GENETIC DIVERSITY OF SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH) AND ITS WILD RELATIVES IN NAMIBIA USING SSR AND RAPD ANALYSES

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
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BY

LYDIA NDINELAO NGHISHIKUNGU- HORN

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SUPERVISOR: DR. P. M. CHIMWAMUROMBE

CO-SUPERVISORS: PROF. DR.T. ALTMANN
DR. M. KANDAWA-SCHULZ

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By
Lydia Ndinelao Nghishikungu- Horn

Supervisor: Dr. P. M. Chimwamurombe (University Of Namibia, Biology Department)

Co-Supervisors: Prof. Dr.T. Altmann (Potsdam University,Germany)
Dr. M. Kandawa-Schulz (University Of Namibia, Chemistry Department)

April 2007

ABSTRACT

Sorghum bicolor (L.) Moench is the fifth most important cereal crop in the world, and in Namibia, sorghum is the second most important. The genetic resources of sorghum remain unexplored in Namibia. This could lead to a loss of genetic diversity as wild relatives and foreign varieties could interbreed with landraces, resulting in an undesired alteration of its genetic make-up. This study was conducted to evaluate the genetic diversity among the Namibian *Sorghum bicolor* accessions stored at NBRI (National Botanical Research Institute), two relatives (*Sorghum halepense)* and four improved varieties (Macia, Pato, Larsvyt 46-85 and ZSV/30). The objectives were (i) to assess the genetic diversity of *Sorghum bicolor* germplasm stored at the National Botanical Research Institute (NBRI) and (ii) to understand the germplasm genetic relationships among land races, wild relatives and improved cultivars using simple sequence repeat (SSR) and random amplified polymorphic DNAs (RAPDs). UPGMA (Unweighted Paired Group Method Using Arithmetic Average) based results revealed a measure of similarity among genotypes (r) =38 for RAPD analyses and at r =18 for SSR respectively for 24 genotypes. The measure of the magnitude of similarity of (r) = 5 were obtained for 63 genotypes using SSR, an ordination method, nonmetric multidimensional scaling (2D maps), confirmed the UPGMA results with stress values ranging from 0.05 to 0.2. Simpson's diversity index (D) calculated for SSR analysis for 63 genotypes was: 1-D = 0.98 while RAPD and SSR on 24 genotypes was 1-D = 0.96. Shannon Wiener index was H′= 3.844 for SSR analysis on 63 genotypes and H′= 3.152 for RAPD and SSR on 24 genotypes. Two new primers (CD230935 and CD231028) designed have amplified mostly all the genotypes analyzed in this study. It was also concluded that there is a genetic variation among *Sorghum bicolor* at the gene level in Namibia. The study also revealed that RAPD and SSR techniques can be used in the evaluation of genetic diversity in sorghum species.

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SYMBOLS AND ABBREVIATIONS

AFLPs	Amplified Fragment Length Polymorphisms
APS	Advanced Photographic System
bp	Base pairs
DNA	Deoxy ribo-Nucleic Acid
EB	Extraction buffer
EB	Elution buffer
FAO	Food and Agricultural Organization
ICRISAT	International Crop Research Institute for the Semi Arid Tropics
ID	Identity
IPP	Intellectual Property Protection
IUCN	World Conservation Union
mg	Milligram
ml	Milliliter
NBRI	National Botanical Research Institute
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PRIMER	Polymouth Routines in Multivariate Ecological Research
RAPDs	Random Amplified Polymorphic DNAs
RFLPs	Restriction Fragment Length Polymorphisms

SADC	Southern Africa Development Communities
SDS	Sodium Dodecyl Sulfate
SNPs	Single Nucleotide Polymorphisms
SSRs	Simple Sequence Repeats
STS	Sequence Target Site
TE	Tris EDTA
TEA	Tris EDTA Acetic acid
TEMED	Tetramethylenediamine
UNAM	University of Namibia
UPGMA	Unweighted Paired Group Method Using Arithmetic Average
US	Unites States
μl	Micro liter
MAWRD	Ministry of Agriculture, Water and Rural Development

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DEDICATIONS

This thesis is dedicated to the future generations of Namibia, who would take up biotechnology field to develop their nation sustainably. I would also like to dedicate a part of this study to my beloved brother Phillip-Hanyemunange Shikambe Hamunyela, who passed away in a horrific accident at Ludritz on 07 June 2002, you inspired me brother, may your soul rest in eternal peace. I would also like to dedicate my work to my beloved grand-mother Helena Nghituvali Molongela, who supported my ideas to continue with my studies after completing high school. I am proud today, because you stood by me “meekulu”. I wish you could be around grand mother to see where I am now. May your soul rest in eternal peace, granny.

DECLARATIONS

I Lydia Ndinelao Nghishikungu Horn, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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CHAPTER 1

1 INTRODUCTION

Sorghum bicolor is the fifth most important grain crop in the world after wheat, maize, rice, and barley, and the second most important cereal crop (after maize) in sub-Saharan Africa (Zidenga, 2004). It consists of cultivated and wild species (Ayana *et al.,* 2002). *Sorghum bicolor, bicolor* (2n=20) is a sub-species which is a taxon that includes agronomical important grain races, that is, bicolor caudatum, durra, guinea and kafir, several hybrid races and working groups (Assar *et al.,* 2005) . According to Atokple (2004), sorghum after millet (*Pennisetum glaucum*) is essential to diets of poor people in the semi-arid tropics where drought cause frequent failures of other crops. Sorghum is an annual and mostly wind pollinated cereal that is known to be predominantly selfing (Dje *et al*., 2004). In Namibia, sorghum is ranked number two after pearl millet which is the staple food source for the majority (50%) population (Rohrbach, 1999). Sorghum is the staple food for the rural population; its flour is used to make a traditional brew (Omalodu) that is very important in Namibia. The seeds are used for making snacks by boiling. Some sorghum types have high sugar content and their fresh sweet stalks are used for chewing as with sugar cane (Ghebru *et al.,* 2002). In 1991, the Ministry of Agriculture, Water and Rural Development (Namibia) in joint collaboration with the ICRISAT Center Patancheru (India) and SADC/ICRISAT in Bulawayo (Zimbabwe) launched a germplasm collection mission in the northern Namibia (Rao *et al.,* 1991) in which about 123 sorghum accessions were collected. The accessions were taken to the

National Botanical Research Institute (NBRI) for preservation as seed in the gene bank (Matanyaire, 1998). Subsequent to the collection mission was the establishment of a national crop improvement program, which focused on improving of the major food crops including sorghum. So far research has been concentrated on yield trials. The diversity present in Namibian sorghum landraces was initially suggested by their morphological variation and phenotypic characteristics (Rao *et al.,* 1991). In the past, indirect estimates of similarity based on morphological information have been widely used in many species including sorghum (Assar *et al.*, 2005). However, morphological variation could not be relied on as a reflecting tool for real genetic variation because of genotype-environment interactions (Assar *et al.*, 2005). Another limiting factor with morphological variation lies with the largely unknown genetic control of polygenetically inherited morphological and agronomic traits (Assar *et al.,* 2005). Despite all that, little or nothing is known about the genetic diversity of the local sorghum accessions in Namibia, which could impede efficient utilization and management of the genetic resources of sorghum. In addition, literature on the curation of sorghum is very limited. It was also reported by Dahlberg *et al. (*1996) that a lack of information on *Sorghum bicolor,* both at molecular and morphological level in the world.

In recent years, since the introduction of crop improvement program in Namibia in 1991, new cultivars that are improved for high drought resistance, early maturity and high yielding have been introduced. According to Matanyaire (1998), many farmers have turned to new innovations and adopted these newly improved lines of *Sorghum bicolor* (Macia) and pearl millet (*Pennisetum glaucum)* (Okashana 1 and 2). This shift may result in a considerable loss of sorghum landraces and associated genetic variability in Namibia. This is true as research done elsewhere has indicated their concerns that the diversity among sorghum crop might have dropped, as stipulated by Buckler IV *et al.* (2001). Buckler IV *et al.* (2001) also reported that the drop in diversity is substantially great at the genes that have been involved in domestication for a longer period of time.

Genetic diversity study 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 (Mohammadi and Prasanna, 2003). The Peak International Conservation body, IUCN (World Conservation Union) recognizes the need to conserve the biological diversity on earth for the reason that one species on earth does not have the right to drive others to extinction, analogous to abhorrence of genocide among human populations (Frankham *et al.,* 2002). The IUCN therefore recognizes the need for conservation at the levels of genetic diversity, which is a crucial factor in species conservation. There are key articles of IUCN that were passed 1983 on plant genetic resources; these have been outlined by (Smith and Shands, 1996). Smith and Shands (1996) have also indicated the importance of germplasm conservation and that greater efforts are required to conserve and utilize genetic diversity in sorghum. These points are also stressed in the Convention on Biological Diversity (CBD) *article 15* which heralds a change in the hitherto informal international exchanges of germplasm (Smith and Shands, 1996). Assar *et al*. (2005) stated that the assessment of the genetic diversity within cultivated crops has a strong influence on plant breeding strategies as well as on conservation of genetic resources because of the usefulness in the characterization of individuals, accessions and cultivars. This information is said to be of importance, when it is comes to determining duplications in germplasm collections and for the choice of parental genotypes in breeding programs (Assar *et al.*, 2005). Therefore the evaluation of Namibian *Sorghum bicolor* accessions or germplasm diversity could be an important tool of information on sorghum accessions. As reported by Mohammadi and Prasanna (2003), germplasm usually carry important traits that need to be rescued. These traits can be used in agronomic evaluation and in crop improvement. For example in unforeseen incidents like the outbreak of diseases and some natural phenomenon like the drought, the stored accessions could provide the genes of resistance to these phenomena. This was the case in the USA as reported by Gowda *et al*. (1993) where maize fields consisting of the same genetic make up were infested by a disease (downy mildew). Nothing could be done to prevent the disease from spreading. The problem was only solved later when a resistant gene was discovered from the maize varieties from Africa.

Knowledge about germplasm diversity and genetic relationships among breeding materials could be a valuable aid in crop improvement strategies. This information has been revealed by many writers like Mohammadi and Prasanna (2003). In this respect, efforts have also been made to predict the prospects of developing superior genotypes from a cross by the measurements of genetic similarity or genetic distance between the parents (Korzun, 2004). The latter could then be used as an estimation of expected genetic variances in different sets of segregation progenies derived from different crosses (Korzun, 2004). The information on the germplasm genetic characteristics would not only be useful for the Namibian crop improvement program, but also provide the needed information on genetic diversity and relationships among local accessions. Moreover, the information on the relationship among accessions is essential for the improvement of crop production and the management of the genetic resources (Chantereau *et al.,* 1993, Anas 2004). This is true as outlined by Chantereau *et al.* (1993) that evaluation of germplasm is the first step in germplasm utilization, meaning that germplasm is useless unless it is evaluated. Anas (2004) has also outlined that a population need to be characterized for the amount and type of genetic variability it contains for the effective conservation, selection and use of crop genetic resources.

In the past, traditional or conventional method have been used to characterize germplasm and usually these methods are not reliable and take long to bring results, that is why new techniques have been developed to characterize and evaluate germplasm at the DNA level. DNA mapping is a very quick way to analyze and compare the DNA sequences of any living organism (Bramel *et al.*, 1993). For the purpose of this study, two methods; Randomly Amplified Polymorphic DNAs (RAPDs) and Simple Sequence Repeat (SSRs) were used for the reasons that they have been used successfully in similar research. Polymerase Chain Reaction (PCR) is a technique to exponentially amplify invitro a small quantity of a specific nucleotide sequence in the presence of template sequence, two primers that hybridize to opposite strands and flank the region of interest in the target DNA, a thermo stable (*Taq*) DNA polymerase. The two techniques chosen RAPDs and SSRs are PCR-based.

It is against this background that sorghum accessions from six different regions in Namibia were obtained from National Botanical Research Institute (NBRI) to be used in this study. These include wild relatives from Kunene region in Namibia, landraces from Kunene, Okavango, Ohangwena, Omusati, Oshana, Otjozodjupa, Oshikoto and Caprivi regions in Namibia. Two more wild relatives of sorghum were collected near UNAM campus in Windhoek and were identified at NBRI as S*orghum halepense.* Additionally from ICRISAT Zimbabwe in collaboration with the Ministry of Agriculture, Water and Forestry (Namibia) were four cultivars (Macia, Pato, Larsvyt 46-85 and ZSV/30) were collected. These accessions have originated from Zambia, Mozambique, Latin America and Zimbabwe respectively were investigated in this study of the genetic diversity of *Sorghum bicolor* (L) Moench in Namibia using microsatellite analysis.

