore materials divided according to their genetic background. These clusters then further divided into smaller groups. Most of the accessions were scattered into different clusters regardless of their geographical origin while some accessions from the same region clustered together, this shows that there was no much differentiation between the sub populations i.e. the geographical regions indicating similarity in the sorghum cultivars found in these sub populations. There are so many sub clusters dividing from the main clusters and this suggests that there is high level of diversity in these Tanzanians accessions.

Looking at the smaller sub clusters beginning with the upper part of the figure 6 we have about three sub clusters and looking at the groups individually a lot of information can be got in the way the accessions are clustering, for example, the first group that is consisting of most of the Marcia accession of sorghum, its observed that this group have materials majorly from Dodoma and Morogoro and all are varieties, the only material that is not from this area is Marcia 83-0 which is from Dar salaam and is also a variety. This accession may have the same genetic composition with another type of sorghum in this cluster or there could have been some mislabeling. The second cluster in this group is composed also by materials majorly from Dodoma and Morogoro and they are all landraces apart from one accession which is a variety and this is Pato Red 213-0, so this

cluster is majorly composed of landraces and this accession which is a variety most probably was raised from these landraces. The other group that has Mtwama Mweupe is composed of materials majorly from Babati and Kilimanjaro regions and all these accessions are varieties with one or two landraces and therefore this cluster is composed mainly of varieties and therefore materials in this cluster also clustered on the basis of genotype and not location.

Looking at the other major cluster the one in the lower region it is observed that there was three major sub clusters and looking at the sub clusters critically we get a lot of information on how the materials clustered. Starting with the first group on our left it is observed that the materials are from Dodoma, Morogoro, Kilimanjaro and also one accession from Mtwara, all these materials are landraces apart from two of them which are varieties and these are Hakika and Roman and a further study can probably show that these varieties were raised from these landraces. Another cluster in this group also shows that materials are majorly landraces with just one accession being a variety. The materials are also majorly from Dodoma and Morogoro. The last cluster on the far right in this lower group also reveals that the materials clustered on the basis of their genotype in that all the materials were also landraces with just one accession Serena 7-0 being a variety.

Although the materials do not cluster according to their geographical location we see that they cluster according to their genetic background i.e. the landraces clustered together while the varieties also clustered together.

As a measure of genetic diversity, the number of alleles and their frequency was analyzed. Using the powermarker software, the average number of alleles detected in this study was 8.0833 with a range of 3 to 22 alleles per locus (table 2). The high number of

alleles and the wide range of alleles per locus indicate high genetic diversity in the sorghum germplasm which is also revealed by the high value of the genetic diversity index (0.69). In a study of genetic diversity in Eritrean sorghum landraces using SSR markers, the number of alleles per locus observed ranged from 7 to 28 with an average of 13.9 alleles per locus (Ghebru *et al.*, 2002). This shows that Eritrean sorghum has a larger diversity than that of Tanzania.

Most of the accessions given the same name or similar identification characters by farmers were grouped together regardless of their origin for example Marcia which is a variant sorghum accession, (fig 8), this shows that these sorghum samples might have originated from the same origin and therefore their genetic make up might be the same regardless of the region from which they were collected.

For the intrapopulation diversity analysis six accessions were randomly picked and four plants were accessed and the results are as in (fig 9), the within diversity exists though not as much as among the accessions as analyzed by the allelobin and the Darwin software.

The materials were majorly from different regions in Dodoma, Morogoro, Kilimanjaro, Babati which is in Manyara and Mtwara all these are different regions with different climatic conditions and therefore this can be exploited in breeding programs. Bioclimatic variables for the 92 accession records were extracted from the climatic datasets using DIVA GIS software. Each point had 19 bioclimatic climatic variables; only variables related to rainfall and temperature were used in the study. This showed that Dodoma is generally a dry region with a rainfall distribution of between 415-802mm (figure 11). Morорgoro on the other hand has a rainfall of between 1035-1265, Manyara has rainfall

distribution of between 803-1034, Mtwara 1035-1265 and Kilimanjaro the highest distribution of between 1266-1618.The temperatures in these areas also vary (figure 12) and hence these areas are all distinct in their climate and rainfall patterns and therefore they can be a very good source for materials to be used in breeding programs. By looking at the two major clusters and their sub clusters it is also observed that the materials are clustering according to their environmental conditions for example materials in the upper cluster which are generally varieties require less amount of rainfall as compared to materials in the lower cluster. The sub clusters in the upper region have a temperature and rainfall with the following ranges, the first sub cluster has a temp range of 23.1-26.0, and a rainfall of 551-994, the second one has a range of 21.7-25.2 and a rainfall of 912-1355, the last of this group had a range of 15.6-24.7 and rainfall range of 582-926, looking at the other major cluster the lower one the following are the temperature and rainfall ranges, for the first sub cluster in this group the temperature range is 20.9-24.8,and a rainfall of 892-1111, the second one has a temp range of 576-998 and the last one a range of 18.2-25.9 and rainfall of 769-1415. By observing the upper major cluster generally the materials require less amount of rainfall with the exception of the second sub clusters and this cluster is mainly composed of landraces. The lower main cluster on the other hand generally requires more amount of rainfall with a few exceptions on the second cluster which have several varieties. By looking at these results it can be concluded that the varieties generally require less amount of rainfall with a few exceptions as compared to the landraces. This shows that the varieties are able to withstand drought conditions than the landraces.

Another observation is that materials from Mororgoro and Dodoma are very widely distributed throughout the clusters as compared to other materials like from Kilimanjaro, this shows that materials from Dodoma and Morogoro are very genetically diverse and can be a very good source when looking for materials that can withstand drought conditions and also be high yielding considering Dodoma is a very dry region and Morogoro a very high yielding area and therefore these materials can be further be improved and used in breeding programs. Materials in the lower cluster can also be a very good source for materials to be used in the breeding programs because generally these materials are landraces and a lot of important traits can be found in these materials.

Table 2: A summary of the number of alleles, diversity indices and average heterozygosity and polymorphic information content of each locus of sorghum.

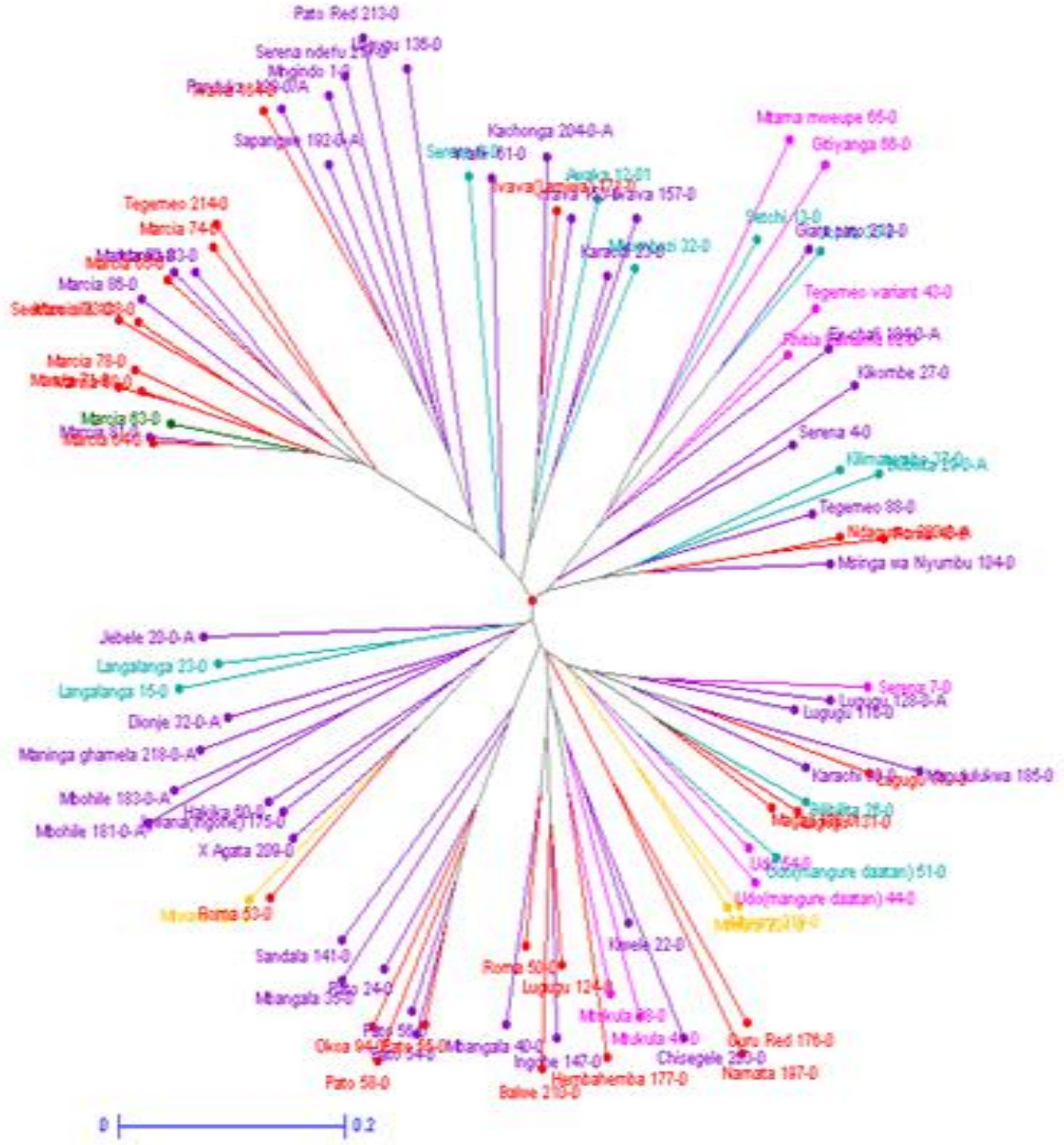
GenotypeNo	SampleSize	No. of obs.	AlleleNo	GeneDiversity	Heterozygosity	PIC
7.0000	92.0000	92.0000	4.0000	0.7137	0.4348	0.6634
10.0000	92.0000	92.0000	10.0000	0.8315	0.0000	0.8107
15.0000	92.0000	92.0000	8.0000	0.7908	0.9565	0.7616
11.0000	92.0000	91.0000	7.0000	0.6748	0.5165	0.6170
4.0000	92.0000	92.0000	3.0000	0.4237	0.0109	0.3823
8.0000	92.0000	92.0000	7.0000	0.7113	0.0109	0.6719
17.0000	92.0000	92.0000	9.0000	0.8009	0.1522	0.7745
31.0000	92.0000	92.0000	11.0000	0.8418	0.4674	0.8239
16.0000	92.0000	92.0000	9.0000	0.7386	0.8804	0.7060
5.0000	92.0000	92.0000	3.0000	0.5180	0.4130	0.4254
26.0000	92.0000	91.0000	22.0000	0.8844	0.0659	0.8747
6.0000	92.0000	92.0000	4.0000	0.3438	0.0543	0.3207
13.0000	92.0000	91.8333	8.0833	0.6894	0.3302	0.6527

