ASSESSMENT OF KENYAN SORGHUM DIVERSITY USING MORPHOLOGICAL CHARACTERS AND MOLECULAR TECHNIQUES

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

To my husband John Kisilu, Son Mark and daughter Sarah for your support, understanding and patience, during this study. I thank God for your love and kindness.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ANOVA: Analysis of variance

AMOVA: Analysis of molecular variance

PCA: Principal component analysis

DNA: Deoxyribonucleic acid

PCA: Principal component analysis

PCoA: Principal coordinate analysis

PIC: Polymorphic Information content

RNA: Ribonucleic acid

RNase-A: Ribonuclease-A

SSR: Simple sequence repeats

ABSTRACT

Characterization of the available Kenyan sorghum genetic diversity is important to understand the dynamics of the genetic resources and to improve and sustain sorghum productivity. The main aim of this study was to assess the extent and structure of diversity in sorghum landraces from Kenya. Phenotypic and genotypic data was used to assess diversity in 148 entries consisting of accessions from Western, Turkana, Coast and Eastern regions and breeders lines. Morphological characterization showed high yields among the accessions as revealed by the means of panicle branches (43), panicle length (21cm), and grain weight (1.5g). Late maturity was also observed as shown by the mean number of days to 50% flowering (88 days). Positive correlations were found between all the traits except with days to 50% flowering. All traits showed significant differences. Number of panicle branches had the highest Broad-sense heritability of 0.957. The phenotypic PCA scatter plot clustered the accessions into three groups while the cluster analysis gave two major groups subdivided into four sub-clusters.

Molecular characterization using 39 SSR/microsatellite markers gave an average PIC value of 0.54 indicating high polymorphism. The mean diversity index per locus was 0.57. The markers gave a total of 349 alleles (8.9 alleles per locus). In AMOVA all variation components were highly significant ($P < 0.001$). Diversity among the accessions within the populations was the highest at 56%. The variability within individual accessions contributed 39% and variability among geographical origins contributed only 5% of the total diversity. FST value of 0.05 was observed. A high inbreeding level ($F_{IS} = 0.59$) and a high heterozygozity deficiency ($F_{IT} = 0.61$) were observed showing an increased degree of allele fixation and high homozygosity. The genotypic cluster analysis grouped the accessions into three clusters, with a genetic distance range of 0.4 to 0.8. PCoA separated the accessions into three groups. Over all, the study revealed a high diversity among the different sorghum germplasm from Kenya. However, there was no appreciable genetic difference between the sorghum sub-populations.

Keywords: Genetic diversity, Germplasm, Phenotypic, Genotypic,
Sorghum, SSR/microsatellites, Morphological.

CHAPTER 1: INTRODUCTION

1.1 Background information

Sorghum (*Sorghum bicolor* (L.) Moench is the fifth most important grain crop worldwide after maize (*Zea mays*), wheat (*Triticum species*), rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.) in terms of both production and area planted (FAOSTAT, 2004; FAO 1995a) and the second highly produced cereal in African after maize (Gerda and Christopher 2007). It is a widely distributed crop that can grow in both temperate and tropical zones (National academy of sciences, 1996). Sorghum is well adapted to growing in dry and marginalized areas (Gerda and Christopher 2007) with annual rainfalls ranging from 350 to 750 mm hence (Markus and Gurling, 2006; FAO 1995a).

Due to its ability to adapt to hard environments, and to an astounding array of soils, sorghum is 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). It is a staple food grain for millions of people in the semi-arid areas of the world, particularly in Africa and India (Taylor and Emmambux, 2008). The crop is grown in an area ranging between 44 to 46 million ha worldwide. This includes Africa, Asia, Oceania, and the Americas (fig 1) (Mamoudou *et al*, 2006; Dogget and Prasada Rao, 1995; FAO, 1995a). Ethiopia is considered the centre of origin (Dogget and Prasada Rao, 1995).

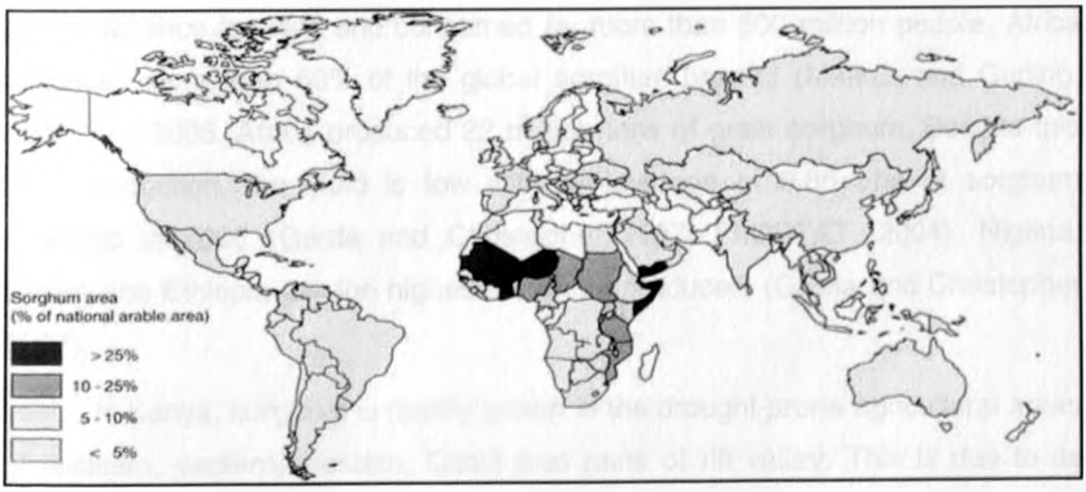


Figure 1: The global Sorghum producing areas (FAO, 1995a)

In areas with deep soils and assured rainfall sorghum yields can reach 2-2.5 t/ha, but in many low-rainfall areas yields are less than 500 kg/ha. Worldwide, the productivity between regions varies a lot due to the degree of commercialization and the corresponding adoption of new technologies. The average global yields are 0.8 t/ha in Africa, 1.2 t/ha in Asia, over 4 t/ha in America, and over 5 t/ha in Europe (fig 2) (FAOSTAT, 2004). The current total global yields are between 50 million to 60 million Metric tons (USDA, 2009)

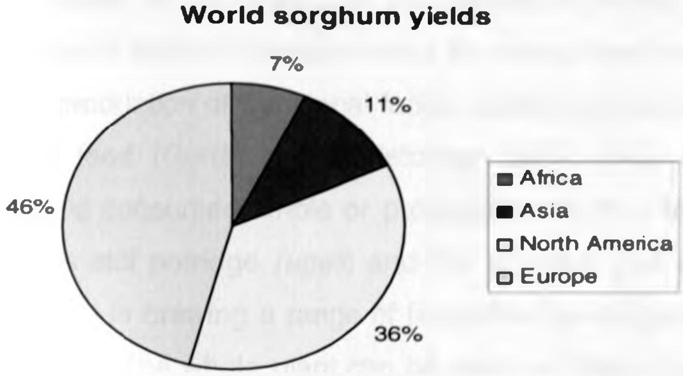


Figure 2: The world sorghum yields. Source of data: FAOSTAT, 2004

The bulk of African sorghum production is concentrated in areas where rainfall is less than 500mm due to its drought tolerance. These are the Northern parts of Africa, drier parts of the Western and Central African countries, the semi arid parts of East Africa and the drier western parts of Southern Africa (Taylor and Emmambux, 2008). It is produced in more than 30 countries in Africa, mainly by subsistence farmers and consumed by more than 500 million people. Africa produces more than 50% of the global sorghum harvest (Markus and Gurling, 2006). In 2005, Africa produced 22 million tons of grain sorghum. Despite this high production, the yield is low with an average of 0.85 t/ha of sorghum recorded in 2006 (Gerda and Christopher 2007; FAOSTAT, 2004). Nigeria, Sudan and Ethiopia are the highest sorghum producers (Gerda and Christopher 2007).

In Kenya, sorghum is mainly grown in the drought-prone agricultural areas of northern, eastern, western, Coast and parts of rift valley. This is due to its tolerance to drought, infertile soil and high temperatures (EPZ, 2005). Sorghum is grown largely under rain fed conditions. It contributes highly to food security for many subsistent farmers due to its capacity to give yields in the harsh environments where other crops grow or yield poorly (CFC and ICRISAT, 2004). Many poor families rely on sorghum for food and nutritional security, and when there are better production conditions, they also earn additional income from selling the surplus produce.

More than 35% of sorghum in the world is grown for human consumption while the rest is used for animal feed, alcohol production and industrial products (Mamoudou *et al*, 2006). In the developed world, sorghum is produced by commercial farmers, predominantly for animal feed but in Africa, it is mainly used in the production of traditional foods, traditional and commercial beer, as well as animal feed (Gerda and Christopher 2007; FAO, 1995a). Sorghum grain in Kenya is consumed whole or processed into flour from which traditional meals such as stiff porridge *(ugali)* and thin porridge *(uji)* are prepared. The grain is also used in brewing a range of fermented beverages as well as feed for poultry and cattle. The whole plant can be used as forage, hay or silage. Stems can be used for building, fencing and broom making (National academy of sciences, 1996).

1.2 Problem statement

Many farmers in dry land areas of Kenya prefer maize to sorghum, mainly because maize does not need a lot of labor in bird scaring and also due to consumer preferences. However, due to the problem of climate change, smallholder farmers not yet growing sorghum will need to consider growing it as conditions change, thus better varieties may need to be produced for them.

For a long time, production increase of sorghum in Kenya was mainly achieved by developing more varieties with higher yields through conventional breeding. The sorghum programs top priority in breeding has been the achievement of high and stable grain yield with an improved harvest index and grain quality. Impressive yield gains through cultivar improvement have been realized by utilizing genetic variability for yield components and adaptation traits (Rai *et al.*, 1999). Several improved sorghum varieties adapted to semi-arid and tropic environments are released every year by sorghum breeders. Selection of varieties meeting specific local food and industrial requirements from this great biodiversity is of high importance for food security.

However, many sorghum accessions have been lost or are under serious risk hence genetic diversity within primary gene pools has been decreasing at an alarming rate due to modern agricultural practices. The consequences of these losses are a high risk of genetic erosion.

1.3 Justification

A comprehensive knowledge of germplasm diversity and genetic relationships among cultivated sorghum is an invaluable aid in the crop improvement strategies. Understanding of the genetic relationships among sorghum germplasm can be particularly useful in planning crosses, in assigning lines to specific heterotic groups, and for precise identification with respect to plant varietal protection. Analysis of genetic diversity in germplasm collections can facilitate reliable classification of accessions with possible utility for specific breeding purposes (Mohammadi and Prasanna, 2003). Germplasm assessment will lead to development of better sorghum varieties, which can escape drought, tolerate low soil fertility and resist pests and diseases hence increase productivity under low-input conditions. Germplasm assessment will provide a foundation for making informed decisions regarding the management and utilization of genetic resources.

More importantly, diversity data generated from the analyses can potentially be employed as a tool for mining germplasm collections for genomic regions associated with adaptive or agronomically-important traits (i.e. genes that are important in adaptation to local environments or are associated with phenotypes selected by farmers or breeders). Studies of the associations between genes and environmental variables may guide germplasm conservationists and breeders to target specific environments where traits of interests may be found.

There is an increasing demand for the establishment of molecular profiles for each of germplasm accession, so that specific germplasm accession can be selected for various crop breeding purposes. Diversity assessment of sorghum germplasm in Kenya needs to dissect the genetic difference between the genotypes thereby enabling the formulation of effective strategies for conservation, germplasm management, and selection of appropriate parents for sorghum breeding programs.

The aim of this study is therefore to unlock the genetic potential of Kenyan sorghum by assessing both phenotypic and molecular diversity. The study will involve determination of the similarities and differences among the selected sorghum accessions, establishment of the genetic relationships between the accessions and assessment of the distribution of genetic diversity among sorghum accessions from different geographic origins in Kenya. The results of this study will enhance utilization of the sorghum landrace collections. This will assist in establishing core collections that can be used to facilitate the maintenance, evaluation, and use of the collection by curators, breeders, and farmers. Ultimately the results will provide a sorghum germplasm database.

1.4 Objectives of the Study

1.4.1 General objective

To assess the genetic diversity of sorghum accessions from Kenya using phenotypic traits and SSR markers.

1.4.2 Specific objectives

- To determine the distribution of genetic diversity through phenotyping among and between sorghum accessions from different geographic origins.
- To determine the genetic diversity among the collected sorghum accessions through genotyping and establish the relationships between them.

1.5 Hypothesis

There are statistically significant differences in the diversity among and within sorghum accessions from different geographic locations of Kenya.

CHAPTER 2: LITERATURE REVIEW

2.1 Sorghum description and taxonomy

The genus *Sorghum* belongs to the family Poaceae (grass) (Gramene, 2009) and the tribe Andropogoneae (Wharton, 2005). It is divided into four species: *Sorghum halepense* (L.) Pers (Johnson grass), *S. propinquum* (K.) Hitch, *S. almum* (Columbus grass) and *S. bicolor* (L.) Moench. (Wharton, 2005). *S. bicolor* encompasses all the domesticated forms, the wild sorghums and stabilized weedy derivatives derived from inter-breeding between domesticated sorghums and their closest wild relatives (Syngenta Foundation for Sustainable Agriculture, 2007; Wharton, 2005). *S. bicolor* is further subdivided into three sub species: *S. bicolor ssp. arundinaceum* (common wild sorghum), *S. bicolor ssp. Bicolor* (grain sorghum), and *S.bicolor ssp. drummondii* (Sudan grass). Sorghum was domesticated in the north-east Africa around 3000 years ago from wild *Sorghum bicolor* subsp. *arundinaceum.*

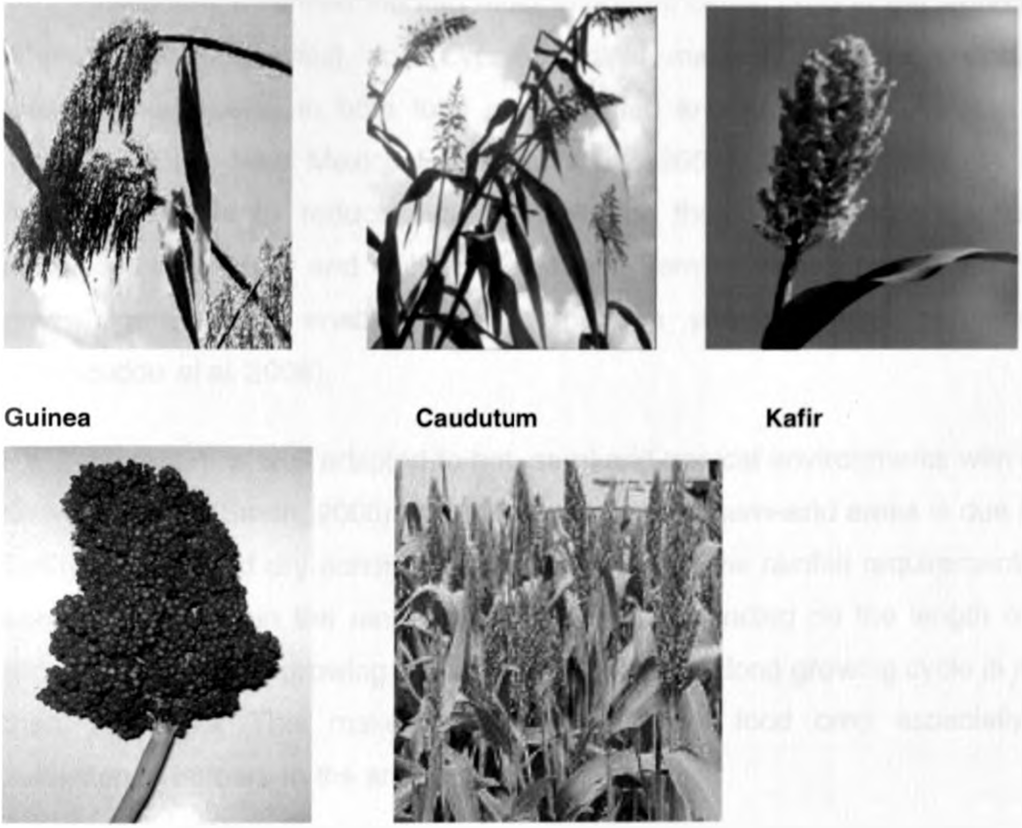
Sorghum bicolor is a genetically variable member of the grass family which looks very similar to maize and sugar cane. It is an annual or short-term perennial plant that ranges in height from less than 1 m to 4 m. Some cultivars can grow up to 6 m in height (Mamoudou *et al*, 2006). The stems are strong and can be juicy or dry, sweet or bitter (Wharton, 2005). Sorghum has deep and finely branched roots. The leaves are long (0.3-1.4 m) and wide (10-13 cm) (Mamoudou *et al*, 2006). They are arranged alternatively at different angles on the stem with long overlapping sheaths attached at the nodes (Wharton, 2005). The sheath in some plants is covered with a waxy bloom (Ikisan, 2000). The leaves are waxy and have the ability to roll when the plant is moisture stressed. This helps the plant to be more drought resistant than other grain crops.

Sorghum panicle is 8-40 cm long and 2 to 20 cm or more wide, with either a short and compact or loose and open shape. The panicle bears many spikelets. A spikelet is the unit of inflorescence. They are arranged in pairs on the panicle where by one spikelet is fertile and sessile and the other is sterile and pedicellate except the terminal sessile spikelet which is accompanied by two pediceled spikelets. The sessile spikelet bears the grain (Ikisan, 2000). Mature glumes of the spikelets are either red, reddish brown, straw coloured, yellowish or sometimes flushed with dark red or reddish brown (FAO, 1995a). The seed may be enclosed by the glumes or may protrude from it, being just visible to almost completely exposed (Ikisan, 2000).The sorghum kernel varies in colour from white through shades of red and brown to pale yellow to deep purple-brown (FAO, 1990).

Many varieties designed for cultivation are dwarf breeds, specially designed for easy harvest. However, in Africa, traditional tall sorghum is still grown (Smith, 2008). Sorghum is known under a variety of names: great millet and guinea corn in West Africa, kafir corn in South Africa, dura in Sudan, mtama in eastern Africa, jowar in India and kaoliang in China. In the United States it is usually referred to as milo or milo-maize (FAO, 1990).

Figure 3: The sorghum plant

There are five basic cultivated races: bicolor, guinea, caudatum, kafir, and durra, and hybrid races that combine the characteristics of any two or more of the basic races. These races have been selected for specific local environments thus occupying a wide range of ecological habitats. Each race has a defined geographical distribution with intermediate races occurring along the edges of these zones. Guinea race is found in West African. Moving eastward we find guinea-caudatum and then caudatum. In East Africa Guinea-kafir is dominant but both guinea and kafir are also grown. Durra-caudatum is grown in northern Nigeria with some durra to the north in Niger, and caudatum to the south and east (Wharton, 2005).



Guinea **Caudutum** **Kafir**

Durra **Bicolor**

Figure 4: Sorghum races

2.2 Sorghum distribution

Sorghum originated in Ethiopia and has spread to other parts of Africa, India, South East Asia, Australia and the United States (Tawanda, 2004; ICRISAT, 2005). According to the Syngenta Foundation for Sustainable Agriculture (2007), sorghum spread from Ethiopia to West Africa across Sudan. Also from Ethiopia, sorghum was taken to East Africa from where it was taken to India. Sorghum races in India are closely related to those in Northeast Africa. From India it was taken to China in the early Christian era. From West Africa, sorghum was distributed to USA and other parts of the world through slave trade around mid 19th Century.

2.3 Sorghum production

Sorghum is ranked the fifth most important cereal crop in the world after wheat (*Triticum species*), rice (*Oryza species),* maize (*Zea mays*) and barley (*Hordeum vulgadre*) in both total area planted and production (Agrama and Tuinstra, 2004; New Mexico State University, 2000). Sorghum is a C4 crop hence it is able to reduce evapotranspiration thus conserving water under conditions of drought and high temperatures. Some varieties posses the "stay green" genes that enable them to perform photosynthesis permanently (Mamoudou *et al*, 2006).

Sorghum is well adapted to hot, semi-arid tropical environments with 400-600mm rainfall (Smith, 2008). Its value in hot, arid or semi-arid areas is due to its ability to withstand dry conditions (Tawanda, 2004).The rainfall requirements for sorghum vary within the range of 350-700 mm depending on the length of the growing cycle, short growing cycle is 90 days and the long growing cycle is more than 130 days. This makes it a very important food crop especially for subsistence farmers in the arid regions of the world.

Due to its ability to adapt to different climatic conditions, sorghum is able to grow at an altitude ranging from sea level to 1,000 m above sea level. It is also found in temperate regions and at altitudes of up to 2300 meters in the tropics (Mamoudou *et al*, 2006). It is deep rooted and grows on a soil PH ranging between pH 5 and pH 8.5. It has adapted to a wide range of soils, especially the heavy soils commonly found in the tropics (Mamoudou *et al*, 2006). The crop can withstand temperatures above 38 ^{0}C, but the best yields are realized at the temperature range of 24-27 ^{0}C. Most sorghum landraces take 90-120 days to mature. Sorghum is a short-day plant. It starts head differentiation in 39 days with a day length of 14 hours.

According to Food Agricultural Organization report 440,000 square kilometres worldwide were devoted to sorghum production in 2004 (FAOSTAT, 2004).The area under Sorghum cultivation in Africa is 16%, Asia 36%, Central- and South America 21% and USA 20% (International Starch Institute, 2001). The average area under sorghum cultivation in Kenya is 0.12 million ha (FAO, 1995). This includes semi-arid regions of eastern, northeastern, Rift valley, coast and western region. The crop performs better in areas between 500 metres and 1700 metres above sea level (EPZ, 2005) in Kenya and is grown under rain fed conditions. The Kenyan rainfall is bimodal, with an average seasonal rainfall of 250-400 mm (Keating *et al*, 1992) and an average annual rainfall of 718 mm (ICRISAT, 1991).

Development and dissemination of new technologies such as improved varieties, higher input use, better resource management and disease/pest control has led to a rise of sorghum yields (as for most crops) over the years in most of the global producing areas, with the exception of Africa where yields fell by 14 % during the 1980s before rising once more in the early 1990. The use of hybrid seed, fertilizer and irrigation has ensured a steady increase of yields in the developed countries (FAO, 1995b). The world annual average production for sorghum has been recorded as 61million tones. The United States, India, Nigeria, Mexico, Sudan, and China currently produce the most grain sorghum (FAO, 1995b).

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 under rain-fed conditions (New Mexico State University, 2000). Irrigated hybrid sorghums in the United States produce 4,500-6,500 kg/ha (FAO, 1995b). According to Wharton (2001), the annual grain production in Africa amounts to 89 million tones, and this accounts for 14% of the total cereal production.

The average grain yield for sorghum in Kenya was recorded as 700 kg/ha by the ministry of agriculture in 1996 (MOA Kenya, 1996). In a research done by the Export Processing Zones Authority (EPZ, 2005 it was recorded that the farm production went up from 118,227 tons in 2002 to 126,433 tons in 2003. This was attributed to rainfall changes where by sorghum as an indigenous Kenyan crop provides food security and is becoming a suitable alternative in many places where maize crop fails. Under farmers' conditions, grain yields of 925 kg/ha have been recorded but this is still low relativ e to yields of more than 5,000 kg/ha commonly reported from experimental plots in this region (Ngugi *et al*, 2002).

Table 1: Kenya Sorghum Production Statistics 2002-2003

Province	**Production (Metric Tonnes)**		**Value (Billion KShs.)**	
	2002	**2003**	**2002**	**2003**
Central	44	34	1,204,000	1,202,500
Nyanza	12,973	12,139	290,000,000	270,794,000
Western	11,00	8,341	25,66,600	18,350,860
Coast	398	70	1,672	31,164,200
Eastern	45,211	33,601	331,876,560	443,726,600
Rift valley	6,413	9,843	47,067,927	72,353,525
Total	**76,539**	**64,023**	**697,386,087**	**72,492,68**

Source: Ministry of Agriculture; Crop Development Division 2003

2.4 Sorghum genetic resources

Sorghum bicolor is a diploid plant with a base chromosome number of n=x=10 (2n=20). It is a sexually reproducing, highly self-pollinating crop. Its cross-fertilization rate is very low at around 6% (Charrier *et al*, 2001).

Sorghum represents one of the largest germplasm collections, comprising more than 42,000 accessions (Huang, 2004; Dahlberg *et al*, 2002). The large diverse germplasm provides great opportunities for improvement of the plant adaptation and other agronomic traits (Huang, 2004). The economic value of genetic resources has been accorded such importance that international institutions have been established to maintain germplasm from different varieties of agriculturally significant crop species (Moritz et al, 1999). A large size of the sorghum landrace collection is held at the International Crops Research Institute for the Semi-Arid Tropics, The National Plant Germplasm System (NPGS) of the United States also maintains a large collection of sorghum germplasm (Rai *et al.*, 1999).

2.5 Sorghum utilization

Sorghum is a staple food for millions of people in India and Africa, however, livestock feeding accounts for most of the U.S. sorghum usage (Agrama and Tuinstra, 2004). In the US, sorghum grain is used primarily as a maize substitute for livestock feed because their nutritional values are very similar. Sorghum straw (stem fibres) can also be used as biodegradable packaging. It does not accumulate static electricity, so it is also being used in packaging materials for sensitive electronic equipment.

Generally in Africa and Kenya in particular, sorghum is used in making boiled porridge or gruel and malted beverages including beer. The stover is used for fuel, fodder, thatching and fencing. The grain is also a principle feed ingredient for both cattle and poultry (M'Ragwa *et al*, 1997). Currently, 12% of grain sorghum production in the US is used to make ethanol which is used as energy.

2.6 Sorghum production constraints

Although sorghum is the Africa's contribution to the world's top crops, it receives merely a fraction of the attention it requires and produces just a fraction of what it could. It is inadequately supported considering its vast and untapped potential hence it is relatively undeveloped. Sorghum has a lot of undeveloped and underutilized genetic potential. Sorghum crop also suffers from lack of status. Indeed it is termed as a "coarse" grain, "animal feed," and "food of the peasant classes.

Low food value is also a drawback for sorghum production in terms of food quality. Sorghum grain has tannins, which when eaten, depress the body's ability to absorb and use nutritional ingredients such as proteins. The protein quality is also a problem. A large proportion of the protein is prolamine, an alcohol-soluble protein that has low digestibility in humans. Sorghum processing is more difficult than that of wheat, rice, or maize (National academy of sciences, 1996).

The causes of low sorghum yields include losses due to biotic stresses such as diseases, insect pests and *Striga, and* abiotic factors such as drought, high temperatures and low soil fertility (Ngugi *et al* 2002).

The success in genetic enhancement of unimproved cultivars of sorghum can be affected by a combination of several factors, such as availability of genetic resources, inheritance and stability of the desired traits, simplicity and effectiveness of screening techniques, access to test environments, availability of technical manpower, financial and material resources (Rai *et al.*, 1999).

Sorghum production has also been affected by policy biases towards certain favored staple crops and general policy neglect for development of the crop. This makes it difficult for farmers to adopt new varieties and complimentary management practices for improving productivity and reducing risks. It leads also to underdeveloped marketing services for this crop. Despite the increasing threat of climate change and growing prospects of warmer, variable and drier climate futures, there is lack of clear policy and government commitment for the development of sorghum.

2.7 Genetic diversity

Diversity is the variation in living organisms within a given ecosystem (Chandra *et al*, 2001). Genetic variation is recognized as one of the three fundamental levels of biodiversity, the other two being ecological diversity and species diversity (Moritz et al, 1999).

Genetic diversity is the variety of genes found among the individuals within a species. It is the raw material available to plant breeders (Peter *et al.*, 2004). Plant genetic resources comprise the diversity of genetic material contained in traditional varieties and modern cultivars, as well as crop wild relatives and other wild plant species. These genetic resources can be used, now or in the future, for food and agriculture. They include resources which contribute to people's livelihoods by providing food, medicine, feed for domestic animals, fibre, clothing, shelter, energy and a multiple of other products and services. The genetic variability of plants results from interaction of mutation, selection and random migration. Mutation pressure and selection pressure are major factors changing the level of genetic equilibrium. Geographical, ecological and reproductive isolation have all had marked effects on level of genetic diversity within species (Mehmood *et al.*, 2008).

Crop diversity is one of the most fundamentally important resources for human life on earth. It provides the natural, biological basis of our ability to grow the food required today, as well as to meet the challenges of population growth, changing climates and constantly evolving pests and diseases. Agricultural robustness and sustainability everywhere depends on diversity. Yet this diversity, contained and stored in seeds, is at risk of disappearing (Global crop diversity trust, 2006). Modern cultivars developed by scientific plant breeding for modern intensive agriculture have very high genetic uniformity. Genetic uniformity makes the crops vulnerable to disease and pest epidemics as well as natural catastrophes. An example is the production of hybrid maize with limited diversity in the Corn Belt of the USA which led to the epidemic of southern leaf blight in1970. All the hybrid maize cultivars adopted and produced in the 1960s had the cytoplasmic male sterility gene. This led to the loss of about 15% of the US maize crop in the early 1970s, because the cultivars were susceptible to the new race of *Helminthosporium maydis* (Liu *et al.*, 2003).

Therefore the conservation and sustainable use of plant genetic resources are essential to the sustainable development of agricultural production hence the knowledge of crop genetic diversity in the cultivated crops is very important.

2.8 Methods of assessing genetic diversity

Information about genetic diversity and genetic relatedness among germplasm is a fundamental element in plant breeding (Siddiqui and Naz, 2009). Accurate assessment of genetic diversity can be used in diverse applications including analysis of genetic variability in cultivars, identifying diverse parental combinations 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 or groups of individuals or populations is analyzed by a specific method or a combination of methods. Diverse data sets have been used by researchers to analyze genetic diversity in crop plants. They include pedigree data, passport data–morphological data, agronomic performance data, biochemical data obtained by analysis of isozymes, storage proteins and molecular (DNA-based) marker data.

The choice of diversity study methods depends on the objectives of the experiment, the level of resolution required, available resources and technological infrastructure, and the operational and time constraints. For accurate and unbiased estimates of genetic diversity, adequate attention has to be devoted to sampling strategies, utilization of various data sets on the basis of the understanding of their strengths and constraints, choice of genetic distance measure, clustering procedures, and the objective determination of genetic relationships (Mohammadi and Prasanna, 2003).

Different markers have been used for analysis of genetic diversity in germplasm accessions, breeding lines, and populations (Siddiqui and Naz, 2009). They include morphological markers, seed protein markers, isozymes, mitochondrial DNA and DNA Molecular Markers. The application of any type of marker in the assessment of diversity among accessions will depend on the crop species, technical expertise, lab equipment and cost, suitability for the specific study and the desired results (Chandra *et al*, 2001).

In this study, morphological markers and DNA molecular Markers have been selected for evaluation of sorghum diversity. Molecular markers rely on a DNA assay while morphological markers rely on visible traits (Tawanda, 2004).

2.8.1 Morphological characteristics

Morphological measurements are often obtained in the field or from field specimens (Gaines *et al*, 1999). They discriminate between individuals based on physical characteristics for instance, maturity cycle, growth habit, leaf shape, hairiness, nature of corolla and panicle/pod/fruit size (Van der Maesen, 1990, Gaines *et al*, 1999). Morphological traits may be ecologically adaptive meaning they are good indicators of genetic variation, local differentiation, or ecotypes. However they are often influenced by the environment (Siddiqui and Naz, 2009).

In phenotypic diversity studies, data on quantitative and qualitative characters is recorded as days to 50% flowering (days), plant height (cm), peduncle exertion (cm), panicle length (cm), panicle width (cm), number of basal tillers, grain size (mm), texture and colour, 100-seed weight (g) glumes colour etc (Grenieret *et al*, 2001).

2.8.2 DNA Molecular Markers

More recently, DNA-based marker systems have been used successfully in DNA fingerprinting of plant genome and in genetic diversity studies (Agrama and Tuinstra, 2004). These are identifiable DNA sequences found at specific regions of the genome and transmitted by the standard laws of inheritance from one generation to the next.

There are many types of molecular markers which have been developed and applied. This include, restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNA (RAPD), Sequence Tagged Sites (STS), microsatellites also known as simple sequence repeats (SSR) and single nucleotide polymorphisms (SNPs) (Tawanda, 2004; Chandra *et al*, 2001). For molecular diversity assessment, SSR markers will be used in this study.

2.8.2.1 SSRs (Microsatellites)

Microsatellites (SSRs) are short repeated DNA sequences in the genome, 2 to 4 nucleotides in length for instance, . . . (GCC) 17 . . .) (Tawanda, 2004; Reisch, 1998).

They are PCR-based markers. The results from Agrama 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 useful for the estimation of genetic similarity among diverse genotypes of sorghum because they display high levels of polymorphism. Sorghum diversity studies have been carried out using molecular markers at Ethiopia and Eritrea (Tawanda, 2004).

The disadvantages of SSRs are that they require polyacrylamide gel electrophoresis. They also give information only about a single locus per assay, although multiplexing of several markers is possible. Another problem often encountered with the microsatellites, is the failure of some genotypes to show amplification for some SSR primer pairs. It is not certain whether the lack of data is due to "null alleles" or it is missing data (Mohammadi and Prasanna, 2003).