1.1 Objectives

- The main objectives of this study were to:

    1. Determine the genetic diversity of *Sorghum bicolor* germplasm stored at the National Botanical Research Institute (NBRI) which has not been done at the molecular level previously.
    2. To understand the germplasm genetic relationships among land races, wild relatives and improved cultivars using RAPD and SSR marker techniques.

1.2 Justification

The studies on *Sorghum bicolor* genetic diversity at molecular level have never been carried out in Namibia. The accessions at NBRI could provide important genetic traits that could be used in crop improvement in Namibia. At the moment however, there is no information on the genetic relationships of these Namibian sorghum accessions and its wild relatives. This means that if the germplasm remains unevaluated for diversity, it cannot be utilized efficiently in crop improvement programs.

1.3 Research Hypotheses

There are genetic variations between Namibia’s *Sorghum bicolor* landraces, wild relatives and improved cultivars based on the fact that they descended from different regions of Namibia, and morphologically they look different.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Importance of genetic diversity studies and the effects of genetic pollution

Indigenous crop varieties all over the world are faced with the threat of genetic pollution as it has been reported by Bunders *et al.* (1996). Genetic erosion is the process acting on natural and domesticated species as well. In nature genetic theories predict that inbreeding between members of small populations will reveal deleterious recessive alleles especially dangerous in out-breeding animal populations. But loss of heterozygosity reduces the adaptive potential of every population (Caro and Laurenson, 1999).

According to Bunders *et al.* (1996), genetic resources pollution is on an increase all over the world, and this needs an urgent attention to stop genetic erosion to guarantee stable agricultural production in the future. This can be achieved through the conservation of the genetic resources of food crops (Anas 2001). There are different ways through which genetic erosion could be reduced. Agrama and Tuinstra (2004) have outlined one way in which genetic erosion could be addressed. Through studying the genetic diversity of the local accessions and documenting their identities, one could keep the record of the accessions (Agrama and Tuinstra, 2004). Studying the genetic diversity of the local accessions can be done through many ways that have been developed for these specific tasks. Kurzun (2004) has outlined some tools such as the molecular marker system. Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Site (STS), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeat (SSRs) or Microsatellites, Single Nucleotide Polymorphisms (SNPs), just to mention a few, have been developed and applied to a range of crop species including cereals such as sorghum (Anas, 2004).

According to Alwala *et al.(*2006) the underlying goal for studying genetic diversity and interrelationships among germplasm collections is to eventually use that information to facilitate the development of better performing varieties of the cultivated species. DNA diversity mapping among others can be used to identify and protect the sorghum germplasm in countries of which Namibia is not an exception, as it has been used successfully in similar studies using different crops such as sorghum, maize, cowpea and millet (Rao e*t al.,* 1991). By using these techniques, breeders can quickly and accurately choose superior individual plants to cross, thus speeding the overall process of genetic improvement. Genetic improvement of sorghum can help to enhance on-farm yield by developing high yielding, early maturing and drought tolerant varieties (Zidenga, 2004; Rao *et al.,* 1991). Moreover, DNA mapping can be used to track down some diseases in plants for example in sorghums, sorghum downy mildew is one of the most destructive diseases not only in sorghum, but also in maize (Gowda *et al.*, 1993). Knowing the gene that causes resistance or susceptibility to downy mildew will be useful for the breeders to study this gene and to come up with techniques of controlling the disease. Genetic mapping is a very important tool in agricultural research and breeding, and molecular markers could be used to provide a better description of the level of diversity in a population (Bramel-Cox *et al.*, 1993). Genotype identification and genetic diversity studies are vital for plant breeding programmes to have sufficient diversity available to allow for the production of new varieties. This is supported by Korzun (2004) who indicated that new varieties that are aimed towards the improvement of crop productivity and able to withstand damage from biotic and abiotic factors can be introduced through genetic diversity studies and improvement. The commonly used molecular marker techniques are the SSR and RAPD and these have been used in many studies on sorghum diversity (Bramel-Cox *et al.*, 1993).

2.2 Simple Sequence Repeat (SSRs)

Microsatellites or SSR technique is a powerful tool for studying genetic diversity in crop plants like sorghum. This is because it utilizes hypervariable regions of the genome comprised of tandemly repeated simple sequences (Anas, 2004). SSRs (Simple Sequence Repeat) remain the best despite the development of new marker systems. This has been supported by Korzun (2004) who stated that valuable markers have been generated from RAPDs and Amplified Fragment Length Polymorphisms (AFLP). However, SSRs which have been developed more recently for major crop plants have been predicted to lead to even more rapid advances in both marker development and implementation in breeding programs (Korzun, 2004).

SSRs are known to be abundant and highly polymorphic in eukaryotic genomes (Rafalski *et al*., 1993). Because of their high polymorphic ability, SSR are widely used for genetic diversity studies (Kuleung *et al.,* 2006). SSRs markers are regarded as important due to the fact that they are co-dominant and highly informative and they generally display a high level of polymorphism and are amenable to automated genotyping strategies (Agrama and Tuinstra, 2003). According to Agrama and Tuinstra (2003), SSRs can be amplified by PCR and can efficiently detect DNA polymorphism and no radioisotopes required in the detection of SSR markers because sequence polymorphism can be detected by separation in agarose gels stained with 0.5% Ethidium

Bromide as well as on polyacrylamide gelS. According to Sghaier and Mohamed (2005), SSRs are becoming the markers of choice for fingerprinting and genetic diversity studies in a wide range of living organisms.

2.3 Random Amplified Polymorphic DNAs (RAPDs)

RAPDs were introduced in 1990 (Weisinger *et al*., 1995). However, the RAPD method does not require any prior knowledge of sequence information. It produces fingerprint-like patterns of variable complexity which distinguishes genotypes. The polymorphisms detected by this method were called RAPDs (Weisinger *et al*., 1995). RAPD reactions are PCR-based, but they amplify segments of DNA which are essentially unknown. Often, PCR is used to amplify a known sequence of DNA. Thus, the scientist chooses the sequence he or she wants to amplify, then designs and makes primers which will anneal to sequences flanking the sequence of interest.

RAPD technology provides researchers with a quick and efficient screen for DNA sequence based polymorphisms at a very large number of loci Weisinger *et al*. (1995). RAPD technology is well suited to DNA fingerprinting although it has some set backs such as a lack of reproducibility due to mismatch annealing (Antonio *et al.,* 2004). According to Rafalski *et al*. (1993), RAPD bands linked to a trait of interest could be easily identified by using two pooled DNA samples, one from individuals that express the trait and the other from individuals that do not. Therefore any polymorphism between the two pools should be linked to the trait.

Table 1: Comparison of RAPDs and SSRs systems in cereals. Korzun (2004)

Feature	**RAPDs**	**SSRs**
DNA required (ng/µl)	0.02	0.05
DNA quality	High	Moderate
PCR-based	Yes	Yes
Number of polymorphic loci analyzed	1.5-50	1.0-3.0
Easy of use	Easy	Easy
Amenable to automation	Moderate	High
Reproducibility	Unreliable	High
Development cost	Low	High
Cost per analysis	Low	Low

2.4 Effectiveness of SSRs and RAPDs methods on genetic diversity studies

According to Korzun (2004), Agrama and Tuinstra (2003) many methods of molecular markers have been developed, these include the SSRs and RAPDs. However, SSRs have some additional advantages over the other techniques because it is relatively simple and can be automated and most of the SSR markers are monolocus and show Mendelian inheritance. SSR markers are also highly informative. However, the main short-coming of SSR method is that, it is expensive and not usually affordable as outlined by Agrama and Tuinstra (2004).

Several efforts have been made to use SSR markers in plants, to study genetic diversity, characterize germplasm, and evaluate population dynamics and were found to be effective (Bramel-Cox *et al.*, 1993). Moreover, SSR are highly polymorphic and are particularly useful for the estimation of genetic similarity among diverse genotypes of sorghum. A set of fifteen (15) microsatellites (SSR) markers have been developed for sorghum genotypes and these allow a fast of sorghum genotype assessment (Ghebru *et al.*, 2002).

Ghebru *et al*. (2002) stated that studies of SSRs have shown that SSR loci give good discrimination between closely related individuals in some cases even if only a few loci were employed. This is true as it was also experienced in a study examining the change in diversity between wild relatives and domesticated grass species (Buckler IV *et al.*, 2001).

On the other hand, RAPDs are dominant markers and their patterns can be used in variety identification. Strain identification is one of the uses of DNA fingerprinting (Agrama and Tuinstra, 2004). RAPDs are not only useful in the detection of important traits in crops but also are very important in the detection of disease resistant genes like genes linked to anthracnose resistant genes in sorghum (Singh *et al.,* 2006). Despite these advantages, RAPDs like SSRs also have some shortcomings. Even though major bands from the RAPD protocol are highly reproducible, minor bands can be difficult to repeat due to the random priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers (Weisinger *et al.*, 1995; Agrama and Tuinstra, 2004). According to Zidenga (2004), since the mid 1980s, genome identification and selection has progressed rapidly with the help of PCR technology. A large number of marker protocols that are rapid and require only small quantities of DNA have been developed.

Even though many PCR based marker methods have been developed, two widely used PCR-based markers are those described above. The choice of a molecular marker technique depends on its reproducibility, cost, effectiveness and simplicity Kurzun (2004) (Table 3). Many studies carried out on the analysis of genetic diversity of different crops revealed a lot of information for example, a study on diversity in twenty eight (28) Eritrean landraces of *Sorghum bicolor* using a high throughput SSR based strategy was used and the results indicated amplification products and polymorphism in almost all of the plants (Ghebru *et al.*, 2002).

Similar studies have indicated that SSR loci give good discrimination between closely related individuals in some cases even when only a few loci were employed (Ghebru *et al.*, 2002). The SSR primer pairs were used in the experiment with the aim of estimating the genetic diversity and determines the genetic relationship among Sudanese sorghum accessions, the study provided a first detailed analysis and quantification of genetic diversity in Sudanese sorghum germplasm (Assar *et al.,* 2005). The data was also in agreement with findings from other research on sorghum diversity as reported by (Ghebru *et al.,* 2002; Weisinger *et al.*, 1995).

SSRs and RAPDs were also used by Agrama and Tuinstra (2003) in a study determining phylogenetic diversity and relationship among sorghum accessions. The study proved that the SSR markers provide the most powerful assay for discriminating genetic diversity among sorghum accessions (Agrama and Tuinstra, 2003). Moreover, RAPDs and SSRs markers do not require radioactive isotopes; therefore they are regarded as ideal and can be efficiently used by researchers in developing countries (Agrama and Tuinstra, 2003).

In another study that was determining polymorphisms among 36 sorghum lines, high profiles (good quality) amplicons were obtained by means of SSR primers and low profiles (poor quality) amplicons by means of RAPD primers (Smith, 2000). It was concluded that SSRs could be used to help genetic conservation management and to support intellectual property protection (IPP) (Smith, 2000). Similar studies in a study to investigate the genetic diversity among thirteen (13) temperate adapted sorghum lines using SSRs, detected polymorphisms in seventeen (17) primer pairs used (Brown *et al.,* 1996). Smith (2000) also reported on thirteen (13) SSRs primers that were used in a study to reveal moderate to high levels of diversity among a group of nine (9) sorghum lines of different racial classification and from different geographic origins.

These research works have just shown that it is possible and efficient to use SSR and RADP markers in the studying of genetic diversity among *Sorghum bicolor* (L.) Moench accessions from different regions and racial backgrounds.

2.5 DNA Fingerprinting

DNA fingerprinting is defined as a method for a simultaneous detection of many highly variable DNA loci by hybridization of specific multilocus probes to electrophoretically separated restriction fragments (Weisinger *et al*., 1995). DNA fingerprinting is important for that it exhibits a great potential as a tool for a wide range of areas in plants and fungal research. These include genotype identification, population genetics, taxonomy, plant breeding and diagnostics and epidemiology of plant, animal and human pathogenic fungi (Weisinger *et al*., 1995). DNA sequencing is applied for evaluating medium and long distance relationships in phylogeny. However it is also used for population studies (Weisinger *et al*., 1995).