Table 3: Showing the sorghum accessions, their origin and their category.

S.No	Genotype		Location,district-region	Category
1	Awaka 12-01		Kilimatembo, Moshi-Kilimanjaro	Landrace
2	Balwe 210-0		Ipala-Hombolo-Dodoma	Landrace
3	Bilibilita 26-0		Kilimatembo, Moshi vijijini-Kilimanjaro	Landrace
4	Bilibilita 29-0-A		Kilimatembo, Moshi vijijini-Kilimanjaro	Landrace
5	Chisegele 203-0		Kibaoni-Gairo, Kilosa-Morogoro	Landrace
6	Dionje 32-0-A		Kihondo/Melela, Mvomero - Morogoro	Landrace
7	Ex-chali 184-0-A		Mkata station, Kilosa-Morogoro	Landrace
8	Giant pato 212-0		Kizinga Mikese-Morogoro	Variety
9	Gitiyanga 66-0		Mdoruu-Babati	Landrace
10	Guru Red 176-0		Mkoyo Hombolo-Dodoma	Landrace
11	Hakika 60-0		Ari/Ilonga, Kilosa-Morogoro	Variety
12	Hembahemba 177-0		Hombolo Bwawani-Dodoma	Landrace
13	Igwana(Ingohe) 175-0		kibaoni/Gairo, Kilosa-Morogoro	Landrace
14	Ingohe 147-0		Ukwamani/Gairo, Kilosa-Morogoro	Landrace
15	Ivava 153-0		Ukwamani/Gairo, Kilosa-Morogoro	Landrace
16	Ivava 157-0		kibaoni/Gairo, Kilosa-Morogoro	Landrace
17	Ivava 164-0		Chamwino-Dodoma	Landrace
18	Ivava(Lamiwa) 171-0		Hombolo/Bwawani-Dodoma	Landrace
19	Jebele 20-0-A		Madudu/Dumila, Mvomero-Morogoro	Landrace
20	Kachonga 204-0-A		Ukwamani Gairo, Kilosa Morogoro	Landrace
21	Karachi 23-0		Mikese Fulwe-Morogoro	Landrace
22	Karachi 30-0		Madudu/Dumila, Mvomero-Morogoro	Landrace
23	Kikombe 27-0		Mikese Fulwe-Morogoro	Landrace
24	Kilimatembo 37-0		Kilimatembo, Moshi vijijini-Kilimanjaro	Landrace
25	Kiwele 22-0		kipogoro, Mmvomero-Morogoro	Landrace
26	Langalanga 15-0		Sango Lowas,Moshi-Kilimanjaro	Landrace
27	Langalanga 23-0		Sango Lowas,Moshi-Kilimanjaro	Landrace
28	Lugugu 110-0		Mkoyo Hombolo-Dodoma	Landrace
29	Lugugu 116-0		kibaoni/Gairo, Kilosa-Morogoro	Landrace
30	Lugugu 124-0		Hombolo Bwawani-Dodoma	Landrace
31	Lugugu 128-0-A		Ukwamani/Gairo, Kilosa-Morogoro	Landrace
32	Lugugu 131-0		Msanga-Dodoma	Landrace

33	Lugugu 136-0		Madudu/Dumila, Mvomero-Morogoro	Landrace
34	Magaji 180-0		Mkoyo/Hombolo-Dodoma	Landrace
35	Maninga ghamela 218-0-A		Mikese Koo-Morogoro	Landrace
36	Mapululukwa 185-0		Kibaoni-Gairo, Kilosa-Morogoro	Landrace
37	Marcia 62-0		Ari/Ilonga, Kilosa-Morogoro	Variety
38	Marcia 63-0		Asa Dar-Dar es Salaam	Variety
39	Marcia 64-0		Ari Hombolo-Dodoma	Variety
40	Marcia 65-0		Hombolo/Bwawani-Dodoma	Variety
41	Marcia 69-0		Ipala-Dodoma	Variety
42	Marcia 71-0		Mtumba-Dodoma	Variety
43	Marcia 73-0		Chamwino-Dodoma	Variety
44	Marcia 74-0		Msanga-Dodoma	Variety
45	Marcia 78-0		Mkoyo-Dodoma	Variety
46	Marcia 81-0		Mkata station, Kilosa-Morogoro	Variety
47	Marcia 83-0		Nyang'ambwe/Mikese-Morogoro	Variety
48	Marcia 86-0		Kizinga Mikese-Morogoro	Variety
49	Mbangala 35-0		Kihondo/Melela, Mvomero - Morogoro	Landrace
50	Mbangala 40-0		Madudu/Dumila, Mvomero-Morogoro	Landrace
51	Mbohile 181-0-A		Dumila, Mvomero-Morogoro	Landrace
52	Mbohile 183-0-A		Madudu/Dumila, Mvomero-Morogoro	Landrace
53	Mkombozi 32-0		Burunge,Moshi Vijijini-Kilimanjaro	Variety
54	Mngindo 1-0		Mikese/Fulwe-Morogoro	Landrace
55	Msinga wa Nyumbu 104-0		Madudu/Dumila, Mvomero-Morogoro	Landrace
56	Mtama mweupe 65-0		Rhotia Kainam-Babati	Landrace
57	Mtukula 38-0		Mdoruu-Babati	Landrace
58	Mtukula 41-0		Rhotia Kainam-Babati	Landrace
59	Mtwara 219-0		Mtwara(region)	Landrace
60	Mtwara 220-0		Mtwara(region)	Landrace
61	Mtwara 221-0		Mtwara(region)	Landrace
62	Namata 197-0		Mkoyo-Hombolo-Dodoma	Landrace
63	Ndagumo 200-0-A		Ipala Hombolo-Dodoma	Landrace
64	Okoa 94-0		Chamwino-Dodoma	Variety
65	Panduka 199-0/A		ukwanani Gairo, Kilosa-Morogoro	Landrace
66	Pato 24-0		Ilonga /Ari, Kilosa-Morogoro	Variety
67	Pato 54-0		Ilonga/Ari, Kilosa-Morogoro	Variety
68	Pato 55-0		Hombolo Bwawani-Dodoma	Variety
69	Pato 56-0		Ari/Hombolo, Kilosa Morogoro	Landrace