2.9 Combining morphological and SSR markers for sorghum diversity assessment

The traditional approach to characterization and evaluation is based on morphological features (Torkpo *et al* 2006). Studies have shown that phenotypic divergences among and within landrace populations are related to geographical distance between the areas of origin. Therefore, eco-geographical information can be used to classify the phenotypic diversity of an entire collection (Grenieret *et al*, 2001).

Analysis of phenotypic performance in the field in combination with molecular analysis provides useful information to increase the efficiency in plant breeding programs. The understanding of between and within population genetic variation and how it is partitioned on the basis of geographic origin will help in improving sampling (Kiambi *et al*, 2005). The improvement of sorghum depends upon the utilization of genetic variability in landraces originally maintained by traditional agricultural practices (Grenieret *et al*, 2001). Genetic improvement of sorghum can help farmers in semi-arid areas where sorghum is a key food crop. In the past, studies have been devoted to assessing patterns of sorghum genetic variation based on morphology or pedigree. However, complex quantitatively inherited traits are difficult to trace based solely on morphology. For this reason, DNA-based methods should be employed together with morphological methods in studies of sorghum genetic diversity and in genetic improvement of the crop (Tawanda, 2004).

CHAPTER 3: PHENOTYPIC DIVERSITY ASSESSMENT OF KENYAN SORGHUM ACCESSIONS USING MORPHOLOGICAL MARKERS

Summary

The main aim of the study was to determine the distribution of phenotypic genetic diversity among and between sorghum accessions from different geographic origins of Kenya. Morphological data was used to assess diversity in 148 sorghum accessions collected from Western, Turkana, Coast, Eastern and breeders' material. Most of the accessions were high yielding as revealed by the means of panicle branches (43), panicle length (21cm), and grain weight (1.5g). Majority of the sorghums were late maturing and tall as shown by the mean number of days to 50% flowering (88 days), number of leaves and nodes. Turkana and coast sorghums had similarities in maturity, height and panicle length. Positive correlations were formed between all the traits except with days to 50% flowering. The analysis of variance showed high significant differences in all traits except in days to flowering which showed low significant different at $P< 0.001$. Number of panicle branches had the highest Broad-sense heritability (0.957). The first two Eigen values in PCA explained 67% of the total variance. The scatter plot clustered the accessions into three groups. Phenotypic cluster analysis gave two major groups subdivided into four sub clusters.

3.1 Introduction

In normal conventional breeding, selection of cultivars is done using morphological traits (Chris and Jason, 2002). The traditional approach to characterization and evaluation is based on morphological features (Torkpo *et al* 2006). Many studies have been devoted to assessing patterns of sorghum genetic variation based on morphology or pedigree (Agrama and Tuinstra, 2004).

Studies have shown that phenotypic divergences among and within landrace populations are related to geographical distance between the areas of origin. The measurement of morphological variation is the most easily obtained indicator of genetic diversity. Morphological characters may be ecologically adaptive meaning they are good indicators of genetic variation, local differentiation, or ecotypes and can be used to classify the phenotypic diversity (Grenieret *et al*, 2001). The disadvantage of morphological variation is that they are influenced by the environment (Siddiqui and Naz, 2009)

In phenotypic diversity studies, data on quantitative and qualitative characters is recorded as days to 50% flowering (days), plant height (cm), peduncle exertion (cm), panicle length (cm), panicle width (cm), number of basal tillers, grain size (mm), texture and colour, 100-seed weight (g) glumes colour etc (Grenieret *et al*, 2001).

In this study, the phenotypic data were recorded using Days to 50% flowering, number of nodes at maturity, number of leafs at maturity, panicle length in cm, 100 grain weight in grams.

3.2 Materials and Methods

3.2.1 Collection of germplasm

A total of 148 Sorghum accessions comprising of landraces, farmer varieties and breeders' varieties were evaluated. The landraces and farmer varieties were collected from the sorghum growing areas of Kenya, namely, Turkana, eastern, coast and western regions. The breeders' varieties were obtained from Kenya Agricultural Research Institute (KARI) Katumani, KARI Lanet and KARI Kakamega (appendix 1)

3.2.2 Experimental site and design

The accessions were planted for evaluation of phenotypic data at Kenya Agricultural Research Institute, Embu on 7th march 2008. Embu is a moist mid altitude area located in Agro-ecological Zone III. The area receives an annual rainfall of above 500mm. The altitude ranges between 1150-1750 metres above seas level with a temperature range of 20 - 25 ^{0}C.

The accessions were replicated 3 times using the "Balanced Lattice" design of 14x14. The plot for each accession consisted of three rows, 3m long with a spacing of 75 cm between rows and 20 cm between plants. Basal (DAP) fertilizer application was done at the rate of 18Kg N and 46 Kg P_2O_5 at planting. The first weeding was done two weeks after germination and the second weeding was done at boot stage.

3.2.3 Selection of plants and scoring

Scoring of phenotypic characters was done in the middle row of each plot. Three plants per accession were used for scoring and the mean obtained. The following parameters were recorded: Days to 50% flowering (Days fl), number of nodes at maturity (Node no), number of leafs at maturity (Leaf no), Panicle length in cm (Pan len), 100 grain weight in grams (Gwht). The data was recorded using the excel Data-sheet

3.3 Phenotypic data analysis

GenStat Discovery Edition 3 was used to calculate the means, coefficient of variation (CV%), least significant difference at 5% level (LSD $_{0.05}$) and correlations (r) for the quantitative data. Analysis of variance (ANOVA) was calculated for quantitative characters using the one way (in randomized blocks) method in the GenStat software.

Broad-sense heritability (H^2) was calculated as $H^2 = \sigma^2_g / \sigma^2_p = \sigma^2_g/[\sigma^2_g + \sigma^2_e/r]$ where σ^2_g is the genetic (or genotypic) variance, σ^2_p is the phenotypic variance, σ^2_e is the plot residual or error variance from the ANOVA and r is the number of replications. Values of H are between 0 and 1.

The GenStat Discovery Edition 3 was also used to calculate the principal component analysis (PCA) from the quantitative data.

Morphological quantitative data was subjected to hierarchical cluster analysis using the R 2.6.1-win32.exe software (R Development Core Team 2008). This was done through the complete linkage method in the R software.

3.4 Phenotypic results

3.4.1 Means, least significance difference and coefficient of variations

The table of means (table 2) indicate that most accessions flowered late with a mean of 88 days to 50% flowering. The mean grain weight was relatively high at 1.5g per 100 seeds. Both the number of leaves and number of nodes at maturity was 9 for the majority of the sorghum accessions. Most of the accessions had 43 panicle branches and this was high. The mean panicle length was 21.07cm long which was also high.

Coast accessions were the latest maturing (99 days) followed by Turkana (92 days). Eastern Kenya accessions and breeders' material were earliest maturing (85days). The coast accessions had also the highest grain weight of 1.7 g, the highest number of panicle branches (55) and panicle length (24.6cm). Turkana and coast sorghums had the highest of number of nodes and leaves (10). Turkana follows Coast in panicle length (22cm). It was observed that there was a lot of similarity between Turkana and Coast accessions.

Results in appendix 2 revealed a high variability between the accessions in the days to flowering with a range of 66 to 117 days and this was found in western materials. The 100 grain weight ranged between 0.4 g (Kimiiru white) and 2.9 g (Kimiiru brown) both of Eastern province. Some accessions had only 5 leaves and nodes (Gakuru and Boke of western) while others had 13 leaves and nodes (Nyabururi of Turkana). The turkana sorghums had the widest range of leaves and nodes (7-13). Accession Naliba of Turkana had the highest panicle branch number of 70 while the lowest panicle branch number was 20 branches. Turkana accessions had the highest range of panicle branch numbers (26-70). The panicle length ranged between 5.6cm for accession Jowi jawomo of Western to 50.1cm for accession Nakualem of Turkana. The highest range of panicle length was found in turkana sorghums (10.6cm-50.1cm).

Table 2 also indicates that the highest coefficient of variation (CV %) was in the 100 grain weight (40.4%) with a least significance difference (LSD $_{0.05}$) of 0.97, followed by panicle branch number (39.1%) with LSD $_{0.05}$ of 21.67. This indicates that the two traits within the accessions were highly influenced by environmental conditions of the trial. The lowest CV% was in days to 50%

flowering (15.2%) with an LSD $_{0.05}$ of 10.03 at 5% level indicating less environmental influence on flowering days. However the CV% values revealed where a bit higher than expected.

Table 2: Table of means for the 148 sorghum accessions collected from coast, eastern, western, Turkana and breeders evaluated at KARI Embu in 2008

	Days Fl	**Gwht (gm)**	**Leaf no.**	**Node no**	**Pan br no.**	**Pan len(cm)**
Western	89	1.6	8	8	41	20.95
Turkaka	92	1.4	10	10	40	22.09
Coast	99	1.7	10	10	55	24.6
Eastern	85	1.5	9	9	46	19.9
Breeders(kak)	85	1.4	8	8	42	21.26
MEANS	**88**	**1.5**	**9**	**9**	**43.2**	**21.07**
LSD (5% Level)	**21.1**	**0.97**	**3.26**	**3.25**	**21.67**	**10.03**
CV%	**15.2**	**40.4**	**23.5**	**23.4**	**31.9**	**29.6**

3.4.2 The correlations between quantitative traits

Table 3 shows a very high positive and significant correlation of r = 0.999** between number of nodes and number of leaves. There were negative correlations between all the traits with 100 grain weight and also between number of panicle branches and days to flowering. There were positive and significant correlations between leaf number and days to flowering as well as node number and days to flowering with correlation indices of r = 0.555** and r = 0.557** respectively.

Panicle length and days to flowering were not significantly related (r = 0.069) and there was no relationship between panicle branch number and days to flowering (r-0.009). The panicle branch number and leaf number were slightly related with a positive correlation of r = 0.101 but the panicle length and leaf number were not significantly related (r = 0.015). There was also a very low correlation between panicle branch number and number of nodes (r = 0.102) and between panicle length and number of nodes with an index of r = 0.017. The panicle length and panicle branch number had a low positive correlation of r = 0.278. Generally the correlations between traits in this study were very low.

Table 3: Correlation coefficients between various traits scored in the 148 accessions collected from all sorghum growing areas of Kenya

Days fl	1.000					
Gwht(gm)	-0.168	1.000				
Leaf no.	0.555**	-0.230	1.000			
Node no	0.557**	-0.230	0.999**	1.000		
Pan br no	-0.009	-0.066	0.101	0.102	1.000	
Pan len	0.069	-0.058	0.015	0.017	0.278**	1.000
	Days fl	**Gwht**	**Leaf no.**	**Node no**	**Pan br no.**	**Pan len**

** Significant at 1% level

3.4.3 Analysis of variance

As shown in table 4, there was a very high significant difference in 100 grain weight (gm), leaf number at flowering, the number of nodes at flowering, the number of panicle branches and the panicle length between the accessions at $P \leq 0.001$. The results also show some significant difference in the number of days to 50% flowering ($P \leq 0.001$).

Table 4: Analysis of variance for phenotypic traits scored in 148 sorghum accessions collected from sorghum growing areas of Kenya

Source of variation	**m.s.**	**v.r.**	**F pr.**
Days fl	243.2	1.41	0.007*
Gwht	0.5804	1.60	<.001**
Leaf no.	8.857	2.15	<.001**
Node no	8.806	2.15	<.001**
Pan br no	288.2	1.58	<.001**
Pan len	79.24	2.03	<.001**

** = Highly significant at $P \leq 0.001$.   *= Significant at $P \leq 0.001$.

3.4.4 Broad sense heritability (H2)

The highest heritability was found in the number of panicle branches (0.957) and days to 50% flowering (0.943). Panicle length had also a good heritability of 0.868. Grain weight had a very low heritability of 0.13 (table 5).

Table 5: Broad-Sense Heritability for phenotypic traits scored in 148 sorghum accessions collected from sorghum growing areas of Kenya

Source of variation	**Heritability (H^2)**	**Calculations from ANOVA table**
Days fl	0.943	$H^2 = \sigma^2_g / \sigma^2_p$ = [m.s_g - ms$_e$/r] / [m.s_g - ms$_e$/r + v.r /r] Where: m.s_g - ms$_e$/r = σ^2_g m.s_g - ms$_e$/r + v.r /r=$\sigma^2_g + \sigma^2_e = \sigma^2_p$ ms$_g$ = genetic mean square m.s_e = error mean square v.r = σ^2_p = residue , r = number of replications
Gwht	0.131	
Leaf no.	0.423	
Node no	0.422	
Pan br no	0.957	
Pan len	0.868	

3.4.5 Principal component analysis (PCA)

Figure 5 is a Principle component scatter plot in which the accessions were clustered into one big and two smaller groups, A, B and C in regard to their quantitative physical characters. Two accessions did not cluster with the rest. These were Naliba from Turkana (48) and kimiiru white from Eastern (89).

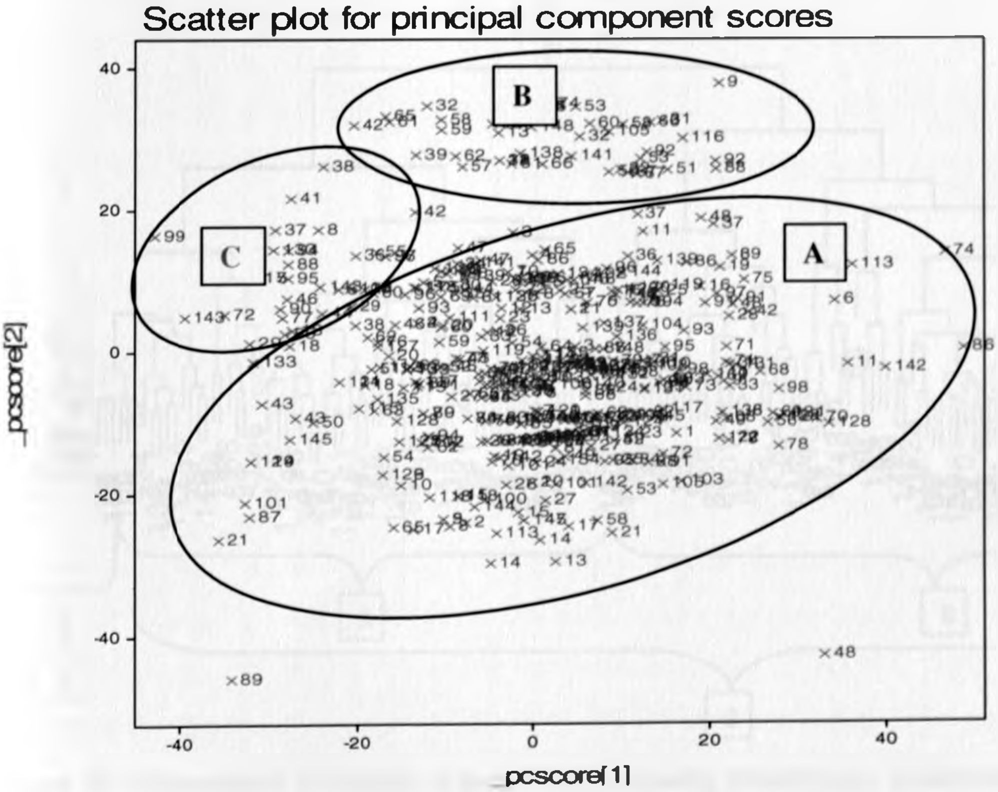


Figure 5: Principle component scatter plot showing phenotypic distance estimates among all the entries

3.4.6 Phenotypic relationships and distances among the accessions

The accessions were subjected to hierarchical clustering using the complete linkage method in the R software to determine their phenotypic distances and relationships.

In figure 6, the 148 accessions showed clustering into two groups, one cluster containing very few accessions (1) and the other cluster having most of the accessions (2). The second cluster was further subdivided into two sub-clusters each putting the accessions into three phenotypic groups. The dendrogram indicates that the accessions did not cluster according to their geographic origins or sub populations but were probably clustered together according to races.

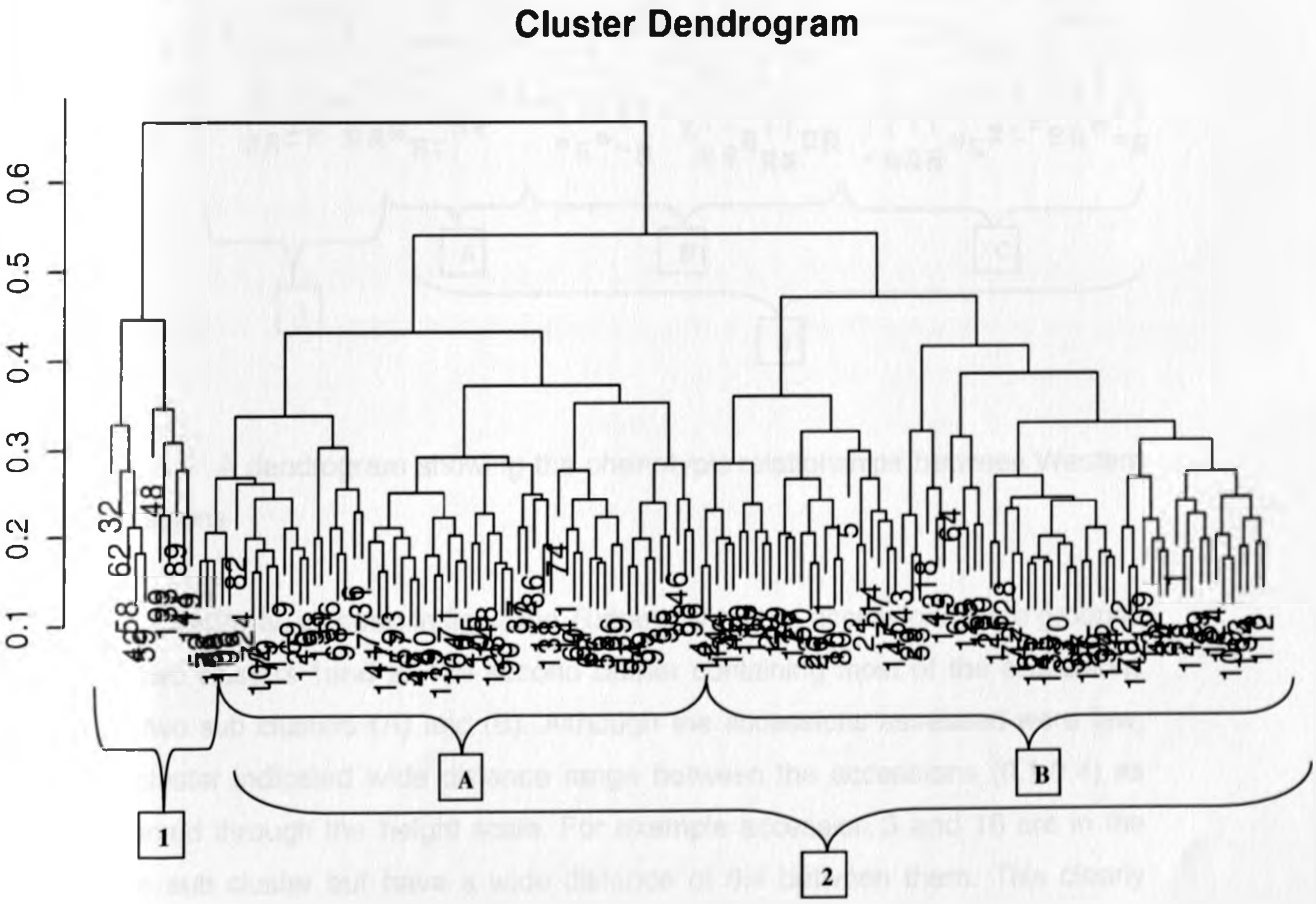


Figure 6: Hierarchical complete linkage tree showing phenotypic relationships between all the 148 accessions

The western province accessions were also clustered into two clusters 1 and 2 with cluster two having most of the accessions (fig 7). The second cluster was subdivided into three sub clusters (A, B, C, D) giving the western province accessions four phenotypic groups hence a higher diversity within this subpopulation was observed. However the distance between the accessions was low with a range of 0.1 – 0.3 revealing a moderate variability among individual accessions.

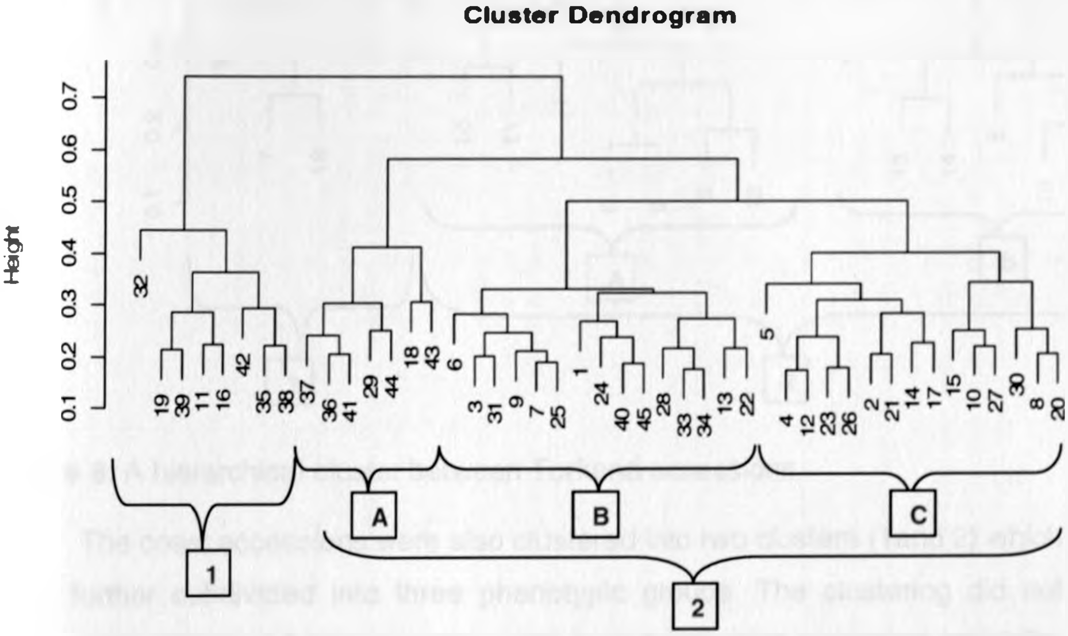

Cluster Dendrogram

Figure 7: A dendrogram showing the phenotypic relationships between Western accessions

Similarly, as seen in fig 8, the Turkana sorghum accessions were grouped into two clusters 1and 2. The second cluster containing most of the accessions had two sub clusters (A) and (B). Although the accessions assessed were few, the cluster indicated wide distance range between the accessions (0.1-0.4) as observed through the height scale. For example accession 3 and 16 are in the same sub cluster but have a wide distance of 0.4 between them. This clearly shows high inter-accession variability within the germplasm from Turkana.

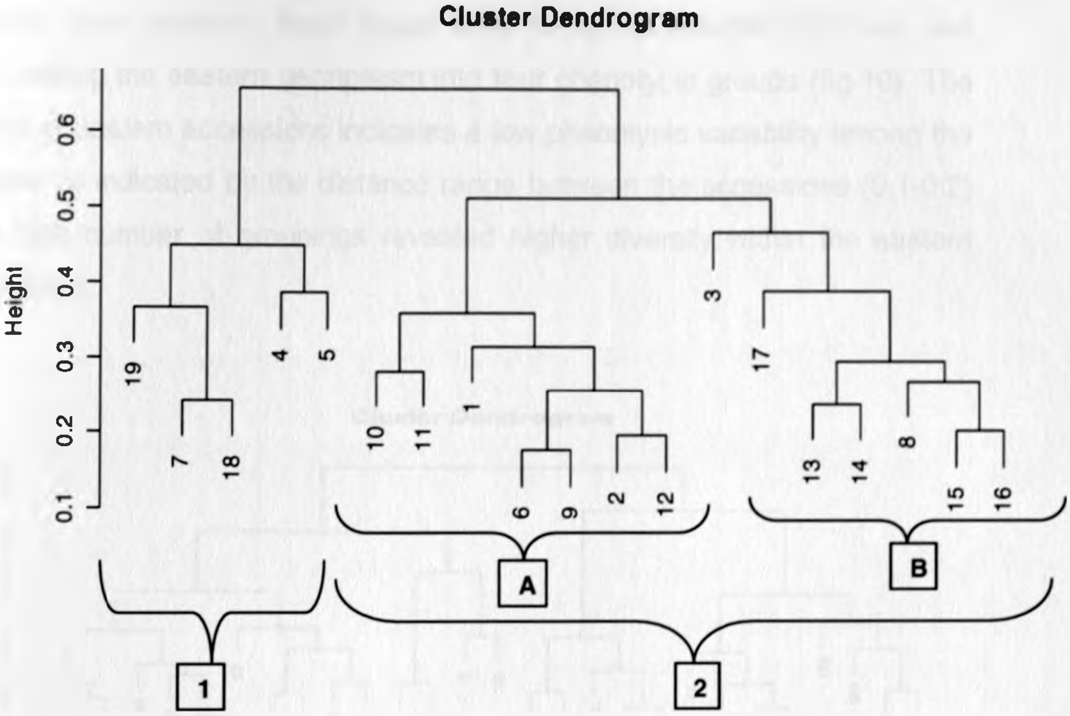


Figure 8: A hierarchical cluster between Turkana accessions

The coast accessions were also clustered into two clusters (1and 2) which were further subdivided into three phenotypic groups. The clustering did not show wide distances between accessions hence low inter-accession variability among the germplasm from coast region (fig 9).

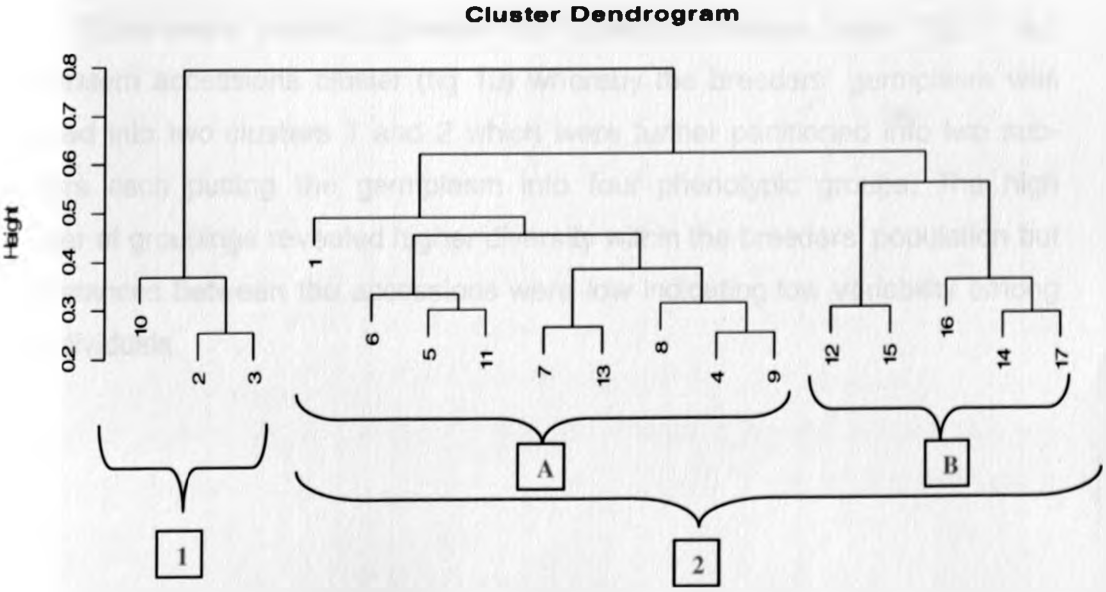


Figure 9: A dendrogram of phenotypic relationships between Coast accessions

Two major groups (1) and (2) were formed in cluster analysis for accessions from eastern. Each group was further subdivided into two sub clusters putting the eastern germplasm into four phenotypic groups (fig 10). The clustering of eastern accessions indicates a low phenotypic variability among the individuals as indicated by the distance range between the accessions (0.1-0.2) but the high number of groupings revealed higher diversity within the eastern subpopulation.

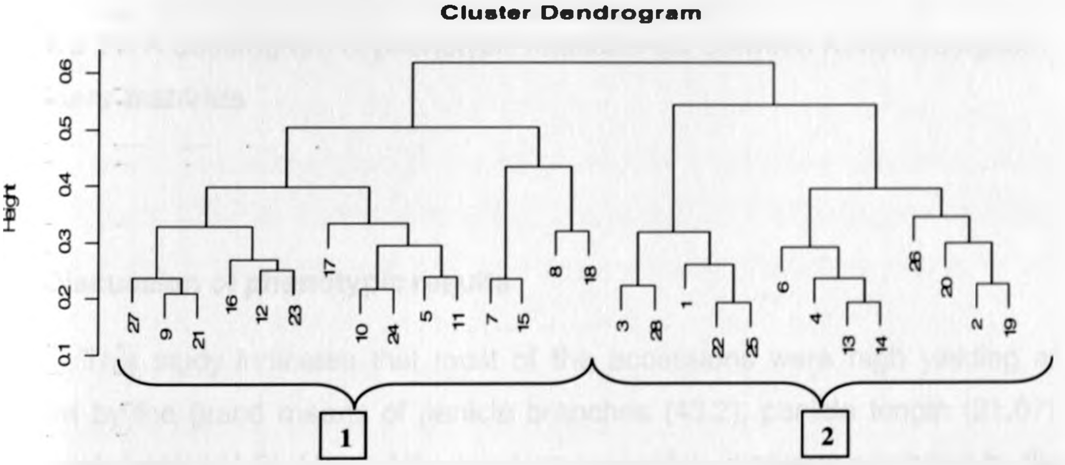


Figure 10: A dendrogram of phenotypic relationships between eastern accessions

There was a similarity between the breeders' varieties cluster (fig11) and the eastern accessions cluster (fig 10) whereby the breeders' germplasm was grouped into two clusters 1 and 2 which were further partitioned into two sub-clusters each putting the germplasm into four phenotypic groups. The high number of groupings revealed higher diversity within the breeders' population but the distances between the accessions were low indicating low variability among the individuals.

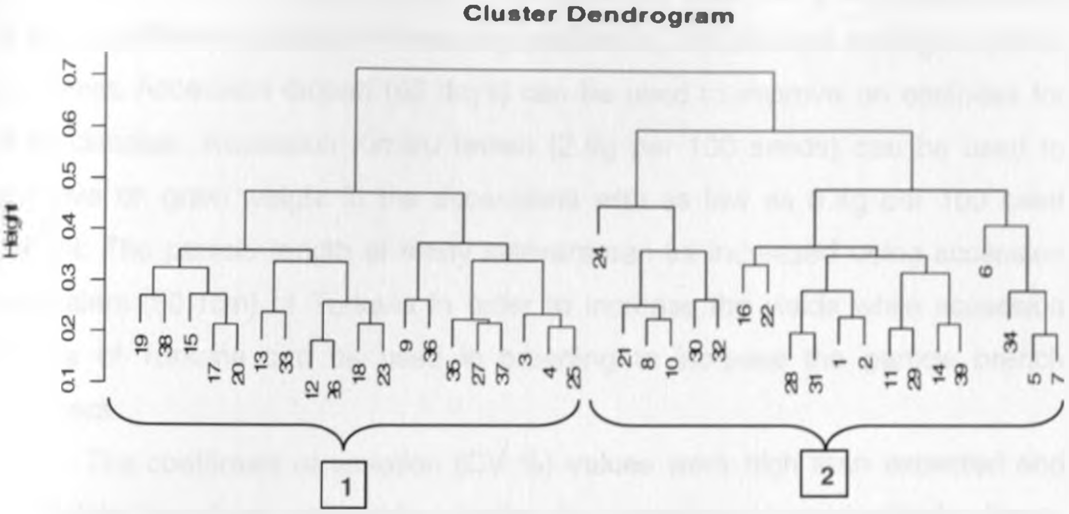


Figure 11: A dendrogram of phenotypic relationships between Kenyan sorghum breeders' materials

3.5 Discussion of phenotypic results

This study indicates that most of the accessions were high yielding as shown by the grand means of panicle branches (43.2), panicle length (21.07), and grain weight (1.5). Most of the sorghums were late maturing as shown by the number of days to 50% flowering, and tall as indicated by number of leaves and nodes at flowering.

It was observed that there was a lot of similarity between Turkana and Coast accessions in regarding late maturity (92 and 99 days respectively), number of nodes (10) and panicle length (22cm and 24.6cm respectively). Eastern and breeders' material were earliest maturing (85days). The coast accessions were high yielding as observed in grain weight (1.7 g), number of panicle branches (55) and panicle length (24.6cm).

The highest range of the days to flowering was found in western materials with a range of 66 to 117 days. Eastern province had the highest range of 100 grain weight (0.4 g - 2.9 g). The Turkana sorghums had the widest range of leaves and nodes (7-13). Turkana accessions had the highest range of panicle branch numbers (26-70). The highest range of panicle length was also found in Turkana sorghums (10.6cm-50.1cm).