According to Antonio *et al*. (2004), the analysis of genetic diversity and relatedness between or within different species, populations and individuals is a major task for many disciplines of biological science. Therefore it has been appreciated that the development of molecular markers based on polymorphisms found in protein or DNA has contributed significantly to research in variety of disciplines for example in taxonomy, phylogeny, ecology, genetics and plant breeding (Antonio *et al*., 2004). This is because each individual's DNA sequence is unique and therefore the DNA information could be exploited for any study of genetic diversity and related relationships between organisms (Weisinger *et al*., 1995). Weisinger *et al*. (1995) outlined the two strategies in which fingerprints are obtained:

1. The first method is the classical method which is a hybridization-based fingerprinting and it involves cutting of genomic DNA with a restriction enzyme, electrophoretic separation of the DNA fragments that have resulted according to their sizes followed by the detection of polymorphic multilocus banding patterns through hybridization with a labeled complementary DNA sequence (probe).
2. The second method is the Polymerase Chain Reaction (PCR). The reaction is cycled involving template denaturation, primer annealing, and the extension of the annealed primers by DNA polymerase until enough copies are made for further analysis. PCR based fingerprinting involves the invitro amplification of a particular DNA sequences with the help of specifically or arbitrarily chosen oligonucleotides (primers) and a thermostable DNA polymerase (Antonio *et al*., 2004). The amplified fragments are then separated through electrophoresis and the detection of polymorphic banding patterns by staining (Antonio *et al*., 2004).

According to Weisinger *et al*. (1995), the level of polymorphism revealed by DNA fingerprinting with simple repetitive oligonucleotides probes depends on some factors like the investigated species, the repeated sequence motif used for hybridization and the restriction enzyme. *Sorghum bicolor* has got twenty pairs of chromosome numbers (2n =20) as it is the case with maize (Jeong *et al*., 2005). This represents a reasonable choice as the standard for a unified chromosome nomenclature for use by the sorghum research community (Jeong *et al*., 2005). However, mapping information has indicated that a higher proportion of markers are duplicated in maize than in sorghum. For example, Antonio *et al.* (2004) found that 44% of restriction fragment length polymorphism markers detected more bands in maize than in sorghum; conversely, only 7% of markers detected more bands in sorghum than in maize.

Many plant species investigated by DNA fingerprinting are crops that have been domesticated and streamlined for thousands of years for example by inbreeding, thereby reducing genetic variability. High level of inbreeding is reflected by low level of variability in multilocus fingerprints (Weisinger *et al*., 1995).

2.6 The role of crop domestication and farmer variety (landraces) in crop genetic diversity

Crop domestication has contributed to a great loss of genetic diversity among cultivated crops (Buckler IV *et al*., 2001). Domestication has radically changed many grass species, and human selection is responsible for moving the grass species towards similar phenotypes and adaptations. Buckler IV *et al*. (2001) outlined that it is likely that many of the same genes have been involved in all of the domestication events, but the origin of the genetic variation, which allows for the modification of these species is still remaining unclear. Grasses are said to have been prominent among the crops that have gone through domestication and provided the majority of the world’s food (Buckler IV *et al*., 2001).

During domestication, traits of interest have been selected and used in breeding of these important grasses (Buckler IV *et al*., 2001). It is believed that the domesticated grasses have major differences in genome structure, diversity and life history even though the process of domestication occurred over the same 500 to 10 000 year period (Buckler IV *et al*., 2001). However, in all the major cereal complexes, (maize in America, wheat barley, oats and rye in the Near East, rice in Asia, sorghum and millet in Africa) the first plant selectors desired the same sets of traits (Buckler IV *et al*., 2001). Plant selectors selected wild grasses that flowered in short days and produced small, naturally dispersed seed (Buckler IV *et al*., 2001).

Crop genetic diversity which comprises farmer varieties, represent a vast and unique dimension of agrobiodiversity that is relevant in small scale agriculture (Gari /FAO, 2003). This diversity provides farmers with further options and strategies for agriculture and food security as varieties differ in aspects such as drought resistance, pest susceptibility, maturing time, yield potential, labor demand, market value as well as nutritional properties (Gari/FAO, 2003). According to Scurrah-Ehrhart (2005), the genetic diversity characteristics of indigenous African cereals are vital to the present and future food security of Sub-Saharan households and nations. However, the nature and usefulness of these resources and how they are managed are not well or even understood by the local communities (Scurrah-Ehrhart, 2005).

2.7 Importance of genetic diversity studies

According to Mohammadi and Prasanna (2003), an understanding of genetic relationships among inbred lines or pure lines can be particularly useful in planning crosses, in assigning lines to specific heterotic groups and for precise identification with respect to plant varietal protection. Once genetic diversity in germplasm collections have been analyzed, reliable classification of accessions and identification of subset of core accessions with possible utility for specific breeding purposes is easily carried out (Mohammadi and Prasanna, 2003).

Traditional or conventional cereal breeding is time consuming and it is dependent on environmental conditions (Smith *et al*., 2002) therefore breeding of a new variety takes between eight and twelve years (Korzun, 2004), even then, release of an improved variety cannot be guaranteed always. This has been a case for example in many studies where estimation of similarity in sorghum and other crop species based on morphological information has been carried out (Assar *et al*., 2005). However, morphological variation was found to be unreliable as it does not reliably reflect the real genetic variation because of genotype-environment interactions and the largely unknown genetic control of polygenically inherited morphological and agronomic traits (Assar *et al*., 2005). For the stated reasons, breeders are interested in new techniques that could make breeding procedures more efficient for example the possibility offered by molecular marker technology.

Genetic diversity among plants (in germplasm) is important because it provides an opportunity in selection of superior plants that can be used as parents in cultivar development programs (Anas, 2004). On the other hand breeding progress depends on the selection and utilization of the most appropriate germplasm and particular breeding method(s), which can be applied to the germplasm (Maunder, 1991). With increased technology, DNA analyzing techniques have been established to enable breeders to locate specific DNA genes or chromosome regions that correspond to particular production traits (Agrama and Tuinstra, 2004).

2.8 Importance of germplasm evaluation and wild relative conservation

The survival of mankind has always depended upon the use of the earth's resources. However, in recent decades, these resources have come under threat from a rapidly increasing human population and from natural and manmade disasters. The world generally has started to focus on the notion that mankind should take responsibility for the ongoing care and management of these precious resources **(**Drew /FAO, 1997). Since food and its production is one of the essential ingredients of mankind's existence, conservation of these resources is regarded as a particularly vital concept for those working in the field of plant sciences (Drew/FAO, 1997). However, for these resources to be effectively utilized there is a need to depend on the availability of crop genetic diversity Ghebru *et al.* (2002).

Farmer varieties or landraces are known for their diverse characteristics and have been used for diverse breeding programs (Ghebru *et al.*, 2002). Moreover, crop diversity is known to be high in the region of their origin and usually collection in such areas can be important sources of material for crop improvement (Ghebru *et al.*, 2002). Each of the major food crops is vital to the lives and culture of millions of people (Mann, 2004). In cereal crops, traditional varieties and wild relatives usually contain most of its gene pool; and these are the raw genetic materials out of which plant breeders develop new varieties. Therefore these resources need to be looked well after, since plant breeders depend on these and they understand their dependency on agricultural biodiversity of which they are worried about its loss (Mann, 2004). For these reason, this study has aims of analyzing the genetic diversity among the germplasm of sorghum at NBRI, improved varieties from some SADC countries and wild relatives collected from Namibia whose genetic variability is not known.

2.9 Data analysis in genetic diversity

2.9.1 Cluster analysis

Cluster analysis refers to a group of multivariate techniques whose primary purpose are to group individuals or objects based on the characteristics they possess (Mohammadi and Prasanna 2003; Jongman *et al*., 1995). Individuals with similar descriptions are mathematically gathered into the same cluster (Mohammadi and Prasanna (2003);

Jongman *et al*., 1995) and the resulting clusters of individuals are expected to exhibit high internal homogeneity and high external heterogeneity (between clusters). Individuals within a cluster would be closer when plotted geometrically and different clusters would be further apart (Mohammadi and Prasanna, 2003) depending on the successfulness of the classification. According to Jongman *et al.* (1995), distance-based clustering method (hierarchical clustering) is being the commonest used in analysis of genetic diversity in crop species.

The use of multivariate statistical algorithms is an important technique for classifying germplasm, ordering variability for a large number of accessions or analyzing genetic relationships among breeding materials. According to Mohammadi and Prasanna (2003) and Jongman *et al*. (1995), the multivariate analytical techniques analyze simultaneously multiple measurements on each individual under investigation, and these are widely used in analysis of genetic diversity irrespective of the dataset i.e. morphological, biochemical or molecular marker data. In this study a cluster analysis which is an example of multivariate method as outlined by Mohammadi and Prasanna (2003), was used to analyze the data.

According to Jongman *et al*. (1995), cluster analysis is an explicit way of identifying groups in raw data and helps us to find structure in the data. The aims of classification are outlined by (Jongman *et. al*., 1995) as follows:

- To give information on the concurrence of species (internal data structure)
- To establish community types for descriptive studies (syntaxonomy and mapping)
- To detect relations between communities and the environment by analysis of the groups formed by the cluster analysis with respect to the environmental variables (external analysis).

2.9.2 Multivariate methods

Computer-based sorting methods can be used to classify taxa or sites showing similar attributes into groups (Jongman *et al*., 1995). These methods simply indicate the degree of similarity or dissimilarity in species composition between stations, or at the same station over time. In contrast to diversity indices, the multivariate methods preserve species identity and are generally regarded as more sensitive in detecting changing community patterns (Towned, 2001). Multi-dimensional scaling (MDS) attempts to construct a map of the sampling sites in which the more similar two samples are in terms of species abundance, biomass, the nearer they are to each other on the map (Towned, 2001). According to Jongman *et al*. (1995), multi-dimensional scaling is popular, because it depends only on rank information rather than quantitative values and use statements like: Sample one is more similar to sample two than it is to sample three. MDS can be in the form of 2D (two dimensional) or 3D (three dimensional).

2.9.3 Average linkage clustering

This method is popular in ecology and in systematic (taxonomy) (Jongman *et al*., 1995). The between-group (dis) similarity is defined as the average (dis) similarity between all possible pairs of members. The algorithm maximizes the cophenetic correlation, the correlation between the original (dis)similarity and the (dis)similarities between samples can be derived from the dendrogram. According to Jongman *et al*. (1995), for any sample pair, it is the lowest dissimilarity (or highest similarity) required to join them in the dendrogram (Jongman *et al*., 1995; Towned, 2001).

Clusters are created using different methods such as the UPGMA (Unweighted Paired Group Method Using Arithmetic Average) is the commonly adopted clustering algorithm and can be calculated using different soft-ware such as PRIMER-E 5 for Windows or NTSYS software has been used in similar studies by El-Alwany (1997); Reinthaler (2004). The clustering method was used in a study reported by Mohammadi and Prasanna (2003), attempting to utilize different datasets and multivariate methods in analysis of genetic diversity in crop plants. Five clustering methods, namely UPGMA, UPGMC (Unweighted Paired Group Method Using Centroids), Single Linkage, Complete Linkage and Median were compared for their utility in revealing genotype associations in barley germplasm accessions. According to Mohammadi and Prasanna (2003); Towned (2001), the study found that UPGMA and UPGMC almost were comparable with a relatively high level of accuracy in accordance with pedigrees, compared to other methods. In another experiment UPGMA, UPGMC, Single Linkage, Complete Linkage, Ward's method and Principal Component Analysis (PCA) were compared in assessing diversity in dent and popcorn maize inbred lines based on intersimple sequence repeat polymorphism (Mohammadi and Prasanna, 2003) where UPGMA gave most consistent results with PCA clearly separated the dent corn lines from the popcorn germplasm.

Mohammadi and Prasanna (2003) reported on the experiment where maize accessions were grouped on the basis of agronomic and morphological characteristics, several clustering methods was compared. However, the UPGMA method was generally consistent with regard to the allocation of clusters when different types and number of characters were used. The study also found that the UPGMA had revealed high co-phenetic correlation coefficient in comparison to UPGMC. These results revealed that the UPGMA clustering method could be the suitable method that can be used in genetic diversity studies hence its use in this study.