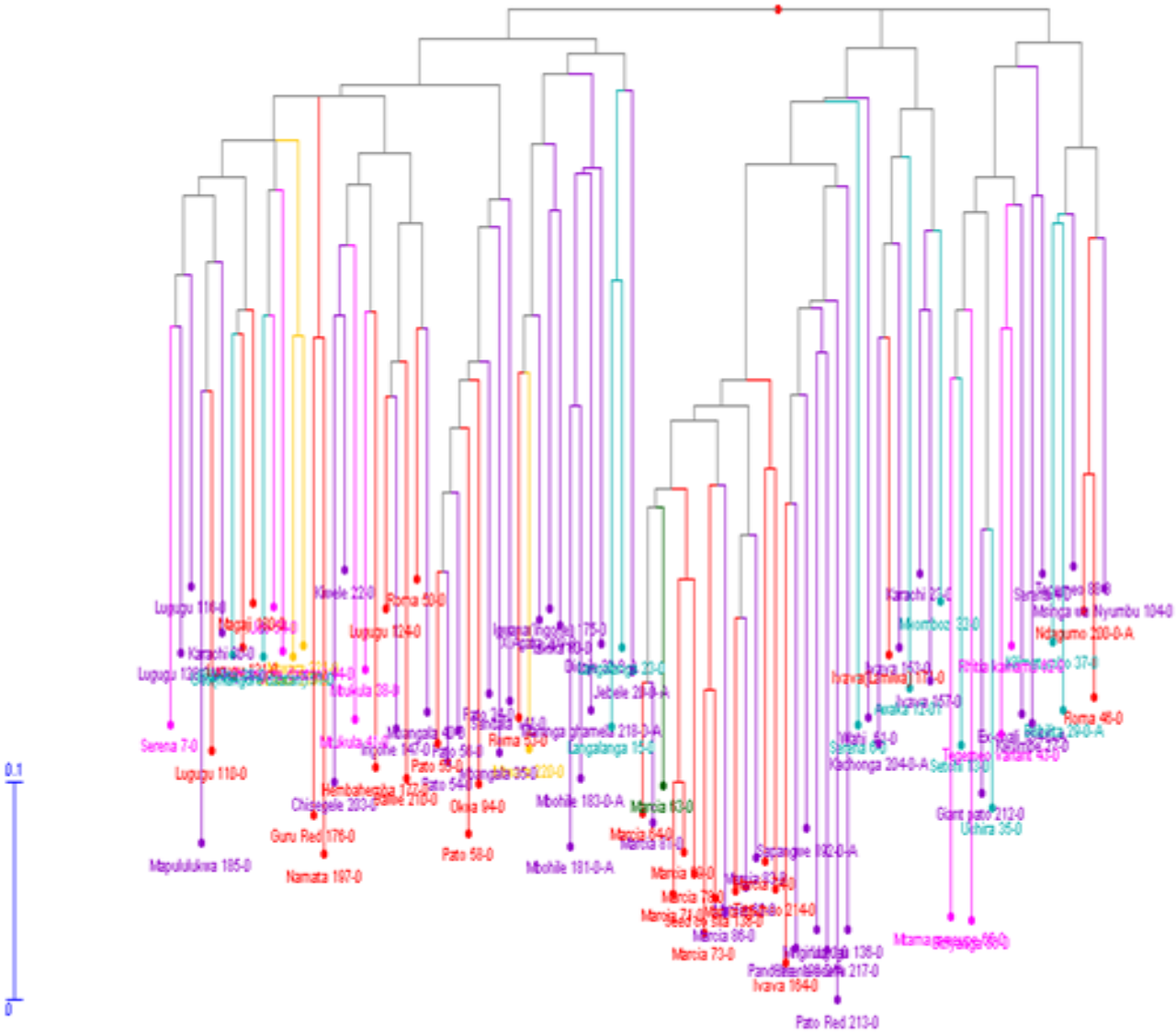
70	Pato 58-0		Chamwino-Dodoma	Variety
71	Pato Red 213-0		Kizinga Mikese-Morogoro	Variety
72	Rhitia kainamu 62-0		Rhotia Kainam-Babati	Landrace
73	Roma 46-0		Ipala-Dodoma	Variety
74	Roma 50-0		Hombolo/Bwawani-Dodoma	Variety
75	Roma 53-0		Chamwino-Dodoma	Variety
76	Sandala 141-0		Ukwamani/Gairo, Kilosa-Morogoro	Landrace
77	Sapangwe 192-0-A		Kibaoni-Gairo, Kilosa-Morogoro	Landrace
78	Seed co sila 138-0		Gift (Hombolo)-Dodoma	Variety
79	Serena 4-0		Kibaoni Gairo, Kilosa-Morogoro	Variety
80	Serena 6-0		Pomuan A, Moshi-Kilimanjaro	Variety
81	Serena 7-0		Babati(region)	Variety
82	Serena ndefu 217-0		Kibaoni Gairo, Kilosa-Morogoro	Variety
83	Setchi 13-0		Setcheda, Moshi Vijijini-Kilimanjaro	Landrace
84	Tegemeo 214-0		Mkoyo Hombolo-Dodoma	Variety
85	Tegemeo 88-0		Msimba Seed Farm, Kilosa-Morogoro	Variety
86	Tegemeo variant 43-0		Magugu-Babati	Landrace
87	Uchira 35-0		Uchira, Moshi-Kilimanjaro	Landrace
88	Udo 54-0		Mdoruu-Babati	Landrace
89	Udo(mangure daatan) 44-0		Rhotia Kainam-Babati	Landrace
90	Udo(mangure daatan) 51-0		Kilimatembo-Moshi	Landrace
91	Wahi 61-0		Ari/Ilonga, Kilosa-Morogoro	Variety
92	X Agata 209-0		Mkata station, Kilosa-Morogoro	Landrace



KEY

	Morogoro
	Kilimanjaro
	Dodoma
	Mtwara
	Dar es salam
	Babati

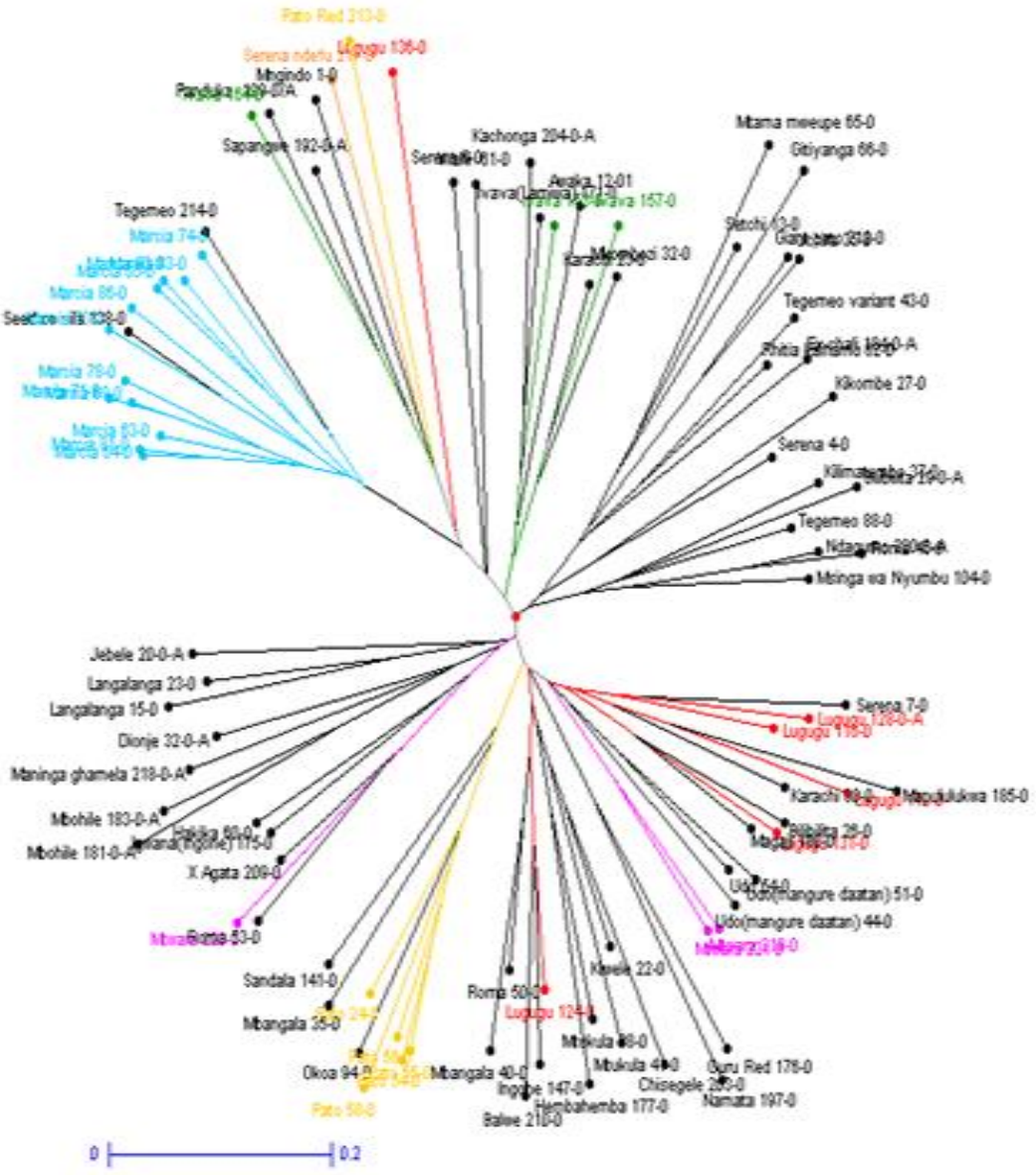
Figure 6: showing how the sorghum accessions clustered and their origin



KEY

	Morogoro
	Kilimanjaro
	Dodoma
	Mtwara
	Dar es salam
	Babati

Figure 7: Horizontal representation of sorghum accessions, their names and origin



KEY

	Marcia
	Lugugu
	Ivava
	Mtwara
	Pato

Figure 8: Sorghum accessions given similar names by farmers cluster together.