The flowering range (66-117days) was very wide giving an opportunity to breed for different durations of maturity suitable for the different ecological zones in Kenya. Accession Gopari (66 days) can be used to improve on earliness for drier climates. Accession Kimiiru brown (2.9g per 100 seeds) can be used to improve on grain weight in the accessions with as low as 0.4g per 100 seed weight. The panicle length of many cultivars can be increased using accession Nakualem (50.1cm) of Turkana in order to increase the yields while accession Naliba of Turkana can be used in breeding to increase the panicle branch numbers.

The coefficient of variation (CV %) values were high than expected and this might have been caused by planting the experiment in mid altitude climate with lower temperatures. Most sorghum cultivars come from areas with high temperatures for instance Turkana and Coast, therefore lower temperatures might have affected their growth habits or morphological characteristics. Grain weight (CV = 40.4%) and panicle branch number (CV = 39.1%) were the most influenced by the prevailing conditions hence the least stable traits. Days to 50% flowering with a lower CV = 15.2% showed a higher stability against the environmental influence. The high CV% results could also have been caused by using only one trial to evaluate the accessions.

The highest positive correlation coefficient was 0.99 between number of nodes and the number of leaves. This indicates lack of senescence at maturity (stay green). Similar observations were made by Elangovand *et al* (2007) with a correlation index of 0.9 between plant height and number of leaves. The negative correlations between all the morphological traits with 100 grain weight and all the other quantitative traits indicates that the more the days to flowering, panicle length, branch numbers, nodes and leaves, the less the weight of grains. The insignificant negative correlation (r = -0.009) between number of panicle branches and days to flowering show that the flowering date does not determine much the number of the branches the panicle will have. The positive and significant correlations between leaf number and days to flowering as well as node number and days to flowering ($r = 0.555^{**}$ and $r = 0.557^{**}$ respectively) indicates that tall plants with more leaves and nodes take long to flower and are late maturing. Panicle length was slightly positively affected by the flowering date (r = 0.069), this was also observed in the means whereby the more the days to flowering the longer the panicle especially Turkana and Coast sorghums. Both the number of leaves and number of nodes had a small positive effect on panicle branch numbers (r = 0.101and r = 0.102 respectively) but not much effect on panicle length (r = 0.015 and r = 0.017 respectively). The positive low correlation between panicle length and panicle branch numbers (r = 0.278) shows that the long panicle will have more branches but the influence of branch numbers by panicle length in low.

Generally the correlations between traits in this study were very low. The data from these results is only based on one trial conducted in one location. In addition the low correlations may have been caused by the lack of data from a second trial.

The analysis of variance showed variation among accessions in all the traits and reveals a high phenotypic diversity between the accessions. While characterizing sorghum germplasm from Karnataka, India Elangovand *et al* (2007) observed similar variation in all the traits.

Broad sense heritability values indicated that the number of panicle branches (0.957), days to 50% flowering (0.943) and panicle length (0.868) are the traits which contributed highly to the phenotypic variance.

The principal component analysis scatter plot clustered the accessions into three groups consisting of one major group with most of the accessions and two smaller groups.

Two groupings were realized through the cluster analysis. The second major group was further divided into two sub clusters putting the accessions into three phenotypic groups without clear patterns relating them to their respective geographical origins. This indicated clustering according to races. This may be similar to the cluster analyses for Ethiopian landraces which showed no distinct pattern in the clustering of landraces based on geographic origin (Desmae, Hailemichael, 2007). Cluster analysis in the morphological characterization of Tanzanian sorghum revealed two major distinct groups each with two subgroups, unlike the observations in this study (Bucheyeki *et al*, 2007).

Four phenotypic groupings were formed in Western province, Eastern province and plant breeders' clusters revealing diversity within the subpopulations. This may be due to wide area under sorghum cultivation in Western Kenya and introgression from the Ugandan sorghums. An increased germplasm introduction into the Eastern province material by farmers and breeders has being going on hence more diversity within the sub population. Breeders' material has been selected from diverse origins and crossed with different germplasm hence the higher diversity. However there was low inter accession variability in these subpopulations as revealed by close distances between the accessions.

The Turkana accessions cluster analysis showed high inter-accession variability within the germplasm and, this was revealed by the wide distances among individual accessions.

Coast subpopulation cluster analysis indicated a low diversity within and among the accessions as revealed by few clusters and low distances between accessions.

3.6 Conclusions

Western province sorghums had the highest diversity in maturity ranging 66-117 days to flowering. The earliest maturing sorghums were found in Eastern and breeders' material. The latest maturing sorghums were confined in coast province and this is not good for breeding for most parts of the arid and semi arid areas in Kenya.

The days to flowering may have been prolonged by the humid climate of the mid altitude area where the experiment was planted.

Eastern province had the highest diversity in100 grain weight with a range of 0.4 g - 2.9 g. Most of the sorghums with high grain weight were found at coast.

The highest diversity in the number of nodes and leaves was found in the Turkana sorghums (7-13 nodes). Sorghums with many nodes and leaves reduced the panicle length (revealed through correlations) and that reduces grain yields. However these sorghums may be good for animal fodder. Turkana accessions had the highest diversity in panicle branch numbers (26-70) and panicle length (10.6cm-50.1cm).It was noted that Turkana sorghums had high diversity in more traits than the other regions.

Coast sorghum material can be used to improve on yield and eastern sorghums can be used to improve on earliness.

Based on the correlation analysis, the results indicated low correlation coefficient values. There is a possibility of breeding for the positively-correlated traits revealed in this study. According to analysis of variance there is high morphological diversity between the 148 sorghum accessions evaluated hence increased selection basis for the different traits tested in this study.

The overall cluster and PCA scatter plot for days to flowering, grain weight, nodes, leaves, panicle branch number and panicle length traits showed high variability among accessions within the whole population. However the overall cluster did not show differentiation between the subpopulations or clustering according to geographical origins indicating similarity in sorghum cultivars found in all the sub populations. Therefore it was concluded that there was exchange of cultivars by famers from one region to another hence the same type of accessions are found in all the geographical locations but with different local names.

CHAPTER 4: GENOTYPIC ASSESSMENT OF KENYAN SORGHUM ACCESSIONS USING MOLECULAR MARKERS

Summary

The aim of genotypic study was to determine the genetic diversity among the collected sorghum accessions and establish their relationships using SSR molecular markers. Genotypic data was used to characterize diversity in 148 sorghum accessions consisting of cultivars from Western, Turkana, Coast and Eastern regions of Kenya and breeders' varieties using 39 SSR markers.

An average PIC value of 0.536 was observed indicating high levels of polymorphism. A mean diversity index of 0.57 and a total of 349 alleles with an average of 8.9 alleles per locus were observed indicating high gene diversity. Based on AMOVA, all variation components were highly significant at $P < 0.001$. The genetic diversity among accessions within the subpopulations (geographical origins) was the highest at 56.37%. The variability within individual accessions was 38.85% and the variability among geographical origins (subpopulations) was the lowest at 4.78%. A low genetic differentiation (FST) value of 0.048 between the sub-populations was observed. The inbreeding level (FIS = 0.59), as well as the heterozygozity deficiency (FIT = 0.61) observed were high showing an increased degree of allele fixation.

Cluster analysis of the SSR data grouped the accessions into three groups with a wide genetic distance (GD) range of 0.4 to 0.8. Principal coordinate analysis (PCoA) also separated the accessions into three groups.

Overall, the results revealed a high diversity within the accessions but less variability between the subpopulations from the different geographic regions.

4.1 Introduction

Microsatellites or Simple Sequence Repeat (SSRs) markers are short repeated DNA sequences in the genome, 2 to 4 nucleotides in length. These repeat motifs are flanked by conserved nucleotide sequences from which forward and reverse primers can be designed to amplify the DNA section containing the SSR by Polymerase Chain Reaction (PCR) process.

PCR is designed to amplify DNA in an automated, cyclic procedure which results in exponential increases in the quantity of a specific sequence of DNA. Selection of a DNA fragment for amplification is a result of "primer-annealing", in which a primer (5 to about 30 bases long) binds to complementary single-stranded genomic DNA present in the reaction. The primer-DNA complex becomes the starting point for replication of the adjacent DNA sequence by a thermo-stable polymerase supplied in the reaction mixture.

The SSR molecular markers require very small amounts of DNA (Daniel *et al*, 1999). SSR alleles can be separated by gel electrophoresis and visualized by silver-staining, autoradiography or other staining with ethidium bromide under UV-light. SSR analysis is amenable to multiplexing, and allows genotyping to be performed on large numbers of lines. They are reproducible, co-dominant and highly polymorphic markers. They also exhibit uniform genome coverage, and are transferable between mapping populations (Agrama and Tuinstra, 2004). SSRs are highly informative and abundant, occurring on average every 6 - 7 kb. These features have made them useful molecular markers.

Agrama and Tuinstra (2004) applied SSR and RAPD markers in sorghum germplasm analysis to compare suitability for quantifying genetic diversity and their results indicated that SSR markers were highly polymorphic with an average of 4.5 alleles per primer. The RAPD primers were less polymorphic with nearly 40% of the fragments being monomorphic. The results also indicated that the genetic distances calculated from SSR data were highly correlated with the distances based on the geographic origin and race classifications. In another study, Hurtado *et al,* (2008) compared the SSR markers with DArTs in the assessment of genetic diversity and population structure in cassava and found that, although DArTs survey many more loci per reaction than SSRs, they provide less polymorphism information per locus. SSRs revealed greater differentiation than DArTs because they often show high levels of allelic diversity per locus, and as co-dominant markers they can be used to infer genotype information (Hurtado *et al*, 2008).

Therefore SSR markers are useful for the estimation of genetic similarity among diverse genotypes of sorghum because they display high levels of polymorphism and are amenable to automated genotyping strategies. Radioisotopes are not required in the detection of SSR markers, because sequence polymorphism can be detected by separation in agarose gels (Agrama and Tuinstra, 2004). Sorghum diversity studies have been carried out using molecular markers at Ethiopia and Eritrea (Tawanda, 2004).

Simple Sequence Repeat markers were used for all molecular genotyping of the sorghum accessions in this study. Parameters for allele scoring and heterozygosity were allele height, number of base pairs (bp) or allele size and number of alleles per loci.

4.2 Materials and Method

4.2.1 DNA extraction

Thirty nine SSR markers were used to genotype the 148 phenotyped sorghum accessions consisting of cultivars from Western, Turkana, Coast and Eastern regions of Kenya and breeders' varieties (appendix 1).

Sorghum seedlings were grown in a 30mm diameter rehydrated Jiff-belt® pellet soil placed in 6cm diameter wells in plastic trays in a 5x7 format in the laboratory. They were then placed by the window for access to light during germination. DNA was extracted from leaves of two week old sorghum seedlings using a modified CTAB protocol (Mace et al., 2004). DNA bulks comprising 5 different plants from each well were used for diversity analysis.

Using this procedure (two 96 well tube boxes and tube strips (each strip containing 8 tubes) were labeled for orientation. The 96 well strip tube boxes containing one stainless steel ball in each tube were put in liquid Nitrogen to chill the tubes and dry the leaf material. Small pieces (approximately 2cm) of fully expanded sorghum leaves (2 weeks after germination) were collected and transferred into each tube placed in the plates. The tubes were closed using strip caps after filling each 8 tubes to avoid mixing.

The extraction buffer was prepared by adding 170µl ß-mecaptoethanol to 100ml CTAB and then putting in 65°C water bath. The CTAB was heat to soften tissue for grinding and to increase the number cells released after grinding. The strip caps were removed and 450µl of pre heated (65°C) extraction buffer (CTAB) was added into each tube using a multi-channeled pipette and the tubes covered. CTAB raptures and lyses the cells for DNA to come into the solution.

The two chilled plates with leaf material were balanced by weighing then set on the genogrinder machine. The genogrinder machine was run at 1000 strokes per minute for 10 minutes to grind the leaf samples. The macerated substance was incubated for 30 minutes at 65°C in water bath with occasional mixing. The plates were balanced by weighing and centrifuged for 2 minutes at 3500 revolutions per minute (rpm) to make the ground material to settle at the bottom of the strips so that contamination will not take place when opening the caps.

Solvent extraction was done by adding 450µl chloroform: isoamylalcohol (24:1) to each tube using the multi-channeled pipette and inverted twice to mix. The chloroform: isoamylalcohol at this stage also removes the proteins. The plates were centrifuged at 5,000rpm for 15 minutes at low temperature (+4°C). Approximately 450µl of the supernatant was transferred into fresh strip tubes.

Equal volume (450µl) of isopropanol (stored at -20°C) was added and the tubes inverted once to mix. The plates were centrifuged at 5000 rpm for 15 minutes. This was necessary to precipitate the crude DNA pellet. The supernatant was decanted and the DNA pellet air-dried for 30 minutes. 200µl low salt TE buffer was added to each sample to dissolve the DNA and 3µl RNase-A (10mg/ml) added to each of the sample to remove RNAs. The DNA samples were incubated for 30 minutes at 37°C or overnight at room temperature (in the dark).

A second solvent extraction was done by adding 200µl phenol: chloroform: isoamylalcohol (25:24:1) to each sample and inverting twice to mix (phenol removes proteins), then centrifuged at 5,000 rpm for 5 minutes. A fixed volume of 180µl of the top layer was transferred to fresh strip tubes and chloroform:

isoamylalcohol (24:1) added to each tube then inverted twice to mix (chloroform: isoamylalcohol removes any traces of phenol and proteins). The plates were centrifuged at 5,000 rpm for 5 minutes. A fixed volume of top layer (approximately 180µl was transferred to fresh strip tubes.

To purify the DNA, 315µl ethanol:sodiumacetate solution was added to each sample and placed at -20^{0}C for 5 minutes then centrifuged at 5,500 rpm for 5 minutes in +4^{0}C. The supernatant from each sample was decanted and the pellets washed by adding 150µl of 70% ethanol then centrifuging at 5,500 rpm for 5 minutes. The supernatant from each sample was decanted and the pellet air-dried for approximately 30 minutes. The pellet was resuspended 100µl low salt TE buffer.

4.2.2 DNA Quality

DNA quality checks were done by gel electrophoresis. 2 µl of each DNA sample were mixed with 1µl bromo-phenol blue dye and 3µl double distilled water then loaded into 0.8% agarose gel wells stained with ethidium bromide (10mg/ml) which was submerged in an electrophoresis unit, containing 1 x TBE buffer. An electric current of 80V was applied for 20 minutes. Due to the ethidium bromide the DNA fragments emitted a luminous glow under UV light and were photographed using a video capture system (Flowgen IS 1000) (fig12)

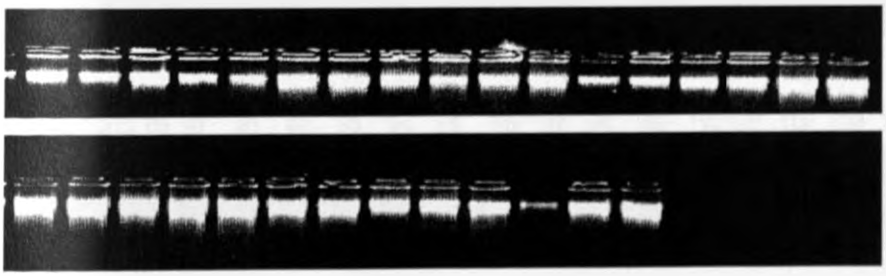


Figure 12: Agarose gel images showing some of the bulk DNA extracted from sorghum accessions.

4.2.3 DNA dilution

This was to ensure uniformity in the amount of DNA samples used. Using the agarose 0.8% gel stained with ethidium bromide (1%), the DNA samples were diluted to 5ng/μl concentration. This was done by adding 40μl double distilled water into 5μl of DNA for each sample to make a 1:10 dilution.

For each sample, 2μl of the diluted DNA were mixed with 1μl bromophenol blue dye and 3μl double distilled water and then they were filled in the wells of submerged gels in an electrophoresis unit, containing 1 x TBE 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 30 minutes.

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 (fig13).

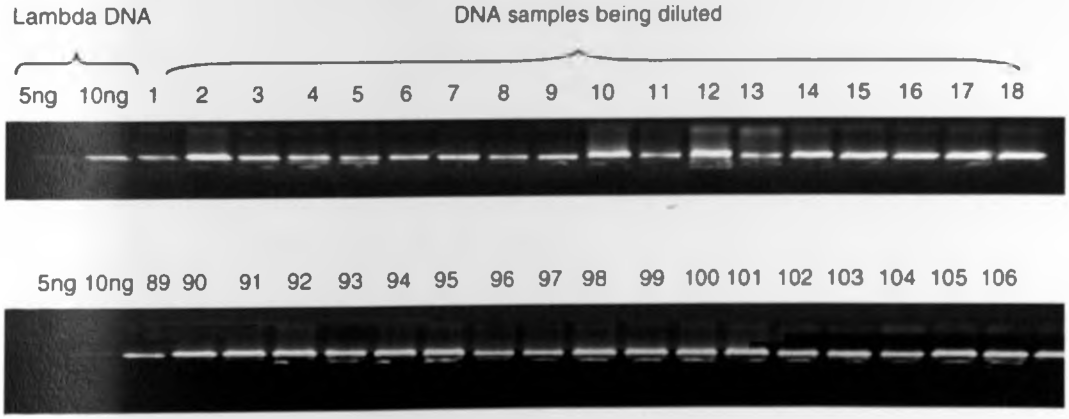


Figure 13: 0.8% Agarose gel images showing DNA being diluted

4.2.4 Optimization of Primers and DNA amplification (PCR)

A set of 39 sorghum SSR primers was used for genotyping (table 6). The SSR markers were chosen based on genome position, repeat size (ranging from di-nucleotide to hexa-nucleotide repeats) and the number of previously reported alleles (ranging from two to six).

Forward primers were labelled with FAM, VIC, NED, PET (PE-Applied Biosystems.), allowing post-PCR pooling of the 39 primer products into ten groups (sets) of four primer products each, with each primer product group being labelled with a different dye.

In order to avoid non amplifications and stuttering, the primers were optimized by varying the PCR reagents as shown in table 6. The annealing temperatures of primers were optimized using the touchdown PCR amplification procedure.

Table 6: Summary of the 39 SSR markers, the multiplex and co-loading sets

No	Marker	Dye	Multiplex set	Chromosome	Repeat motif	References
1	Xtxp040	FAM	Set 1	5	(GGA)7	Kong et al., 2000
2	Xtxp145	VIC	Set 1	9	(AG)22	Bhattramakki et al., 2000
3	mSbCIR286	NED	Set 1	1	(AC)9	Unpublished,Agropolis-Cirad-Genoplante
4	Xcup02	PET	Set 1	6	(GCA)6	Schloss et al., 2002
5	Xtxp010	FAM	Set 2	6	(CT)14	Kong et al., 2000
6	mSbCIR329	VIC	Set 2	10	(AC)8.5	unpublished, Agropolis-Cirad-Genoplante
7	Xtxp114	NED	Set 2	3	(AGG)8	Bhattramakki et al., 2000
8	mSbCIR238	PET	Set 2	2	(AC)26	unpublished, Agropolis-Cirad-Genoplante
9	Xtxp141	FAM	Set 3	7	(GA)23	Bhattramakki et al., 2000
10	Xtxp012	VIC	Set 3	4	(CT)22	Kong et al., 2000
11	mSbCIR223	NED	Set 3	2	(AC)6	unpublished, Agropolis-Cirad-Genoplante
12	Xtxp273	PET	Set 3	8	(TTG)20	Bhattramakki et al., 2000
13	mSbCIR276	FAM	Set 4	3	(AC)9	unpublished, Agropolis-Cirad-Genoplante
14	Xtxp320	VIC	Set 4	1	(AAG)20	Bhattramakki et al., 2000
15	Xcup61	NED	Set 4	3	(CAG)7	Schloss et al., 2002
16	Xtxp021	PET	Set 4	4	(AG)18	Kong et al., 2000
17	Xtxp278	FAM	Set 5	5	(TTG)12	Bhattramakki et al., 2000
18	mSbCIR283	VIC	Set 5	7	(CT)8 (GT)8.5	unpublished,Agropolis-Cirad-Genoplante
19	Xtxp321	NED	Set 5	8	(GT)4+(AT)6+(CT)21	Bhattramakki et al., 2000
20	MSbCIR300	PET	Set 5	5	(GT)9	unpublished, Agropolis-Cirad-Genoplante
21	gpsb067	FAM	Set 6	8	(GT)10	unpublished, Agropolis-Cirad-Genoplante
22	gpsb123	VIC	Set 6	8	(CA)7+(GA)5	unpublished, Agropolis-Cirad-Genoplante
23	sbAGB02	NED	Set 6	5	(AG)35	Taramino et al., 1997
24	Xisep0310	PET	Set 6	2	(CCAAT)4	unpublished, ICRISAT
25	mSbCIR240	FAM	Set 7	8	(TG)9	unpublished, Agropolis-Cirad-Genoplante
26	Xgap84	VIC	Set 7	2	(AG)14	Brown et al., 1996
27	Xtxp015	NED	Set 7	10	(TC)16	Kong et al., 2000
28	Xcup63	PET	Set 7	2	(GGATGC)4	Schloss et al., 2002
29	mSbCIR306	FAM	Set 8	1	(GT)7	unpublished, Agropolis-Cirad-Genoplante
30	Xcup53	VIC	Set 8	1	(TTTA)5	Schloss et al., 2002
31	mSbCIR248	NED	Set 8	10	(GT)7.5	unpublished, Agropolis-Cirad-Genoplante
32	mSbCIR262	PET	Set 8	7	(CATG)3.25	unpublished, Agropolis-Cirad-Genoplante
33	Xgap72	FAM	Set 9	9	(AG)16	Brown et al., 1996
34	mSbCIR246	VIC	Set 9	5	(CA)7.5	unpublished, Agropolis-Cirad-Genoplante
35	Xtxp265	NED	Set 9	9	(GAA)19	Bhattramakki et al., 2000
36	Xtxp136	PET	Set 9	10	(GCA)5	Bhattramakki et al., 2000
37	Xcup14	FAM	Set 10	3	(AG)10	Schloss et al., 2002
38	Xtxp057	VIC	Set 10	9	(GT)21	Bhattramakki et al., 2000
39	Xgap206	NED	Set 10	6	(AC)13/(AG)20	Brown et al., 1996

DNA amplification reactions were carried out in a Micro Amp Optical 384-well Reaction plate (Applied Bio systems) in a total volume of 10µl buffer mix containing $Mgcl_2$, dNTPs, DNA, forward and reverse primers and Taq polymerase except for marker Xtxp145 which had a total reaction volume of 8µl according to the optimization (table 7).

Table 7: Optimization protocols for the primers used in the PCR reactions.

Marker	**Water (µl)**	**Mgcl$_2$ 10mM/µl**	**Buffer 10X**	**dNTPS 2mM/µl**	**Enzyme 5U/µl**	**Primer (Forward +reverse) p/µl**	**DNA 5ng/µl**	**Total reaction vol (µl)**
Xtxp040	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp145	1.46	2	1	0.5	0.04	1	2	8
MSbCIR286	3.96	1.5	1	0.5	0.04	1	2	10
Xcup02	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp010	3.96	1.5	1	0.5	0.04	1	2	10
MSbCIR329	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp114	3.96	1.5	1	0.5	0.04	1	2	10
MSbCIR238	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp141	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp012	3.96	1.5	1	0.5	0.04	1	2	10
mSbCIR223	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp273	3.96	1.5	1	0.5	0.04	1	2	10
mSbCIR276	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp320	3.96	1.5	1	0.5	0.04	1	2	10
Xcup61	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp021	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp278	3.96	1.5	1	0.5	0.04	1	2	10
mSbCIR283	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp321	3.96	1.5	1	0.5	0.04	1	2	10
MSbCIR300	3.96	1.5	1	0.5	0.04	1	2	10
gpsb067	3.96	1.5	1	0.5	0.04	1	2	10
gpsb123	3.96	1.5	1	0.5	0.04	1	2	10
sbAGB02	3.96	1.5	1	0.5	0.04	1	2	10
Xisep310	3.96	1.5	1	0.5	0.04	1	2	10
mSbCIR240	3.96	1.5	1	0.5	0.04	1	2	10
Xgap84	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp015	3.96	1.5	1	0.5	0.04	1	2	10
Xcup063	3.96	1.5	1	0.5	0.04	1	2	10
mSbCIR306	3.96	1.5	1	0.5	0.04	1	2	10
Xcup53	3.96	1.5	1	0.5	0.04	1	2	10
mSbCIR248	3.96	1.5	1	0.5	0.04	1	2	10
mSbCIR262	3.96	1.5	1	0.5	0.04	1	2	10
Xgap72	3.96	1.5	1	0.5	0.04	1	2	10
mSbCIR246	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp265	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp136	3.96	1.5	1	0.5	0.04	1	2	10
Xcup14	3.96	1.5	1	0.5	0.04	1	2	10
Xtxp57	3.96	1.5	1	0.5	0.04	1	2	10
Xgap206	3.96	1.5	1	0.5	0.04	1	2	10

The PCR reactions for the 39 primers were run using the BTX623 standard DNA. The reactions were carried out using the Gene Amp PCR systems 9600 thermocycler (Applied Biosystems) and touch-down PCR amplification. The PCR temperature profile consisted of 5 minutes initial template denaturisation step at 95°C, followed by 35 cycles of 35 seconds at 95°C, 1 minute at primer annealing temperature and 30 seconds at 72°C, this was followed by a final primer extension of 72°C for 20 minutes. The final extension temperature was extended for 30 minutes for primers with a lot of stutter bands, to reduce the probability of false scoring of stutter bands as alleles.

Two micro litres (2µl) of PCR product mixed with 2µl bromo-phenol blue dye and 2µl double distilled water were run on 2.5% agarose gel stained with ethidium bromide to check for amplification (fig 14). The PCR products were then stored at 4°C prior to electrophoresis.

4.2.5 Capillary electrophoresis

Parameters for allele scoring and heterozygosity were allele height, number of base pairs (bp) and number of alleles per loci. All the thirty nine SSR markers were used for this study. 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 was to facilitate the accurate sizing of the microsatellite allele to within ± 0.3 base pairs (Buhariwalla and Crouch 2004).

Co- loading sets shown in table 7 were optimized and multiplexed post-PCR based on dye label, fragment size and fluorescence to reduce the unit cost of high throughput genotyping. 1 ml of formamide (HI-DI) (PE-Applied Biosystems) was mixed with 12µl ROX-labelled GS500LIZ-3730 size standard (PE-Applied Biosystems).

Then 0.7-0.9µl of the PCR products (depending on the intensity of the bands on agarose gel) was loaded mixed with 7.5µl of the HIDI-LIZ mixture into Micro Amp Optical 384-well Reaction plates (Applied Bio systems). DNA fragments were denatured and size-fractioned using capillary electrophoresis on an ABI 3730 automatic DNA sequence (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).

A control sample, accession BTx623 was included during the PCR for each SSR marker and during each capillary electrophoresis run, to verify the repeatability the PCR and capillary electrophoresis. The Allelobin software was used to check the quality of markers.

4.3 Molecular data analysis

The data generated from Allelobin was analysed using PowerMarker version 3.25, Darwin Version 5.25 and the Arlequin version 2.0 softwares. The PowerMarker was used to calculate the Polymorphic Information Content (PIC), expected (gene diversity) and observed heterozygosity, genotype and allele numbers and genotype and allele frequencies for each marker.

PIC values give the measure of the usefulness of each marker in distinguishing one individual from another. The PIC values are affected by the number and frequency of alleles. Genetic diversity was measured in terms of number of alleles per locus.

Analysis of molecular variance (AMOVA) was done in order to assess overall distribution of diversity or the population structure using Arlequin version 2.0 software (Schneider et al. 2000) whereby the molecular distances were calculated using the pairwise differences method according to *Weir, B. S., 1996*. The population differentiation was elucidated using F-statistics provided in the Arlequin software.

Distance matrices were calculated on the basis of Rogers modified distance (Wright, 1978) between accessions and their geographic origins as described by Nei (1973). To determine the genetic relationships and differentiation, the 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 a dissimilarity index was calculated by simple matching. The distances were computed for microsatellite data and trees constructed using the Un-weighted neighbour- joining method provided in Darwin Version 5.25 software.

Darwin Version 5.25 software was used to calculate the principal co-ordinate analysis (PCoA).

4.4 Genotypic evaluation results

4.4.1 DNA Amplification through Polymerase Chain Reaction

When the PCR products were ran on 2.5% agarose gel, the gel images showed that the primers amplified well (fig 14).

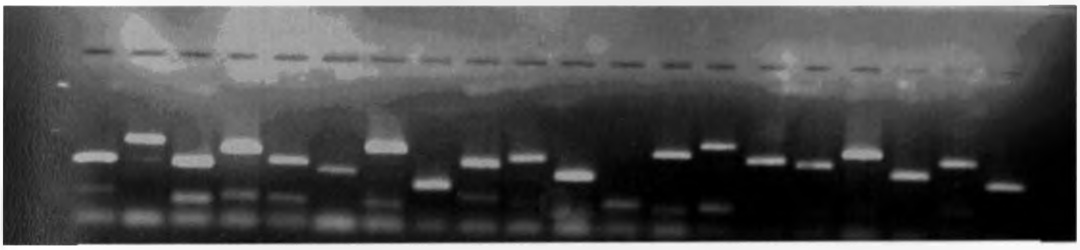


Figure 14: A 2.5% Agarose gel image showing some of the amplifications of the optimised primers using the extracted genomic DNA .

The PCR products were then ran on the sequencer to check the quality and height of the peaks. The electrophenograph peaks had good heights of above 500 Relative Frequency Units. Some markers were able to show homozygous alleles (one allele) as well as heterozygous alleles (more than one allele) on different accessions thus indicating polymorphism. This also revealed heterozygozity in some of the accessions as shown in figure 15.

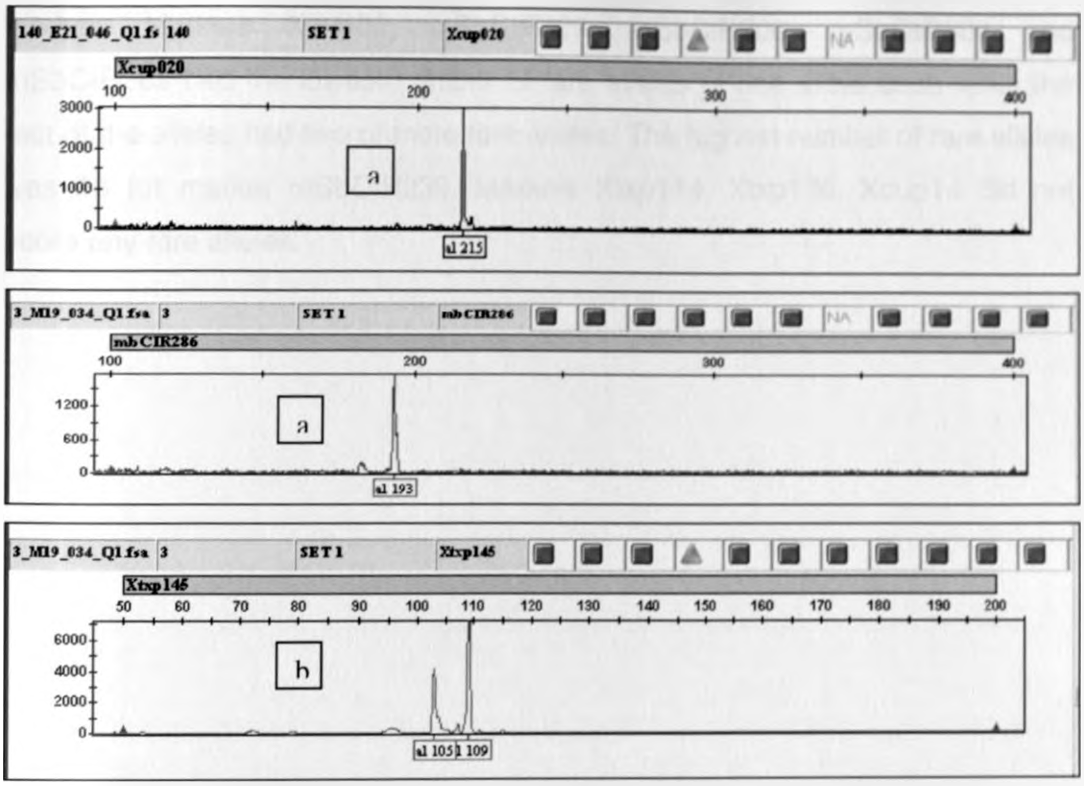


Figure 15: Genotype plot electrophenographs showing the homozygote allele states (a) and the heterozygote allele state (b) (ABI 3730 capillary sequencer)

4.4.2 Marker characterization

4.4.2.1 Marker qualification

Results in table 8 indicate that the marker characterization through the allelobin software revealed a total of 349 alleles giving an average of 8.9 alleles per marker. The highest quality index was 0.5160 which was scored by Marker mSbCIR238 which had also the highest number of 16 rare alleles. Marker Xtxp278 had the lowest quality index of 0.0876. The highest number of alleles was found in marker Xtxp012 with a total of 24 alleles. However this marker had the lowest percentage of abundant allele (13.93%). This was followed by a total of 22 alleles scored by mSbCIR238. The lowest number of alleles was 2 in marker Xcup14.