2.9.4 Diversity indices

Species diversity can be quantified using different diversity indices. Diversity indices are useful because they enable comparison of species within a community (alpha diversity), between communities or habitats (beta diversity) and among communities over geographic area (gamma diversity) (Pereira, 2002). A diversity index is a mathematical measure of species diversity in a community (Pereira, 2002). Diversity indices provide important information about rarity and commonness of species in a community.

Two nonparametric Indices or diversity indices (Shannon Wiener and Simpson's) are used in genetic diversity analyzes. Shannon index also called Shannon-Weaver index (SDI) or Shannon-Wiener index) H′ is defined as measure of intra-population genetic diversity (Chao and Jenshen, 2002). The index ($H' = -\sum (Pi)(\ln Pi)$) where p_i is proportion of the *i*th type in the population, *ln* is the natural logarithm of *Pi*. According to Pereira (2002); Chao and Jenshen (2002), Shannon index takes into account the number of species (abundance) and the evenness of the species. The index is increased either by having more unique species, or by having greater species evenness. Richness is the number of species per sample. Evenness is a measure of the relative abundance of the different species making up the richness in an area. According to Palmer and Young (2002), the Shannon-Wiener index calculates the uncertainty of predicting the type of another isolate from that population. The information theory of H′ predicts: How difficult would it be to correctly predict the species of the next individual collected? The lower the value of H′, the greater the probability that the next individual encountered will be the same as the previous species. Shannon Wiener index suits well in the analysis of genetic diversity as it relatively insensitive to the bias produced by the failure to detect heterozygous (Pereira, 2002).

Simpson's diversity (D) is calculated as: $D = \sum ni (ni-1)/N (N-1)$, where N = Total number of organisms in the survey, *ni* = the number of individuals for each species. Simpson Diversity Index is a mathematical formula that takes into account species richness and evenness (Pereira, 2002). Simpson's index of diversity predicts the probability that two specimens picked at random in a community of infinite size will be the same species? And probability of picking two organisms at random that are different. Simpson's index of diversity (1-D) ranges from 0 (low diversity) to almost 1 (high diversity). Therefore the greater the value, the greater the sample diversity. With Simpson's Reciprocal Index (1/D) the value of the index starts with 1 as the lowest possible figure. The higher the value, the greater the diversity (Pereira, 2002).

CHAPTER 3

3 MATERIALS AND METHODS

3.1 Genomic DNA extraction from *Sorghum bicolor* (L) Moench seeds

Sorghum bicolor accessions evaluated in this study were obtained from the National Botanical Research Institute (NBRI) in Namibia, as presented in Appendix (A) table (a) and table (b). Wild relatives were collected from around Windhoek and the improved varieties were from ICRISAT Zimbabwe. Two methods of DNA analysis SSR and RAPD were used to screen 24 accessions at Potsdam University Department of Genetics at the institute für Biochemie und Biologie. The DNA was extracted from *Sorghum bicolor* seed because the time allocated was not sufficient to germinate the seeds before extracting the DNA as the time of stay in Germany was limited to one month.

Sorghum bicolor seeds (6) from each accession was taken and ground to a fine powder in the motor using a pestle. The finer powder was transferred to the eppendorf tubes using a sterilized spatula to scrap all powder into the tubes. Tubes were clearly labelled with the accession number to ensure correct placement of samples. Once the powder was in the eppendorf tubes, an amount of 360 µl Extraction Buffer (EB) was added in order to neutralize the reactions. The tubes were vortexed for 2 seconds each time a reagent was added in order to mix well. An amount of 90 µl of 10% SDS was added and vortexed again for 2 seconds. The two reagents above (EB buffer and 10% SDS) could also be used to transfer the grounded seed powder easily from the motor by simply adding them to the powder while in the motor. Proteinase K (2 µl of 20mg/ml) was added to the each sample, this helps to degrade the protein. Samples were incubated in the oven at 65°C for 20 minutes. The RNAse (1µl of 100mg/ml) was added after incubation. Acetate 3M (240 µl) of (CH_3COOK) pH: 4.8) 4°C was added to precipitate the DNA. Samples were then vortexed for 2 seconds before placing them on the ice cubes for 20 minutes to precipitate the DNA further. Samples where then centrifuged at 14000 rpm in Avanti J-25 centrifuge from Beckman Coulter for 20 minutes to settle the residues and suspend the supernatant. Supernatant (400µl) was transferred to new eppendorf tubes, before adding 2 volumes ethanol 100% (800µl) to the supernatant in order to precipitate the DNA.

The tubes where placed on ice cubes again for 20 minutes in order to get more DNA precipitated. After that the samples were centrifuged for 15 minutes at 14000 rpm to collect the DNA pellet. After that the ethanol was poured out and the axcess alcohol is dried off by facing the tubes down on a tissue paper. The pellet was then washed in 200 µl 70% ethanol, by centrifuge for 5 minutes at 14000 rpm. After centrifuging, the alcohol was poured out taking care not to pour the pellet out, and the axcess alcohol drained off by facing the tubes on a tissue paper.

To make sure that no alcohol remain in the tubes, the tubes where placed open in the oven at 65°C for 15 minutes to evaporate the alcohol. After alcohol had evaporated and no more liquid was left in the tubes, an amount of 110µl TE Buffer (pH 8) was added to dissolve the DNA. The DNA was stored at -20°C until used. The DNA materials from the seed were purified to remove much of the RNAse. The protocol for DNA purification is presented in appendix B.

3.2 Morphological characteristics of *Sorghum bicolor* plants seed

Sorghum bicolor seedlings were monitored in the green house to compare the relationship between seed color and stem colors. The seed colors were estimated using a color chart from Pentel pencils. A table of information was generated. Three weeks after the seed had germinated, the stem colors of the seedlings was evaluated again using the same color chart. The information was added in the same table next to the seed color corresponding to the accession number. The scores were assigned to each accession as follow: if the stem color resembled the seed color a (0) was assigned and if the stem color differs from the seed color a (1) was assigned. The scores were entered into a Microsoft office Excel, 2003 and imported into PRIMER-5 for window statistical software analysis. A dendrogram based on Bray-Curtis similarity was created using PRIMER-5 software.

Sorghum accessions (89) were planted in order to germinate and get the leaf tissue (Figure 1). Out of these accessions, 85 were Namibian obtained from NBRI and 2 were wild relatives see Table (a), Appendix A. Two wild relatives (2) (S*orghum halepense*) were collected from Windhoek near UNAM main campus (entry no. 114 and 115) (Table a Appendix A) and DNA was also extracted from their leaves. In this experiment, many accessions were used because molecular plant breeding requires the genotyping of a large number of individuals for diversity analysis, marker-assisted selection and variety fingerprinting as stated by Mace, (2003). These accessions had been collected from different regions in the country. Accessions entry number 110-113 (Table a), (Macia, Pato, Larsvyt 46-85 and ZSV/30) were the improved varieties from the Ministry of Agriculture (Namibia) in conjunction with ICRISAT-Zimbabwe and have originated from Mozambique, Tanzania, Latin-America and Zimbabwe respectively. These four accessions have been selected randomly from the list of hundreds of varieties that have been on trials in the northern Namibia for the past four years carried by the Division of Plant Production Research in the Ministry of Agriculture, Water and Forestry (Namibia).

3.3 Genomic DNA extraction from leaves of *Sorghum bicolor* (L) Moench

Four seeds from each accession were planted in different pots, labeled with the accession number for easy identification (Figure 1).

Figure 1: *Sorghum bicolor* seedlings in the germinating pots ready for leaf harvesting.

The leaves were harvested from three-week old plants and placed in zip lock plastic bags before being stored at -80°C until use. Genomic DNA was extracted from fresh leaves following the instruction of the extraction kit (Promega quick protocol) as follows: Approximately (40mg) of leaf tissue was ground in liquid nitrogen; an amount of (600 µl) Nuclei Lysis Solution was added and then incubated at 65°C for 15 minutes. Three microliter (µl) of RNase Solution was added before incubating at 37°C for 15 minutes. The samples were cooled to room temperature for 5 minutes. Protein Precipitation Solution (200 µl) was added before vortex. Then the samples were centrifuged at

13,000 g in an Eppendorf mini centrifuge for 3 minutes. The supernatant was transferred to clean tubes containing 600 µl isopropanol at room temperature. The tubes were mixed by inversion five times and then centrifuge at 13,000 g for 1 minute. The supernatant was decanted and an additional (600 µl) room temperature ethanol 70% was added and then centrifuge at 13,000 g for 1 minute. Then the DNA Rehydration Solution (100 µl) was added before rehydrating at 65°C for 1 hour or overnight at 4°C. An Eppendorf BioPhotometer was used to measure the quantity of the DNA material before PCR amplification.

Figure 2: Picture showing *Sorghum halepense* (wild relatives) vegetative and generative parts.

Figure 3: Picture showing *Sorghum bicolor* (land races) vegetative and reproductive parts.

3.4 Generation of a Namibian Map based on the regions from where the accessions were collected

Arch view GIS 3.3 was used to generate the maps (Figure 4 and 5) using the coordinate points as obtained from the NBRI data base system. The regions from which the accessions colleted are presented on the Namibian map presented in Figure 4 and 5 respectively. The accessions from ICRISAT-Zimbabwe and the wild relatives collected from Namibia are not presented on the map because their coordinates were not known.

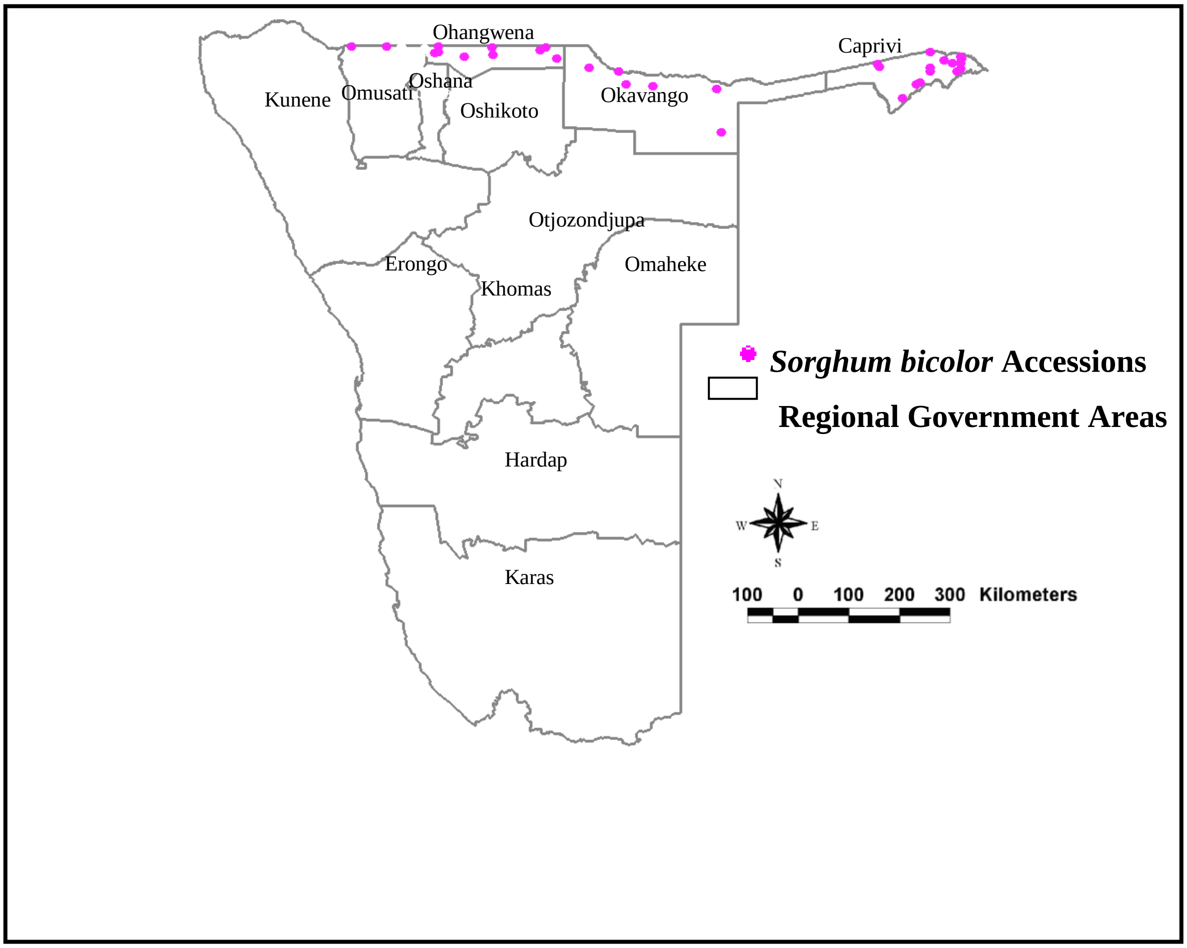

Figure 4: Geographical distribution of the *Sorghum bicolor* accessions analyzed at Potsdam University–Germany.