Figure 9: Results for the intrapopulation study.

Table 4: Showing the accessions, and the average monthly temperature and annual mean rainfall

RECNO	GENOTYPE	CATEGORY	Longitude	Latitude	Altitude	Av Monthly Temp	Annual Mean Rnf
1	Panduka 199-0/A	Landrace	37.0756	-6.7709	474.0000	24.7	997
2	Wahi 61-0	Variety	37.2774	-6.0523	1099.0000	20.9	892
3	Hakika 60-0	Variety	37.2774	-6.0523	1099.0000	20.9	892
4	Setchi 13-0	Landrace	37.3350	-3.4937	719.0000	23.7	582
5	Mtwara 221-0	Landrace	39.0390	-10.9405	235.0000	25.9	944
6	Mtwara 219-0	Landrace	39.0620	-10.7369	443.0000	24.6	978
7	Mtwara 220-0	Landrace	38.7663	-10.9399	306.0000	25.9	930
8	Mbangala 40-0	Landrace	37.4991	-6.2761	502.0000	24.4	998
9	Mbangala 35-0	Landrace	37.5675	-6.4605	368.0000	25.4	935
10	Mbohile 183-0-A	Landrace	37.4991	-6.2761	502.0000	24.4	998
11	Mbohile 181-0-A	Landrace	37.4991	-6.2761	502.0000	24.4	998
12	Ingohe 147-0	Landrace	37.0756	-6.7709	474.0000	24.7	997
13	Sandala 141-0	Landrace	37.0756	-6.7709	474.0000	24.7	997
14	Jebele 20-0-A	Landrace	37.4991	-6.2761	502.0000	24.4	998
15	Namata 197-0	Landrace	35.4144	-5.8815	964.0000	23.1	576
16	Lugugu 124-0	Landrace	35.9941	-5.9131	1106.0000	22.2	764
17	Lugugu 128-0-A	Landrace	37.0756	-6.7709	474.0000	24.7	997
18	Lugugu 116-0	Landrace	36.8829	-6.8292	693.0000	23.5	856
19	Lugugu 136-0	Landrace	37.4991	-6.2761	502.0000	24.4	998
20	Lugugu 110-0	Landrace	35.4144	-5.8815	964.0000	23.1	576
21	Lugugu 131-0	Landrace	36.0645	-5.9976	1015.0000	22.6	764
22	Seed co sila 138-0	Variety	35.5485	-6.3849	1015.0000	22.9	551
23	Udo(mangure daatan) 44-0	Landrace	35.4811	-4.1800	2208.0000	15.6	847
24	Udo 54-0	Landrace	35.6305	-4.3387	1634.0000	19.0	806
25	Udo(mangure daatan) 51-0	Landrace	37.4978	-3.2900	1661.0000	18.2	1415
26	Tegemeo 88-0	Variety	37.1844	-6.5297	495.0000	24.7	926
27	Tegemeo 214-0	Variety	35.4144	-5.8815	964.0000	23.1	576
28	Guru Red 176-0	Landrace	35.4144	-5.8815	964.0000	23.1	576
29	Roma 53-0	Variety	35.7352	-6.1633	1128.0000	22.6	572
30	Roma 50-0	Variety	35.4144	-5.8815	964.0000	23.1	576
31	Roma 46-0	Variety	35.9360	-6.0436	1090.0000	22.5	675
32	Ndagumo 200-0-A	Landrace	35.9360	-6.0436	1090.0000	22.5	675
33	Uchira 35-0	Landrace	37.3222	-3.3345	871.0000	23.0	912

34	Awaka 12-01	Landrace	37.4978	-3.2900	1661.0000	18.2	1415
35	Okoa 94-0	Variety	35.7352	-6.1633	1128.0000	22.6	572
36	Serena 6-0	Variety	37.3222	-3.3345	871.0000	23.0	912
37	Serena 7-0	Variety	35.9763	-4.0732	1183.0000	21.0	768
38	Serena ndefu 217-0	Variety	36.9826	-6.7958	587.0000	24.1	944
39	Serena 4-0	Variety	36.9826	-6.7958	587.0000	24.1	944
40	Kiwele 22-0	Landrace	37.5114	-7.2206	560.0000	24.1	1297
41	Maninga ghamela 218-0-A	Landrace	37.9298	-6.6807	448.0000	24.8	1021
42	Ex-chali 184-0-A	Landrace	36.9999	-6.7889	744.0000	23.2	885
43	Pato 24-0	Variety	37.2192	-6.0626	1038.0000	21.3	864
44	Pato 54-0	Variety	37.2192	-6.0626	1038.0000	21.3	864
45	Pato 56-0	Landrace	37.0756	-6.7709	474.0000	24.7	997
46	Giant pato 212-0	Variety	37.6790	-7.3464	386.0000	25.2	1355
47	Pato Red 213-0	Variety	37.6790	-7.3464	386.0000	25.2	1355
48	Pato 58-0	Variety	35.7352	-6.1633	1128.0000	22.6	572
49	Pato 55-0	Variety	35.4144	-5.8815	964.0000	23.1	576
50	Bilibilita 29-0-A	Landrace	37.4978	-3.2900	1661.0000	18.2	1415
51	Bilibilita 26-0	Landrace	37.4978	-3.2900	1661.0000	18.2	1415
52	Hembahemba 177-0	Landrace	35.4144	-5.8815	964.0000	23.1	576
53	Mtukula 38-0	Landrace	35.6305	-4.3387	1634.0000	19.0	806
54	Mtukula 41-0	Landrace	35.4811	-4.1800	2208.0000	15.6	847
55	Marcia 73-0	Variety	35.7352	-6.1633	1128.0000	22.6	572
56	Marcia 81-0	Variety	36.9999	-6.7889	744.0000	23.2	885
57	Marcia 62-0	Variety	37.2192	-6.0626	1038.0000	21.3	864
58	Marcia 83-0	Variety	38.0948	-6.8238	254.0000	26.0	1055
59	Marcia 69-0	Variety	35.9360	-6.0436	1090.0000	22.5	675
60	Marcia 64-0	Variety	35.4144	-5.8815	964.0000	23.1	576
61	Marcia 74-0	Variety	36.0645	-5.9976	1015.0000	22.6	764
62	Marcia 63-0	Variety	39.1048	-6.9386	117.0000	25.2	1063
63	Marcia 65-0	Variety	35.4144	-5.8815	964.0000	23.1	576
64	Marcia 71-0	Variety	35.8806	-6.1641	1111.0000	22.4	619
65	Marcia 78-0	Variety	35.9532	-5.9086	1099.0000	22.3	756
66	Marcia 86-0	Variety	37.8988	-6.7072	487.0000	24.6	994
67	Dionje 32-0-A	Landrace	37.4991	-6.2761	502.0000	24.4	998
68	Ivava 153-0	Landrace	37.0756	-6.7709	474.0000	24.7	997
69	Ivava(Lamiwa) 171-0	Landrace	35.4144	-5.8815	964.0000	23.1	576
70	Ivava 164-0	Landrace	35.7352	-6.1636	1128.0000	22.6	572
71	Ivava 157-0	Landrace	37.0756	-6.1633	994.0000	21.7	797