The highest percentage of abundant allele was 98.46% (allele 204) in marker Xisep0310 and the lowest was 13.93 % (allele 200) in marker Xtxp12. The total number of rare alleles (alleles with frequencies of less than 5%) was 154 giving an average of 3.95 per marker. This means the average number of common alleles (alleles with frequencies of at least 5%) was 5.3 per SSR marker. Markers Xcup02, mSbCIR329, mSbCIR300, mSbCIR306 and mSbCIR262 had the lowest number of rare alleles of one allele each while the rest of the alleles had two or more rare alleles. The highest number of rare alleles was 16 for marker mSbCIR238. Markers Xtxp114, Xtxp136, Xcup14 did not score any rare alleles.

Table 8: Molecular characteristics of the 39 SSR markers used for genotyping

Marker Name	**Repeat Length**	**Quality Index**	**Total Alleles**	**Min Allele**	**Max Allele**	**Abundant Allele (%)**	**Rare Allele(s) (<=5%)**
Xtxp40	3	0.2462	7	121	142	133 (80.83)	121,127,130,139,142
mSbCIR286	2	0.3929	8	104	128	108 (42.31)	104,110,120,122,128
Xcup02	3	0.2107	4	193	205	193 (75.13)	202
Xtxp10	2	0.3173	9	128	150	138 (27.95)	132,136,144,146,150
mSbCIR329	2	0.1649	6	105	115	109 (48.98)	105
Xtxp114	3	0.1131	4	215	233	215 (58.72)	
mSbCIR238	2	0.516	22	54	110	84 (20.79)	54,56,68,76,78,80,82,86,94,98,100102,104,106,108,110
Xtxp141	2	0.3991	12	137	165	149 (48.97)	137,141,143,147,153
mSbCIR223	2	0.4333	8	98	116	104 (62.90)	98,106,108,112,114,100
Xtxp273	3	0.1311	9	207	231	219 (53.45)	207,210,216,228,231
mSbCIR276	2	0.4395	4	224	230	226 (89.64)	224,230
Xcup61	3	0.1230	6	186	204	198 (81.48)	186,189,201,204
Xtxp21	2	0.2451	10	169	187	171 (49.21)	173,175,183,185,187
Xtxp278	3	0.0876	4	241	253	247 (96.89)	241,250,253
mSbCIR283	2	0.2132	9	113	143	113 (47.68)	135,137,139,141,143
Xtxp321	2	0.1743	16	190	224	198 (36.98)	190,192,194,196,200
mSbCIR300	2	0.2825	4	104	112	106 (63.85)	104
gpsb067	2	0.1999	7	170	184	180 (59.72)	170,174,176,178,182
sbAGB02	2	0.3937	7	93	117	97 (37.95)	101,103,113,115,117
Xisep0310	5	0.0926	3	184	204	204 (98.46)	184,194,199
mSbCIR240	2	0.2077	14	99	161	109 (47.94)	99,103,107,127,129,100,
Xgap84	2	0.1825	17	179	219	183 (59.69)	179,187,189,195,197
Xtxp15	2	0.1457	10	199	227	211 (32.56)	199,207,209,221,227
Xcup63	6	0.1131	4	129	147	147 (86.01)	129,135
mSbCIR306	2	0.2184	3	118	122	122 (62.50)	118
Xcup53	4	0.1547	5	183	199	195 (95.41)	183,187,191,199
mSbCIR248	2	0.2783	5	90	100	100 (63.28)	94,98
mSbCIR262	4	0.4100	3	214	222	218 (77.04)	222
Xgap72	2	0.1916	7	181	197	181 (40.05)	185,191,197
mSbCIR246	2	0.3700	5	93	101	99 (81.63)	93,97
Xtxp136	3	0.0912	3	237	243	237 (50.51)	
Xcup14	2	0.1509	2	204	206	204 (70.21)	
Xtxp57	2	0.1690	14	231	263	247 (23.96)	231,235,237,239,243
Xgap206	2	0.3876	23	101	153	103 (23.01)	101,107,109,111,119
gpsb123	2	0.4497	10	276	294	292 (30.65)	276,278,280,282,294
Xtxp12	2	0.1637	24	162	220	200 (13.93)	162,168,170,172,176
Xtxp145	2	0.2378	13	175	303	213 (47.14)	175,177,179,201,203
Xtxp265	3	0.3377	17	141	231	210 (23.70)	141,186,189,192,195
Xtxp320	3	0.2755	11	260	290	272 (28.31)	266,269,281,284,287
Total			**349**				

4.4.2.2 Major Allele frequencies, gene diversity, heterozygosity and PIC values

According to table 9, the major allele frequencies ranged between 0.14 in marker Xtxp012 to 0.98 for marker Xsep310. The mean allele frequency for the major alleles was 0.55. A total of 349 alleles were observed for all the loci with an average allele number of 8.9487 per locus as it was also observed in allelobin analysis results. The number of alleles per locus ranged from 2 in marker Xcup14 to 24 in marker Xtxp012. Markers Xgap206 and mSbCIR 238 had also high number of alleles of 23 and 22 alleles respectively.

The mean gene diversity (expected heterozygosity) per SSR marker was 0.57 where by markers Xtxp012 Xgap206 had the highest gene diversity values of 0.904 and 0.895 respectively followed by marker mbCIR238 with a gene diversity of 0.87. These three markers also had the highest allele numbers of 24, 23 and 22 alleles respectively as mentioned earlier. Marker Xsep310 and Xtxp278 had the lowest gene diversity of 0.03.

The level of heterozygosity ranged from 0.01 for marker Xcup063, to 0.65 for marker Xtxp265. The average level of heterozygosity was 0.23. The average PIC value for the 39 SSR markers was 0.54 with marker Xtxp012 having the highest PIC value of 0.9 and marker Xsep310 having the lowest PIC value of 0.03.

Table 9: Allelic frequencies, genetic diversity and Heterozygozity values of SSR markers genotyped in 148 accessions

Marker	**Major. Allele. Frequency**	**Genotype No**	**Allele No**	**Gene Diversity**	**Heterozygosity**	**PIC**
Xtxp40	0.8345	10.0000	7.0000	0.2917	0.0608	0.2751
CIR286	0.4218	15.0000	8.0000	0.6429	0.2245	0.5758
Xcup02	0.7448	5.0000	4.0000	0.4112	0.0350	0.3729
Xtxp10	0.2872	19.0000	9.0000	0.8018	0.2297	0.7743
CIR329	0.4797	14.0000	6.0000	0.6749	0.2905	0.6280
Xtxp114	0.5980	6.0000	4.0000	0.5533	0.1284	0.4885
CIR238	0.2432	52.0000	22.0000	0.8804	0.4589	0.8707
Xtxp141	0.4830	24.0000	12.0000	0.7243	0.1837	0.7032
CIR223	0.6750	15.0000	8.0000	0.5071	0.2571	0.4729
Xtxp273	0.5426	16.0000	9.0000	0.6494	0.2248	0.6149
CIR276	0.9054	5.0000	4.0000	0.1731	0.0541	0.1613
Xcup061	0.7931	7.0000	6.0000	0.3374	0.0828	0.2952
Xtxp21	0.4759	17.0000	10.0000	0.7176	0.3034	0.6901
Xtxp278	0.9830	5.0000	4.0000	0.0336	0.0204	0.0334
CIR283	0.4595	18.0000	9.0000	0.6727	0.4595	0.6208
Xtxp321	0.3414	30.0000	16.0000	0.7770	0.2000	0.7490
CIR300	0.6351	7.0000	4.0000	0.5193	0.0743	0.4556
Gpsb067	0.6058	11.0000	7.0000	0.5278	0.3650	0.4502
SbAGB02	0.3818	18.0000	7.0000	0.7080	0.2095	0.6605
Xsep310	0.9831	4.0000	3.0000	0.0334	0.0203	0.0331
CIR240	0.4660	19.0000	14.0000	0.6837	0.2925	0.6361
Xgap84	0.5986	29.0000	17.0000	0.6248	0.4286	0.6126
Xtxp15	0.3209	21.0000	10.0000	0.7869	0.1689	0.7568
Xcup063	0.8571	5.0000	4.0000	0.2485	0.0136	0.2238
CIR306	0.6216	6.0000	3.0000	0.4803	0.1014	0.3772
Xcup53	0.9493	8.0000	5.0000	0.0980	0.0811	0.0967
CIR248	0.6276	9.0000	5.0000	0.5302	0.3931	0.4690
CIR262	0.7432	6.0000	3.0000	0.3927	0.1149	0.3320
Xgap72	0.4223	12.0000	7.0000	0.7122	0.0743	0.6665
CIR246	0.8074	7.0000	5.0000	0.3346	0.0405	0.3170
Xtxp136	0.4899	5.0000	3.0000	0.5570	0.6149	0.4580
Xcup14	0.6892	3.0000	2.0000	0.4284	0.1757	0.3366
Xtxp057	0.2329	32.0000	14.0000	0.8434	0.3904	0.8244
Xgap206	0.2153	52.0000	23.0000	0.9020	0.3942	0.8950
Gpsb123	0.2891	23.0000	10.0000	0.7924	0.2266	0.7635
Xtxp12	0.1413	52.0000	24.0000	0.9114	0.3623	0.9047
Xtxp145	0.4926	21.0000	13.0000	0.6973	0.3824	0.6661
Xtxp265	0.2245	42.0000	17.0000	0.8711	0.6463	0.8586
Xtxp320	0.2813	26.0000	11.0000	0.8052	0.2361	0.7798
Mean	**0.5473**	**17.3333**	**8.9487**	**0.5727**	**0.2313**	**0.5359**

4.4.3 Allele and Genotype frequencies

4.4.3.1 Allele frequency

The total alleles detected per locus were 349 and the overall mean allele frequency per loci was 0.165 (table 10, appendix 3)

Table 10: Number of alleles per locus and their allelic frequencies. (Data summarized from Appendix 3)

Marker	Total Alleles per marker	Average allele Frequency
Xtxp40	7	0.143
CIR286	8	0.125
Xcup02	4	0.250
Xtxp10	9	0.111
CIR329	6	0.167
Xtxp114	4	0.250
CIR238	22	0.045
Xtxp141	12	0.083
CIR223	8	0.125
Xtxp273	9	0.111
CIR276	4	0.250
Xcup061	6	0.167
Xtxp21	10	0.100
Xtxp278	4	0.250
CIR283	9	0.111
Xtxp321	16	0.062
CIR300	4	0.250
Gpsb067	7	0.143
SbAGB02	7	0.143
Xsep310	3	0.333
CIR240	14	0.071
Xgap84	17	0.059
Xtxp15	10	0.100
Xcup063	4	0.250
CIR306	3	0.333
Xcup53	5	0.200
CIR248	5	0.200
CIR262	3	0.333
Xgap72	7	0.143
CIR246	5	0.200
Xtxp136	3	0.333
Xcup14	2	0.500
Xtxp057	14	0.071
Xgap206	23	0.043
Gpsb123	10	0.100
Xtxp12	24	0.042
Xtxp145	13	0.077
Xtxp265	17	0.059
Xtxp320	11	0.090
Total	**349**	**0.165**

When the alleles were arranged into allele frequency ranges in table 11, the majority of the alleles, 144 (41.26%) had frequencies of between 0.051-0.1. The other frequency categories with many alleles were 0.11-0.2 which had 28.08% (98 alleles) of the total alleles followed by range 0.01-0.05 with 69 (19.77%) alleles. The rest of the alleles had frequencies of between 0.21 – 0.5 with 38 (10.89%) alleles.

Table 11: Allele frequency ranges

Average Frequency range	Number of Alleles	Percentage %
<0.01	0	0
0.01-0.050	69	19.77
0.051-0.1	144	41.26
0.11-0.2	98	28.08
0.21 – 0.5	38	10.89
>0.5	0	
Total	**349**	**100**

4.4.3.2 Genotype frequency

A total of 676 genotypes were detected with an average of 17.33 genotypes per loci. The highest number of genotypes was detected by markers mSbCIR238, Xgap206 and Xtxp12 with 52 genotypes each. The lowest number of genotypes was three for marker Xcup14. Genotype frequencies ranged between 0.019 and 0.333 with an overall mean genotype frequency of 0.101(table 12, appendix 4).

Table 12: The number of genotypes and average genotypic frequencies per locus detected by 39 SSR markers (Data summarized from appendix 4)

Marker	GenotypeNo	**Average genotype Frequency**
Xtxp40	10	0.100
CIR286	15	0.067
Xcup02	5	0.200
Xtxp10	19	0.052
CIR329	14	0.071
Xtxp114	6	0.167
CIR238	52	0.019
Xtxp141	24	0.042
CIR223	15	0.067
Xtxp273	16	0.063
CIR276	5	0.200
Xcup061	7	0.143
Xtxp21	17	0.059
Xtxp278	5	0.200
CIR283	18	0.056
Xtxp321	30	0.033
CIR300	7	0.143
Gpsb067	11	0.091
SbAGB02	18	0.056
Xsep310	4	0.250
CIR240	19	0.053
Xgap84	29	0.034
Xtxp15	21	0.048
Xcup063	5	0.200
CIR306	6	0.167
Xcup53	8	0.125
CIR248	9	0.111
CIR262	6	0.167
Xgap72	12	0.083
CIR246	7	0.143
Xtxp136	5	0.200
Xcup14	3	0.333
Xtxp057	32	0.031
Xgap206	52	0.019
Gpsb123	23	0.043
Xtxp12	52	0.019
Xtxp145	21	0.048
Xtxp265	42	0.024
Xtxp320	26	0.038
Total	**676**	
Mean	**17.3333**	**0.101**

Most of the genotypes, 404 (59.76%) had very low frequencies ranging between 0.01 - 0.050. A total of 184 genotypes (27.22%) had frequencies between 0.051 – 0.10 while 81 (11.98%) genotypes detected had frequency ranging between 0.11 – 0.20. A very small percentage of the total genotypes (1.04%) had high frequencies ranging between 0.21 -0.3. There were no genotypes with a frequency of less than 0.01 or exceeding 0.3 (fig 16).

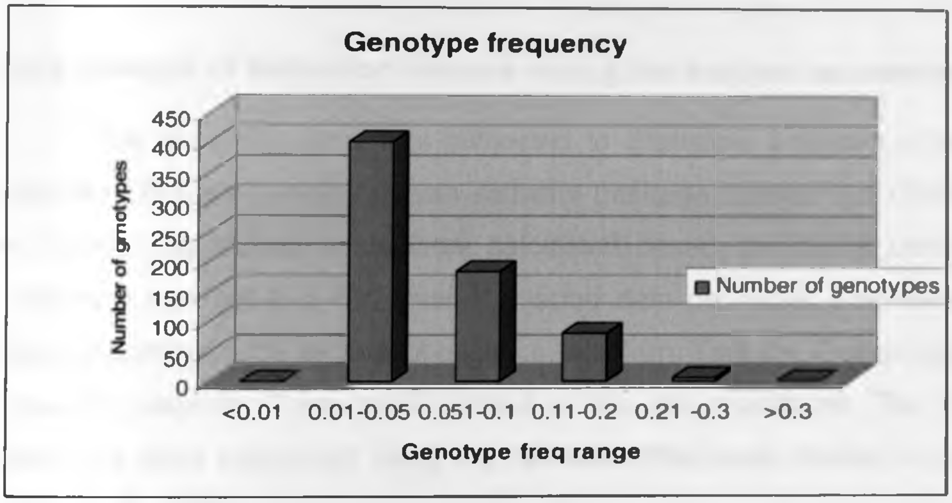


Figure 16: Number of genotypes per average frequency range

4.4.4 Observed heterozygosity, gene diversity (H) and polymorphism

The observed levels of heterozygosity and Nei's unbiased estimate of gene diversity (*H*) for all microsatellites was moderate. The mean Nei's unbiased estimate of gene diversity varied between $H = 0.03$ in marker Xtxp278 to $H = 0.91$ in Xtxp12 which was the highest. Marker Xgap206 had also a high expected heterozygosity of $H = 0.90$. The mean Nei's unbiased estimate of gene diversity across the 39 loci was $H = 0.57$ (table 9).

The average observed heterozygosity level per marker in the accessions was low, 0.237 as compared to the average expected heterozygosity or the mean Nei's unbiased estimate of gene diversity (*H*) per microsatellite which was $H = 0.57$. The observed heterozygosity levels ranged between 0.01 in marker Xcup063 to 0.65 in marker Xtxp265. The observed heterozygosity values across the markers were also lower than the values for Nei's unbiased estimate of gene diversity. There were major differences between the observed heterozygosity and the Nei's unbiased estimate of gene diversity for all the microsatellite markers which indicates inbreeding among accessions or a low percentage of cross pollination (table 9).

The average PIC value for the 39 SSR markers was 0.54 with marker Xtxp012 having the highest polymorphism of 0.9 and marker Xsep310 having the lowest polymorphism value of 0.03 (table 9).

4.4.5 Analysis of Molecular Variance among the sorghum accessions

The genotypic data was subjected to Statistical analyses of molecular variance (AMOVA) using Arlequin software package, version 2.0 (Schneider *et al*, 2000). Significance tests were calculated based on 10,000 permutations. Tolerance was set to a 5% level of missing data per locus. The analysis was done according to the regions of origin i.e. Western, Turkana, Coast, Eastern and breeders' material. These were termed as the sub-populations. The molecular distances were calculated using the pairwise differences method according to *Weir, B. S., 1996*.

4.4.5.1 Population structure

Data in table 13 shows a clear genetic differentiation both among regions, among individuals within the regions and within the individuals. The F statistics indicated very high significance in all the tests with significance at P=<0.000.

The variation among the regions (sub-populations) was very low with a variance component of 0.41544 but higher among the individuals within the regions with a variance component of 4.89634. The within individual variance was moderate with a variance component of 3.37500.

Of the total diversity, 56.37% was attributed to variation among accessions within the sub-populations, 38.85% was attributed to differences within the accessions while only 4.78% was attributed to variation among the regions.

The value of F_{IS} (level of inbreeding) was 0.59196 and this was almost equal to the value of F_{IT} (level of non heterozygosity) which was 0.61148, indicating a relatively high level of inbreeding as well as high number of homozygotes as expected in sorghum due to self pollination. The level of genetic differentiation (Fst) among the sub-populations was low at 0.04782.

Table 13: AMOVA design and results for all the accessions showing variance and significance measures calculated according to weir, Weir, B.S. 1996

Source of variation	**Variance components**	**Percentage of variation**	**P-value**	**Fst**
Among sub-populations	0.41544 Va	4.78	<0.000	
Among individuals within sub-populations	4.89634 Vb	56.37	<0.000	0.04782
Within individuals	3.37500 Vc	38.85	<0.000	
Fixation Indices				
FIS	0.59196			
FST	0.04782			
FIT	0.61148			

In table 14, the population specific F-statistics indicate that Western, Eastern and the breeders' material had almost the same in-breeding coefficient value. Sorghum material from Turkana had the highest FIS and accessions from coast had the lowest. There was a very high significance in the F tests for the five sub-populations.

Table 14: Population specific FIS indices (10100 permutations)

Pop#	**Name**	**FIS**	**P (Rand FIS>=Obs FIS)**
1	Western_Accessions	0.59870	0.000000
2	Turkana_Accessions	0.65132	0.000000
3	Coast_Accessions	0.54789	0.000000
4	Eastern_Accessions	0.60720	0.000000
5	Breeders_Accessions	0.56722	0.000000

4.4.5.2 Genetic diversity among the sorghum accessions

Table 15 indicates that a total of 1,073 alleles were detected in all the accessions in the five regions with an average number of 27.51 alleles per accession. The average number of alleles per sub-population ranged between 4.44 for Turkana and 6.48 for breeders material.

Western sorghum accessions had 90 gene copies, the highest number whereas coast had 34 gene copies, the lowest. A total of 296 gene copies with a mean of 59.2 per population were detected. All the populations had a high number of polymorphic loci with ranging between 26 and 33.

The mean expected and observed heterozygozity values for all the sub-populations were almost equal. The overall mean expected heterozygozity of 1.146 was higher than the overall mean observed heterozygozity of 0.544 for all the accessions. The theta (H) for all the five populations had positive values indicating deficiency of heterozygotes.

Table 15: Intra population Molecular diversity parameters calculated according to *Nei, M., 1987.*

Sub-population	**Western**	**Turkana**	**Coast**	**Eastern**	**Breeders'**	**Mean**	**Total**
No. of polym. loci	33	26	29	33	30	30.200	
No. of gene copies	90	38	34	56	78	59.2	296
Number of alleles	6.28	4.44	4.79	5.51	6.48	27.51	1,073
Mean expected heterozygosity*(He)*	0.558	0.497	0.5597	0.531	0.574	0.544	
MeanObserved heterozygosity *(Ho)*	0.229	0.172	0.273	0.218	0.254	1.146	
Theta(H) under the infinite-allele model	1.264	0.987	1.271	1.131	1.349	1.206	

4.4.6 Genetic relationships and distances among the sorghum accessions

4. 4.6.1 Cluster analysis

The genetic distance was calculated to determine the relationships among the accessions. The pair-wise genetic distance matrix was calculated based on dissimilarity index, simple matching using the Darwin V.5.25 software. The genetic distances ranged between GD=0.4 to GD=0.8. Most of the accessions had a GD of 0.7.

The distance matrix of the pair-wise genetic distances between accessions was subjected to un-weighted pair-group analysis (UPGMA) using the un-weighted Neighbour joining tree construction in Darwin V.5.25.

Figure 17 is a dendrogram of all the accessions showing clustering into three major groups. As shown by the dendrogram, the color codes indicate that most of the accessions were scattered into different clusters regardless of their geographic origin while a few accessions from the same region clustered together.

The accessions from Turkana were found mainly in the second cluster of the dendrogram except accession 53(Lopook) which was placed in the first cluster and accessions 49(Naliba B), 55 (Emaritoit) and 58 (Naliba C) which were grouped in cluster three. All the other landraces from Western, Coast, Eastern and even the breeders' material were placed in all the three clusters. One cultivar from the breeders' material, accession 119 (N-12) was clustered together with the Turkana material.

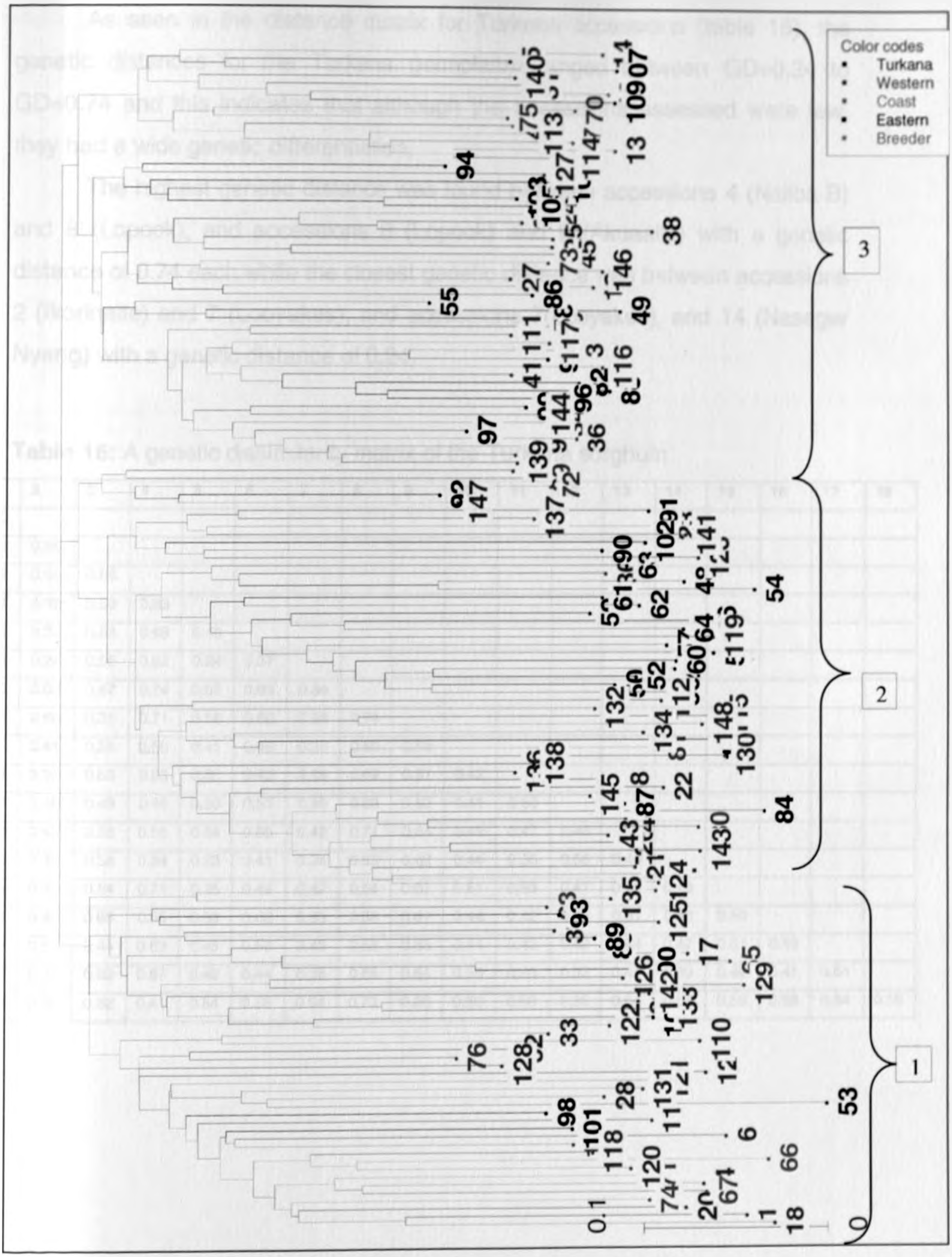


Figure 17: Neighbour joining hierarchical vertical tree showing distances between the accessions from different origins (Dissimilarity minimum value = 0.4; dissimilarity maximum value = 0.8).

As seen in the distance matrix for Turkana accessions (table 16), the genetic distances for the Turkana germplasm ranged between GD=0.24 to GD=0.74 and this indicates that although the accessions assessed were few, they had a wide genetic differentiation.

The highest genetic distance was found between accessions 4 (Naliba B) and 8 (Lopook), and accessions 8 (Lopook) and 9 (Akuaaite) with a genetic distance of 0.74 each while the closest genetic distance was between accessions 2 (Ikorinaite) and 7 (Looyakes), and accessions 7(Looyakes), and 14 (Naseger Nyang) with a genetic distance of 0.24.

Table 16: A genetic dissimilarity matrix of the Turkana sorghum

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
2	0.50																	
3	0.56	0.55																
4	0.59	0.54	0.65															
5	0.47	0.32	0.53	0.63														
6	0.56	0.32	0.53	0.68	0.40													
7	0.44	0.24	0.56	0.62	0.24	0.37												
8	0.60	0.63	0.67	0.74	0.62	0.65	0.64											
9	0.62	0.60	0.31	0.71	0.58	0.60	0.58	0.74										
10	0.42	0.41	0.55	0.56	0.41	0.49	0.38	0.60	0.56									
11	0.58	0.35	0.53	0.65	0.35	0.42	0.29	0.69	0.51	0.42								
12	0.58	0.46	0.49	0.46	0.50	0.51	0.49	0.68	0.60	0.41	0.50							
13	0.44	0.47	0.55	0.55	0.44	0.55	0.42	0.71	0.64	0.49	0.47	0.46						
14	0.46	0.35	0.58	0.69	0.23	0.41	0.24	0.63	0.62	0.44	0.36	0.56	0.44					
15	0.53	0.49	0.54	0.71	0.35	0.49	0.47	0.64	0.67	0.51	0.53	0.47	0.41	0.45				
16	0.54	0.40	0.65	0.65	0.38	0.42	0.35	0.68	0.67	0.44	0.42	0.49	0.51	0.38	0.50			
17	0.54	0.51	0.44	0.63	0.45	0.50	0.46	0.68	0.55	0.44	0.42	0.42	0.44	0.47	0.51	0.59		
18	0.38	0.38	0.62	0.67	0.40	0.44	0.36	0.65	0.64	0.35	0.40	0.53	0.41	0.33	0.49	0.41	0.51	
19	0.62	0.50	0.62	0.41	0.54	0.58	0.54	0.73	0.65	0.50	0.60	0.35	0.54	0.58	0.56	0.56	0.54	0.59

Figure 18 indicates that accession Lopook was very far from the rest .The cluster showed three groupings among the accessions.

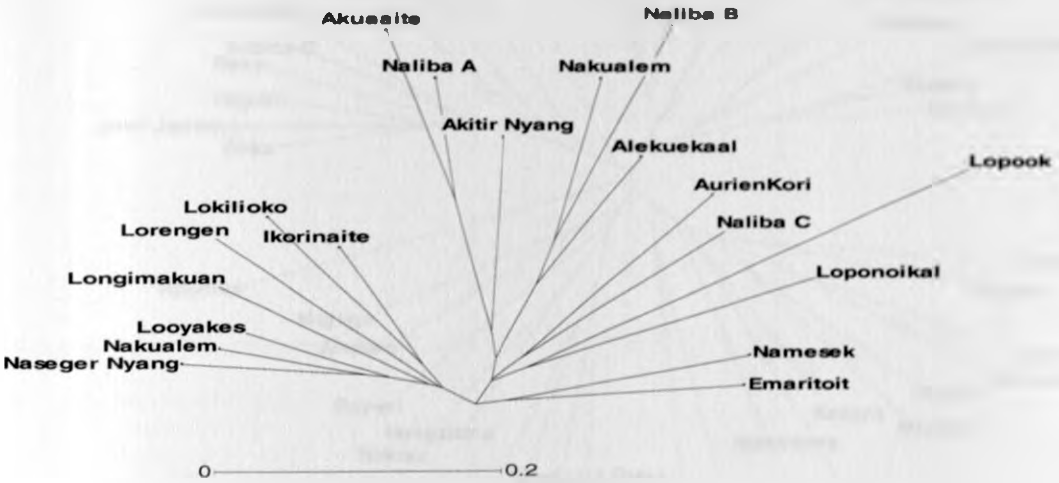


Figure 18: Neighbour joining root dendrogram of genetic relations among the 19 Turkana accessions

The cluster analysis of the western germplasm partitioned the accessions into four major clusters which were supposedly in accordance with the races (fig 19). This shows a higher genetic diversity among them. The most distant accession was Nyaimbo.

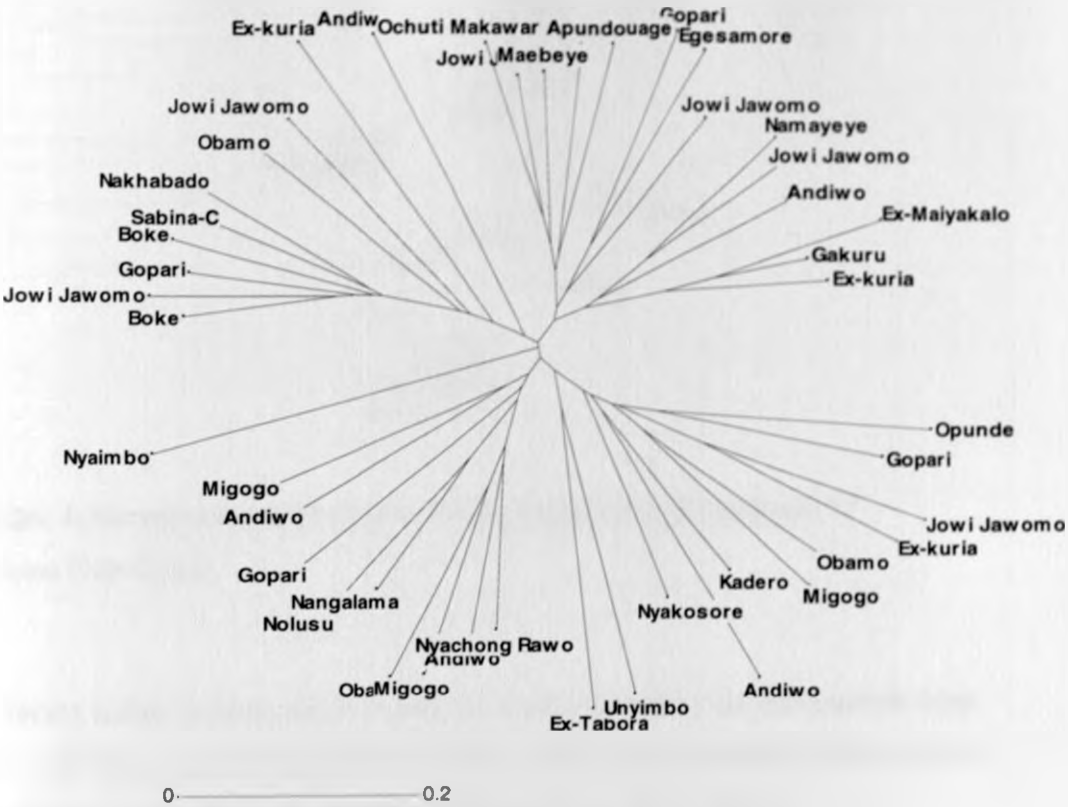


Figure 19: Neighbour joining hierarchical radial dendrogram of genetic relations among the 45 western accessions (Darwin V.5.25)

The coast germplasm was only grouped into 2 main clusters which were not very distinct showing less diversity (fig 20). A third cluster with only one accession, Kibiriti-A was formed. Kibiriti-A was the only accession genetically far away from the rest.