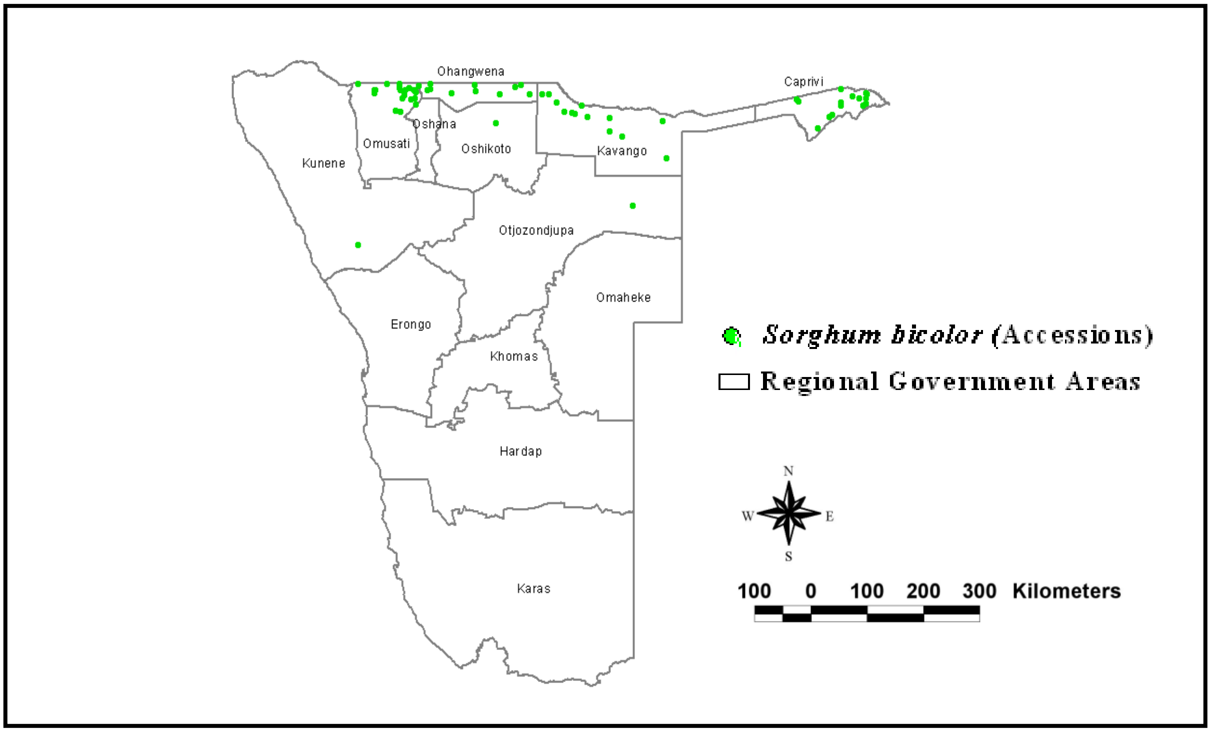

Figure 5: Geographical distribution of the *Sorghum bicolor* accessions analyzed at University of Namibia.

3.5 Electrophoresis of DNA and PCR products

Genomic DNA was quantified on 1% agarose gel while the PCR products were analyzed on a 2% agarose gel and polyacrylamide gel stained with 0.5% ethidium bromide (Appendix C). Electrophoresis was run at 90 volt for 55 minutes. Genomic DNA pictures were kept on a gel documentation system (Bio zym at Potsdam and UVIdoc gel documentation system at UNAM). A protocol for direct purification of PCR products from NucleoSpin extract 2 was used to purify the DNA from the seeds, see appendix B.

3.6 RAPD Amplification

RAPD primers (4) used in this test are presented in Table 1. All 24 DNA samples from the seed were analyzed with the four primers presented in Table 2. The following procedures were followed: (a mixture for one sample) master mix (MM) (10 μl) and 1μl primer was added to a flat cap strip and DNA template (2 μl). The flat cap strips were sealed with a thermo well sealers clear polyethylene to prevent the samples from spilling over and from evaporation during PCR. Samples were placed in the PCR machine at for 2 hours and 30 minutes (see profile at Table 3). Different PCR machines were used (Eppendorf Mastercycler at UNAM and Gradient Cycler at Potsdam). After PCR analysis DNA products were screened on a agarose gel stained with 0.5% ethidium bromide. RAPD amplification was repeated two times.

Table 2: Characteristics of the RAPD primers analyzed.

RAPD locus	Size bp	Sequences	%GC count	Annealing Temperature °C
88R	23	5′ -ATT TAA GTG AAA CTG TCG CCC AG-3′	43.5	67,3
45R	26	5′ -TAA ATT TAA AGT AAA TAA AAT GGT GG-3′	19.2	60,2
933R	21	5′ GCA TCA AGA GTC GCC ACT TCC-3′	57.1	69,0
51R	23	5′ -TTT CTT ACT TTC TAT CCT CGA GAC C-3′	40.0	63,4

Table 3: PCR amplification profile.

Preheat the lid 105˚C		
	Temperature (˚C)	Time
Step 1	92	2 minutes
Step 2	92	10 seconds
Step 3	55	10 seconds
Step 4	72	1 min 30 seconds
Step 5	Go to 2, 40 times	
Step 6	72	2 min
Step 7	4	Until use
Step 8	End	

3.7 SSR (Micro satellite) Amplification

Sorghum genomic DNA was used in the SSR analysis. Four primer pairs were used (in Table 4 sequence written 5′ to 3′). Two of these SSR primers (CD231028 and CD230935) were designed using Primer 3 software (www.cgi V0.2) (a free software online). The primers were ordered from EUROGENTEC S.A, Belgium. The instruction of using them was followed according to the manufacturer's guidelines. An amount of (20 µl) was taken from the forward (F) and reverse (R) primers each and pipetted into one Eppendorf tube to make up (40 µl). Distilled water (160 µl) was added to the solution and then mixed by vortexing for 3 seconds. DNA (2 µl) was taken from each sample of the 24 samples of 10.0 ng/µl into flat cap strips. An amount of (10 µl) from the primer mixture was added to each sample.

Two pairs of SSR primers were used on 24 DNA samples at Potsdam University, Germany where PCR products was analyzed for 2 hours and 30 minutes in the Lycor machine (DNA) analyzer on 6.5% polyacrylamide gel. While at UNAM four pairs of SSR markers were used on 63 DNA samples and the PCR products were analyzed on 2% superfine agarose gel. Both tests were repeated twice to confirm the results obtained.

Table 4: Characteristics of the SSR primer analyzed.

Name of Primers	Size bp	Flanking Sequences	%GC	Annealing Temperature °C
	18	F: CGCTCTCGGGATGAATGA	56	56
Sb6-84	23	R: TAACGGACCACTAACAAATGATT	35	62
	21	F: TGCTTGTGAGAGTGCCTCCCT	57	66
Sb6-342	24	R: TGAACCTGCTGCTTTAGTCGATG	50	72
	20	F: CGC-GGC-GAT-AGT-GAA-TTA-GT	50	60
CD231028	20	R: AGCAGCGTAGTGGTCAGCTC	60	64
	20	F: TCATCCTCCACAACGGCGTC	60	64
CD230935	20	R: AGAGA-TAT-GCG-TGG-CCG-GAT-GC	60	64

3.8 DATA ANALYSES

3.8.1 Generation of the tables for the band scores obtained

In order to get an idea of how many bands were generated using specific primers; a frequency distribution table for the bands per primer was created. For each primer used both RAPD and SSR primers, the pictures obtained after PCR electrophoresis were analyzed. The bands obtained at each base pair on the lambda DNA (bp) comparing with the 100bp DNA ladder were scored and assigned letters to them. The band that appeared first above all the others was given a code as (A) while the lowest band that appeared last in the line given a low code for example (J) depending on the alphabetical order and the number of bands that occur before it. The bands for the primers used in one reaction were combined and added together to come up with a frequency distribution table. In this case three tables were created. For example, in the SSR reaction at Potsdam University, two primer pairs (Sb6-84 and Sb6-342) were used. This was applied to both SSR analyses at UNAM and RAPD at Potsdam University-Germany. Percentage % count for each band was also determined.

3.8.2 Cluster analysis

Data analysis was done by a multivariate method to estimate the genetic distance among accessions as described by Mohammadi and Prasanna (2003). Primer-E 5 for Windows (Plymouth Routines in Multivariate Ecological Research) software was used (PRIMER-

E Ltd. 2001). Cluster analysis which is an example of multivariate method as outlined by Mohammadi and Prasanna (2003); Jongman *et al*. (1995) was used. Genetic diversity was estimated by scoring the DNA bands on the photograph obtained from electrophoresis to generate binary data tables allocating a (1) point where the band is present and (0) point when the band is absent. The scores were then entered in Microsoft office excel (2003) and exported to the Primer-E 5 for Window software (2001). The construction of dendrogram, objectively reflecting genetic relationships, mainly depends on the number of bands scored. Genetic similarity were computed by Bray-Curtis Similarity method using group average linkage and subjected to clustered (UPGMA) and ordination (MDS) analysis in Primer-E 5.

3.8.3 Diversity indices

Genetic diversity among genotypes were calculated by Shannon Wiener and Simpson' diversity indices (Appendix D, table c-f). The bands obtained per genotype using different primers (RAPD and SSR) were added together as total evidence per single genome. Then the genetic diversity was calculated based on total binary score profile.

CHAPTER 4

4 RESULTS

4.1 DNA extraction from seed and leaves

All together (UNAM and Potsdam), DNA was successfully extracted from 87 genotypes (Figure 6). Genomic DNA average yield concentration was 10ng quantified using the Eppendorf bio photometer.

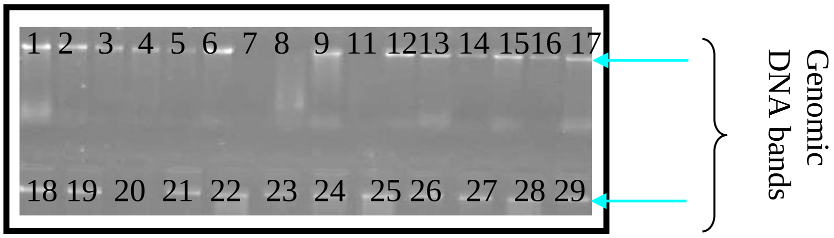

Figure 6: Photograph of electrophoresis results of some genotypes of Sorghum genomic DNA on 0.5% ethidium bromide –stained 1% agarose gel. The horizontal numbers on the photograph represents the sorghum genotypes from which the genomic DNA extracted. The arrows indicate the white bands of sorghum genomic DNA revealed during electrophoresis on 1% agarose gel. The empty lines (7 and 11) contain no white bands; this means that no genomic DNA was yielded from those genotypes.

4.2 RAPD analysis

Each of the 4 RAPD primers used gave an amplification product in all of the 24 accessions. There were 205 total bands observed from all 4 primers scored for the 24 accessions. There were no specific bands unique to one genotype observed in this analysis. However few bands were shared among certain genotypes and not with other genotypes when different primers were used. For example in Figure 7, different banding pattern was observed at 700 bp observed with accession 5,6,7,10,36 and 45. However, most bands were shared across the accessions (Figure 7). As it is presented in Fig. 4.2-4.5, the majority of bands were observed at approximately 600 bp and 100 bp. where a total number of 70 (90) bands were observed. The 70 (90%) bands were commonly shared among the genotypes. A total number of 7 (10) bands were observed between 800 bp and 600 bp. The 7 (10%) bands were not common among the accessions.