72	Langalanga 23-0	Landrace	37.3171	-3.3172	996.0000	21.8	1111
73	Langalanga 15-0	Landrace	37.3171	-3.3172	996.0000	21.8	1111
74	Magaji 180-0	Landrace	35.9532	-5.9086	1099.0000	22.3	756
75	Kachonga 204-0-A	Landrace	37.0756	-6.7709	474.0000	24.7	997
76	Karachi 23-0	Landrace	37.8988	-6.7072	487.0000	24.6	994
77	Karachi 30-0	Landrace	37.4991	-6.2761	502.0000	24.4	998
78	Gitiyanga 66-0	Landrace	35.6305	-4.3387	1634.0000	19.0	806
79	Mkombozi 32-0	Variety	37.3494	-3.3426	811.0000	23.4	857
80	Kilimatembo 37-0	Landrace	37.3222	-3.3345	871.0000	23.0	912
81	Sapangwe 192-0-A	Landrace	36.9826	-6.7958	587.0000	24.1	944
82	Chisegele 203-0	Landrace	36.9826	-6.7958	587.0000	24.1	944
83	Igwana(Ingohe) 175-0	Landrace	36.9826	-6.7958	587.0000	24.1	944
84	Mapululukwa 185-0	Landrace	36.9826	-6.7958	587.0000	24.1	944
85	X Agata 209-0	Landrace	36.9999	-6.7889	744.0000	23.2	885
86	Balwe 210-0	Landrace	35.9360	-6.0436	1090.0000	22.5	675
87	Msinga wa Nyumbu 104-0	Landrace	37.4991	-6.2761	502.0000	24.4	998
88	Mngindo 1-0	Landrace	37.8988	-6.7072	487.0000	24.6	994
89	Mtama mweupe 65-0	Landrace	35.4811	-4.1800	2208.0000	15.6	847
90	Rhitia kainamu 62-0	Landrace	35.4811	-4.1800	2208.0000	15.6	847
91	Tegemeo variant 43-0	Landrace	35.6963	-3.9858	1067.0000	21.6	849
92	Kikombe 27-0	Landrace	37.8988	-6.7072	487.0000	24.6	994

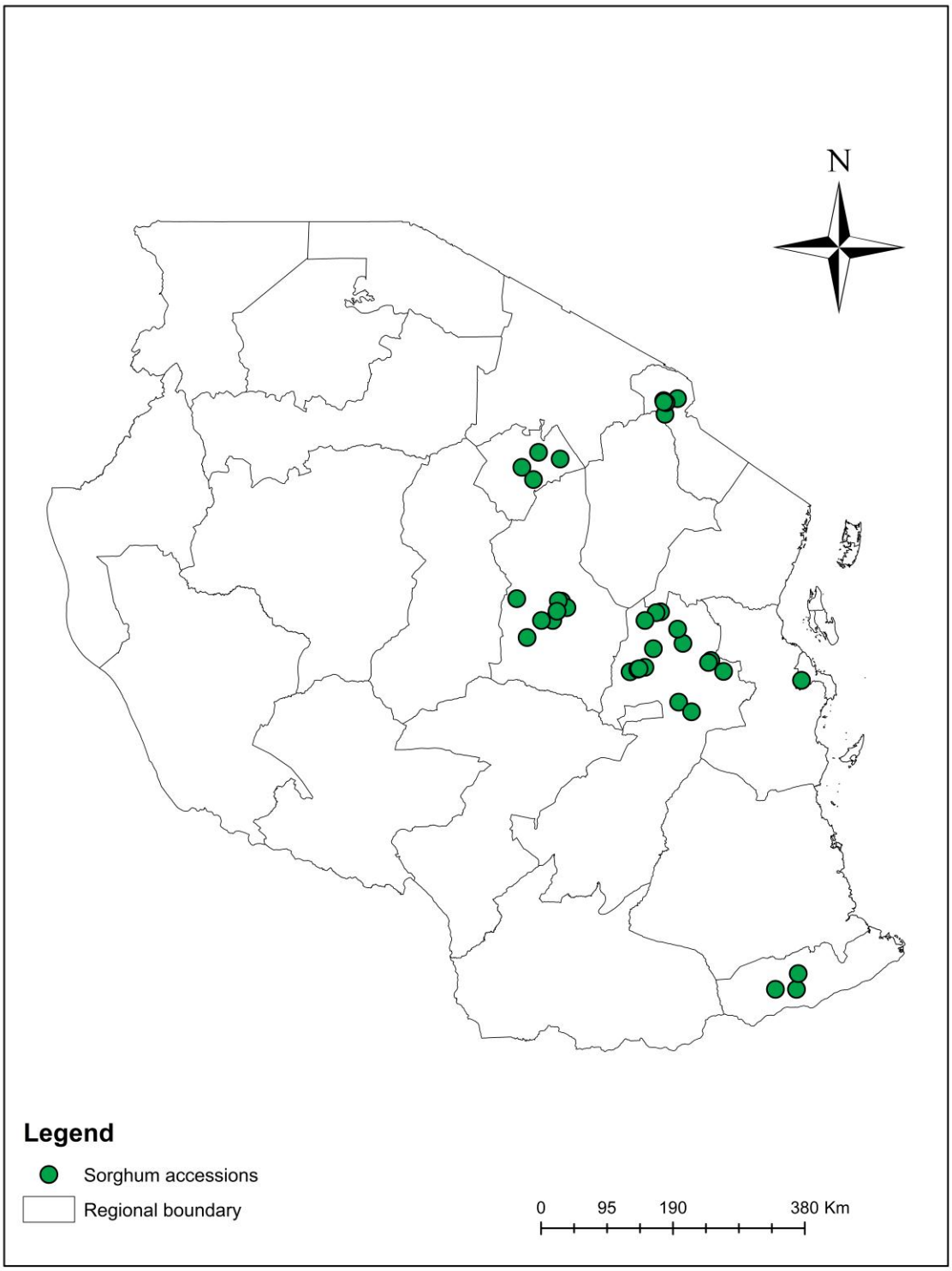


Figure 10: Showing sorghum accessions and where they were collected

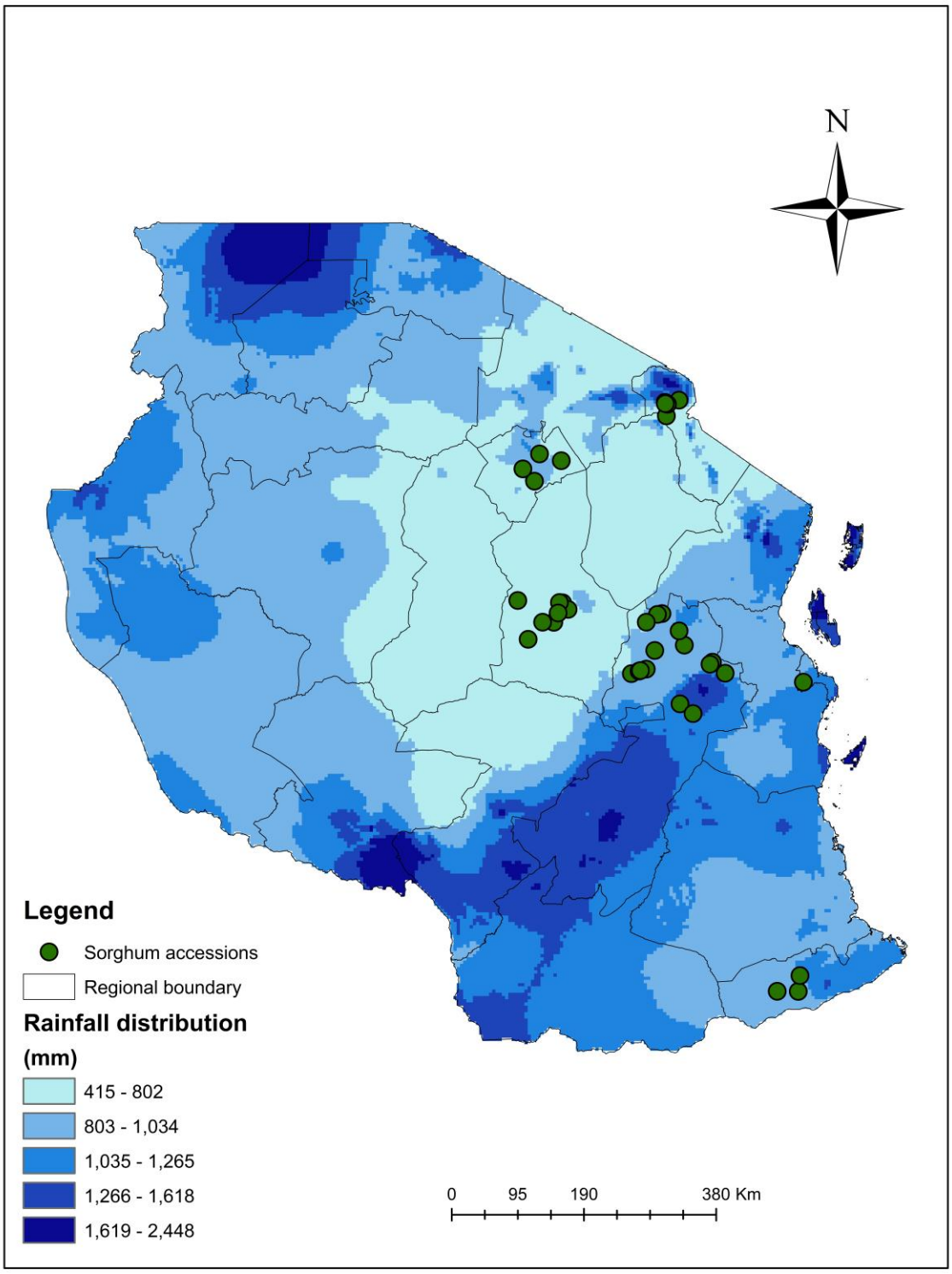


Figure 11: Showing the accessions and the rainfall distribution of the area which they were collected