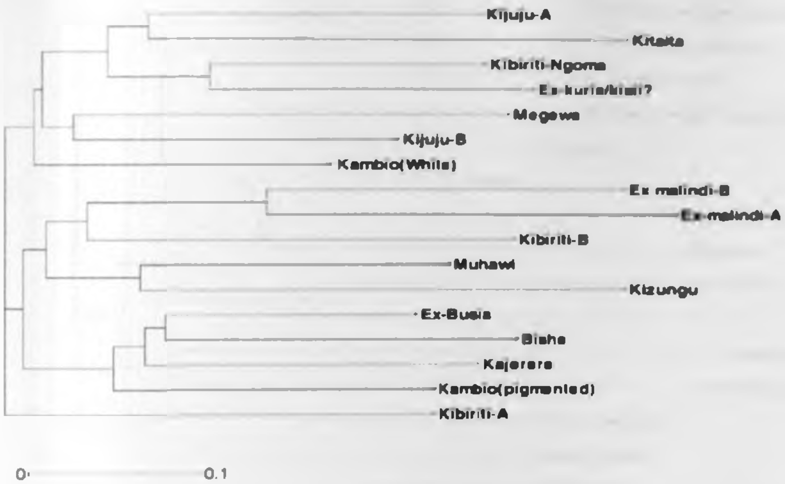


Figure 20: A hierarchical dendrogram showing relationships between 17 accessions from Coast

Three major groups were formed in cluster analysis for accessions from eastern indicating racial segregation (fig 21). This was repeatedly observed in almost all the cluster analysis for the accessions from other regions.

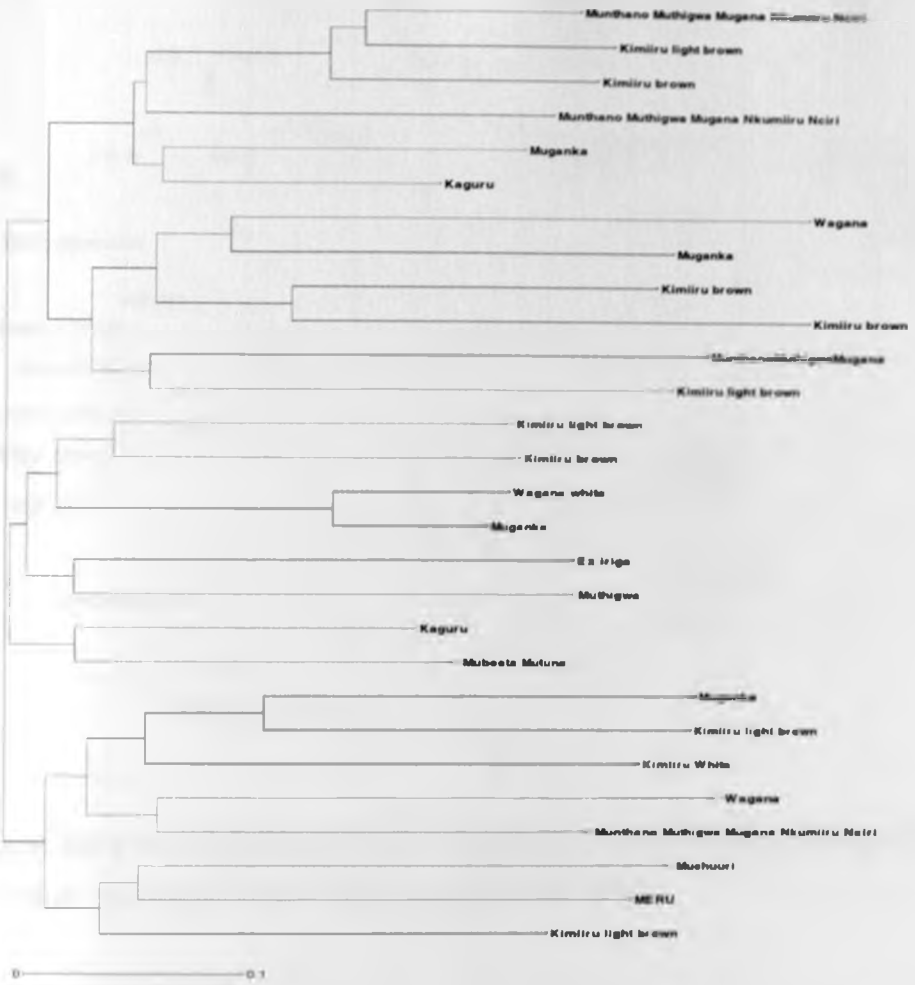


Figure 21: A neighbour joining tree showing genetic distance among 28 accessions from Eastern

Breeders' varieties were diverse according to fig 22. They were clustered into two groups depending on the breeding programme where they were developed. Cluster one (1) consisted mainly of varieties from KARI Kakamega and KARI Lanet except one accession from KARI Katumani , IS3697XICSVIII(IN). This cluster was further partitioned into three sub-clusters; sub-cluster 'a' consisting of KARI Kakamega sorghum with only one Lanet accession (L-5) and the KARI Katumani accession IS3697XICSVIII(IN), sub-cluster 'b' which had only two KARI Lanet sorghums (IS25567 and Damoga) and the rest from KARI Kakamega, sub-cluster 'c' which contained KARI Lanet sorghum material only. The second cluster (2) consisted of mainly Katumani varieties with a few Lanet varieties. (fig 22).

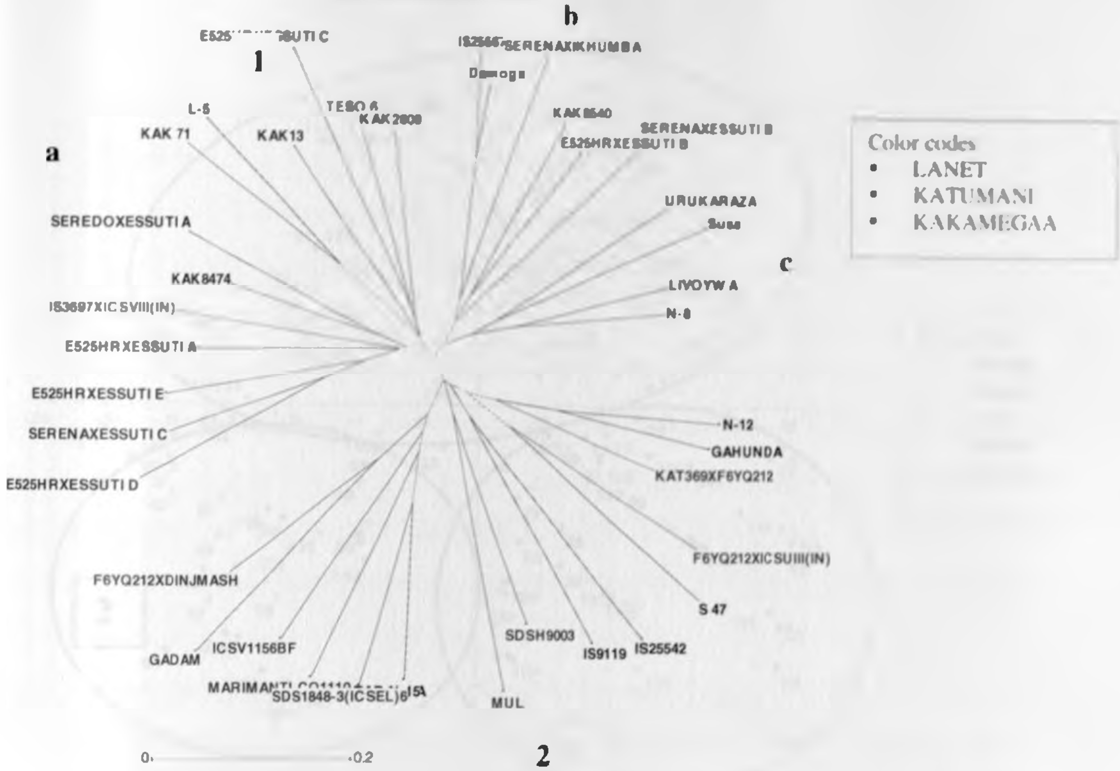


Figure 22: A radial dendrogram showing genetic relationships between 39 Breeders' germplasm from different research stations

4.4.6.2 Principal co-ordinate Analysis of all the accessions based on genetic distance estimates

The dissimilarity distant matrix was subjected to the principal co-ordinate analysis (PCoA). A scatter plot (fig 23) of the first and second axes of non-metric multi-dimensional scaling (MDS) revealed three inter-relationship groupings among accessions. This confirmed the patterns of cluster analysis.

The accessions generally clustered on the basis of their geographical origins as seen in the scatter plot. The accessions from Turkana were found in cluster 2 except accession Alekuikaal (55) which was in cluster 3. Most of the western and Eastern accessions were in cluster 3 and a few in cluster 1. Accessions from coast were placed in clusters 1 and 3 but accessions Ex-malindi-B (80) and Kimiiru brown (81) were found together with Turkana material in cluster 2. All breeders' materials were widely distributed in all the clusters.

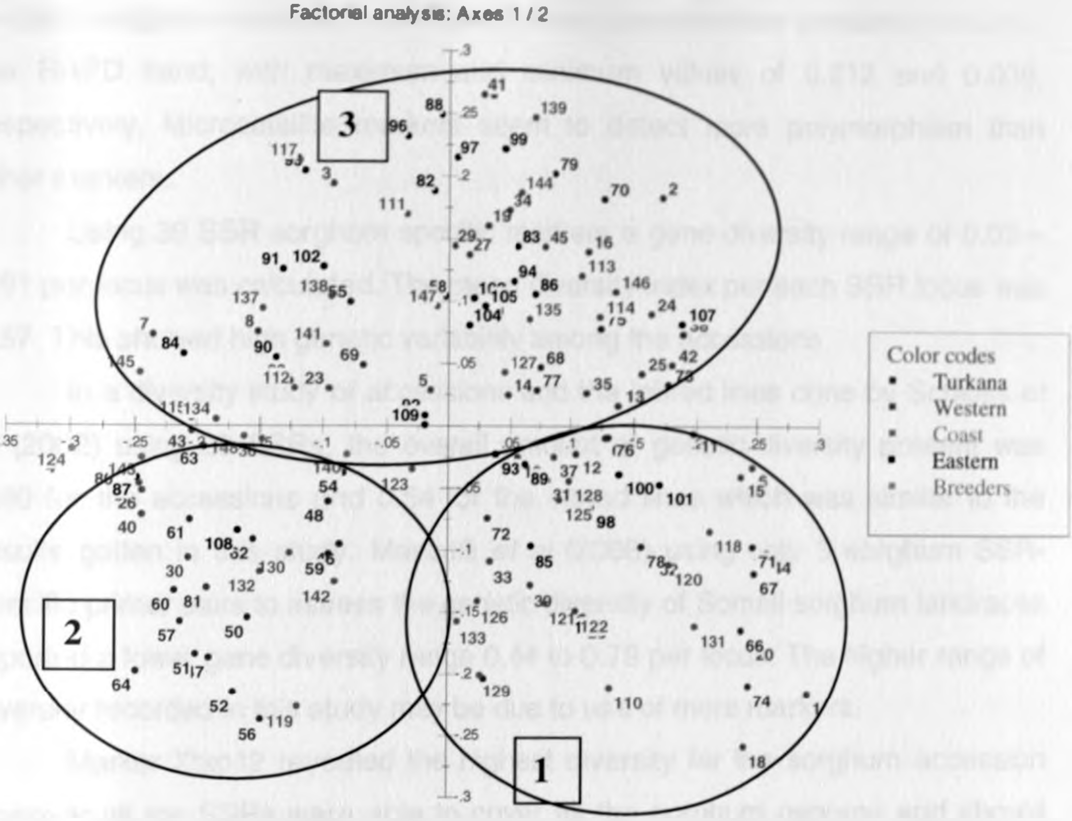


Figure 23: Principle co-ordinate scatter plot showing genetic distance estimates

4.5 Discussion of genotypic results

Each of the 39 primer pairs that we employed gave amplification products in almost all of the accessions. All the 39 SSR markers that were used in this study showed high PIC values ranging between 0.033 and 0.904 with an average PIC value of 0.536 indicating high levels of polymorphism in the assessed sorghum germplasm.

In previous studies, similar mean values of polymorphism rate for SSR loci were reported by Brown *et al*, 1996 (0.54) and Kong et al., 2000 (0.69). Agrama and Tuinstra (2004) detected the same high polymorphism with SSRs in sorghum germplasm analysis. Their polymorphism results indicated that SSR markers were highly polymorphic with an average of 4.5 alleles per primer. Geleta *et al* 2005 while doing genetic diversity analysis in sorghum using AFLP, SSR and morphological traits markers recorded mean PIC values of 0.65 (AFLPs) and 0.46 (SSRs). Tao *et al*, 1992 while working on DNA polymorphisms in grain sorghum recorded a low overall mean polymorphism frequency of 0.117 per RAPD band, with maximum and minimum values of 0.212 and 0.039, respectively. Microsatellite markers seem to detect more polymorphism than other markers.

Using 39 SSR sorghum specific markers a gene diversity range of 0.03 – 0.91 per locus was calculated. The mean diversity index per each SSR locus was 0.57. This showed high genetic variability among the accessions.

In a diversity study of accessions and the inbred lines done by Schloss *et al* (2002) using 29 SSRs, the overall amount of genetic diversity present was 0.60 for the accessions and 0.54 for the inbred lines which was similar to the results gotten in this study. Manzelli *et al* (2006) using only 5 sorghum SSR-specific primer pairs to assess the genetic diversity of Somali sorghum landraces reported a lower gene diversity range 0.44 to 0.79 per locus. The higher range of diversity recorded in this study may be due to use of more markers.

Marker Xtxp12 revealed the highest diversity for the sorghum accession however all the SSRs were able to cover all the sorghum genome and should represent the genetic diversity among these sorghum lines.

As a measure of genetic diversity, the number of alleles and their frequency was analyzed. The average number of alleles per locus detected in this study was 8.9 with a range of 2 to 24 alleles across the loci. In total 349 alleles were observed for the Kenyan sorghum collections. The high number of alleles detected and the wide range of alleles per locus indicate high genetic diversity in the sorghum germplasm as it is also revealed by the high value of genetic diversity index (0.57).

The number of alleles observed in this study was higher than the average number of alleles observed by Agrama and Tuinstra (2004) which was 4.5 alleles per primer. In a study of genetic diversity of Eritrean sorghum landraces using SSR markers, a total of 208 alleles were observed, 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). Menz *et al* (2004) got an average of 7.8 alleles among sorghum inbred lines which was almost equal to the mean value calculated in this study, but they only detected between 2 and 19 alleles, a lower range compared to this study. However Perumal *et al* (2007) recorded between 4 and

21 alleles using seven SSRs, which was similar to the number of alleles in this study but higher than those observed by Menz *et al* (2004).

It was observed that the majority of the alleles, had allele frequencies of between 0.051-0.1. The highest observed frequency of an individual allele per locus was 0.5. This was similar to the observation made by Ghebru *et al* (2002) in the diversity assessment of Eritrean sorghum landraces.

The expected heterozygosity *(He)* across all the 39 loci was higher than the observed hetrozygosity *(Ho)* indicating low cross pollination rate among the accessions as expected in sorghum. The average observed heterozygosity level per locus in the accessions was low, 0.237 with range of 0.01 to 0.65 as compared to the observed heterozygosity among the Eritrean sorghums ranging from 0.7% to 26.5% with an average of 13.9% per locus (Ghebru *et al*, 2002).

The analysis of molecular variance (AMOVA) showed all variation components to be highly significant ($P < 0.001$). Most of the genetic diversity occurred among accessions within the sub-populations with a variance component of 4.89634 thus contributing 56.37% of the total diversity. This demonstrates that accessions among the populations are not under selection processes and there is a continuous exchange of genes between the sorghum cultivars in the sub-populations. The SSR variability within individual accessions was also high with a variance component of 3.37500 constituting 38.85% of the total diversity but the lowest variability was found among geographical origins having a variance component of only 0.41544 hence contributing 4.78% of the genetic diversity in the population structure. This indicates uniformity in Kenyan sorghum sub-populations.

These results were in agreement with those obtained previously by Djė et al. (2000) using microsatellite markers whereby 70% of the total genetic diversity occurred among accessions but the variation among geographic origins, accounted for less than 15% of the total genetic diversity. Elsewhere, Nkongolo and Nsapato (2003) observed that the within-region (among accessions) variations accounted for 96.43% of the total molecular variance but there were low variations related to agroecological differences in Malawian sorghum. In contrast the highest percentage (45%) of SSR variability in the Eritrean sorghum diversity assessment was attributed to variability among populations followed by within-population (40.9%) then between accessions (10.2%) differentiation (Ghebru *et al*, 2002).

The genetic differentiation (FST = 0.048) value observed in this study, although lower than reported for other sorghum populations, was significant. The low genetic differentiation (FST) value observed in this study between the sub-populations indicates less differentiation hence similarity in diversity among Kenyan sorghum sub-populations. This was also observed in the low value of variability between geographical origins having a variance component of only 0.41544. Ghebru *et al* (2002) recorded a similar low FST (0.50) but Djè et al. (2000) reported a higher FST = 0.68 for landraces on the basis of only three different SSR loci.

A high relative inbreeding level (F_{IS} = 0.59), was observed which would be expected as a consequence of self-fertilization and it shows a high degree of relatedness (or less heterozygotes) among the sorghum accessions studied. This may also be due to a lot of selection for uniformity exercised by breeders and farmers.

Similar high values of inbreeding coefficient (F_{IS} = 0.70) were obtained using both alloenzyme and microsatellite markers in cultivated sorghum sampled *in situ* in North-Western Morroco (Djè *et al*, 2000). The Eritrean sorghums showed a lower level of inbreeding (FSC=0.55) and hence high levels of heterozygosity.

The high value of heterozygosity deficiency (F_{IT} = 0.61) observed indicated a high percentage of homozygotes among the sub-populations studied. Both the F_{IS} and the F_{IT} values observed show an increased degree of allele fixation. This might be because the local farmers and breeders practice a lot of selection, hence effective population size are reduced, therefore increasing the opportunity for fixation of alleles.

Although the Turkana sorghums showed the highest level of allelic fixation (F_{IS} = 0.65), generally the sorghums from all the five populations had high heterozygote deficiencies indicating an increased level of inbreeding and hence high levels of homozygosity in Kenyan sorghum. Due to geographical isolation, the sorghums from Turkana have not interacted with sorghums from the other geographical regions thus maintaining their original genes, hence the high allelic fixation.

The P (theta) values for all the sub-populations were positive indicating a high deficiency of heterozygotes.

Breeders' material and Western accessions had the highest average number of alleles which were 6.48 and 6.28 alleles per accession respectively. The lowest number of alleles was found in Turkana sorghum with an average of 4.44 alleles per accession. This may be due to use of few accessions (19) from Turkana.

Associations among the 148 sorghum accessions illustrated a genetic distance (GD) range of 0.4 to 0.8. This indicates a wide range of genotypic variability among the accessions. Boureima *et al*, (2009) got a dissimilarity distance range of between 0.0344 and 0.8906. Geleta *et al* 2005 got an average pairwise genetic distance estimates of 0.57 for morphological traits, 0.62 for AFLPs and 0.60 for SSRs.

In cluster analysis the accessions were divided into three groups with accessions from Western, Coast, Eastern and breeders' material scattered across all the groups. Only the Turkana materials were able to group into their own sub-cluster. This study showed a tendency of clustering based on the accessions race rather than geographical regions. Similar clustering was also observed by Boureima *et al*, (2009) and Menz *et al* (2004) whereby the major clustering of lines corresponded to sorghum race–working groups.

The dendrogram formed indicates similarity in genetic makeup among the sub-populations but high diversity among the accessions. It was attributed to movement of sorghum cultivars by farmers across the geograghphical regions through exchange of seed. The Turkana sorghum maintained their own genetic makeup due to less movement of germplasm from other regions therefore showing a tendency to cluster together.

The within region dendrograms clustered the sorghum accessions from each region generally into either two or three clusters similar to the overall cluster. However there was a clear clustering of the breeders' material according to their breeding programmes with a few exceptions probably due to exchange of breeding lines between the programmes. The Turkana sorghums also showed a wide genetic distance range (0.24-0.74) indicating high variability between the accessions.

The principal coordinate analysis PCoA based on genetic distance (GD) estimates determined by SSR data separated the accessions into three groups as it was also seen in the cluster analysis. It was observed that sorghum materials from same geographical origin were relatively clustering in the same axis as it has been recorded by Boureima *et al* (2009).

4.6 Conclusion

The SSR loci used in this study were able to amplify all the DNA samples for each accession and the markers showed high levels of polymorphism hence they would be useful as a tool in categorizing the sorghum germplasm. This also suggests that Kenyan sorghums contain high polymorphism.

The high value of gene diversity index per locus and the high number of observed alleles for most of the loci which were similar to other studies meant that there is a high genetic variability among the Kenyan sorghum germplasm.

Marker Xtxp12 was the best SSR for analyzing genotypic diversity for it revealed the highest diversity. However all the SSRs were able to cover all the sorghum genome.

Low cross pollination rate among the accessions observed through the high expected heterozygosity / gene diversity value implied that Kenyan germplasm is highly self pollinated as expected in sorghum.

There is high genetic diversity among Kenyan accessions within the geographical origins as revealed by AMOVA and clustering due to exchange of genes between the sorghum cultivars.

However there is low genetic difference between the geographical origins as revealed by AMOVA and clustering due the exchange of seed between farmers from the different sorghum growing areas bringing uniformity in Kenyan sorghum sub-populations.

There is a high allele fixation in Kenyan sorghum cultivars seen through the high homozygosity percentage as revealed by F_{IS} and the F_{IT} values. This is because of selection by the local farmers and breeders reducing effective population size, therefore increasing the opportunity for fixation of alleles.

The lowest number of alleles was found in Turkana sorghum with an average of 4.44 alleles per accession. This may be due to use of few accessions (19) from Turkana.

Western sorghums have many alleles because the accessions were collected over a broader area were many (45). They also grow in an area bordering the Uganda, providing an additional opportunity for the introgression of foreign genetic material.

Breeders' varieties also had many alleles because they have been selected from diverse origins and crossed with different germplasm.

Turkana germplasm has maintained its genes and therefore the sorghums are slightly genetically different from sorghum from the other regions. This is seen through the tendency to cluster together in the overall cluster and also the few alleles observed and this can be attributed to lack of introduction of genes from other regionS. The Turkana sorghums also had high inter-accession Variabil)ty seen in the wide GD on the Turkaja dendrogram. These germplasm can be used as a selection base for rare important traits.

CHAPTER 5: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion and conclusions

According to phenotypic diversity results, Kenyan sorghum germplasm are generally high yielding but late maturing. The maturity period may have been prolonged by the humid climate of Embu area. Western province sorghums had the highest diversity in maturity ranging 66-117 days to flowering but Eastern and breeders' sorghums had the earliest maturing accessions while the latest maturing sorghums were confined in coast province. Eastern province had the highest diversity in100 grain weight with a range of 0.4 g - 2.9 g but most of the sorghums with high grain weight were found at coast.

Turkana sorghums had highest diversity in the number of nodes and leaves (7-13 nodes), panicle branch numbers (26-70) and panicle length (10.6cm-50.1cm). Low correlation coefficient values were revealed.

The molecular data indicates that the SSR loci used in this study were able to amplify all the DNA samples for each accession and the markers showed high levels of polymorphism hence they would be useful as a tool in categorizing the sorghum germplasm. This also suggests that Kenyan sorghums contain high polymorphism. The high value of gene diversity index per locus and the high number of observed alleles for most of the loci which were similar to other studies meant that there is a high genetic variability among the Kenyan sorghum germplasm.

The results by both ANOVA and AMOVA which shows all variation components to be highly significant, confirm that there is high morphological and molecular diversity among the 148 sorghum accessions evaluated hence increased selection basis for the different traits tested in this study.

The cluster analysis results for both phenotypic and genotypic results also show a high variability among accessions within the population as seen in the whole population phenotypic and genotypic clusters. Both clusters separated the 148 accessions into three groups which were not based on geographical origins.

Therefore both molecular and morphological results revealed that there was no much differentiation between the sub-populations (geographical origins) indicating similarity in sorghum cultivars found in all the sub populations.

Diversity analysis for both data sets revealed the highest diversity among breeders' and western sorghum material. The western sorghums were collected over a broader area. Also, Western sorghums grow in an area bordering the Uganda, providing an additional opportunity for the introgression of foreign genetic material. Breeders' material has been selected from diverse origins and crossed with different germplasm hence the higher diversity.

Turkana germplasm has maintained its genetic makeup and therefore the sorghums are slightly genetically different from sorghum from the other regions and this can be attributed to lack of introduction of genes from other regions. The Turkana germplasm also showed wide genetic distances between the sorghums as revealed by both phenotypic and genotypic clusters for the subpopulation. This indicates a high variability between the Turkana accessions. This germplasm can be used as a selection base for rare important traits.

Over all, both the morphological and molecular diversity analyses revealed high variability among sorghum germplasm from Kenya but the absence of appreciable genetic difference between the different sub-populations.

In conclusion, our results demonstrate that molecular analysis and morphological characterization can be applied complimentary to assess the genetic diversity and phylogenetic relationship of plant species.

5.2 General Recommendations

1. This study clearly revealed that Kenyan sorghum germplasm has a high diversity and a wide genetic background. This diversity can be tapped in making new and better varieties.

2. It also indicates that the Kenyan sorghum growing regions contain almost same type of cultivars except some differences in a few traits. Almost all the accessions are late maturing and this must be improved through breeding for earliness since most of them were high yielding.

3. Turkana sorghum landraces can be used as a selection base for rare important traits.

4. Kenyan sorghums need a broader characterization at both molecular and agricultural levels, including the molecular mapping of important traits because many SSR loci are significantly linked to important agronomic traits that can be used in future crop improvement. (Upadhyaya *et al*, 2008). More trials instead of one should be done for better morphological characterization because this was not possible in this study due to time and financial shortage.

5. Conservation strategies should focus on the available sorghum landraces to avoid genetic erosion.

6. Additional breeding must be done to improve the available diversity of the Kenyan germplasm through introduction, crosses and hybrid breeding techniques.

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APPENDICES

Appendix 1: The 148 sorghum accessions assessed

Acc no	Acc Name	Region	District	Latitude	Longitude	Acc no	Acc Name	Region	District	Latitude	Longitude
1	Ex-kuria	Western	Gucha	-1.1487	34.59013	75	Ex-Busia	Coast	Tana river	-2.43385	40.5116
2	Gakuru	Western				76	Kambio(White)	Coast	Malindi	-3.01443	40.1376
3	Egesamore	Western	Gucha	-0.7246	34.64032	77	Kajerere	Coast	Malindi	-3.01443	40.1376
4	Jowi Jawomo	Western	Homabay	-0.59613	34.55227	78	Megewa	Coast	Malindi	-3.01443	40.1376
5	Migogo	Western	Homabay	-0.59613	34.55227	79	Kambio(pigmented)	Coast	Malindi	-3.01443	40.1376
6	Andiwo	Western	Homabay	-0.59613	34.55227	80	Ex-malindi-A	Coast	Malindi	-3.22328	40.1263
7	Gopari	Western	Homabay	-0.59613	34.552267	81	Ex malindi-B	Coast	Malindi	-3.22328	40.1263
8	Obamo	Western	Migori	-0.99892	34.28493	82	Kimiiru brown	Eastern	Meru central	-0.04626	37.672
9	Jowi Jawomo	Western	Migori	-0.99892	34.28493	83	Kimiiru brown	Eastern	Meru central	-0.0397	37.6890
10	Obamo	Western	Homabay	-0.59173	34.60727	84	Kimiiru brown	Eastern	Meru central	-0.0323	37.7126
11	Gopari	Western	Homabay	-0.73388	34.46333	85	Kimiiru light brown	Eastern	Meru central	-0.0323	37.7126
12	Nyaimbo	Western	Homabay	-0.59613	34.552267	86	Kimiiru light brown	Eastern	Meru central	-0.01695	37.7310
13	Migogo	Western	Homabay	-0.6492	34.444217	87	Kimiiru brown	Eastern	Meru central	-0.00441	37.7639
14	Gopari	Western	Homabay	-0.70405	34.414983	88	Kimiiru light brown	Eastern	Meru central	-0.00441	37.7639
15	Kadero	Western	Homabay	-0.70405	34.414983	89	Kimiiru White	Eastern	Meru central	-0.00441	37.7639
16	Jowi Jawomo	Western	Homabay	-0.74316	34.343617	90	Kimiiru light brown	Eastern	Meru central	0.02196	37.805
17	Ex-Tabora	Western	Migori	-0.78786	34.2279	91	MunthanoMuthigwaMugana	Eastern	Meru North	0.17518	37.9603
18	Jowi Jawomo	Western	Migori	-0.81635	34.20225	92	Mubeeta Mutune	Eastern	Meru North	0.17518	37.9603
19	Gopari	Western	Migori	-0.81635	34.20225	93	Mugana Nkumiiru Nciri	Eastern	Meru North	0.15771	37.9853
20	Migogo	Western	Migori	-0.823	34.189617	94	Kaguru	Eastern	Meru North	0.15063	37.9881
21	Boke	Western	Migori	-0.823	34.189617	95	Mugana Nkumiiru Nciri	Eastern	Meru North	0.15063	37.9881
22	Andiwo	Western	Migori	-0.823	34.189617	96	Mugana Nkumiiru Nciri	Eastern	Meru North	0.12908	37.9950
23	Ex-kuria	Western	Kuria	-1.1487	34.590133	97	Kaguru	Eastern	Meru North	0.1146	37.9986
24	Ex-kuria	Western	Kuria	-1.24335	34.6016	98	Muthigwa	Eastern	Meru North	0.1146	37.9986
25	Andiwo	Western	Migori	-0.99891	34.284933	99	Muganka	Eastern	Meru South	-.272633	37.6475
26	Boke	Western	Migori	-0.99891	34.284933	100	Muganka	Eastern	Meru South	-0.2698	37.6183

27	Jowi Jawomo	Western	Suba	-0.76001	34.098383	101	Ex iriga	Eastern	Meru South	-0.2698	37.6183
28	Obamo	Western	Migori	-0.99891	34.284933	102	Muganka	Eastern	Meru South	-0.2669	37.6305
29	Nyaguage	Western	Migori	-0.80866	34.121567	103	Muganka	Eastern	Meru South	-0.27775	37.6881
30	Jowi Jawomo	Western	Migori	-0.99891	34.284933	104	Kimiiru light brown	Eastern	Meru South	-0.27775	37.6881
31	Andiwo	Western	Suba	-0.55918	34.2564	105	Wagana white	Eastern	Meru South	-0.27931	37.7464
32	Nyakosore	Western	Suba	-0.55918	34.2564	106	Wagana	Eastern	Meru South	-0.27931	37.7464
33	Andiwo	Western	Suba	-0.42253	34.181883	107	MERU	Eastern	Meru South	-0.31178	37.8354
34	Apundo	Western	Suba	-0.42253	34.181883	108	Gakathi	Eastern	Meru South	-0.27931	37.7464
35	Nyachong Rawo	Western	Suba	-0.42253	34.181883	109	Muchuuri	Eastern	Meru South	-0.27931	37.7464
36	Ochuti Makawar	Western	Suba	-0.42253	34.181883	110	S 47	Lanet	Breeders		
37	Nolusu	Western	Busia	0.15476	0.1547667	111	N-8	Lanet	Breeders		
38	Ex-Maiyakalo	Western	Busia	0.15476	34.0355	112	Susa	Lanet	Breeders		
39	Unyimbo	Western	Busia	0.15476	34.0355	113	Damoga	Lanet	Breeders		
40	Nakhabado	Western	Busia	-3.49836	38.303467	114	IS25567	Lanet	Breeders		
41	Maebeye	Western	Busia	0.44398	34.631683	115	L-5	Lanet	Breeders		
42	Nangalama	Western	Busia	0.56076	34.2926	116	URUKARAZA	Lanet	Breeders		
43	Sabina-C	Western	Busia	0.5079	34.168683	117	LIVOYWA	Lanet	Breeders		
44	Opunde	Western	Busia	0.09835	33.994083	118	GAHUNDA	Lanet	Breeders		
45	Namayeye	Western	Busia	0.09835	33.994083	119	N-12	Lanet	Breeders		
46	Loponoikal	Turkana	Turkana	2.76468	35.372675	120	IS9119	Lanet	Breeders		
47	Ikorinaite	Turkana	Turkana	2.76468	35.372675	121	GATARANGA	Lanet	Breeders		
48	Naliba A	Turkana	Turkana	2.76468	35.372675	122	N-15	Lanet	Breeders		
49	Naliba B	Turkana	Turkana	2.76468	35.372675	123	IS25542	Lanet	Breeders		
50	Nakualem	Turkana	Turkana	2.76468	35.372675	124	ICSV1156BF	Katumani	Breeders		
51	Lokilioko	Turkana	Turkana	2.76468	35.372675	125	MUL	Katumani	Breeders		
52	Looyakes	Turkana	Turkana	3.85872	34.839413	126	F6YQ212XDINJMASH	Katumani	Breeders		
53	Lopook	Turkana	Turkana	3.85896	34.838991	127	SDSH9003	Katumani	Breeders		
54	Akuaaite	Turkana	Turkana	3.85895	34.839038	128	KAT369XF6YQ212	Katumani	Breeders		
55	Emantoit	Turkana	Turkana	3.85853	34.83897	129	GADAM	Katumani	Breeders		
56	Longimakuan	Turkana	Turkana	3.85848	34.839135	130	MARIMANTI-CO1110	Katumani	Breeders		
57	Alekuekaal	Turkana	Turkana	3.85848	34.839135	131	F6YQ212XICSUIII(IN)	Katumani	Breeders		
58	Naliba C	Turkana	Turkana	3.11861	36.04444	132	IS3697XICSVIII(IN)	Katumani	Breeders		
59	Naseger Nyang	Turkana	Turkana	3.11861	36.04444	133	SDS1848-3(ICSEL)6	Katumani	Breeders		