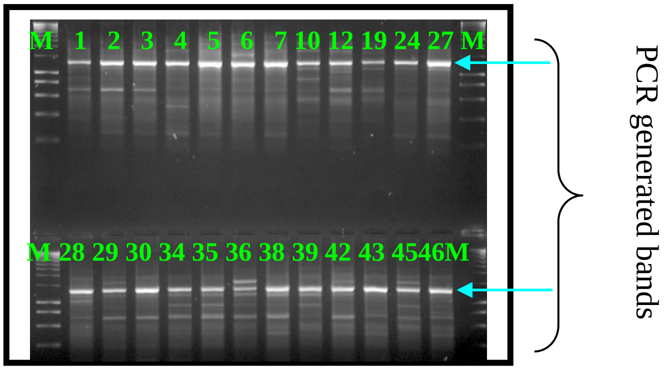

Figure 7: PCR amplification products obtained with RAPD primer 51R. The number on the labels indicates the 24 genotypes analysed at Potsdam University-Germany. The first and last line marked by M both top row and bottom row represent the marker (size standard) of 100 bp in length. The arrows indicate the bands obtained during PCR amplification stained with 0.5% Ethidium bromide on 2% agarose gel.

Amplification was achieved in all genotypes analyzed and a total number of 77 bands were observed using primer 51R. Different banding patterns were observed with accessions 5,6,7,10,36 and 45 respectively where a band of about 800 bp was observed. Moreover, accessions 1,2,3,12,28,29,30,34,35,36,38 and 42 share the same band at about 600 bp. Few faint bands were also some among genotypes and they were not considered in scoring (Fig.7).

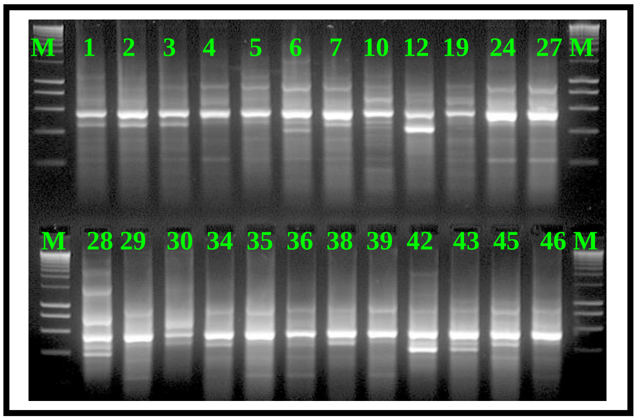

Figure 8: PCR amplification products obtained with RAPD primer 88R. The number on the labels indicates the 24 genotypes analysed at Potsdam University-Germany. The first and last line marked by M both top row and bottom row represent the marker (size standard) of 100 bp in length. The arrows indicate the bands obtained during PCR amplification stained with 0.5% Ethidium bromide on 2% agarose gel. Amplification was achieved in all genotypes analyzed and a total number of 77 bands were observed.

Different banding patterns were observed with accessions 5,6,7,10,36 and 45 respectively where a band of about 800 bp was observed. Moreover, accessions 1,2,3,12,28,29,30,34,35,36,38 and 42 share the same band at about 600 bp. Few faint bands were also some among genotypes and they were not considered in scoring (Fig 8).

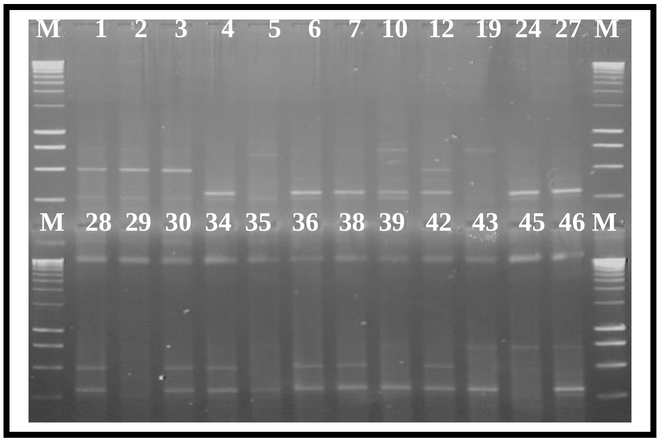

Figure 9: PCR amplification products obtained with RAPD primer 45R. The number on the labels indicates the 24 genotypes analysed at Potsdam University-Germany. The first and last line marked by M both top row and bottom row represent the marker (size standard) of 100 bp in length. The arrows indicate the bands obtained during PCR amplification stained with 0.5% Ethidium bromide on 2% agarose gel.

Amplification was achieved in all genotypes analyzed and a total number of 50 bands were observed. Different banding patterns were observed with accessions 1, 2,3,10,19,43,45 and 46 respectively where a band of about 300 bp was observed. Moreover, the rest of the genotypes share the same band at about 200 bp. Few faint bands were also some among genotypes and they were not considered in scoring (Fig 9).

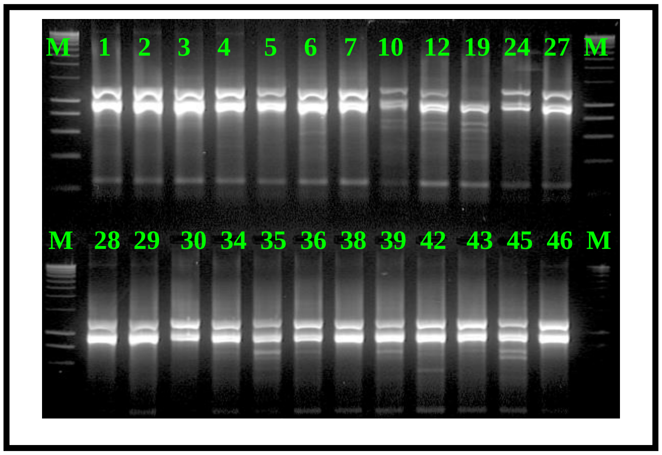

Figure 10: PCR amplification products obtained with RAPD primer 933R. The number on the labels indicates the 24 genotypes analysed at Potsdam University-Germany. The first and last line marked by M both top row and bottom row represent the marker (size standard) of 100 bp in length. The arrows indicate the bands obtained during PCR amplification stained with 0.5% Ethidium bromide on 2% agarose gel. Amplification was achieved in all genotypes analyzed and a total number of 51 bands were observed.

Different banding patterns were observed with accessions 10,12,19,35 and 45 respectively where a double band of about 300 bp was observed. Moreover, the rest of the genotypes share the similar double band at about 500 bp. Few faint ands were also some among genotypes and they were not considered in scoring (Fig 10).

Table 5: Frequency distribution of bands or polymorphisms obtained from RAPD amplification using all four primer set (88R, 45R, 933R, and 51R) to amplify 24 *Sorghum bicolor* genotypes at Potsdam University-Germany.

Bands	A	B	C	D	E	F	G	H	I
Total	54	37	45	36	4	7	9	5	8
%	26	18	21	17	2	3	4	2	3
Observed size range (bp)	800	500	400	300	200	100	± 100	± 100	± 100

On table 5, the observed bands were between ±100 and 800 bp when compared with the 100 bp DNA ladder. The highest number of bands scored was (A) observed at 800 bp with 26% and the lowest were scores (E and H) observed at ± 100 bp with 2%.

4.2.1 SSR analysis

SSR primer pairs (Sb6-84 and Sb6-34) at Potsdam were used to amplify all 24 genotypes. A total amount of 56 bands was generated from the two SSR primer pairs (Table 6).

Table 6: Frequency distribution of bands or polymorphisms obtained from PCR amplification using two SSR primers pairs reverse and forward (Sb6-84 and Sb6-342) at Potsdam University-Germany.

Bands	A	B	C	D	E	F	G	H	I	J
Total	7	5	11	5	11	13	1	1	1	1
%	12.5	9	19.6	9	23.9	19.6	2	2	2	2
Observed size range (bp)	600	500	400	300	200	100	± 100	± 100	± 100	± 100

The two SSR primer pairs were used to analyze the 24 *Sorghum bicolor* genotypes. In total the observed number of bands was 56 observed between 100 bp and 500 bp. The highest number of bands was (E) observed at 200 bp with 23% and the lowest was G, H, I, J observed at ± 100 with 2% (Table 6).

Table 7: Frequency distribution of bands or polymorphisms obtained from PCR amplification using four SSR primers pairs reverse and forward (Sb6-84, Sb6-342, CD230935 and CD231028) at Potsdam University-Germany.

Bands	A	B	C	D	E	F
Total	46	87	100	60	52	10
%	12.95	24.5	28.2	16.9	14.65	2.82
Observed size range (bp)	600	500	400	300	200	± 100

The four SSR primer pairs were used to analyze the 63 *Sorghum bicolor* genotypes. The observed number of bands was between 100bp and 600 bp. The highest number of bands was (C) observed at 400 bp with 28% and the lowest was (F) observed at ± 100 with 2.8% (Table 7).

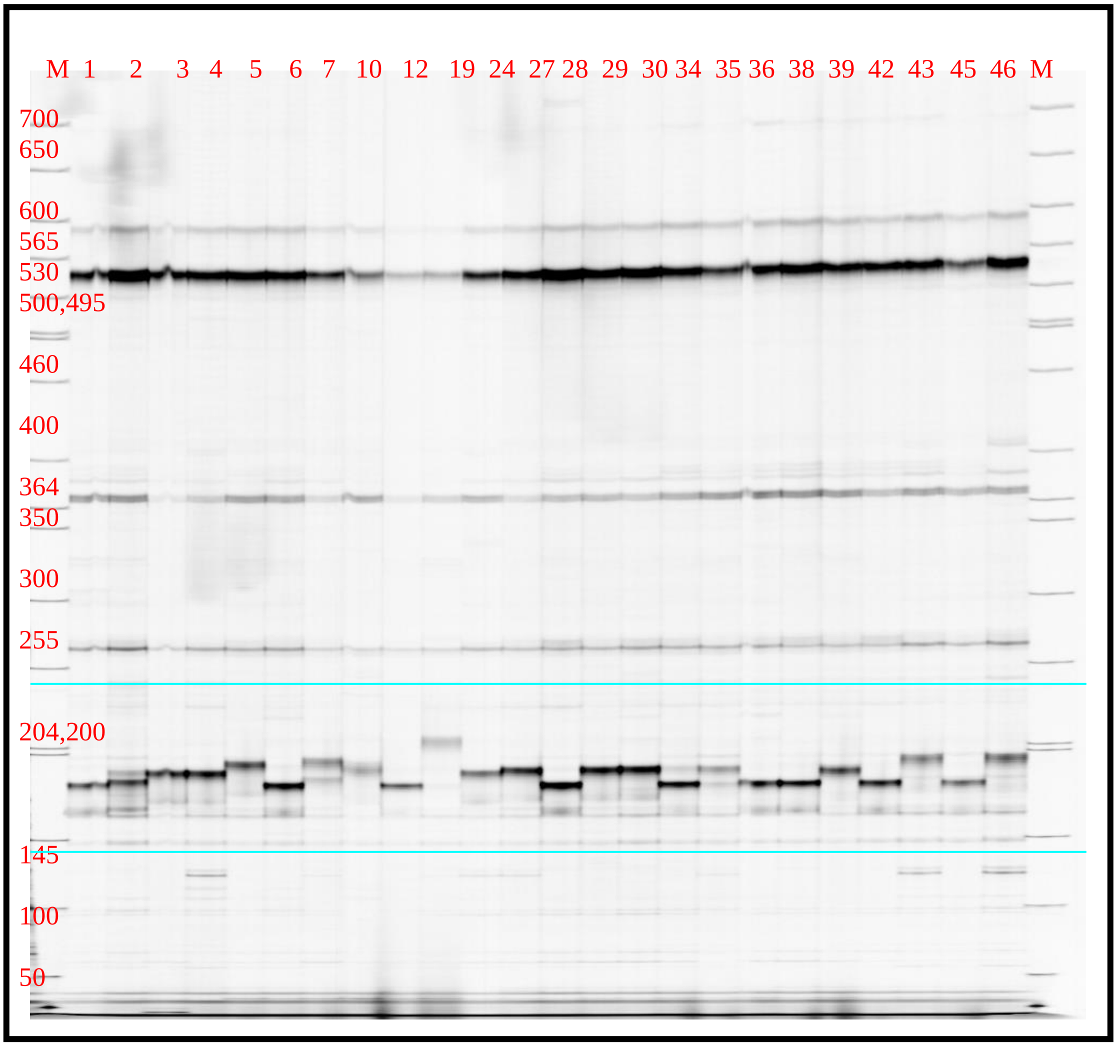

Figure 11: PCR amplification products obtained with SSR primer Sb6-84. The PCR products were analyzed on polyacrylamide gel. The number on the labels indicates the 24 genotypes analysed at Potsdam University-Germany. The first and last lines marked by M represent a DNA ladder (size standard) of 100 bp in length. The two lines demarcated the region where polymorphism was detected. A total number of 26 bands were detected from all 24 genotypes of which 10 were polymorphic. Different banding patterns were observed in all genotypes between 145 and 204 bp length. Accessions number 12 is different from the rest of the other with a distinct band observed at 204 bp. However monomorphic bands were observed at 180 bp for accessions 1, 2, 6, 12, 28, 34, 36, 38, 42 and 45. Accessions 3, 4, 10, 24, 27, 29, 30, 35, and 39 showed a similarity with a band that was observed at 185 bp consistently.