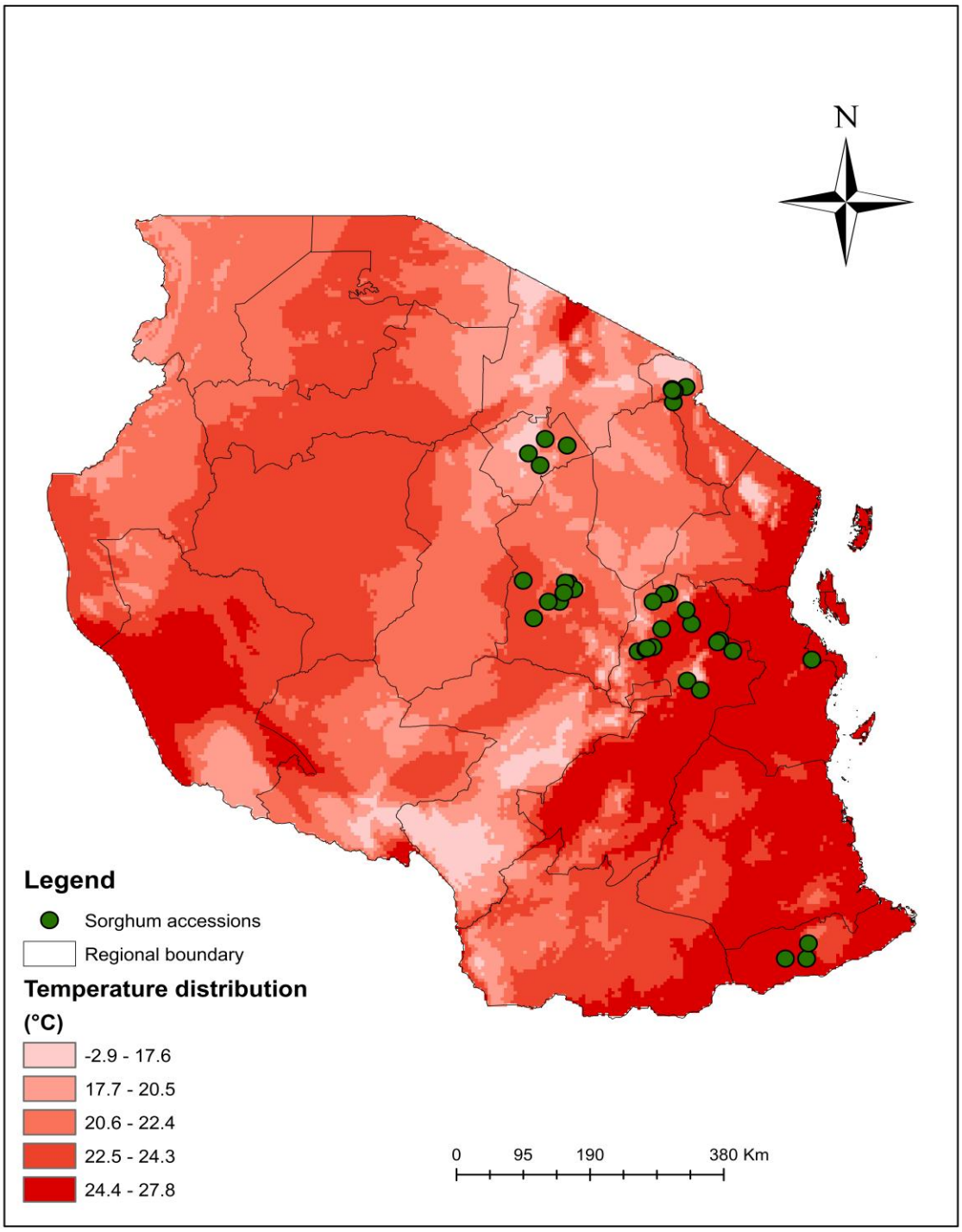


Figure 12: Showing the accessions and the temperature distribution of the area which they were collected

CHAPTER FIVE

CONCLUSIONS AND RECCOMENDATIONS

5.1 Conclusion

The study revealed that there is a considerable amount of genetic diversity among the Tanzania sorghum and this is indicated by the high number of alleles and the clusters generated. The study also reaffirms the power of SSR Markers in the use of genetic diversity. This study also showed that the sorghum accessions in Tanzania did not cluster according to their geographical regions but rather on the bases of their genetic background. The considerable amount of genetic diversity in Tanzania can be tapped and used in breeding programs especially while generating sorghum that can withstand harsh conditions and also high yielding and materials from Morogoro and Dodoma can especially be used in this because it was observed that they are very genetically diverse.

5.2 Recommendations

Further studies can be carried out on sorghum varieties that come from the drier parts like Dodoma and qualitative loci with the genes for the drought resistant can be isolated and put in farmer preferred sorghum that are high yielding and therefore have better varieties that are both high yielding and can be able to withstand harsh conditions. This will be very useful especially in Africa where food security is a big issue in this era of climate change and global warming.

Similar studies should be carried out also in Kenya if we are to mitigate and adapt to the effects of climate change and also meet our millennium development goal of food security.

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APPENDICES

Appendix 1: Pcr components

COMPONENTS	STOCK CONC	FINAL CONC	1rxn vol	100rxns
Mgcl2	50mm	2mm	0.4	28
Buffer	10x	1x	1	70
dnTps	2mm	0.16mm	0.5	35
Enzyme	5u/ul	0.2ul	0.4	28
Forward primer	2Pmol	0.2pmol	0.1	7
Label	2Pmol	0.16	0.4	28
Reverse	2Pmol	0.2pmol	0.5	35
Water			5.7	399
DNA	10ng	30ng	1	
Total			10	

Appendix 2: Genotype frequency

Marker	Allele1	Allele2	Covariance	Count	Freq	2.5% l.b.	97.5% u.b.
CIR223	104	104	0.0012	10	0.1087	0.0543	0.1630
CIR223	104	111	0.0002	9	0.0978	0.0543	0.1739
CIR223	105	105	0.0022	29	0.3152	0.2174	0.4348
CIR223	105	111	0.0007	15	0.1630	0.0761	0.2283
CIR223	106	106	0.0011	8	0.0870	0.0435	0.1413
CIR223	106	111	0.0000	16	0.1739	0.1087	0.2391
CIR223	111	111	0.0010	5	0.0543	0.0109	0.0978
CIR238	70	70	0.0005	4	0.0435	0.0109	0.0761
CIR238	72	72	0.0021	24	0.2609	0.1739	0.3370
CIR238	74	74	0.0018	19	0.2065	0.1522	0.2935
CIR238	75	75	0.0002	2	0.0217	0.0000	0.0435
CIR238	76	76	0.0005	4	0.0435	0.0000	0.0761
CIR238	82	82	0.0003	3	0.0326	0.0000	0.0652
CIR238	83	83	0.0001	1	0.0109	0.0000	0.0326
CIR238	84	84	0.0015	15	0.1630	0.0870	0.2391
CIR238	85	85	0.0013	13	0.1413	0.0870	0.2283
CIR238	89	89	0.0008	7	0.0761	0.0217	0.1522
CIR240	102	107	-0.0001	3	0.0326	0.0000	0.0652
CIR240	102	111	0.0000	3	0.0326	0.0000	0.0652
CIR240	103	109	0.0000	5	0.0543	0.0109	0.1087
CIR240	103	111	0.0000	5	0.0543	0.0109	0.0978
CIR240	104	107	0.0000	1	0.0109	0.0000	0.0326
CIR240	104	109	0.0000	6	0.0652	0.0217	0.1087
CIR240	104	111	0.0000	12	0.1304	0.0870	0.2065
CIR240	105	109	0.0000	1	0.0109	0.0000	0.0326
CIR240	105	111	0.0000	2	0.0217	0.0000	0.0435
CIR240	106	107	0.0001	2	0.0217	0.0000	0.0543
CIR240	106	109	0.0001	12	0.1304	0.0761	0.2174
CIR240	106	111	-0.0001	31	0.3370	0.2500	0.4239
CIR240	107	111	0.0001	4	0.0435	0.0109	0.0978
CIR240	109	109	0.0009	4	0.0435	0.0109	0.0761
CIR240	109	111	0.0006	1	0.0109	0.0000	0.0326
CIR248	101	101	0.0014	17	0.1868	0.1000	0.2667
CIR248	101	91	0.0008	27	0.2967	0.2198	0.4066
CIR248	101	93	0.0004	5	0.0549	0.0000	0.0989
CIR248	101	94	0.0000	2	0.0220	0.0000	0.0543
CIR248	101	95	0.0001	6	0.0659	0.0217	0.1111
CIR248	101	96	0.0000	2	0.0220	0.0000	0.0549