60	AurienKori	Turkana	Turkana	3.11861	36.04444	134	SEREDOXESSUTI	Kakamega	Breeders		
61	Lorengen	Turkana	Turkana	1.96427	36.123173	135	SERENAXESSUTI	Kakamega	Breeders		
62	Akitir Nyang	Turkana	Turkana	1.96427	36.123173	136	KAK8474	Kakamega	Breeders		
63	Namesek	Turkana	Turkana	1.96427	36.123173	137	TESO 6	Kakamega	Breeders		
64	Nakualem	Turkana	Turkana	1.96427	36.123173	138	E525HRXESSUTI	Kakamega	Breeders		
65	Kizungu	Coast	Taita Taveta	-3.4227	38.3175	139	E525HRXESSUTI	Kakamega	Breeders		
66	Kitaita	Coast	Taita Taveta	-3.4227	38.3175	140	KAK13	Kakamega	Breeders		
67	Ex-kuria/kisii	Coast	Kilifi	-3.81785	39.808117	141	E524HRXESSUTI	Kakamega	Breeders		
68	Muhawi	Coast	Lamu	-2.30895	40.9261	142	SERENAXESSUTI	Kakamega	Breeders		
6	Kibiriti-B	Coast	Lamu	-2.30895	40.92613	143	E525HRXESSUTI	Kakamega	Breeders		
70	Bishe	Coast	Lamu	-2.30895	40.92613	144	KAK8540	Kakamega	Breeders		
71	Kibiriti-Ngoma	Coast	Lamu	-2.30895	40.92613	145	E525HRXESSUTI	Kakamega	Breeders		
72	Kibiriti-A	Coast	Lamu	-2.30895	40.92613	146	SERENAXIKHUMBA	Kakamega	Breeders		
73	Kijuju-B	Coast	Lamu	-2.38341	40.801767	147	KAK2809	Kakamega	Breeders		
74	Kijuju-A	Coast	Lamu	-2.38341	40.801767	148	KAK 71	Kakamega	Breeders		

Appendix 2: Table of means for the 148 sorghum accessions evaluated at KARI Embu in 2008

Accn	Genotype	Region	Days Fl	Gwht (gm)	Leaf no.	Node no	Pan br no.	Pan len(cm)
1	Ex-kuria	Western	85	1.8	7	7	51	27.8
2	Gakuru	Western	80	2.5	5	5	36	20.3
3	Egesamore	Western	92	1.3	9	9	47	22.8
4	Jowi Jawomo	Western	92	1.9	6	6	39	19.9
5	Migogo	Western	86	2.8	6	6	29	16.6
6	Andiwo	Western	97	1.7	8	8	59	22.9
7	Gopari	Western	88	1.9	9	9	46	20.3
8	Obamo	Western	88	1.2	6	6	33	16.3
9	Jowi Jawomo	Western	91	2.2	9	9	54	22.3
10	Obamo	Western	79	1.8	7	7	38	18.7
11	Gopari	Western	101	1.2	10	10	57	28.3
12	Nyaimbo	Western	87	2	6	6	39	21.1
13	Migogo	Western	90	1.3	7	7	42	23.9
14	Gopari	Western	66	2.3	6	6	43	24.6
15	Kadero	Western	76	1	7	7	44	15.9
16	Jowi Jawomo	Western	104	1.4	11	11	52	29.2
17	Ex-Tabora	Western	74	2.4	6	6	36	20.9
18	Jowi Jawomo	Western	88	1.5	9	9	21	5.6
19	Gopari	Western	85	1.6	10	10	48	23.6
20	Migogo	Western	83	1.7	6	6	35	15.9
21	Boke	Western	73	2.1	5	5	36	21.3
22	Andiwo	Western	99	1.2	8	8	43	24
23	Ex-kuria	Western	89	2.2	7	7	46	18.7
24	Ex-kuria	Western	84	1.8	9	9	42	26.11
25	Andiwo	Western	88	1.7	9	9	52	21.22
26	Boke	Western	88	2.3	7	7	41	17.3
27	Jowi Jawomo	Western	74	1.7	6	6	43	18.3
28	Obamo	Western	84	1.2	7	7	52	20.6
29	Nyaguage	Western	89	1	9	9	29	14.4
30	Jowi Jawomo	Western	90	1.4	6	6	34	26.4
31	Andiwo	Western	100	1.5	9	9	50	24.2
32	Nyakosore	Western	117	1	14	14	43	27.4
33	Andiwo	Western	83	1.4	8	8	51	17
34	Apundo	Western	84	1.3	8	8	50	20.6
35	Nyachong Rawo	Western	105	1.4	10	10	36	27.2
36	Ochuti Makawar	Western	97	1.3	10	10	39	17.4
37	Nolusu	Western	106	1.1	10	10	45	16.2
38	Ex-Maiyakalo	Western	106	1.6	10	10	27	24
39	Unyimbo	Western	99	1.6	10	10	42	24
40	Nakhabado	Western	76	1.5	8	8	45	25.1
41	Maebeye	Western	90	1.2	10	10	32	16.2
42	Nangalama	Western	106	1.6	12	12	40	19.1
43	Sabina-C	Western	79	1.9	9	9	27	19.9
44	Opunde	Western	95	1.2	11	11	27	14.7
45	Namayeye	Western	82	1.3	8	8	42	24.9
	MEAN	Western	**89**	**1.6**	**8**	**8**	**41**	**20.95**
46	Loponoikal	Turkaka	90	1.8	10	10	26	15.9
47	Ikorinaite	Turkaka	96	1.3	10	10	38	17.3
48	Naliba	Turkaka	81	1.3	12	12	70	13.4
49	Naliba	Turkaka	75	1.3	7	7	55	19.9

50	Nakualem	Turkaka	86	2.3	7	7	32	17.8
51	Lokilioko	Turkaka	94	1.6	10	10	39	26.44
52	Looyakes	Turkaka	82	1.5	9	9	44	21.1
53	Lopook	Turkaka	101	1.5	11	11	52	25.4
54	Akuaaite	Turkaka	94	1.4	10	10	36	26.1
55	Ex-Nambale	Turkaka	91	1.2	9	9	27	10.9
56	Ex-Kidera-2	Turkaka	92	1.4	9	9	58	10.6
57	Alekuekaal	Turkaka	96	1	10	10	42	17.9
58	Ushalak	Turkaka	100	1.5	12	12	44	27.7
59	Naseger Nyang	Turkaka	108	1.6	12	12	34	23.8
60	Oleuro	Turkaka	102	1	11	11	35	22.8
61	Lorengen	Turkaka	102	1.4	11	11	33	19.6
62	Nyabuluri	Turkaka	92	1.6	13	13	29	26.9
63	Nagugu	Turkaka	81	1.1	9	9	34	26.11
64	Nakualem	Turkaka	85	1.2	8	8	36	50.1
	MEAN	Turkaka	**92**	**1.4**	**10**	**10**	**40**	**22.09**
65	Kizungu	Coast	95	1.5	9	9	32	26.9
66	Kitaita	Coast	105	1.1	11	11	45	24.3
67	Ex-kuria/kisii?	Coast	100	1.2	11	11	51	27.7
68	Muhawi	Coast	81	1.5	9	9	62	18.1
69	Kibiriti-B	Coast	85	1.5	9	9	55	26.9
70	Bishe	Coast	81	1.2	8	8	54	23.4
71	Kibiriti-Ngoma	Coast	86	1.5	10	10	57	18
72	Kibiriti-A	Coast	81	1.1	9	9	41	16
73	Kijuju-B	Coast	80	1.4	9	9	51	21.2
74	Kijuju-A	Coast	102	1.9	11	11	62	21.1
75	Ex-Busia	Coast	95	1.1	9	9	57	23.4
76	Kambio(White)	Coast	82	2	8	8	34	16.3
77	Kajerere	Coast	84	1.4	10	10	39	18.44
78	Megewa	Coast	77	1.6	7	7	59	23.8
79	Kambio(pigmented)	Coast	83	2.2	9	9	44	17.11
80	Ex-malindi-A	Coast	76	2.1	7	7	38	19.9
81	Ex malindi-B	Coast	75	1.3	7	7	47	27
	MEAN	Coast	**99**	**1.7**	**10**	**10**	**55**	**24.6**
82	Kimiiru brown	Eastern	78	2.9	9	9	50	25.2
83	Kimiiru brown	Eastern	80	1.7	7	7	43	22
84	Kimiiru brown	Eastern	75	2	8	8	44	25.6
85	Kimiiru light brown	Eastern	83	1.1	8	8	43	22.1
86	Kimiiru light brown	Eastern	96	1	10	10	66	19
87	Kimiiru brown	Eastern	79	1.3	8	8	36	12.6
88	Kimiiru light brown	Eastern	95	2.2	10	10	46	16.7
89	Kimiiru White	Eastern	74	0.4	11	11	37	14.6
90	Kimiiru light brown	Eastern	92	1.3	9	9	42	21.1
91	MunthanoMuthigwaMugana	Eastern	87	1.2	9	9	63	18.4
92	Mubeeta Mutune	Eastern	104	1.3	10	10	60	17.6
93	Munthano Muthigwa Mugana Nkumiiru Nciri	Eastern	90	0.8	10	10	41	16.9
94	Kaguru	Eastern	87	1.2	8	8	35	18.9
95	Munthano Muthigwa Mugana Nkumiiru Nciri	Eastern	86	1.3	8	8	42	20.1
96	Munthano Muthigwa Mugana Nkumiiru Nciri	Eastern	99	2.5	10	10	37	15
97	Kaguru	Eastern	93	1.6	10	10	44	21.8
98	Muthigwa	Eastern	83	1	10	10	63	25

99	Muganka	Eastern	93	1	12	12	39	16.1
100	Muganka	Eastern	75	1.2	7	7	43	18.9
101	Ex iriga	Eastern	69	2.1	7	7	33	17.8
102	Muganka	Eastern	86	1.9	9	9	45	21.1
103	Muganka	Eastern	78	1.3	9	9	53	26
104	Kimiiru light brown	Eastern	89	1.2	11	10	50	18.1
105	Wagana white	Eastern	90	0.8	9	9	56	17.6
106	Wagana	Eastern	79	1.5	9	9	49	27.1
107	MERU	Eastern	83	0.9	6	6	34	19.7
108	Wagana	Eastern	84	1.5	9	9	50	19.3
109	Muchuuri	Eastern	78	2.4	8	8	47	22.9
	MEAN	Eastern	**85**	**1.5**	**9**	**9**	**46**	**19.9**
110	S 47	Breeders(Lan)	82	1.7	9	9	54	18.56
111	N-8	Breeders(Lan)	84	2.1	9	9	46	21.78
112	Susa	Breeders(Lan)	86	1.3	7	7	32	22.2
113	Damoga	Breeders(Lan)	86	1.1	7	7	47	20.8
114	IS25567	Breeders(Lan)	85	1	8	8	37	20.9
115	L-5	Breeders(Lan)	86	2.5	9	9	21	20.7
116	URUKARAZA	Breeders(Lan)	94	1	8	8	38	21.6
117	LIVOYWA	Breeders(Lan)	87	1.1	10	10	33	17.9
118	GAHUNDA	Breeders(Lan)	77	1.6	6	6	31	26.4
119	N-12	Breeders(Lan)	85	1	10	11	36	20
120	IS9119	Breeders(kat)	90	1.2	10	10	51	19.44
121	GATARANGA	Breeders(kat)	80	1.2	8	8	45	23.4
122	N-15	Breeders(kat)	76	1.5	8	8	59	26.3
123	IS25542	Breeders(kat)	91	1.1	9	9	45	22.2
124	ICSV1156BF	Breeders(kat)	76	1.5	7	7	40	11.8
125	MUL	Breeders(kat)	88	1.4	9	9	34	35.8
126	F6YQ212XDINJMASH	Breeders(kat)	81	1.3	8	8	34	14.7
127	SDSH9003	Breeders(kat)	83	1.9	8	8	34	24.22
128	KAT369XF6YQ212	Breeders(kat)	79	1	8	8	50	12.9
129	GADAM	Breeders(kat)	83	1.8	8	8	41	15.1
130	MARIMANTI-CO1110	Breeders(kat)	86	1	10	10	38	27.9
131	F6YQ212XICSUIII(IN)	Breeders(kat)	82	1.1	9	9	64	28
132	IS3697XICSVIII(IN)	Breeders(kat)	82	1.8	8	8	50	25.8
133	SDS1848-3(ICSEL)6	Breeders(kat)	91	0.9	12	12	20	10.9
134	SEREDOXESSUTI	Breeders(kak)	82	1.1	7	7	43	21.9
135	SERENAXESSUTI	Breeders(kak)	82	1.5	8	8	45	22.6
136	KAK8474	Breeders(kak)	90	1.2	6	6	50	22.8
137	TESO 6	Breeders(kak)	87	1.5	10	10	47	23.1
138	E525HRXESSUTI	Breeders(kak)	92	1.3	10	10	52	27.3
139	E525HRXESSUTI	Breeders(kak)	96	1.2	11	11	47	20.7
140	KAK13	Breeders(kak)	81	1.5	10	10	44	20.1
141	E525HRXESSUTI	Breeders(kak)	99	1.8	11	11	38	23.9
142	SERENAXESSUTI	Breeders(kak)	76	1.7	7	7	57	21.8
143	E525HRXESSUTI	Breeders(kak)	92	0.8	8	8	23	14.2
144	KAK8540	Breeders(kak)	87	1.8	6	6	47	24.2
145	E525HRXESSUTI	Breeders(kak)	73	2	6	6	36	16.9
146	SERENAXIKHUMBA	Breeders(kak)	97	1.7	6	6	49	22.7
147	KAK2809	Breeders(kak)	77	1.2	9	9	44	18.8
148	KAK 71	Breeders(kak)	95	0.7	9	9	46	18.9
	MEAN	Breeders(kak)	**85**	**1.4**	**8**	**8**	**42**	**21.26**
	GRAND MEANS		**88**	**1.5**	**9**	**9**	**43.2**	**21.07**

LSD (5% Level)		21.1	0.97	3.26	3.25	21.67	10.03
CV%		15.2	40.4	23.5	23.4	31.9	29.6

Appendix 3: Allele frequency for all the 39 SSR Markers

Marker	Allele	Count	Freq	Variance	SD	2.5% l.b.	97.5% u.b.
Xtxp40	121	2	0.0068	0.00004535	0.0067	0.0000	0.0203
Xtxp40	127	2	0.0068	0.00004535	0.0067	0.0000	0.0203
Xtxp40	130	5	0.0169	0.00010079	0.0100	0.0000	0.0405
Xtxp40	133	247	0.8345	0.00083064	0.0288	0.7770	0.8885
Xtxp40	136	31	0.1047	0.00055363	0.0235	0.0608	0.1554
Xtxp40	139	7	0.0236	0.00014460	0.0120	0.0034	0.0507
Xtxp40	142	2	0.0068	0.00004535	0.0067	0.0000	0.0203
CIR286	104	4	0.0136	0.00009129	0.0096	0.0000	0.0340
CIR286	108	121	0.4116	0.00138138	0.0372	0.3390	0.4831
CIR286	110	7	0.0238	0.00012341	0.0111	0.0035	0.0479
CIR286	120	2	0.0068	0.00002282	0.0048	0.0000	0.0171
CIR286	122	7	0.0238	0.00010027	0.0100	0.0068	0.0445
CIR286	124	124	0.4218	0.00133511	0.0365	0.3503	0.4932
CIR286	126	27	0.0918	0.00050952	0.0226	0.0507	0.1385
CIR286	128	2	0.0068	0.00004596	0.0068	0.0000	0.0205
Xcup02	193	213	0.7448	0.00126821	0.0356	0.6736	0.8138
Xcup02	199	46	0.1608	0.00089494	0.0299	0.1036	0.2218
Xcup02	202	1	0.0035	0.00001214	0.0035	0.0000	0.0107
Xcup02	205	26	0.0909	0.00057793	0.0240	0.0483	0.1408
Xtxp10	128	33	0.1115	0.00058941	0.0243	0.0676	0.1622
Xtxp10	132	1	0.0034	0.00001134	0.0034	0.0000	0.0101
Xtxp10	136	13	0.0439	0.00024948	0.0158	0.0169	0.0777
Xtxp10	138	85	0.2872	0.00114343	0.0338	0.2230	0.3547
Xtxp10	140	50	0.1689	0.00083442	0.0289	0.1149	0.2264
Xtxp10	142	28	0.0946	0.00048738	0.0221	0.0541	0.1419
Xtxp10	144	11	0.0372	0.00023035	0.0152	0.0101	0.0709
Xtxp10	146	1	0.0034	0.00001134	0.0034	0.0000	0.0101
Xtxp10	148	74	0.2500	0.00108428	0.0329	0.1858	0.3142
CIR329	105	1	0.0034	0.00001134	0.0034	0.0000	0.0101
CIR329	107	20	0.0676	0.00035721	0.0189	0.0338	0.1081
CIR329	109	142	0.4797	0.00125270	0.0354	0.4122	0.5473
CIR329	111	81	0.2736	0.00108050	0.0329	0.2128	0.3378
CIR329	113	27	0.0912	0.00045739	0.0214	0.0507	0.1351
CIR329	115	25	0.0845	0.00041975	0.0205	0.0473	0.1284
Xtxp114	215	177	0.5980	0.00143030	0.0378	0.5236	0.6723
Xtxp114	218	85	0.2872	0.00118908	0.0345	0.2196	0.3547
Xtxp114	230	16	0.0541	0.00032266	0.0180	0.0236	0.0912
Xtxp114	233	18	0.0608	0.00036307	0.0191	0.0270	0.1014
CIR238	100	1	0.0034	0.00001165	0.0034	0.0000	0.0103
CIR238	102	12	0.0411	0.00022300	0.0149	0.0138	0.0743
CIR238	104	1	0.0034	0.00001165	0.0034	0.0000	0.0104

CIR238	106	5	0.0171	0.00008009	0.0089	0.0034	0.0374
CIR238	108	1	0.0034	0.00001165	0.0034	0.0000	0.0104
CIR238	110	4	0.0137	0.00006908	0.0083	0.0000	0.0338
CIR238	54	8	0.0274	0.00013560	0.0116	0.0068	0.0517
CIR238	56	9	0.0308	0.00009905	0.0100	0.0136	0.0514
CIR238	68	4	0.0137	0.00004563	0.0068	0.0034	0.0278
CIR238	70	18	0.0616	0.00025545	0.0160	0.0338	0.0952
CIR238	72	71	0.2432	0.00101417	0.0318	0.1824	0.3069
CIR238	74	21	0.0719	0.00030469	0.0175	0.0408	0.1088
CIR238	76	7	0.0240	0.00010162	0.0101	0.0068	0.0451
CIR238	78	9	0.0308	0.00014596	0.0121	0.0102	0.0574
CIR238	80	14	0.0479	0.00021882	0.0148	0.0207	0.0788
CIR238	82	4	0.0137	0.00004563	0.0068	0.0034	0.0278
CIR238	84	49	0.1678	0.00078057	0.0279	0.1156	0.2257
CIR238	86	6	0.0205	0.00011439	0.0107	0.0034	0.0442
CIR238	88	33	0.1130	0.00053412	0.0231	0.0699	0.1610
CIR238	90	13	0.0445	0.00023272	0.0153	0.0170	0.0764
CIR238	94	1	0.0034	0.00001165	0.0034	0.0000	0.0103
CIR238	98	1	0.0034	0.00001165	0.0034	0.0000	0.0104
Xtxp141	137	2	0.0068	0.00004596	0.0068	0.0000	0.0205
Xtxp141	139	20	0.0680	0.00040815	0.0202	0.0310	0.1096
Xtxp141	141	14	0.0476	0.00026224	0.0162	0.0171	0.0811
Xtxp141	143	13	0.0442	0.00025279	0.0159	0.0169	0.0782
Xtxp141	145	34	0.1156	0.00062631	0.0250	0.0685	0.1655
Xtxp141	147	8	0.0272	0.00015693	0.0125	0.0068	0.0544
Xtxp141	149	142	0.4830	0.00146733	0.0383	0.4063	0.5578
Xtxp141	151	38	0.1293	0.00064992	0.0255	0.0811	0.1815
Xtxp141	157	1	0.0034	0.00001149	0.0034	0.0000	0.0103
Xtxp141	159	8	0.0272	0.00015693	0.0125	0.0068	0.0544
Xtxp141	161	13	0.0442	0.00025279	0.0159	0.0169	0.0782
Xtxp141	165	1	0.0034	0.00001149	0.0034	0.0000	0.0103
CIR223	104	189	0.6750	0.00117156	0.0342	0.6058	0.7413
CIR223	106	7	0.0250	0.00008482	0.0092	0.0074	0.0455
CIR223	108	7	0.0250	0.00008482	0.0092	0.0073	0.0441
CIR223	110	51	0.1821	0.00089823	0.0300	0.1259	0.2430
CIR223	112	4	0.0143	0.00007507	0.0087	0.0000	0.0328
CIR223	114	11	0.0393	0.00018030	0.0134	0.0147	0.0683
CIR223	116	9	0.0321	0.00018395	0.0136	0.0073	0.0616
CIR223	98	2	0.0071	0.00002515	0.0050	0.0000	0.0180
Xtxp273	207	4	0.0155	0.00011832	0.0109	0.0000	0.0391
Xtxp273	210	1	0.0039	0.00001491	0.0039	0.0000	0.0122
Xtxp273	213	25	0.0969	0.00057321	0.0239	0.0530	0.1452
Xtxp273	216	10	0.0388	0.00022872	0.0151	0.0117	0.0709
Xtxp273	219	140	0.5426	0.00159338	0.0399	0.4643	0.6202
Xtxp273	222	51	0.1977	0.00100410	0.0317	0.1381	0.2617
Xtxp273	225	19	0.0736	0.00042368	0.0206	0.0365	0.1172
Xtxp273	228	4	0.0155	0.00011832	0.0109	0.0000	0.0394
Xtxp273	231	4	0.0155	0.00008828	0.0094	0.0000	0.0370
CIR276	224	2	0.0068	0.00004535	0.0067	0.0000	0.0203
CIR276	226	268	0.9054	0.00048738	0.0221	0.8615	0.9459
CIR276	228	25	0.0845	0.00044258	0.0210	0.0473	0.1284

CIR276	230	1	0.0034	0.00001134	0.0034	0.0000	0.0101
Xcup061	186	2	0.0069	0.00004723	0.0069	0.0000	0.0211
Xcup061	189	2	0.0069	0.00004723	0.0069	0.0000	0.0210
Xcup061	195	53	0.1828	0.00089926	0.0300	0.1267	0.2431
Xcup061	198	230	0.7931	0.00098897	0.0314	0.7276	0.8517
Xcup061	201	1	0.0034	0.00001181	0.0034	0.0000	0.0106
Xcup061	204	2	0.0069	0.00004723	0.0069	0.0000	0.0210
Xtxp21	169	44	0.1517	0.00074493	0.0273	0.1007	0.2069
Xtxp21	171	138	0.4759	0.00152987	0.0391	0.3986	0.5544
Xtxp21	173	11	0.0379	0.00023978	0.0155	0.0105	0.0714
Xtxp21	175	16	0.0552	0.00019304	0.0139	0.0282	0.0833
Xtxp21	177	42	0.1448	0.00064012	0.0253	0.0959	0.1959
Xtxp21	179	15	0.0517	0.00018369	0.0136	0.0274	0.0799
Xtxp21	181	20	0.0690	0.00030014	0.0173	0.0374	0.1062
Xtxp21	183	1	0.0034	0.00001181	0.0034	0.0000	0.0105
Xtxp21	185	1	0.0034	0.00001181	0.0034	0.0000	0.0105
Xtxp21	187	2	0.0069	0.00004723	0.0069	0.0000	0.0210
Xtxp278	241	3	0.0102	0.00005714	0.0076	0.0000	0.0272
Xtxp278	247	289	0.9830	0.00007902	0.0089	0.9628	0.9966
Xtxp278	250	1	0.0034	0.00001149	0.0034	0.0000	0.0103
Xtxp278	253	1	0.0034	0.00001149	0.0034	0.0000	0.0103
CIR283	113	136	0.4595	0.00108459	0.0329	0.3953	0.5236
CIR283	115	24	0.0811	0.00041212	0.0203	0.0439	0.1216
CIR283	117	21	0.0709	0.00029698	0.0172	0.0405	0.1047
CIR283	119	95	0.3209	0.00082200	0.0287	0.2669	0.3784
CIR283	135	3	0.0101	0.00005637	0.0075	0.0000	0.0270
CIR283	137	2	0.0068	0.00002252	0.0047	0.0000	0.0169
CIR283	139	2	0.0068	0.00004535	0.0067	0.0000	0.0203
CIR283	141	2	0.0068	0.00004535	0.0067	0.0000	0.0203
CIR283	143	11	0.0372	0.00020752	0.0144	0.0101	0.0676
Xtxp321	192	12	0.0414	0.00024978	0.0158	0.0137	0.0753
Xtxp321	194	4	0.0138	0.00007003	0.0084	0.0000	0.0315
Xtxp321	196	1	0.0034	0.00001181	0.0034	0.0000	0.0105
Xtxp321	198	99	0.3414	0.00137226	0.0370	0.2705	0.4138
Xtxp321	200	12	0.0414	0.00022600	0.0150	0.0139	0.0729
Xtxp321	202	8	0.0276	0.00018500	0.0136	0.0068	0.0556
Xtxp321	204	87	0.3000	0.00122235	0.0350	0.2343	0.3706
Xtxp321	206	4	0.0138	0.00009381	0.0097	0.0000	0.0347
Xtxp321	208	21	0.0724	0.00040379	0.0201	0.0347	0.1154
Xtxp321	210	22	0.0759	0.00041215	0.0203	0.0385	0.1182
Xtxp321	212	5	0.0172	0.00008118	0.0090	0.0034	0.0377
Xtxp321	216	4	0.0138	0.00009381	0.0097	0.0000	0.0347
Xtxp321	218	4	0.0138	0.00009381	0.0097	0.0000	0.0347
Xtxp321	220	2	0.0069	0.00004723	0.0069	0.0000	0.0210
Xtxp321	222	3	0.0103	0.00005871	0.0077	0.0000	0.0278
Xtxp321	224	2	0.0069	0.00004723	0.0069	0.0000	0.0210
CIR300	104	2	0.0068	0.00002252	0.0047	0.0000	0.0169
CIR300	106	188	0.6351	0.00147449	0.0384	0.5574	0.7095
CIR300	110	77	0.2601	0.00122054	0.0349	0.1926	0.3311
CIR300	112	29	0.0980	0.00054006	0.0232	0.0541	0.1453
Gpsb067	170	2	0.0073	0.00005289	0.0073	0.0000	0.0224

Gpsb067	172	88	0.3212	0.00100531	0.0317	0.2609	0.3838
Gpsb067	174	1	0.0036	0.00001322	0.0036	0.0000	0.0113
Gpsb067	176	1	0.0036	0.00001322	0.0036	0.0000	0.0113
Gpsb067	178	11	0.0401	0.00021467	0.0147	0.0145	0.0714
Gpsb067	180	166	0.6058	0.00113034	0.0336	0.5399	0.6714
Gpsb067	182	5	0.0182	0.00009081	0.0095	0.0035	0.0396
SbAGB02	101	14	0.0473	0.00023598	0.0154	0.0203	0.0777
SbAGB02	115	6	0.0203	0.00011136	0.0106	0.0034	0.0439
SbAGB02	117	6	0.0203	0.00011136	0.0106	0.0034	0.0439
SbAGB02	93	28	0.0946	0.00053304	0.0231	0.0507	0.1419
SbAGB02	95	106	0.3581	0.00127923	0.0358	0.2872	0.4291
SbAGB02	97	113	0.3818	0.00135504	0.0368	0.3108	0.4527
SbAGB02	99	23	0.0777	0.00044998	0.0212	0.0372	0.1216
Xsep310	194	3	0.0101	0.00005637	0.0075	0.0000	0.0270
Xsep310	199	2	0.0068	0.00002252	0.0047	0.0000	0.0169
Xsep310	204	291	0.9831	0.00007797	0.0088	0.9628	0.9966
CIR240	105	53	0.1803	0.00080859	0.0284	0.1259	0.2379
CIR240	107	8	0.0272	0.00015693	0.0125	0.0068	0.0541
CIR240	109	137	0.4660	0.00131102	0.0362	0.3966	0.5372
CIR240	111	75	0.2551	0.00102659	0.0320	0.1939	0.3197
CIR240	127	1	0.0034	0.00001149	0.0034	0.0000	0.0103
CIR240	129	2	0.0068	0.00004596	0.0068	0.0000	0.0205
CIR240	135	7	0.0238	0.00014654	0.0121	0.0034	0.0507
CIR240	141	1	0.0034	0.00001149	0.0034	0.0000	0.0103
CIR240	149	1	0.0034	0.00001149	0.0034	0.0000	0.0103
CIR240	151	1	0.0034	0.00001149	0.0034	0.0000	0.0103
CIR240	155	2	0.0068	0.00002282	0.0048	0.0000	0.0171
CIR240	157	2	0.0068	0.00004596	0.0068	0.0000	0.0205
CIR240	161	1	0.0034	0.00001149	0.0034	0.0000	0.0103
CIR240	99	3	0.0102	0.00003400	0.0058	0.0000	0.0236
Xgap84	179	1	0.0034	0.00001149	0.0034	0.0000	0.0103
Xgap84	181	21	0.0714	0.00030080	0.0173	0.0405	0.1069
Xgap84	183	176	0.5986	0.00103289	0.0321	0.5345	0.6610
Xgap84	185	18	0.0612	0.00025216	0.0159	0.0338	0.0946
Xgap84	187	4	0.0136	0.00006816	0.0083	0.0000	0.0308
Xgap84	189	6	0.0204	0.00011286	0.0106	0.0034	0.0442
Xgap84	193	14	0.0476	0.00016968	0.0130	0.0238	0.0748
Xgap84	195	11	0.0374	0.00021029	0.0145	0.0135	0.0680
Xgap84	197	12	0.0408	0.00019691	0.0140	0.0169	0.0709
Xgap84	199	9	0.0306	0.00014403	0.0120	0.0101	0.0574
Xgap84	201	1	0.0034	0.00001149	0.0034	0.0000	0.0103
Xgap84	207	6	0.0204	0.00011286	0.0106	0.0034	0.0439
Xgap84	209	1	0.0034	0.00001149	0.0034	0.0000	0.0103
Xgap84	211	4	0.0136	0.00004502	0.0067	0.0034	0.0274
Xgap84	213	5	0.0170	0.00005588	0.0075	0.0034	0.0338
Xgap84	217	2	0.0068	0.00002282	0.0048	0.0000	0.0170
Xgap84	219	3	0.0102	0.00003400	0.0058	0.0000	0.0236
Xtxp15	199	6	0.0203	0.00013419	0.0116	0.0000	0.0473
Xtxp15	207	2	0.0068	0.00004535	0.0067	0.0000	0.0203
Xtxp15	209	4	0.0135	0.00006725	0.0082	0.0000	0.0304
Xtxp15	211	95	0.3209	0.00130136	0.0361	0.2500	0.3919