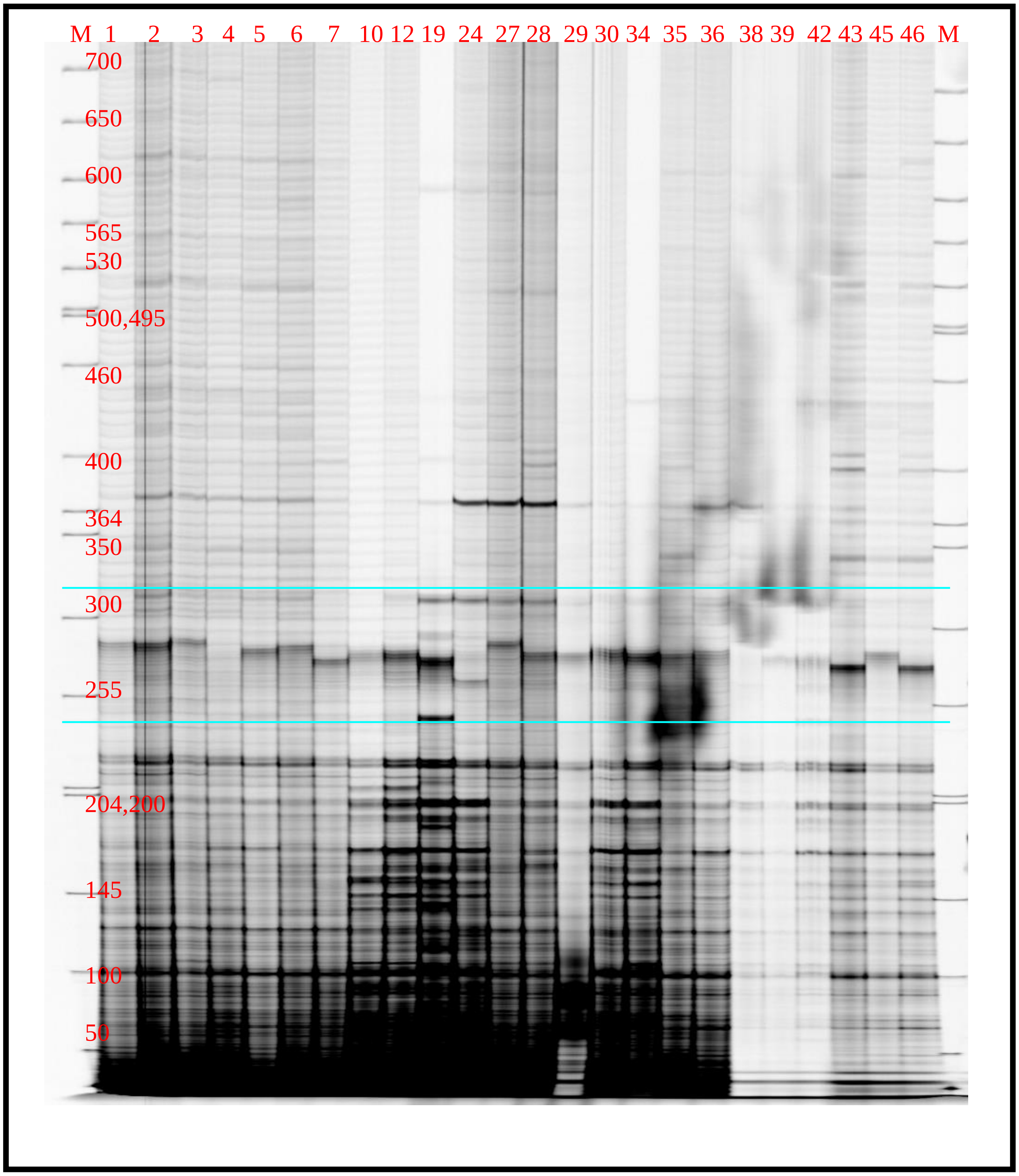

Figure 12: PCR amplification products obtained with SSR primer Sb6-342. The PCR products were analyzed on polyacrylamide gel. The number on the labels indicates the 24 genotypes analysed at Potsdam University-Germany. The first and last lines marked by M represent a DNA ladder (size standard) of 100 bp in length. The two lines demarcated the region where polymorphism was detected. A total number of 30 bands were detected from all 24 genotypes of which 10 were polymorphic. Different banding patterns were observed with in all genotypes between 250 and 322 bp length on the DNA ladder. Accessions number 19 is different from the rest of the other with 3 distinct bands observed at 208-245 bp on the DNA ladder. However monomorphic bands were observed at 330 bp and 380 for accessions 24, 27, 28 and 38. The rest of the accessions showed the bands shared among all other genotypes.

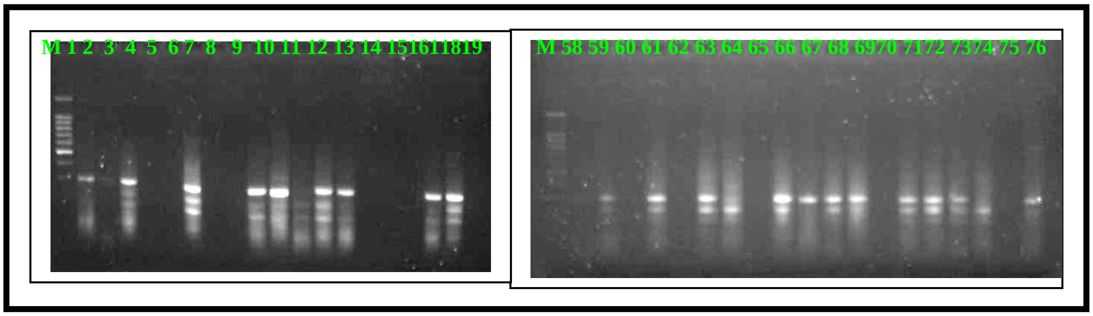

Figure 13: PCR amplification products from the 63 sorghum genotypes analyzed at UNAM amplified with SSR primer Sb6-342. The PCR products were analyzed on 2% superfine agarose gel stained with 0.5% µl Ethidium bromide. The number on the labels indicates the genotypes analysed. The (M) represent a DNA ladder (size standard) of 100bp in length. A total number of 109 bands were detected from all 63 genotypes. No amplification was observed consistently with some genotypes as indicated by empty rows on the figure. The banding patterns were observed between 100 bp and 200 bp in length on the DNA ladder. All amplified genotypes showed the same banding patterns with this primer pairs (Sb6-342).

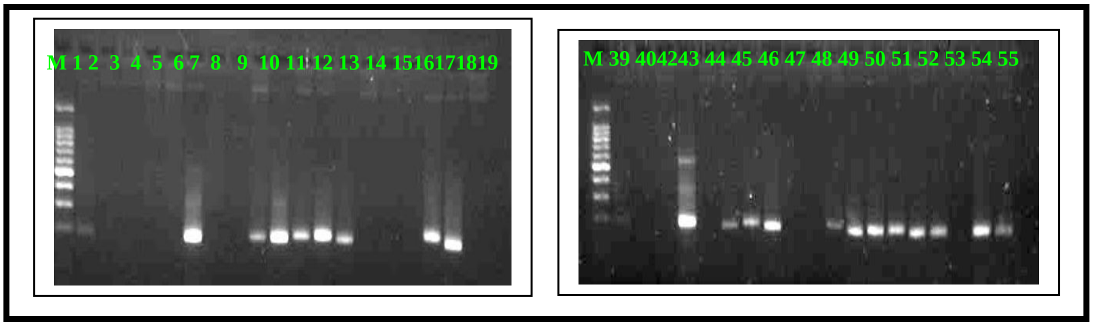

Figure 14: PCR amplification products from the 63 sorghum genotypes analyzed at UNAM amplified with SSR primer Sb6-84. The PCR products were analyzed on 2% superfine agarose gel stained with 0.5% Ethidium bromide. The number on the labels indicates the genotypes analysed. The (M) represent a DNA ladder (size standard) of 100 bp in length. A total number of 56 bands were detected from all 63 genotypes. No amplification was observed consistently with some genotypes as indicated by empty rows on the figure. The banding patterns were observed between 100 bp and 200 bp in length on the DNA ladder. This primer revealed only one band of different sizes in most of the accession. However, genotype number 7 and 43 showed unique bands at 200 bp and 400 bp.

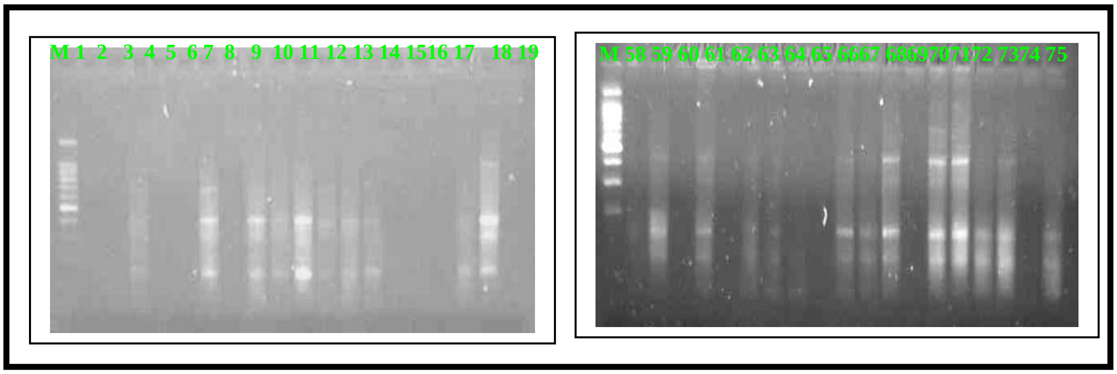

Figure 15: PCR amplification products from the 63 sorghum genotypes analyzed at UNAM amplified with SSR primer CD230935. The PCR products were analyzed on 2% superfine agarose gel stained with 0.5% Ethidium bromide. The number on the labels indicates the genotypes analysed. The (M) represent a DNA ladder (size standard) of 100 bp in length. A total number of 82 bands were detected from all 63 genotypes. No amplification was observed consistently with some genotypes as indicated by empty rows on the figure. The banding patterns were observed between 100 bp and 400 bp in length on the DNA ladder. Different bandings was observed among genotypes except for genotype number 7, 38, 70 and 71 that showed monomorphic bands at 300 bp and 100 bp.

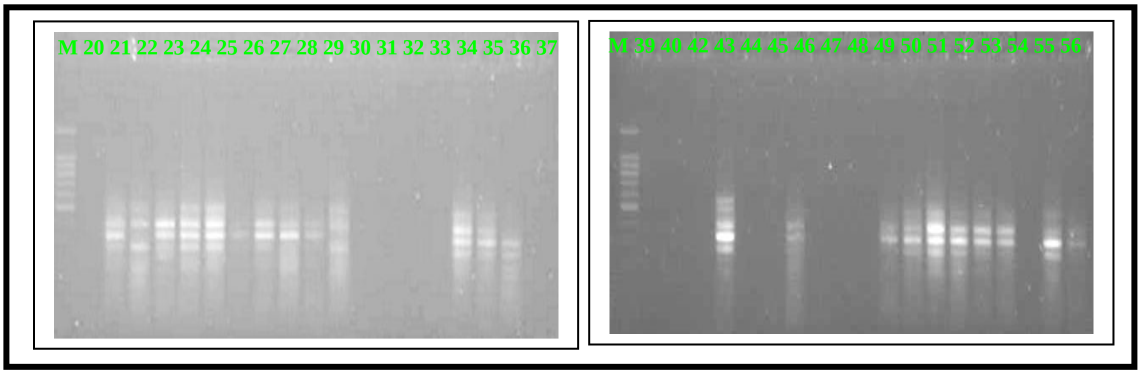

Figure 16: PCR amplification products from the 63 sorghum genotypes analyzed at UNAM amplified with SSR primer CD231028. The PCR products were analyzed on 2% superfine agarose gel stained with 0.5% Ethidium bromide. The number on the labels indicates the genotypes analysed. The (M) represent a DNA ladder (size standard) of 100 bp in length. A total number of 108 bands were detected from all 63 genotypes. No amplification was observed consistently with some genotypes as indicated by empty rows on the figure. The banding patterns were observed between 100 bp and 300bp in length on the DNA ladder. Different bandings was observed among genotypes except for genotype number 7, 38, 70 and 71 that showed monomorphic bands at 300bp and 100bp.