CIR248	88	93	0.0000	2	0.0220	0.0000	0.0549
CIR248	91	91	0.0016	18	0.1978	0.1087	0.2747
CIR248	91	93	0.0004	3	0.0330	0.0000	0.0659
CIR248	93	93	0.0009	6	0.0659	0.0326	0.1099
CIR248	95	95	0.0005	3	0.0330	0.0000	0.0879
xcup53	186	186	0.0010	9	0.0978	0.0435	0.1630
xcup53	195	195	0.0021	67	0.7283	0.6304	0.8261
xcup53	195	196	0.0013	1	0.0109	0.0000	0.0326
xcup53	196	196	0.0015	15	0.1630	0.0978	0.2283
xgap72	182	182	0.0008	7	0.0761	0.0326	0.1304
xgap72	184	184	0.0027	41	0.4457	0.3370	0.5326
xgap72	185	185	0.0002	2	0.0217	0.0000	0.0543
xgap72	188	188	0.0005	4	0.0435	0.0109	0.0870
xgap72	190	190	0.0020	23	0.2500	0.1739	0.3261
xgap72	190	192	0.0003	1	0.0109	0.0000	0.0326
xgap72	191	191	0.0003	3	0.0326	0.0000	0.0761
xgap72	192	192	0.0012	11	0.1196	0.0652	0.2065
xgap84	181	181	0.0003	2	0.0217	0.0000	0.0435
xgap84	181	193	0.0001	1	0.0109	0.0000	0.0326
xgap84	183	183	0.0019	20	0.2174	0.1304	0.3152
xgap84	183	187	0.0001	1	0.0109	0.0000	0.0326
xgap84	183	193	0.0008	1	0.0109	0.0000	0.0326
xgap84	183	195	0.0003	4	0.0435	0.0109	0.0761
xgap84	184	184	0.0004	3	0.0326	0.0000	0.0652
xgap84	184	193	0.0001	1	0.0109	0.0000	0.0326
xgap84	185	185	0.0005	4	0.0435	0.0000	0.0978
xgap84	187	187	0.0006	5	0.0543	0.0217	0.0870
xgap84	187	191	0.0000	1	0.0109	0.0000	0.0435
xgap84	191	191	0.0009	8	0.0870	0.0326	0.1522
xgap84	191	195	0.0001	2	0.0217	0.0000	0.0543
xgap84	193	193	0.0021	25	0.2717	0.1630	0.3587
xgap84	193	195	0.0004	3	0.0326	0.0000	0.0761
xgap84	195	195	0.0012	10	0.1087	0.0543	0.1848
xgap84	196	196	0.0001	1	0.0109	0.0000	0.0326
xtxp010	129	129	0.0005	3	0.0326	0.0109	0.0652
xtxp010	129	140	0.0001	1	0.0109	0.0000	0.0326
xtxp010	129	142	0.0001	3	0.0326	0.0000	0.0652
xtxp010	129	144	0.0000	1	0.0109	0.0000	0.0326
xtxp010	129	149	0.0000	1	0.0109	0.0000	0.0326
xtxp010	129	150	0.0002	2	0.0217	0.0000	0.0435
xtxp010	133	133	0.0002	2	0.0217	0.0000	0.0543

xtxp010	137	137	0.0004	3	0.0326	0.0000	0.0761
xtxp010	137	140	0.0001	1	0.0109	0.0000	0.0326
xtxp010	137	150	0.0001	1	0.0109	0.0000	0.0326
xtxp010	140	140	0.0013	12	0.1304	0.0761	0.1848
xtxp010	140	142	0.0003	1	0.0109	0.0000	0.0326
xtxp010	140	144	0.0000	1	0.0109	0.0000	0.0326
xtxp010	140	146	0.0000	1	0.0109	0.0000	0.0217
xtxp010	140	149	0.0001	1	0.0109	0.0000	0.0326
xtxp010	140	150	0.0004	1	0.0109	0.0000	0.0435
xtxp010	141	142	0.0000	1	0.0109	0.0000	0.0326
xtxp010	141	149	0.0000	2	0.0217	0.0000	0.0435
xtxp010	141	150	0.0000	1	0.0109	0.0000	0.0326
xtxp010	142	142	0.0011	7	0.0761	0.0217	0.1196
xtxp010	142	146	0.0001	1	0.0109	0.0000	0.0326
xtxp010	142	149	0.0000	5	0.0543	0.0109	0.1087
xtxp010	142	150	0.0002	12	0.1304	0.0652	0.1957
xtxp010	143	143	0.0006	5	0.0543	0.0109	0.0870
xtxp010	143	150	0.0001	4	0.0435	0.0109	0.0761
xtxp010	144	144	0.0002	1	0.0109	0.0000	0.0326
xtxp010	146	146	0.0003	2	0.0217	0.0000	0.0435
xtxp010	146	149	0.0000	1	0.0109	0.0000	0.0326
xtxp010	149	149	0.0004	1	0.0109	0.0000	0.0326
xtxp010	149	150	0.0002	1	0.0109	0.0000	0.0326
xtxp010	150	150	0.0014	13	0.1413	0.0761	0.2283
xtxp012	191	238	0.0000	2	0.0217	0.0000	0.0543
xtxp012	193	193	0.0003	1	0.0109	0.0000	0.0326
xtxp012	193	238	0.0000	6	0.0652	0.0217	0.1304
xtxp012	195	195	0.0010	6	0.0652	0.0109	0.1196
xtxp012	195	201	0.0002	1	0.0109	0.0000	0.0326
xtxp012	195	238	0.0002	30	0.3261	0.2391	0.4239
xtxp012	195	284	0.0000	1	0.0109	0.0000	0.0326
xtxp012	197	197	0.0005	1	0.0109	0.0000	0.0326
xtxp012	197	238	0.0000	15	0.1630	0.0761	0.2391
xtxp012	197	284	0.0000	1	0.0109	0.0000	0.0326
xtxp012	199	199	0.0005	2	0.0217	0.0000	0.0435
xtxp012	199	238	0.0001	11	0.1196	0.0543	0.1739
xtxp012	201	201	0.0004	1	0.0109	0.0000	0.0326
xtxp012	201	238	0.0000	11	0.1196	0.0543	0.1630
xtxp012	207	238	0.0000	1	0.0109	0.0000	0.0326
xtxp012	238	284	0.0000	2	0.0217	0.0000	0.0543
xtxp136	237	237	0.0004	3	0.0326	0.0109	0.0652

xtxp136	237	241	0.0001	3	0.0326	0.0000	0.0761
xtxp136	238	238	0.0016	37	0.4022	0.3152	0.4783
xtxp136	238	241	0.0013	35	0.3804	0.2935	0.4674
xtxp136	241	241	0.0014	14	0.1522	0.0652	0.2174
xtxp265	175	175	0.0005	4	0.0440	0.0110	0.0761
xtxp265	180	180	0.0001	1	0.0110	0.0000	0.0333
xtxp265	181	202	0.0000	1	0.0110	0.0000	0.0330
xtxp265	184	184	0.0002	1	0.0110	0.0000	0.0330
xtxp265	184	191	0.0000	1	0.0110	0.0000	0.0333
xtxp265	184	204	0.0000	1	0.0110	0.0000	0.0330
xtxp265	190	190	0.0006	5	0.0549	0.0110	0.1196
xtxp265	190	213	0.0000	1	0.0110	0.0000	0.0333
xtxp265	191	191	0.0003	2	0.0220	0.0000	0.0543
xtxp265	193	193	0.0005	4	0.0440	0.0110	0.0787
xtxp265	194	194	0.0001	1	0.0110	0.0000	0.0326
xtxp265	196	196	0.0001	1	0.0110	0.0000	0.0341
xtxp265	197	197	0.0002	2	0.0220	0.0000	0.0444
xtxp265	201	201	0.0001	1	0.0110	0.0000	0.0326
xtxp265	201	213	0.0000	1	0.0110	0.0000	0.0333
xtxp265	204	204	0.0018	19	0.2088	0.1413	0.2935
xtxp265	207	207	0.0016	16	0.1758	0.1087	0.2556
xtxp265	210	210	0.0014	13	0.1429	0.0761	0.2069
xtxp265	210	213	0.0001	1	0.0110	0.0000	0.0333
xtxp265	213	213	0.0004	3	0.0330	0.0000	0.0652
xtxp265	216	216	0.0006	5	0.0549	0.0110	0.0978
xtxp265	219	219	0.0001	1	0.0110	0.0000	0.0230
xtxp265	221	221	0.0002	2	0.0220	0.0000	0.0556
xtxp265	224	224	0.0002	2	0.0220	0.0000	0.0543
xtxp265	227	227	0.0001	1	0.0110	0.0000	0.0326
xtxp265	230	230	0.0001	1	0.0110	0.0000	0.0333
xtxp273	210	210	0.0006	5	0.0543	0.0217	0.0870
xtxp273	220	220	0.0016	71	0.7717	0.7065	0.8587
xtxp273	220	223	0.0009	4	0.0435	0.0000	0.0870
xtxp273	220	226	0.0002	1	0.0109	0.0000	0.0435
xtxp273	223	223	0.0010	9	0.0978	0.0435	0.1413
xtxp273	226	226	0.0003	2	0.0217	0.0000	0.0435