Xtxp15	213	70	0.2365	0.00112870	0.0336	0.1723	0.3007
Xtxp15	215	58	0.1959	0.00090475	0.0301	0.1385	0.2568
Xtxp15	217	23	0.0777	0.00042716	0.0207	0.0405	0.1216
Xtxp15	219	26	0.0878	0.00049571	0.0223	0.0473	0.1351
Xtxp15	221	11	0.0372	0.00023035	0.0152	0.0101	0.0709
Xtxp15	227	1	0.0034	0.00001134	0.0034	0.0000	0.0101
Xcup063	129	2	0.0068	0.00004596	0.0068	0.0000	0.0205
Xcup063	135	2	0.0068	0.00004596	0.0068	0.0000	0.0205
Xcup063	141	38	0.1293	0.00074248	0.0272	0.0782	0.1858
Xcup063	147	252	0.8571	0.00080985	0.0285	0.7973	0.9088
CIR306	118	4	0.0135	0.00006725	0.0082	0.0000	0.0304
CIR306	120	108	0.3649	0.00140601	0.0375	0.2905	0.4392
CIR306	122	184	0.6216	0.00142946	0.0378	0.5473	0.6959
Xcup53	183	3	0.0101	0.00003355	0.0058	0.0000	0.0236
Xcup53	187	6	0.0203	0.00006570	0.0081	0.0068	0.0372
Xcup53	191	2	0.0068	0.00002252	0.0047	0.0000	0.0169
Xcup53	195	281	0.9493	0.00022233	0.0149	0.9189	0.9764
Xcup53	199	4	0.0135	0.00004442	0.0067	0.0034	0.0270
CIR248	100	182	0.6276	0.00096978	0.0311	0.5680	0.6884
CIR248	90	74	0.2552	0.00078757	0.0281	0.2007	0.3103
CIR248	92	30	0.1034	0.00049695	0.0223	0.0621	0.1493
CIR248	94	3	0.0103	0.00003493	0.0059	0.0000	0.0238
CIR248	98	1	0.0034	0.00001181	0.0034	0.0000	0.0105
CIR262	214	69	0.2331	0.00103670	0.0322	0.1723	0.2973
CIR262	218	220	0.7432	0.00110680	0.0333	0.6757	0.8074
CIR262	222	7	0.0236	0.00012177	0.0110	0.0034	0.0473
Xgap72	181	125	0.4223	0.00159133	0.0399	0.3446	0.5000
Xgap72	183	24	0.0811	0.00045777	0.0214	0.0439	0.1250
Xgap72	185	1	0.0034	0.00001134	0.0034	0.0000	0.0101
Xgap72	187	62	0.2095	0.00107317	0.0328	0.1453	0.2736
Xgap72	189	71	0.2399	0.00115206	0.0339	0.1757	0.3074
Xgap72	191	11	0.0372	0.00023035	0.0152	0.0101	0.0709
Xgap72	197	2	0.0068	0.00004535	0.0067	0.0000	0.0203
CIR246	101	28	0.0946	0.00057869	0.0241	0.0473	0.1419
CIR246	93	13	0.0439	0.00027230	0.0165	0.0135	0.0777
CIR246	95	15	0.0507	0.00026798	0.0164	0.0203	0.0845
CIR246	97	1	0.0034	0.00001134	0.0034	0.0000	0.0101
CIR246	99	239	0.8074	0.00099351	0.0315	0.7432	0.8649
Xtxp136	237	145	0.4899	0.00083249	0.0289	0.4324	0.5473
Xtxp136	240	132	0.4459	0.00066506	0.0258	0.3953	0.4966
Xtxp136	243	19	0.0642	0.00018902	0.0137	0.0372	0.0912
Xcup14	204	204	0.6892	0.00115060	0.0339	0.6216	0.7568
Xcup14	206	92	0.3108	0.00115060	0.0339	0.2432	0.3784
Xtxp057	231	1	0.0034	0.00001165	0.0034	0.0000	0.0104
Xtxp057	235	4	0.0137	0.00009254	0.0096	0.0000	0.0345
Xtxp057	237	2	0.0068	0.00002314	0.0048	0.0000	0.0172
Xtxp057	239	5	0.0171	0.00008009	0.0089	0.0034	0.0374
Xtxp057	241	15	0.0514	0.00022822	0.0151	0.0240	0.0828
Xtxp057	243	9	0.0308	0.00016942	0.0130	0.0069	0.0582
Xtxp057	245	50	0.1712	0.00066707	0.0258	0.1224	0.2245
Xtxp057	247	68	0.2329	0.00089521	0.0299	0.1769	0.2925

Xtxp057	249	38	0.1301	0.00065807	0.0257	0.0822	0.1824
Xtxp057	251	40	0.1370	0.00073936	0.0272	0.0856	0.1939
Xtxp057	253	53	0.1815	0.00074780	0.0273	0.1293	0.2378
Xtxp057	255	4	0.0137	0.00006908	0.0083	0.0000	0.0315
Xtxp057	257	1	0.0034	0.00001165	0.0034	0.0000	0.0104
Xtxp057	263	2	0.0068	0.00004659	0.0068	0.0000	0.0208
Xgap206	101	1	0.0036	0.00001322	0.0036	0.0000	0.0112
Xgap206	103	59	0.2153	0.00108678	0.0330	0.1536	0.2810
Xgap206	107	6	0.0219	0.00010306	0.0102	0.0037	0.0441
Xgap206	109	4	0.0146	0.00010500	0.0102	0.0000	0.0370
Xgap206	111	2	0.0073	0.00002625	0.0051	0.0000	0.0185
Xgap206	113	22	0.0803	0.00043246	0.0208	0.0417	0.1232
Xgap206	115	35	0.1277	0.00066677	0.0258	0.0786	0.1812
Xgap206	117	18	0.0657	0.00034145	0.0185	0.0326	0.1051
Xgap206	119	11	0.0401	0.00021467	0.0147	0.0146	0.0719
Xgap206	121	7	0.0255	0.00011511	0.0107	0.0073	0.0489
Xgap206	123	11	0.0401	0.00013475	0.0116	0.0185	0.0647
Xgap206	125	30	0.1095	0.00055185	0.0235	0.0657	0.1583
Xgap206	127	1	0.0036	0.00001322	0.0036	0.0000	0.0113
Xgap206	133	3	0.0109	0.00006572	0.0081	0.0000	0.0294
Xgap206	135	6	0.0219	0.00012970	0.0114	0.0036	0.0471
Xgap206	137	8	0.0292	0.00018026	0.0134	0.0072	0.0580
Xgap206	139	6	0.0219	0.00007642	0.0087	0.0072	0.0401
Xgap206	141	16	0.0584	0.00034807	0.0187	0.0252	0.0977
Xgap206	143	7	0.0255	0.00014175	0.0119	0.0038	0.0515
Xgap206	145	5	0.0182	0.00009081	0.0095	0.0036	0.0396
Xgap206	147	10	0.0365	0.00023003	0.0152	0.0107	0.0688
Xgap206	149	4	0.0146	0.00005172	0.0072	0.0036	0.0299
Xgap206	153	2	0.0073	0.00002625	0.0051	0.0000	0.0185
Gpsb123	276	2	0.0078	0.00006056	0.0078	0.0000	0.0244
Gpsb123	278	2	0.0078	0.00006056	0.0078	0.0000	0.0246
Gpsb123	280	2	0.0078	0.00006056	0.0078	0.0000	0.0242
Gpsb123	282	10	0.0391	0.00020170	0.0142	0.0150	0.0691
Gpsb123	284	32	0.1250	0.00064087	0.0253	0.0781	0.1769
Gpsb123	286	22	0.0859	0.00049162	0.0222	0.0444	0.1326
Gpsb123	288	34	0.1328	0.00080824	0.0284	0.0787	0.1908
Gpsb123	290	73	0.2852	0.00142467	0.0377	0.2131	0.3598
Gpsb123	292	74	0.2891	0.00142241	0.0377	0.2176	0.3633
Gpsb123	294	5	0.0195	0.00013435	0.0116	0.0000	0.0462
Xtxp12	162	6	0.0217	0.00012785	0.0113	0.0036	0.0468
Xtxp12	168	3	0.0109	0.00006478	0.0080	0.0000	0.0292
Xtxp12	170	8	0.0290	0.00017770	0.0133	0.0071	0.0580
Xtxp12	172	7	0.0254	0.00016600	0.0129	0.0036	0.0540
Xtxp12	174	27	0.0978	0.00054764	0.0234	0.0547	0.1466
Xtxp12	176	2	0.0072	0.00005213	0.0072	0.0000	0.0224
Xtxp12	178	8	0.0290	0.00017770	0.0133	0.0071	0.0584
Xtxp12	182	2	0.0072	0.00002587	0.0051	0.0000	0.0182
Xtxp12	186	1	0.0036	0.00001303	0.0036	0.0000	0.0112
Xtxp12	188	6	0.0217	0.00012785	0.0113	0.0036	0.0471
Xtxp12	190	13	0.0471	0.00028586	0.0169	0.0177	0.0833
Xtxp12	192	29	0.1051	0.00056325	0.0237	0.0603	0.1549

Xtxp12	194	30	0.1087	0.00051825	0.0228	0.0652	0.1547
Xtxp12	196	38	0.1377	0.00062403	0.0250	0.0896	0.1879
Xtxp12	198	24	0.0870	0.00039154	0.0198	0.0500	0.1278
Xtxp12	200	39	0.1413	0.00078736	0.0281	0.0896	0.1978
Xtxp12	202	16	0.0580	0.00031696	0.0178	0.0255	0.0956
Xtxp12	204	2	0.0072	0.00005213	0.0072	0.0000	0.0224
Xtxp12	206	3	0.0109	0.00006478	0.0080	0.0000	0.0292
Xtxp12	208	2	0.0072	0.00005213	0.0072	0.0000	0.0224
Xtxp12	214	2	0.0072	0.00005213	0.0072	0.0000	0.0224
Xtxp12	216	4	0.0145	0.00005099	0.0071	0.0035	0.0294
Xtxp12	218	3	0.0109	0.00003853	0.0062	0.0000	0.0252
Xtxp12	220	1	0.0036	0.00001303	0.0036	0.0000	0.0111
Xtxp145	175	11	0.0404	0.00024479	0.0156	0.0115	0.0746
Xtxp145	177	2	0.0074	0.00005367	0.0073	0.0000	0.0227
Xtxp145	179	2	0.0074	0.00005367	0.0073	0.0000	0.0229
Xtxp145	201	1	0.0037	0.00001342	0.0037	0.0000	0.0114
Xtxp145	203	3	0.0110	0.00003965	0.0063	0.0000	0.0254
Xtxp145	205	36	0.1324	0.00038482	0.0196	0.0954	0.1728
Xtxp145	207	51	0.1875	0.00067413	0.0260	0.1383	0.2407
Xtxp145	211	16	0.0588	0.00035302	0.0188	0.0255	0.0971
Xtxp145	213	134	0.4926	0.00159454	0.0399	0.4130	0.5688
Xtxp145	215	12	0.0441	0.00022898	0.0151	0.0181	0.0761
Xtxp145	227	1	0.0037	0.00001342	0.0037	0.0000	0.0114
Xtxp145	235	2	0.0074	0.00005367	0.0073	0.0000	0.0227
Xtxp145	303	1	0.0037	0.00001342	0.0037	0.0000	0.0114
Xtxp265	174	50	0.1701	0.00091389	0.0302	0.1122	0.2313
Xtxp265	186	3	0.0102	0.00005714	0.0076	0.0000	0.0272
Xtxp265	189	10	0.0340	0.00017724	0.0133	0.0102	0.0612
Xtxp265	192	9	0.0306	0.00016716	0.0129	0.0068	0.0582
Xtxp265	195	7	0.0238	0.00010027	0.0100	0.0068	0.0448
Xtxp265	198	13	0.0442	0.00018338	0.0135	0.0203	0.0719
Xtxp265	201	21	0.0714	0.00025452	0.0160	0.0411	0.1054
Xtxp265	204	23	0.0782	0.00027074	0.0165	0.0476	0.1122
Xtxp265	207	2	0.0068	0.00002282	0.0048	0.0000	0.0170
Xtxp265	210	66	0.2245	0.00065213	0.0255	0.1735	0.2755
Xtxp265	213	47	0.1599	0.00046246	0.0215	0.1190	0.2041
Xtxp265	216	23	0.0782	0.00022446	0.0150	0.0507	0.1088
Xtxp265	219	12	0.0408	0.00012750	0.0113	0.0204	0.0646
Xtxp265	222	4	0.0136	0.00006816	0.0083	0.0000	0.0310
Xtxp265	225	1	0.0034	0.00001149	0.0034	0.0000	0.0103
Xtxp265	228	2	0.0068	0.00002282	0.0048	0.0000	0.0170
Xtxp265	231	1	0.0034	0.00001149	0.0034	0.0000	0.0103
Xtxp320	260	79	0.2743	0.00115330	0.0340	0.2103	0.3425
Xtxp320	263	15	0.0521	0.00028257	0.0168	0.0211	0.0868
Xtxp320	266	4	0.0139	0.00007100	0.0084	0.0000	0.0317
Xtxp320	269	6	0.0208	0.00011755	0.0108	0.0034	0.0448
Xtxp320	272	81	0.2813	0.00124708	0.0353	0.2118	0.3507
Xtxp320	275	39	0.1354	0.00065632	0.0256	0.0878	0.1866
Xtxp320	278	37	0.1285	0.00066904	0.0259	0.0810	0.1815
Xtxp320	281	6	0.0208	0.00014166	0.0119	0.0000	0.0483
Xtxp320	284	6	0.0208	0.00011755	0.0108	0.0034	0.0448

Xtxp320	287	8	0.0278	0.00016343	0.0128	0.0068	0.0552
Xtxp320	290	7	0.0243	0.00015263	0.0124	0.0035	0.0496

Appendix 4: Genotype frequency for all the 39 SSR Markers

Marker	**Allele1**	**Allele2**	**Covariance**	**Count**	**Freq**	**2.5% l.b.**	**97.5% u.b.**
Xtxp40	121	121	0.0000	1	0.0068	0.0000	0.0203
Xtxp40	127	127	0.0000	1	0.0068	0.0000	0.0203
Xtxp40	130	130	0.0001	2	0.0135	0.0000	0.0338
Xtxp40	130	133	0.0001	1	0.0068	0.0000	0.0203
Xtxp40	133	133	0.0008	119	0.8041	0.7365	0.8649
Xtxp40	133	136	0.0005	7	0.0473	0.0135	0.0811
Xtxp40	133	139	0.0001	1	0.0068	0.0000	0.0203
Xtxp40	136	136	0.0006	12	0.0811	0.0405	0.1284
Xtxp40	139	139	0.0001	3	0.0203	0.0000	0.0473
Xtxp40	142	142	0.0000	1	0.0068	0.0000	0.0203
CIR286	104	104	0.0001	2	0.0136	0.0000	0.0340
CIR286	108	108	0.0014	49	0.3333	0.2585	0.4110
CIR286	108	110	0.0001	1	0.0068	0.0000	0.0205
CIR286	108	122	0.0001	1	0.0068	0.0000	0.0205
CIR286	108	124	0.0010	18	0.1224	0.0743	0.1769
CIR286	108	126	0.0002	3	0.0204	0.0000	0.0473
CIR286	110	110	0.0001	2	0.0136	0.0000	0.0340
CIR286	110	124	0.0000	2	0.0136	0.0000	0.0340
CIR286	120	124	0.0000	2	0.0136	0.0000	0.0342
CIR286	122	122	0.0001	1	0.0068	0.0000	0.0205
CIR286	122	124	0.0000	4	0.0272	0.0068	0.0544
CIR286	124	124	0.0013	48	0.3265	0.2517	0.4014
CIR286	124	126	0.0002	2	0.0136	0.0000	0.0340
CIR286	126	126	0.0005	11	0.0748	0.0340	0.1216
CIR286	128	128	0.0000	1	0.0068	0.0000	0.0205
Xcup02	193	193	0.0013	104	0.7273	0.6525	0.7986
Xcup02	193	199	0.0008	4	0.0280	0.0068	0.0567
Xcup02	193	202	0.0000	1	0.0070	0.0000	0.0214
Xcup02	199	199	0.0009	21	0.1469	0.0915	0.2071
Xcup02	205	205	0.0006	13	0.0909	0.0483	0.1408
Xtxp10	128	128	0.0006	13	0.0878	0.0473	0.1351
Xtxp10	128	138	0.0002	2	0.0135	0.0000	0.0338
Xtxp10	128	148	0.0001	5	0.0338	0.0068	0.0676
Xtxp10	132	138	0.0000	1	0.0068	0.0000	0.0203
Xtxp10	136	136	0.0002	5	0.0338	0.0068	0.0676
Xtxp10	136	138	0.0001	2	0.0135	0.0000	0.0338
Xtxp10	136	140	0.0000	1	0.0068	0.0000	0.0203
Xtxp10	138	138	0.0011	32	0.2162	0.1486	0.2838
Xtxp10	138	140	0.0003	6	0.0405	0.0135	0.0743
Xtxp10	138	142	0.0002	1	0.0068	0.0000	0.0203
Xtxp10	138	148	0.0004	9	0.0608	0.0270	0.1014
Xtxp10	140	140	0.0008	20	0.1351	0.0811	0.1892
Xtxp10	140	142	0.0001	3	0.0203	0.0000	0.0473
Xtxp10	142	142	0.0005	10	0.0676	0.0338	0.1081
Xtxp10	142	144	0.0000	1	0.0068	0.0000	0.0203

Xtxp10	142	146	0.0000	1	0.0068	0.0000	0.0203
Xtxp10	142	148	0.0001	2	0.0135	0.0000	0.0338
Xtxp10	144	144	0.0002	5	0.0338	0.0068	0.0676
Xtxp10	148	148	0.0011	29	0.1959	0.1351	0.2635
CIR329	105	109	0.0000	1	0.0068	0.0000	0.0203
CIR329	107	107	0.0004	7	0.0473	0.0135	0.0811
CIR329	107	109	0.0002	5	0.0338	0.0068	0.0676
CIR329	107	113	0.0000	1	0.0068	0.0000	0.0203
CIR329	109	109	0.0013	52	0.3514	0.2770	0.4324
CIR329	109	111	0.0007	20	0.1351	0.0811	0.1959
CIR329	109	113	0.0002	5	0.0338	0.0068	0.0676
CIR329	109	115	0.0002	7	0.0473	0.0135	0.0878
CIR329	111	111	0.0011	29	0.1959	0.1351	0.2635
CIR329	111	113	0.0001	2	0.0135	0.0000	0.0338
CIR329	111	115	0.0001	1	0.0068	0.0000	0.0203
CIR329	113	113	0.0005	9	0.0608	0.0270	0.1014
CIR329	113	115	0.0000	1	0.0068	0.0000	0.0203
CIR329	115	115	0.0004	8	0.0541	0.0203	0.0946
Xtxp114	215	215	0.0014	80	0.5405	0.4595	0.6216
Xtxp114	215	218	0.0010	17	0.1149	0.0676	0.1689
Xtxp114	218	218	0.0012	34	0.2297	0.1689	0.2973
Xtxp114	230	230	0.0003	7	0.0473	0.0203	0.0811
Xtxp114	230	233	0.0000	2	0.0135	0.0000	0.0338
Xtxp114	233	233	0.0004	8	0.0541	0.0203	0.0946
CIR238	100	76	0.0000	1	0.0068	0.0000	0.0208
CIR238	102	102	0.0002	4	0.0274	0.0068	0.0552
CIR238	102	106	0.0000	1	0.0068	0.0000	0.0208
CIR238	102	72	0.0001	1	0.0068	0.0000	0.0207
CIR238	102	88	0.0000	2	0.0137	0.0000	0.0345
CIR238	104	72	0.0000	1	0.0068	0.0000	0.0208
CIR238	106	106	0.0001	1	0.0068	0.0000	0.0208
CIR238	106	70	0.0000	1	0.0068	0.0000	0.0208
CIR238	106	84	0.0000	1	0.0068	0.0000	0.0208
CIR238	108	72	0.0000	1	0.0068	0.0000	0.0208
CIR238	110	110	0.0001	1	0.0068	0.0000	0.0208
CIR238	110	72	0.0000	1	0.0068	0.0000	0.0208
CIR238	110	86	0.0000	1	0.0068	0.0000	0.0208
CIR238	54	54	0.0001	2	0.0137	0.0000	0.0345
CIR238	54	70	0.0000	2	0.0137	0.0000	0.0345
CIR238	54	76	0.0000	1	0.0068	0.0000	0.0207
CIR238	54	88	0.0000	1	0.0068	0.0000	0.0208
CIR238	56	70	0.0000	3	0.0205	0.0000	0.0476
CIR238	56	72	0.0000	3	0.0205	0.0000	0.0476
CIR238	56	80	0.0000	2	0.0137	0.0000	0.0345
CIR238	56	86	0.0000	1	0.0068	0.0000	0.0208
CIR238	68	72	0.0000	4	0.0274	0.0068	0.0556
CIR238	70	70	0.0003	3	0.0205	0.0000	0.0476
CIR238	70	74	0.0000	3	0.0205	0.0000	0.0476
CIR238	70	78	0.0000	1	0.0068	0.0000	0.0208
CIR238	70	82	0.0000	2	0.0137	0.0000	0.0345
CIR238	72	72	0.0010	25	0.1712	0.1103	0.2345

CIR238	72	74	0.0001	3	0.0205	0.0000	0.0476
CIR238	72	88	0.0001	4	0.0274	0.0068	0.0552
CIR238	72	90	0.0001	2	0.0137	0.0000	0.0345
CIR238	72	98	0.0000	1	0.0068	0.0000	0.0208
CIR238	74	74	0.0003	4	0.0274	0.0068	0.0552
CIR238	74	76	0.0000	1	0.0068	0.0000	0.0208
CIR238	74	82	0.0000	1	0.0068	0.0000	0.0208
CIR238	74	84	0.0001	1	0.0068	0.0000	0.0207
CIR238	74	88	0.0000	2	0.0137	0.0000	0.0345
CIR238	74	90	0.0000	1	0.0068	0.0000	0.0208
CIR238	74	94	0.0000	1	0.0068	0.0000	0.0208
CIR238	76	76	0.0001	1	0.0068	0.0000	0.0208
CIR238	76	80	0.0000	1	0.0068	0.0000	0.0208
CIR238	76	88	0.0000	1	0.0068	0.0000	0.0208
CIR238	78	78	0.0001	2	0.0137	0.0000	0.0345
CIR238	78	84	0.0000	4	0.0274	0.0068	0.0552
CIR238	80	80	0.0002	3	0.0205	0.0000	0.0476
CIR238	80	84	0.0000	5	0.0342	0.0068	0.0680
CIR238	82	88	0.0000	1	0.0068	0.0000	0.0208
CIR238	84	84	0.0008	17	0.1164	0.0680	0.1712
CIR238	84	88	0.0001	2	0.0137	0.0000	0.0345
CIR238	84	90	0.0000	2	0.0137	0.0000	0.0342
CIR238	86	86	0.0001	2	0.0137	0.0000	0.0345
CIR238	88	88	0.0005	10	0.0685	0.0338	0.1111
CIR238	90	90	0.0002	4	0.0274	0.0068	0.0552
Xtxp141	137	137	0.0000	1	0.0068	0.0000	0.0205
Xtxp141	139	139	0.0004	9	0.0612	0.0270	0.1020
Xtxp141	139	141	0.0000	1	0.0068	0.0000	0.0205
Xtxp141	139	151	0.0000	1	0.0068	0.0000	0.0205
Xtxp141	141	141	0.0003	5	0.0340	0.0068	0.0676
Xtxp141	141	143	0.0000	1	0.0068	0.0000	0.0205
Xtxp141	141	145	0.0000	1	0.0068	0.0000	0.0205
Xtxp141	141	149	0.0001	1	0.0068	0.0000	0.0205
Xtxp141	143	143	0.0003	5	0.0340	0.0068	0.0676
Xtxp141	143	149	0.0001	1	0.0068	0.0000	0.0205
Xtxp141	143	165	0.0000	1	0.0068	0.0000	0.0205
Xtxp141	145	145	0.0006	14	0.0952	0.0479	0.1429
Xtxp141	145	147	0.0000	1	0.0068	0.0000	0.0205
Xtxp141	145	149	0.0003	3	0.0204	0.0000	0.0473
Xtxp141	145	157	0.0000	1	0.0068	0.0000	0.0205
Xtxp141	147	147	0.0002	3	0.0204	0.0000	0.0473
Xtxp141	147	149	0.0001	1	0.0068	0.0000	0.0205
Xtxp141	149	149	0.0015	61	0.4150	0.3378	0.4966
Xtxp141	149	151	0.0003	9	0.0612	0.0270	0.1020
Xtxp141	149	159	0.0001	2	0.0136	0.0000	0.0342
Xtxp141	149	161	0.0001	3	0.0204	0.0000	0.0476
Xtxp141	151	151	0.0006	14	0.0952	0.0483	0.1429
Xtxp141	159	159	0.0002	3	0.0204	0.0000	0.0476
Xtxp141	161	161	0.0003	5	0.0340	0.0068	0.0676
CIR223	104	104	0.0012	79	0.5643	0.4823	0.6454
CIR223	104	106	0.0000	6	0.0429	0.0141	0.0786

CIR223	104	108	0.0001	4	0.0286	0.0069	0.0580
CIR223	104	110	0.0007	12	0.0857	0.0429	0.1357
CIR223	104	112	0.0001	1	0.0071	0.0000	0.0221
CIR223	104	114	0.0001	6	0.0429	0.0140	0.0791
CIR223	104	98	0.0000	2	0.0143	0.0000	0.0362
CIR223	106	110	0.0000	1	0.0071	0.0000	0.0219
CIR223	108	112	0.0000	1	0.0071	0.0000	0.0219
CIR223	108	116	0.0000	2	0.0143	0.0000	0.0362
CIR223	110	110	0.0009	19	0.1357	0.0803	0.1942
CIR223	112	112	0.0001	1	0.0071	0.0000	0.0219
CIR223	114	114	0.0002	2	0.0143	0.0000	0.0360
CIR223	114	116	0.0000	1	0.0071	0.0000	0.0219
CIR223	116	116	0.0002	3	0.0214	0.0000	0.0500
Xtxp273	207	207	0.0001	2	0.0155	0.0000	0.0391
Xtxp273	210	213	0.0000	1	0.0078	0.0000	0.0242
Xtxp273	213	213	0.0006	9	0.0698	0.0305	0.1172
Xtxp273	213	216	0.0000	1	0.0078	0.0000	0.0242
Xtxp273	213	219	0.0003	5	0.0388	0.0078	0.0758
Xtxp273	216	216	0.0002	3	0.0233	0.0000	0.0534
Xtxp273	216	219	0.0001	3	0.0233	0.0000	0.0534
Xtxp273	219	219	0.0016	59	0.4574	0.3721	0.5420
Xtxp273	219	222	0.0007	10	0.0775	0.0328	0.1269
Xtxp273	219	225	0.0002	4	0.0310	0.0075	0.0640
Xtxp273	222	222	0.0010	18	0.1395	0.0827	0.2016
Xtxp273	222	225	0.0001	3	0.0233	0.0000	0.0530
Xtxp273	222	231	0.0000	2	0.0155	0.0000	0.0394
Xtxp273	225	225	0.0004	6	0.0465	0.0152	0.0853
Xtxp273	228	228	0.0001	2	0.0155	0.0000	0.0397
Xtxp273	231	231	0.0001	1	0.0078	0.0000	0.0244
CIR276	224	224	0.0000	1	0.0068	0.0000	0.0203
CIR276	226	226	0.0005	130	0.8784	0.8243	0.9257
CIR276	226	228	0.0004	7	0.0473	0.0135	0.0811
CIR276	226	230	0.0000	1	0.0068	0.0000	0.0203
CIR276	228	228	0.0004	9	0.0608	0.0270	0.1014
Xcup061	186	186	0.0000	1	0.0069	0.0000	0.0210
Xcup061	189	189	0.0000	1	0.0069	0.0000	0.0210
Xcup061	195	195	0.0009	21	0.1448	0.0897	0.2041
Xcup061	195	198	0.0009	11	0.0759	0.0345	0.1216
Xcup061	198	198	0.0010	109	0.7517	0.6803	0.8194
Xcup061	198	201	0.0000	1	0.0069	0.0000	0.0210
Xcup061	204	204	0.0000	1	0.0069	0.0000	0.0210
Xtxp21	169	169	0.0007	16	0.1103	0.0621	0.1644
Xtxp21	169	171	0.0004	11	0.0759	0.0345	0.1233
Xtxp21	169	177	0.0001	1	0.0069	0.0000	0.0210
Xtxp21	171	171	0.0015	61	0.4207	0.3403	0.5000
Xtxp21	171	173	0.0001	1	0.0069	0.0000	0.0210
Xtxp21	171	175	0.0002	1	0.0069	0.0000	0.0210
Xtxp21	171	177	0.0004	3	0.0207	0.0000	0.0479
Xtxp21	173	173	0.0002	5	0.0345	0.0069	0.0685
Xtxp21	175	175	0.0002	1	0.0069	0.0000	0.0210
Xtxp21	175	177	-0.0001	13	0.0897	0.0476	0.1379

Xtxp21	177	177	0.0006	12	0.0828	0.0411	0.1301
Xtxp21	177	179	0.0000	1	0.0069	0.0000	0.0210
Xtxp21	179	179	0.0002	1	0.0069	0.0000	0.0210
Xtxp21	179	181	-0.0001	12	0.0828	0.0414	0.1310
Xtxp21	181	181	0.0003	4	0.0276	0.0068	0.0556
Xtxp21	183	185	0.0000	1	0.0069	0.0000	0.0210
Xtxp21	187	187	0.0000	1	0.0069	0.0000	0.0210
Xtxp278	241	241	0.0001	1	0.0068	0.0000	0.0207
Xtxp278	241	247	0.0001	1	0.0068	0.0000	0.0205
Xtxp278	247	247	0.0001	143	0.9728	0.9452	0.9932
Xtxp278	247	250	0.0000	1	0.0068	0.0000	0.0207
Xtxp278	247	253	0.0000	1	0.0068	0.0000	0.0205
CIR283	113	113	0.0011	42	0.2838	0.2162	0.3581
CIR283	113	115	0.0002	4	0.0270	0.0068	0.0541
CIR283	113	117	0.0002	4	0.0270	0.0068	0.0541
CIR283	113	119	0.0005	41	0.2770	0.2095	0.3514
CIR283	113	137	0.0000	1	0.0068	0.0000	0.0203
CIR283	113	143	0.0001	2	0.0135	0.0000	0.0338
CIR283	115	115	0.0004	8	0.0541	0.0203	0.0946
CIR283	115	119	0.0001	4	0.0270	0.0068	0.0541
CIR283	117	117	0.0003	4	0.0270	0.0068	0.0541
CIR283	117	119	0.0001	9	0.0608	0.0270	0.1014
CIR283	119	119	0.0008	19	0.1284	0.0811	0.1824
CIR283	119	135	0.0000	1	0.0068	0.0000	0.0203
CIR283	119	137	0.0000	1	0.0068	0.0000	0.0203
CIR283	119	143	0.0001	1	0.0068	0.0000	0.0203
CIR283	135	135	0.0001	1	0.0068	0.0000	0.0203
CIR283	139	139	0.0000	1	0.0068	0.0000	0.0203
CIR283	141	141	0.0000	1	0.0068	0.0000	0.0203
CIR283	143	143	0.0002	4	0.0270	0.0068	0.0541
Xtxp321	192	192	0.0002	5	0.0345	0.0069	0.0685
Xtxp321	192	198	0.0001	1	0.0069	0.0000	0.0210
Xtxp321	192	204	0.0001	1	0.0069	0.0000	0.0210
Xtxp321	194	194	0.0001	1	0.0069	0.0000	0.0210
Xtxp321	194	200	0.0000	1	0.0069	0.0000	0.0210
Xtxp321	194	208	0.0000	1	0.0069	0.0000	0.0210
Xtxp321	196	198	0.0000	1	0.0069	0.0000	0.0210
Xtxp321	198	198	0.0014	42	0.2897	0.2183	0.3636
Xtxp321	198	200	0.0001	1	0.0069	0.0000	0.0210
Xtxp321	198	204	0.0006	11	0.0759	0.0347	0.1197
Xtxp321	198	222	0.0000	1	0.0069	0.0000	0.0210
Xtxp321	200	200	0.0002	4	0.0276	0.0068	0.0556
Xtxp321	200	208	0.0000	1	0.0069	0.0000	0.0210
Xtxp321	200	210	0.0000	1	0.0069	0.0000	0.0210
Xtxp321	202	202	0.0002	4	0.0276	0.0068	0.0556
Xtxp321	204	204	0.0012	34	0.2345	0.1655	0.3034
Xtxp321	204	208	0.0001	2	0.0138	0.0000	0.0347
Xtxp321	204	210	0.0001	3	0.0207	0.0000	0.0479
Xtxp321	204	212	0.0000	2	0.0138	0.0000	0.0347
Xtxp321	206	206	0.0001	2	0.0138	0.0000	0.0347
Xtxp321	208	208	0.0004	8	0.0552	0.0207	0.0959