Appendix 3: Allele frequency

Marker	Allele	Count	Freq	Variance	SD	2.5% l.b.	97.5% u.b.
CIR223	104	29	0.1576	0.00117730	0.0343	0.0815	0.2174
CIR223	105	73	0.3967	0.00215844	0.0465	0.3152	0.4728
CIR223	106	32	0.1739	0.00108901	0.0330	0.1196	0.2283
CIR223	111	50	0.2717	0.00096958	0.0311	0.2120	0.3261
CIR238	70	8	0.0435	0.00045204	0.0213	0.0109	0.0870
CIR238	72	48	0.2609	0.00209583	0.0458	0.1630	0.3478
CIR238	74	38	0.2065	0.00178120	0.0422	0.1196	0.2935
CIR238	75	4	0.0217	0.00023116	0.0152	0.0000	0.0543
CIR238	76	8	0.0435	0.00045204	0.0213	0.0109	0.0761
CIR238	82	6	0.0326	0.00034288	0.0185	0.0109	0.0761
CIR238	83	2	0.0109	0.00011686	0.0108	0.0000	0.0326
CIR238	84	30	0.1630	0.00148326	0.0385	0.0870	0.2391
CIR238	85	26	0.1413	0.00131889	0.0363	0.0652	0.1957
CIR238	89	14	0.0761	0.00076411	0.0276	0.0326	0.1196
CIR240	102	6	0.0326	0.00016566	0.0129	0.0109	0.0652
CIR240	103	10	0.0543	0.00026326	0.0162	0.0272	0.0924
CIR240	104	19	0.1033	0.00044530	0.0211	0.0598	0.1467
CIR240	105	3	0.0163	0.00008572	0.0093	0.0000	0.0380
CIR240	106	45	0.2446	0.00067903	0.0261	0.1957	0.2935
CIR240	107	10	0.0543	0.00026326	0.0162	0.0217	0.0870
CIR240	109	33	0.1793	0.00086138	0.0293	0.1087	0.2337
CIR240	111	58	0.3152	0.00063312	0.0252	0.2717	0.3696
CIR248	101	76	0.4176	0.00140465	0.0375	0.3297	0.4835
CIR248	88	2	0.0110	0.00005905	0.0077	0.0000	0.0278
CIR248	91	66	0.3626	0.00163422	0.0404	0.2989	0.4389
CIR248	93	22	0.1209	0.00086588	0.0294	0.0449	0.1833
CIR248	94	2	0.0110	0.00005905	0.0077	0.0000	0.0275
CIR248	95	12	0.0659	0.00049564	0.0223	0.0222	0.1067
CIR248	96	2	0.0110	0.00005905	0.0077	0.0000	0.0222
xcup53	186	18	0.0978	0.00095931	0.0310	0.0435	0.1522
xcup53	195	135	0.7337	0.00209423	0.0458	0.6576	0.7989
xcup53	196	31	0.1685	0.00149322	0.0386	0.1033	0.2500
xgap72	182	14	0.0761	0.00076411	0.0276	0.0326	0.1304
xgap72	184	82	0.4457	0.00268529	0.0518	0.3587	0.5543
xgap72	185	4	0.0217	0.00023116	0.0152	0.0000	0.0543
xgap72	188	8	0.0435	0.00045204	0.0213	0.0109	0.0870
xgap72	190	47	0.2554	0.00203772	0.0451	0.1793	0.3370

xgap72	191	6	0.0326	0.00034288	0.0185	0.0109	0.0761
xgap72	192	23	0.1250	0.00115932	0.0340	0.0652	0.2011
xgap84	181	5	0.0272	0.00025781	0.0161	0.0000	0.0598
xgap84	183	46	0.2500	0.00186082	0.0431	0.1522	0.3261
xgap84	184	7	0.0380	0.00036825	0.0192	0.0109	0.0815
xgap84	185	8	0.0435	0.00045204	0.0213	0.0000	0.0870
xgap84	187	12	0.0652	0.00060358	0.0246	0.0217	0.1359
xgap84	191	19	0.1033	0.00091789	0.0303	0.0380	0.1522
xgap84	193	56	0.3043	0.00212409	0.0461	0.2120	0.3967
xgap84	195	29	0.1576	0.00117730	0.0343	0.0978	0.2228
xgap84	196	2	0.0109	0.00011686	0.0108	0.0000	0.0326
xtxp010	129	14	0.0761	0.00052781	0.0230	0.0326	0.1196
xtxp010	133	4	0.0217	0.00023116	0.0152	0.0000	0.0543
xtxp010	137	8	0.0435	0.00039297	0.0198	0.0054	0.0870
xtxp010	140	31	0.1685	0.00131600	0.0363	0.1033	0.2554
xtxp010	141	4	0.0217	0.00011301	0.0106	0.0054	0.0489
xtxp010	142	37	0.2011	0.00106686	0.0327	0.1359	0.2554
xtxp010	143	14	0.0761	0.00064596	0.0254	0.0326	0.1250
xtxp010	144	4	0.0217	0.00017208	0.0131	0.0000	0.0489
xtxp010	146	7	0.0380	0.00030917	0.0176	0.0054	0.0652
xtxp010	149	13	0.0707	0.00038879	0.0197	0.0326	0.1196
xtxp010	150	48	0.2609	0.00144602	0.0380	0.1957	0.3370
xtxp012	191	2	0.0109	0.00005779	0.0076	0.0000	0.0272
xtxp012	193	8	0.0435	0.00027482	0.0166	0.0163	0.0815
xtxp012	195	44	0.2391	0.00103251	0.0321	0.1848	0.2935
xtxp012	197	18	0.0978	0.00048672	0.0221	0.0543	0.1359
xtxp012	199	15	0.0815	0.00048896	0.0221	0.0380	0.1196
xtxp012	201	14	0.0761	0.00040966	0.0202	0.0435	0.1033
xtxp012	207	1	0.0054	0.00002922	0.0054	0.0000	0.0163
xtxp012	238	78	0.4239	0.00035059	0.0187	0.3967	0.4565
xtxp012	284	4	0.0217	0.00011301	0.0106	0.0054	0.0489
xtxp136	237	9	0.0489	0.00041705	0.0204	0.0163	0.0870
xtxp136	238	109	0.5924	0.00159082	0.0399	0.5054	0.6739
xtxp136	241	66	0.3587	0.00137796	0.0371	0.2880	0.4348
xtxp265	175	8	0.0440	0.00046180	0.0215	0.0109	0.0879
xtxp265	180	2	0.0110	0.00011943	0.0109	0.0000	0.0333
xtxp265	181	1	0.0055	0.00002986	0.0055	0.0000	0.0165
xtxp265	184	4	0.0220	0.00017583	0.0133	0.0055	0.0543
xtxp265	190	11	0.0604	0.00059384	0.0244	0.0165	0.1044
xtxp265	191	5	0.0275	0.00026341	0.0162	0.0000	0.0611
xtxp265	193	8	0.0440	0.00046180	0.0215	0.0110	0.0769

xtxp265	194	2	0.0110	0.00011943	0.0109	0.0000	0.0330
xtxp265	196	2	0.0110	0.00011943	0.0109	0.0000	0.0444
xtxp265	197	4	0.0220	0.00023621	0.0154	0.0000	0.0543
xtxp265	201	3	0.0165	0.00014796	0.0122	0.0000	0.0389
xtxp265	202	1	0.0055	0.00002986	0.0055	0.0000	0.0165
xtxp265	204	39	0.2143	0.00182000	0.0427	0.1154	0.2889
xtxp265	207	32	0.1758	0.00159242	0.0399	0.1304	0.2637
xtxp265	210	27	0.1484	0.00135820	0.0369	0.0778	0.1978
xtxp265	213	9	0.0495	0.00042597	0.0206	0.0111	0.0989
xtxp265	216	10	0.0549	0.00057062	0.0239	0.0000	0.1087
xtxp265	219	2	0.0110	0.00011943	0.0109	0.0000	0.0341
xtxp265	221	4	0.0220	0.00023621	0.0154	0.0000	0.0543
xtxp265	224	4	0.0220	0.00023621	0.0154	0.0000	0.0549
xtxp265	227	2	0.0110	0.00011943	0.0109	0.0000	0.0333
xtxp265	230	2	0.0110	0.00011943	0.0109	0.0000	0.0330
xtxp273	210	10	0.0543	0.00055863	0.0236	0.0109	0.0978
xtxp273	220	147	0.7989	0.00159852	0.0400	0.7120	0.8587
xtxp273	223	22	0.1196	0.00102608	0.0320	0.0598	0.1848
xtxp273	226	5	0.0272	0.00025781	0.0161	0.0000	0.0543