Xtxp321	208	210	0.0000	1	0.0069	0.0000	0.0210
Xtxp321	210	210	0.0004	8	0.0552	0.0207	0.0966
Xtxp321	210	212	0.0000	1	0.0069	0.0000	0.0210
Xtxp321	212	212	0.0001	1	0.0069	0.0000	0.0211
Xtxp321	216	216	0.0001	2	0.0138	0.0000	0.0347
Xtxp321	218	218	0.0001	2	0.0138	0.0000	0.0347
Xtxp321	220	220	0.0000	1	0.0069	0.0000	0.0211
Xtxp321	222	222	0.0001	1	0.0069	0.0000	0.0210
Xtxp321	224	224	0.0000	1	0.0069	0.0000	0.0210
CIR300	104	110	0.0000	2	0.0135	0.0000	0.0338
CIR300	106	106	0.0015	90	0.6081	0.5270	0.6892
CIR300	106	110	0.0011	4	0.0270	0.0068	0.0541
CIR300	106	112	0.0004	4	0.0270	0.0068	0.0541
CIR300	110	110	0.0012	35	0.2365	0.1689	0.3041
CIR300	110	112	0.0002	1	0.0068	0.0000	0.0203
CIR300	112	112	0.0005	12	0.0811	0.0405	0.1284
Gpsb067	170	170	0.0001	1	0.0073	0.0000	0.0224
Gpsb067	172	172	0.0010	22	0.1606	0.1007	0.2248
Gpsb067	172	178	0.0001	1	0.0073	0.0000	0.0226
Gpsb067	172	180	0.0009	40	0.2920	0.2174	0.3704
Gpsb067	172	182	0.0000	3	0.0219	0.0000	0.0504
Gpsb067	174	180	0.0000	1	0.0073	0.0000	0.0226
Gpsb067	176	180	0.0000	1	0.0073	0.0000	0.0226
Gpsb067	178	178	0.0002	3	0.0219	0.0000	0.0504
Gpsb067	178	180	0.0001	4	0.0292	0.0071	0.0597
Gpsb067	180	180	0.0011	60	0.4380	0.3556	0.5214
Gpsb067	182	182	0.0001	1	0.0073	0.0000	0.0227
SbAGB02	101	101	0.0002	4	0.0270	0.0068	0.0541
SbAGB02	101	95	0.0001	4	0.0270	0.0068	0.0541
SbAGB02	101	97	0.0001	2	0.0135	0.0000	0.0338
SbAGB02	115	115	0.0001	2	0.0135	0.0000	0.0338
SbAGB02	115	95	0.0000	1	0.0068	0.0000	0.0203
SbAGB02	115	97	0.0000	1	0.0068	0.0000	0.0203
SbAGB02	117	117	0.0001	2	0.0135	0.0000	0.0338
SbAGB02	117	95	0.0000	2	0.0135	0.0000	0.0338
SbAGB02	93	93	0.0005	12	0.0811	0.0405	0.1284
SbAGB02	93	95	0.0002	1	0.0068	0.0000	0.0203
SbAGB02	93	97	0.0002	2	0.0135	0.0000	0.0338
SbAGB02	93	99	0.0000	1	0.0068	0.0000	0.0203
SbAGB02	95	95	0.0013	41	0.2770	0.2095	0.3514
SbAGB02	95	97	0.0008	15	0.1014	0.0541	0.1486
SbAGB02	95	99	0.0002	1	0.0068	0.0000	0.0203
SbAGB02	97	97	0.0014	46	0.3108	0.2365	0.3851
SbAGB02	97	99	0.0002	1	0.0068	0.0000	0.0203
SbAGB02	99	99	0.0004	10	0.0676	0.0338	0.1081
Xsep310	194	194	0.0001	1	0.0068	0.0000	0.0203
Xsep310	194	204	0.0001	1	0.0068	0.0000	0.0203
Xsep310	199	204	0.0000	2	0.0135	0.0000	0.0338
Xsep310	204	204	0.0001	144	0.9730	0.9459	0.9932
CIR240	105	105	0.0008	18	0.1224	0.0743	0.1781
CIR240	105	109	0.0005	9	0.0612	0.0270	0.1020

CIR240	105	111	0.0003	4	0.0272	0.0068	0.0548
CIR240	105	127	0.0000	1	0.0068	0.0000	0.0205
CIR240	105	99	0.0000	3	0.0204	0.0000	0.0473
CIR240	107	107	0.0002	3	0.0204	0.0000	0.0473
CIR240	107	109	0.0001	1	0.0068	0.0000	0.0205
CIR240	107	111	0.0000	1	0.0068	0.0000	0.0205
CIR240	109	109	0.0013	52	0.3537	0.2789	0.4345
CIR240	109	111	0.0006	18	0.1224	0.0743	0.1769
CIR240	109	135	0.0001	1	0.0068	0.0000	0.0205
CIR240	109	141	0.0000	1	0.0068	0.0000	0.0205
CIR240	109	155	0.0000	2	0.0136	0.0000	0.0340
CIR240	109	161	0.0000	1	0.0068	0.0000	0.0205
CIR240	111	111	0.0010	26	0.1769	0.1156	0.2381
CIR240	129	129	0.0000	1	0.0068	0.0000	0.0205
CIR240	135	135	0.0001	3	0.0204	0.0000	0.0476
CIR240	149	151	0.0000	1	0.0068	0.0000	0.0205
CIR240	157	157	0.0000	1	0.0068	0.0000	0.0205
Xgap84	179	183	0.0000	1	0.0068	0.0000	0.0205
Xgap84	181	181	0.0003	4	0.0272	0.0068	0.0548
Xgap84	181	183	0.0002	12	0.0816	0.0408	0.1293
Xgap84	181	201	0.0000	1	0.0068	0.0000	0.0205
Xgap84	183	183	0.0010	62	0.4218	0.3401	0.5000
Xgap84	183	185	0.0001	11	0.0748	0.0340	0.1216
Xgap84	183	187	0.0000	2	0.0136	0.0000	0.0340
Xgap84	183	189	0.0001	1	0.0068	0.0000	0.0205
Xgap84	183	193	0.0001	11	0.0748	0.0340	0.1216
Xgap84	183	195	0.0001	3	0.0204	0.0000	0.0473
Xgap84	183	197	0.0001	4	0.0272	0.0068	0.0544
Xgap84	183	199	0.0001	4	0.0272	0.0068	0.0544
Xgap84	183	211	0.0000	1	0.0068	0.0000	0.0207
Xgap84	183	213	0.0000	2	0.0136	0.0000	0.0340
Xgap84	185	185	0.0003	3	0.0204	0.0000	0.0473
Xgap84	185	189	0.0000	1	0.0068	0.0000	0.0205
Xgap84	187	187	0.0001	1	0.0068	0.0000	0.0205
Xgap84	189	189	0.0001	2	0.0136	0.0000	0.0342
Xgap84	193	193	0.0002	1	0.0068	0.0000	0.0205
Xgap84	193	197	0.0000	1	0.0068	0.0000	0.0205
Xgap84	195	195	0.0002	4	0.0272	0.0068	0.0548
Xgap84	197	197	0.0002	3	0.0204	0.0000	0.0473
Xgap84	197	199	0.0000	1	0.0068	0.0000	0.0205
Xgap84	199	199	0.0001	2	0.0136	0.0000	0.0340
Xgap84	207	207	0.0001	2	0.0136	0.0000	0.0340
Xgap84	207	209	0.0000	1	0.0068	0.0000	0.0205
Xgap84	207	219	0.0000	1	0.0068	0.0000	0.0207
Xgap84	211	213	0.0000	3	0.0204	0.0000	0.0476
Xgap84	217	219	0.0000	2	0.0136	0.0000	0.0340
Xtxp15	199	199	0.0001	3	0.0203	0.0000	0.0473
Xtxp15	207	207	0.0000	1	0.0068	0.0000	0.0203
Xtxp15	209	209	0.0001	1	0.0068	0.0000	0.0203
Xtxp15	209	211	0.0000	1	0.0068	0.0000	0.0203
Xtxp15	209	215	0.0000	1	0.0068	0.0000	0.0203

Xtxp15	211	211	0.0013	40	0.2703	0.2027	0.3446
Xtxp15	211	213	0.0005	4	0.0270	0.0068	0.0541
Xtxp15	211	215	0.0003	7	0.0473	0.0135	0.0811
Xtxp15	211	219	0.0002	2	0.0135	0.0000	0.0338
Xtxp15	211	221	0.0001	1	0.0068	0.0000	0.0203
Xtxp15	213	213	0.0011	31	0.2095	0.1486	0.2770
Xtxp15	213	215	0.0003	2	0.0135	0.0000	0.0338
Xtxp15	213	217	0.0001	2	0.0135	0.0000	0.0338
Xtxp15	215	215	0.0009	22	0.1486	0.0946	0.2095
Xtxp15	215	217	0.0001	2	0.0135	0.0000	0.0338
Xtxp15	215	219	0.0001	1	0.0068	0.0000	0.0203
Xtxp15	215	227	0.0000	1	0.0068	0.0000	0.0203
Xtxp15	217	217	0.0004	9	0.0608	0.0270	0.1014
Xtxp15	217	219	0.0000	1	0.0068	0.0000	0.0203
Xtxp15	219	219	0.0005	11	0.0743	0.0338	0.1216
Xtxp15	221	221	0.0002	5	0.0338	0.0068	0.0676
Xcup063	129	129	0.0000	1	0.0068	0.0000	0.0205
Xcup063	135	135	0.0000	1	0.0068	0.0000	0.0205
Xcup063	141	141	0.0007	18	0.1224	0.0743	0.1769
Xcup063	141	147	0.0007	2	0.0136	0.0000	0.0342
Xcup063	147	147	0.0008	125	0.8503	0.7905	0.9048
CIR306	118	118	0.0001	1	0.0068	0.0000	0.0203
CIR306	118	120	0.0000	1	0.0068	0.0000	0.0203
CIR306	118	122	0.0000	1	0.0068	0.0000	0.0203
CIR306	120	120	0.0014	47	0.3176	0.2432	0.3919
CIR306	120	122	0.0014	13	0.0878	0.0473	0.1351
CIR306	122	122	0.0014	85	0.5743	0.4932	0.6554
Xcup53	183	187	0.0000	1	0.0068	0.0000	0.0203
Xcup53	183	195	0.0001	1	0.0068	0.0000	0.0203
Xcup53	183	199	0.0000	1	0.0068	0.0000	0.0203
Xcup53	187	191	0.0000	1	0.0068	0.0000	0.0203
Xcup53	187	195	0.0001	4	0.0270	0.0068	0.0541
Xcup53	191	195	0.0000	1	0.0068	0.0000	0.0203
Xcup53	195	195	0.0002	136	0.9189	0.8716	0.9595
Xcup53	195	199	0.0001	3	0.0203	0.0000	0.0473
CIR248	100	100	0.0010	64	0.4414	0.3630	0.5208
CIR248	100	90	0.0006	42	0.2897	0.2177	0.3655
CIR248	100	92	0.0003	10	0.0690	0.0338	0.1111
CIR248	100	94	0.0000	2	0.0138	0.0000	0.0347
CIR248	90	90	0.0008	15	0.1034	0.0556	0.1575
CIR248	90	92	0.0002	1	0.0069	0.0000	0.0210
CIR248	90	98	0.0000	1	0.0069	0.0000	0.0210
CIR248	92	92	0.0005	9	0.0621	0.0274	0.1034
CIR248	92	94	0.0000	1	0.0069	0.0000	0.0210
CIR262	214	214	0.0010	27	0.1824	0.1216	0.2432
CIR262	214	218	0.0010	14	0.0946	0.0541	0.1419
CIR262	214	222	0.0000	1	0.0068	0.0000	0.0203
CIR262	218	218	0.0011	102	0.6892	0.6149	0.7635
CIR262	218	222	0.0001	2	0.0135	0.0000	0.0338
CIR262	222	222	0.0001	2	0.0135	0.0000	0.0338
Xgap72	181	181	0.0016	60	0.4054	0.3243	0.4865

Xgap72	181	183	0.0002	2	0.0135	0.0000	0.0338
Xgap72	181	189	0.0007	2	0.0135	0.0000	0.0338
Xgap72	181	191	0.0001	1	0.0068	0.0000	0.0203
Xgap72	183	183	0.0005	10	0.0676	0.0338	0.1149
Xgap72	183	189	0.0001	2	0.0135	0.0000	0.0338
Xgap72	185	187	0.0000	1	0.0068	0.0000	0.0203
Xgap72	187	187	0.0011	29	0.1959	0.1351	0.2635
Xgap72	187	189	0.0003	3	0.0203	0.0000	0.0473
Xgap72	189	189	0.0012	32	0.2162	0.1486	0.2838
Xgap72	191	191	0.0002	5	0.0338	0.0068	0.0676
Xgap72	197	197	0.0000	1	0.0068	0.0000	0.0203
CIR246	101	101	0.0006	14	0.0946	0.0541	0.1419
CIR246	93	93	0.0003	6	0.0405	0.0135	0.0743
CIR246	93	95	0.0000	1	0.0068	0.0000	0.0203
CIR246	95	95	0.0003	5	0.0338	0.0068	0.0676
CIR246	95	99	0.0002	4	0.0270	0.0068	0.0541
CIR246	97	99	0.0000	1	0.0068	0.0000	0.0203
CIR246	99	99	0.0010	117	0.7905	0.7230	0.8514
Xtxp136	237	237	0.0008	35	0.2365	0.1689	0.3041
Xtxp136	237	240	0.0007	72	0.4865	0.4054	0.5676
Xtxp136	237	243	0.0002	3	0.0203	0.0000	0.0473
Xtxp136	240	240	0.0007	22	0.1486	0.0946	0.2095
Xtxp136	240	243	0.0000	16	0.1081	0.0608	0.1622
Xcup14	204	204	0.0012	89	0.6014	0.5203	0.6824
Xcup14	204	206	0.0012	26	0.1757	0.1149	0.2365
Xcup14	206	206	0.0012	33	0.2230	0.1554	0.2905
Xtxp057	231	239	0.0000	1	0.0068	0.0000	0.0208
Xtxp057	235	235	0.0001	2	0.0137	0.0000	0.0345
Xtxp057	237	243	0.0000	1	0.0068	0.0000	0.0208
Xtxp057	237	245	0.0000	1	0.0068	0.0000	0.0208
Xtxp057	239	239	0.0001	1	0.0068	0.0000	0.0208
Xtxp057	239	253	0.0000	2	0.0137	0.0000	0.0345
Xtxp057	241	241	0.0002	3	0.0205	0.0000	0.0476
Xtxp057	241	245	0.0000	3	0.0205	0.0000	0.0476
Xtxp057	241	247	0.0001	1	0.0068	0.0000	0.0208
Xtxp057	241	253	0.0000	4	0.0274	0.0068	0.0556
Xtxp057	241	257	0.0000	1	0.0068	0.0000	0.0208
Xtxp057	243	243	0.0002	3	0.0205	0.0000	0.0476
Xtxp057	243	247	0.0000	1	0.0068	0.0000	0.0208
Xtxp057	243	249	0.0000	1	0.0068	0.0000	0.0208
Xtxp057	245	245	0.0007	12	0.0822	0.0408	0.1293
Xtxp057	245	247	0.0001	14	0.0959	0.0493	0.1448
Xtxp057	245	249	0.0001	2	0.0137	0.0000	0.0345
Xtxp057	245	251	0.0001	2	0.0137	0.0000	0.0345
Xtxp057	245	253	0.0002	4	0.0274	0.0068	0.0552
Xtxp057	247	247	0.0009	20	0.1370	0.0822	0.1931
Xtxp057	247	249	0.0002	3	0.0205	0.0000	0.0476
Xtxp057	247	251	0.0002	2	0.0137	0.0000	0.0345
Xtxp057	247	253	0.0002	7	0.0479	0.0139	0.0878
Xtxp057	249	249	0.0007	14	0.0959	0.0490	0.1448
Xtxp057	249	251	0.0001	1	0.0068	0.0000	0.0208

Xtxp057	249	253	0.0001	3	0.0205	0.0000	0.0476
Xtxp057	251	251	0.0007	17	0.1164	0.0680	0.1712
Xtxp057	251	253	0.0002	1	0.0068	0.0000	0.0208
Xtxp057	253	253	0.0007	15	0.1027	0.0552	0.1517
Xtxp057	253	255	0.0000	2	0.0137	0.0000	0.0345
Xtxp057	255	255	0.0001	1	0.0068	0.0000	0.0208
Xtxp057	263	263	0.0000	1	0.0068	0.0000	0.0208
Xgap206	101	139	0.0000	1	0.0073	0.0000	0.0224
Xgap206	103	103	0.0011	24	0.1752	0.1159	0.2426
Xgap206	103	117	0.0001	1	0.0073	0.0000	0.0224
Xgap206	103	135	0.0000	1	0.0073	0.0000	0.0226
Xgap206	103	137	0.0000	1	0.0073	0.0000	0.0224
Xgap206	103	139	0.0000	2	0.0146	0.0000	0.0370
Xgap206	103	141	0.0001	2	0.0146	0.0000	0.0368
Xgap206	103	143	0.0000	2	0.0146	0.0000	0.0368
Xgap206	103	149	0.0000	2	0.0146	0.0000	0.0368
Xgap206	107	107	0.0001	1	0.0073	0.0000	0.0226
Xgap206	107	115	0.0000	3	0.0219	0.0000	0.0507
Xgap206	107	139	0.0000	1	0.0073	0.0000	0.0226
Xgap206	109	109	0.0001	2	0.0146	0.0000	0.0368
Xgap206	111	113	0.0000	2	0.0146	0.0000	0.0368
Xgap206	113	113	0.0004	7	0.0511	0.0150	0.0929
Xgap206	113	115	0.0000	2	0.0146	0.0000	0.0370
Xgap206	113	117	0.0000	1	0.0073	0.0000	0.0226
Xgap206	113	123	0.0000	2	0.0146	0.0000	0.0370
Xgap206	113	125	0.0001	1	0.0073	0.0000	0.0224
Xgap206	115	115	0.0007	12	0.0876	0.0438	0.1377
Xgap206	115	117	0.0000	1	0.0073	0.0000	0.0226
Xgap206	115	119	0.0000	1	0.0073	0.0000	0.0224
Xgap206	115	121	0.0000	1	0.0073	0.0000	0.0224
Xgap206	115	125	0.0001	3	0.0219	0.0000	0.0507
Xgap206	117	117	0.0003	5	0.0365	0.0074	0.0719
Xgap206	117	119	0.0000	1	0.0073	0.0000	0.0226
Xgap206	117	125	0.0000	1	0.0073	0.0000	0.0226
Xgap206	117	133	0.0000	1	0.0073	0.0000	0.0226
Xgap206	117	149	0.0000	1	0.0073	0.0000	0.0226
Xgap206	117	153	0.0000	1	0.0073	0.0000	0.0227
Xgap206	119	119	0.0002	3	0.0219	0.0000	0.0500
Xgap206	119	121	0.0000	1	0.0073	0.0000	0.0226
Xgap206	119	125	0.0000	1	0.0073	0.0000	0.0224
Xgap206	119	139	0.0000	1	0.0073	0.0000	0.0224
Xgap206	121	121	0.0001	1	0.0073	0.0000	0.0226
Xgap206	121	123	0.0000	3	0.0219	0.0000	0.0504
Xgap206	123	125	0.0000	6	0.0438	0.0144	0.0809
Xgap206	125	125	0.0006	9	0.0657	0.0290	0.1103
Xgap206	127	137	0.0000	1	0.0073	0.0000	0.0226
Xgap206	133	133	0.0001	1	0.0073	0.0000	0.0226
Xgap206	135	135	0.0001	2	0.0146	0.0000	0.0368
Xgap206	135	145	0.0000	1	0.0073	0.0000	0.0226
Xgap206	137	137	0.0002	3	0.0219	0.0000	0.0504
Xgap206	139	141	0.0000	1	0.0073	0.0000	0.0226

Xgap206	141	141	0.0003	6	0.0438	0.0143	0.0803
Xgap206	141	143	0.0000	1	0.0073	0.0000	0.0226
Xgap206	143	143	0.0001	2	0.0146	0.0000	0.0368
Xgap206	145	145	0.0001	1	0.0073	0.0000	0.0226
Xgap206	145	147	0.0000	1	0.0073	0.0000	0.0224
Xgap206	145	153	0.0000	1	0.0073	0.0000	0.0226
Xgap206	147	147	0.0002	4	0.0292	0.0071	0.0597
Xgap206	147	149	0.0000	1	0.0073	0.0000	0.0224
Gpsb123	276	276	0.0001	1	0.0078	0.0000	0.0244
Gpsb123	278	278	0.0001	1	0.0078	0.0000	0.0246
Gpsb123	280	280	0.0001	1	0.0078	0.0000	0.0246
Gpsb123	282	282	0.0002	2	0.0156	0.0000	0.0394
Gpsb123	282	284	0.0000	3	0.0234	0.0000	0.0543
Gpsb123	282	286	0.0000	1	0.0078	0.0000	0.0246
Gpsb123	282	288	0.0000	1	0.0078	0.0000	0.0244
Gpsb123	282	290	0.0001	1	0.0078	0.0000	0.0244
Gpsb123	284	284	0.0006	9	0.0703	0.0305	0.1190
Gpsb123	284	286	0.0000	3	0.0234	0.0000	0.0534
Gpsb123	284	288	0.0001	1	0.0078	0.0000	0.0242
Gpsb123	284	290	0.0002	5	0.0391	0.0079	0.0752
Gpsb123	284	292	0.0003	2	0.0156	0.0000	0.0400
Gpsb123	286	286	0.0005	7	0.0547	0.0164	0.0977
Gpsb123	286	290	0.0002	2	0.0156	0.0000	0.0397
Gpsb123	286	292	0.0002	2	0.0156	0.0000	0.0394
Gpsb123	288	288	0.0008	14	0.1094	0.0588	0.1667
Gpsb123	288	292	0.0002	4	0.0313	0.0076	0.0650
Gpsb123	290	290	0.0014	31	0.2422	0.1692	0.3197
Gpsb123	290	292	0.0006	3	0.0234	0.0000	0.0534
Gpsb123	292	292	0.0014	31	0.2422	0.1680	0.3185
Gpsb123	292	294	0.0000	1	0.0078	0.0000	0.0242
Gpsb123	294	294	0.0001	2	0.0156	0.0000	0.0397
Xtxp12	162	162	0.0001	2	0.0145	0.0000	0.0368
Xtxp12	162	192	0.0000	1	0.0072	0.0000	0.0222
Xtxp12	162	200	0.0000	1	0.0072	0.0000	0.0222
Xtxp12	168	168	0.0001	1	0.0072	0.0000	0.0224
Xtxp12	168	178	0.0000	1	0.0072	0.0000	0.0224
Xtxp12	170	170	0.0002	3	0.0217	0.0000	0.0504
Xtxp12	170	174	0.0000	1	0.0072	0.0000	0.0222
Xtxp12	170	202	0.0000	1	0.0072	0.0000	0.0226
Xtxp12	172	172	0.0002	3	0.0217	0.0000	0.0500
Xtxp12	172	190	0.0000	1	0.0072	0.0000	0.0224
Xtxp12	174	174	0.0005	10	0.0725	0.0301	0.1185
Xtxp12	174	186	0.0000	1	0.0072	0.0000	0.0222
Xtxp12	174	192	0.0001	1	0.0072	0.0000	0.0224
Xtxp12	174	194	0.0001	1	0.0072	0.0000	0.0222
Xtxp12	174	198	0.0000	2	0.0145	0.0000	0.0365
Xtxp12	174	202	0.0000	1	0.0072	0.0000	0.0226
Xtxp12	176	176	0.0001	1	0.0072	0.0000	0.0224
Xtxp12	178	178	0.0002	3	0.0217	0.0000	0.0500
Xtxp12	178	196	0.0000	1	0.0072	0.0000	0.0222
Xtxp12	182	190	0.0000	1	0.0072	0.0000	0.0224

Xtxp12	182	194	0.0000	1	0.0072	0.0000	0.0224
Xtxp12	188	188	0.0001	2	0.0145	0.0000	0.0365
Xtxp12	188	192	0.0000	1	0.0072	0.0000	0.0222
Xtxp12	188	196	0.0000	1	0.0072	0.0000	0.0224
Xtxp12	190	190	0.0003	5	0.0362	0.0073	0.0709
Xtxp12	190	200	0.0000	1	0.0072	0.0000	0.0222
Xtxp12	192	192	0.0006	10	0.0725	0.0350	0.1176
Xtxp12	192	194	0.0001	2	0.0145	0.0000	0.0365
Xtxp12	192	196	0.0001	2	0.0145	0.0000	0.0368
Xtxp12	192	198	0.0001	1	0.0072	0.0000	0.0222
Xtxp12	192	200	0.0001	1	0.0072	0.0000	0.0226
Xtxp12	194	194	0.0005	8	0.0580	0.0217	0.1000
Xtxp12	194	196	0.0000	5	0.0362	0.0073	0.0709
Xtxp12	194	198	0.0001	1	0.0072	0.0000	0.0222
Xtxp12	194	200	0.0001	1	0.0072	0.0000	0.0224
Xtxp12	194	202	0.0000	2	0.0145	0.0000	0.0368
Xtxp12	194	206	0.0000	1	0.0072	0.0000	0.0224
Xtxp12	196	196	0.0006	10	0.0725	0.0296	0.1176
Xtxp12	196	198	0.0000	6	0.0435	0.0143	0.0809
Xtxp12	196	200	0.0001	1	0.0072	0.0000	0.0224
Xtxp12	196	216	0.0000	2	0.0145	0.0000	0.0368
Xtxp12	198	198	0.0004	5	0.0362	0.0073	0.0709
Xtxp12	198	200	0.0001	2	0.0145	0.0000	0.0368
Xtxp12	198	202	0.0000	2	0.0145	0.0000	0.0365
Xtxp12	200	200	0.0008	16	0.1159	0.0652	0.1716
Xtxp12	202	202	0.0003	5	0.0362	0.0072	0.0709
Xtxp12	204	204	0.0001	1	0.0072	0.0000	0.0224
Xtxp12	206	206	0.0001	1	0.0072	0.0000	0.0224
Xtxp12	208	208	0.0001	1	0.0072	0.0000	0.0222
Xtxp12	214	214	0.0001	1	0.0072	0.0000	0.0222
Xtxp12	216	218	0.0000	2	0.0145	0.0000	0.0368
Xtxp12	218	220	0.0000	1	0.0072	0.0000	0.0224
Xtxp145	175	175	0.0002	4	0.0294	0.0071	0.0597
Xtxp145	175	213	0.0001	2	0.0147	0.0000	0.0373
Xtxp145	175	303	0.0000	1	0.0074	0.0000	0.0227
Xtxp145	177	177	0.0001	1	0.0074	0.0000	0.0226
Xtxp145	179	179	0.0001	1	0.0074	0.0000	0.0227
Xtxp145	201	203	0.0000	1	0.0074	0.0000	0.0226
Xtxp145	203	213	0.0000	2	0.0147	0.0000	0.0376
Xtxp145	205	205	0.0004	1	0.0074	0.0000	0.0227
Xtxp145	205	207	-0.0002	29	0.2132	0.1462	0.2846
Xtxp145	205	211	0.0000	2	0.0147	0.0000	0.0373
Xtxp145	205	213	0.0004	3	0.0221	0.0000	0.0511
Xtxp145	207	207	0.0007	9	0.0662	0.0290	0.1111
Xtxp145	207	211	0.0001	1	0.0074	0.0000	0.0229
Xtxp145	207	213	0.0006	3	0.0221	0.0000	0.0507
Xtxp145	211	211	0.0004	6	0.0441	0.0145	0.0815
Xtxp145	211	213	0.0002	1	0.0074	0.0000	0.0227
Xtxp145	213	213	0.0016	58	0.4265	0.3433	0.5108
Xtxp145	213	215	0.0001	6	0.0441	0.0145	0.0809
Xtxp145	213	227	0.0000	1	0.0074	0.0000	0.0227

Xtxp145	215	215	0.0002	3	0.0221	0.0000	0.0507
Xtxp145	235	235	0.0001	1	0.0074	0.0000	0.0227
Xtxp265	174	174	0.0009	23	0.1565	0.1014	0.2162
Xtxp265	174	189	0.0000	1	0.0068	0.0000	0.0205
Xtxp265	174	210	0.0002	1	0.0068	0.0000	0.0205
Xtxp265	174	213	0.0002	2	0.0136	0.0000	0.0340
Xtxp265	186	186	0.0001	1	0.0068	0.0000	0.0205
Xtxp265	186	192	0.0000	1	0.0068	0.0000	0.0207
Xtxp265	189	189	0.0002	3	0.0204	0.0000	0.0473
Xtxp265	189	213	0.0000	2	0.0136	0.0000	0.0340
Xtxp265	189	219	0.0000	1	0.0068	0.0000	0.0205
Xtxp265	192	192	0.0002	3	0.0204	0.0000	0.0473
Xtxp265	192	195	0.0000	1	0.0068	0.0000	0.0205
Xtxp265	192	210	0.0000	1	0.0068	0.0000	0.0207
Xtxp265	195	195	0.0001	1	0.0068	0.0000	0.0205
Xtxp265	195	198	0.0000	2	0.0136	0.0000	0.0340
Xtxp265	195	210	0.0000	2	0.0136	0.0000	0.0342
Xtxp265	198	198	0.0002	2	0.0136	0.0000	0.0342
Xtxp265	198	201	0.0000	5	0.0340	0.0068	0.0680
Xtxp265	198	210	0.0001	1	0.0068	0.0000	0.0205
Xtxp265	198	219	0.0000	1	0.0068	0.0000	0.0205
Xtxp265	201	201	0.0003	2	0.0136	0.0000	0.0340
Xtxp265	201	204	-0.0001	8	0.0544	0.0204	0.0946
Xtxp265	201	210	0.0001	2	0.0136	0.0000	0.0340
Xtxp265	201	216	0.0000	2	0.0136	0.0000	0.0342
Xtxp265	204	204	0.0003	2	0.0136	0.0000	0.0342
Xtxp265	204	207	0.0000	1	0.0068	0.0000	0.0205
Xtxp265	204	210	0.0000	7	0.0476	0.0137	0.0833
Xtxp265	204	213	0.0001	1	0.0068	0.0000	0.0205
Xtxp265	204	216	0.0000	1	0.0068	0.0000	0.0207
Xtxp265	204	228	0.0000	1	0.0068	0.0000	0.0207
Xtxp265	207	213	0.0000	1	0.0068	0.0000	0.0205
Xtxp265	210	210	0.0007	10	0.0680	0.0338	0.1096
Xtxp265	210	213	0.0000	23	0.1565	0.1014	0.2177
Xtxp265	210	216	0.0000	7	0.0476	0.0137	0.0828
Xtxp265	210	219	0.0000	2	0.0136	0.0000	0.0340
Xtxp265	213	213	0.0005	4	0.0272	0.0068	0.0544
Xtxp265	213	216	0.0000	7	0.0476	0.0138	0.0828
Xtxp265	213	219	0.0000	3	0.0204	0.0000	0.0476
Xtxp265	216	219	0.0000	5	0.0340	0.0068	0.0676
Xtxp265	216	222	0.0000	1	0.0068	0.0000	0.0205
Xtxp265	222	222	0.0001	1	0.0068	0.0000	0.0205
Xtxp265	222	225	0.0000	1	0.0068	0.0000	0.0205
Xtxp265	228	231	0.0000	1	0.0068	0.0000	0.0205
Xtxp320	260	260	0.0012	30	0.2083	0.1448	0.2759
Xtxp320	260	263	0.0001	1	0.0069	0.0000	0.0213
Xtxp320	260	266	0.0000	1	0.0069	0.0000	0.0211
Xtxp320	260	272	0.0005	6	0.0417	0.0137	0.0764
Xtxp320	260	275	0.0002	7	0.0486	0.0142	0.0845
Xtxp320	260	278	0.0002	2	0.0139	0.0000	0.0350
Xtxp320	260	287	0.0000	2	0.0139	0.0000	0.0350

Xtxp320	263	263	0.0003	5	0.0347	0.0069	0.0685
Xtxp320	263	275	0.0000	3	0.0208	0.0000	0.0483
Xtxp320	263	278	0.0000	1	0.0069	0.0000	0.0211
Xtxp320	266	266	0.0001	1	0.0069	0.0000	0.0211
Xtxp320	266	275	0.0000	1	0.0069	0.0000	0.0213
Xtxp320	269	269	0.0001	2	0.0139	0.0000	0.0350
Xtxp320	269	272	0.0000	2	0.0139	0.0000	0.0350
Xtxp320	272	272	0.0012	34	0.2361	0.1678	0.3056
Xtxp320	272	275	0.0003	1	0.0069	0.0000	0.0213
Xtxp320	272	278	0.0002	4	0.0278	0.0068	0.0563
Xtxp320	275	275	0.0007	13	0.0903	0.0479	0.1389
Xtxp320	275	284	0.0000	1	0.0069	0.0000	0.0211
Xtxp320	278	278	0.0007	14	0.0972	0.0544	0.1479
Xtxp320	278	284	0.0000	1	0.0069	0.0000	0.0211
Xtxp320	278	290	0.0000	1	0.0069	0.0000	0.0213
Xtxp320	281	281	0.0001	3	0.0208	0.0000	0.0483
Xtxp320	284	284	0.0001	2	0.0139	0.0000	0.0350
Xtxp320	287	287	0.0002	3	0.0208	0.0000	0.0479
Xtxp320	290	290	0.0002	3	0.0208	0.0000	0.0483