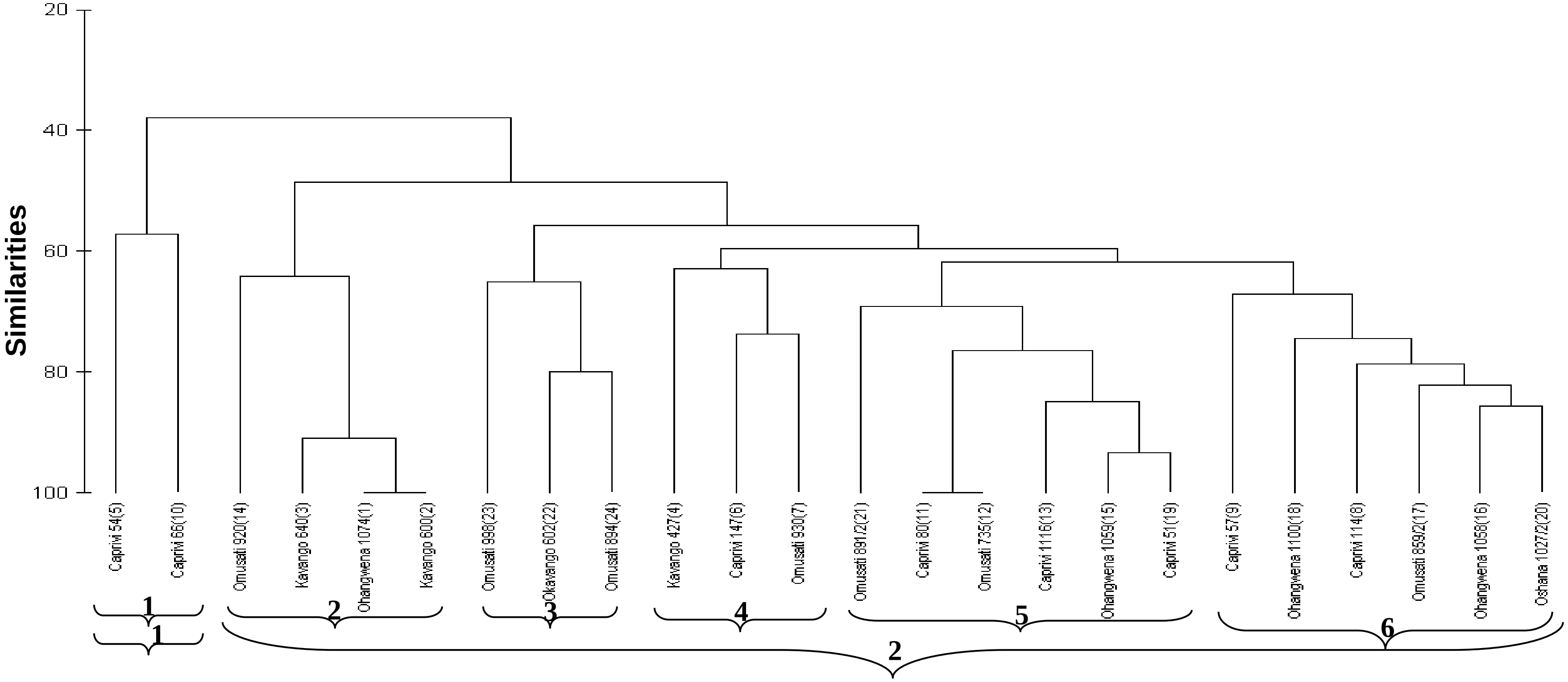

Figure 17: A dendrogram generated by UPGMA clustering showing the similarities among 24 sorghum accessions based on Bray-Curtis similarities. These accessions were amplified with RAPD primers at Potsdam. The numbers on the braces indicates the number of clusters formed. Two major clusters and six sub-clusters were formed. On the labels, the number in parentheses indicates the entry number while the number out of parentheses indicates the accession number as obtained from the NBRI database. The four accessions Ohangwena 107(14), Kavango 600(2), Capri 80(11) and Omusati 735(12) showed

100% similarities in the 2nd major cluster. While the similarities between the accessions in major cluster 1 and 2 was 39% similar.

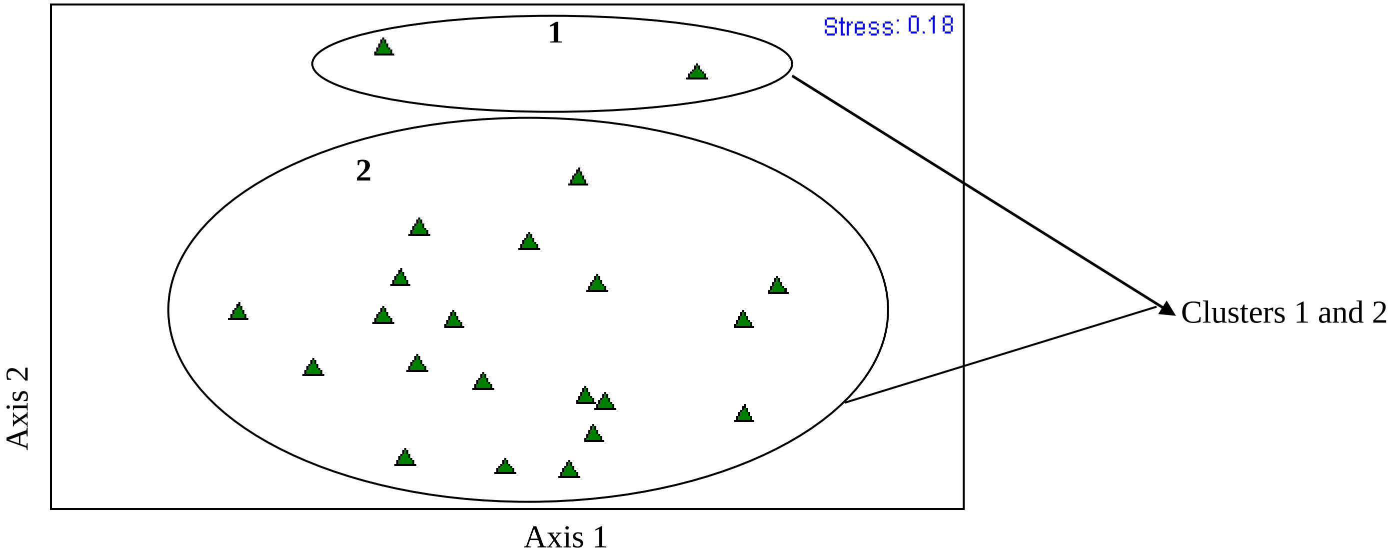

Figure 18: An ordination plot (non metric multidimensional scaling) generate by UPGMA based on Bray-Curtis similarities for RAPD analyses at Potsdam. The triangular symbols on the figure represent the accessions. Two major and 2 have been formed with a stress value of 0.18. Clusters 1 represent the two accessions Caprivi 54(5) and Caprivi 66(10) while all other accessions were grouped in cluster 2. These accessions showed their uniqueness through population differentiation by appearing in different clusters.

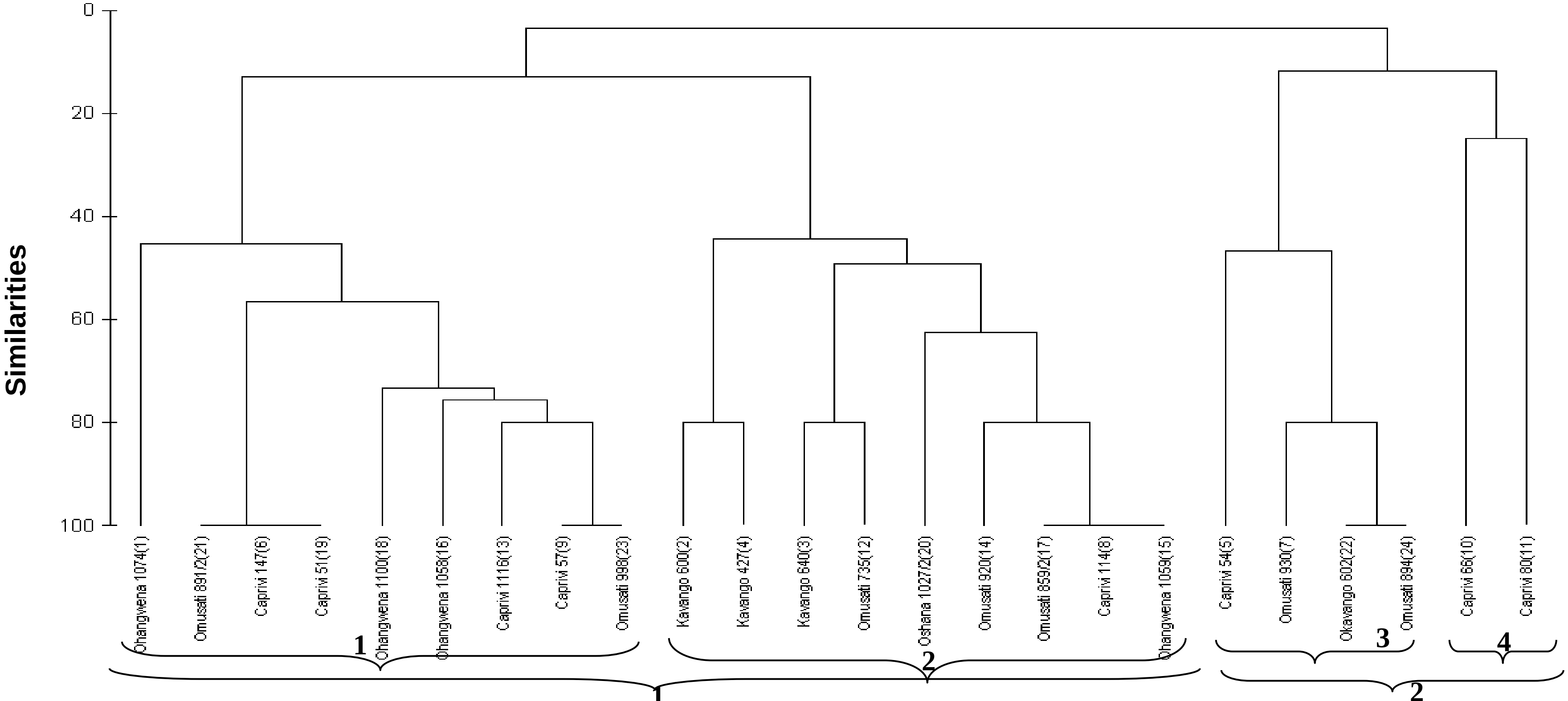

Figure 19: A dendrogram generated by UPGMA clustering showing the similarities among 24 sorghum accessions based on Bray-Curtis similarities. These accessions were amplified with SSR primers at Potsdam. The numbers on the braces indicates the number of clusters formed. Two major clusters and four sub-clusters were formed. On the labels, the number in parentheses indicates the entry number while the number out of parentheses indicates the accession number as obtain from the NBRIdatabase. It is evident from (Fig 19) that accessions Omusati 891/2(21), Caprivi 147(6), Caprivi 51(19), Caprivi 57(9), Omusati 998(23), Omusati 859/2(17), Caprivi 114(8) and ohangwena 1059(15) showed 100% similarities. While in the 2nd major cluster, Accessions Okavango 602(22) and Omusati 894(24) were 100% similar. While the similarities between the accessions in major cluster 1 and 2 was 5% similar. Subclusters are the clusters immerging under the major clusters which are underneath.

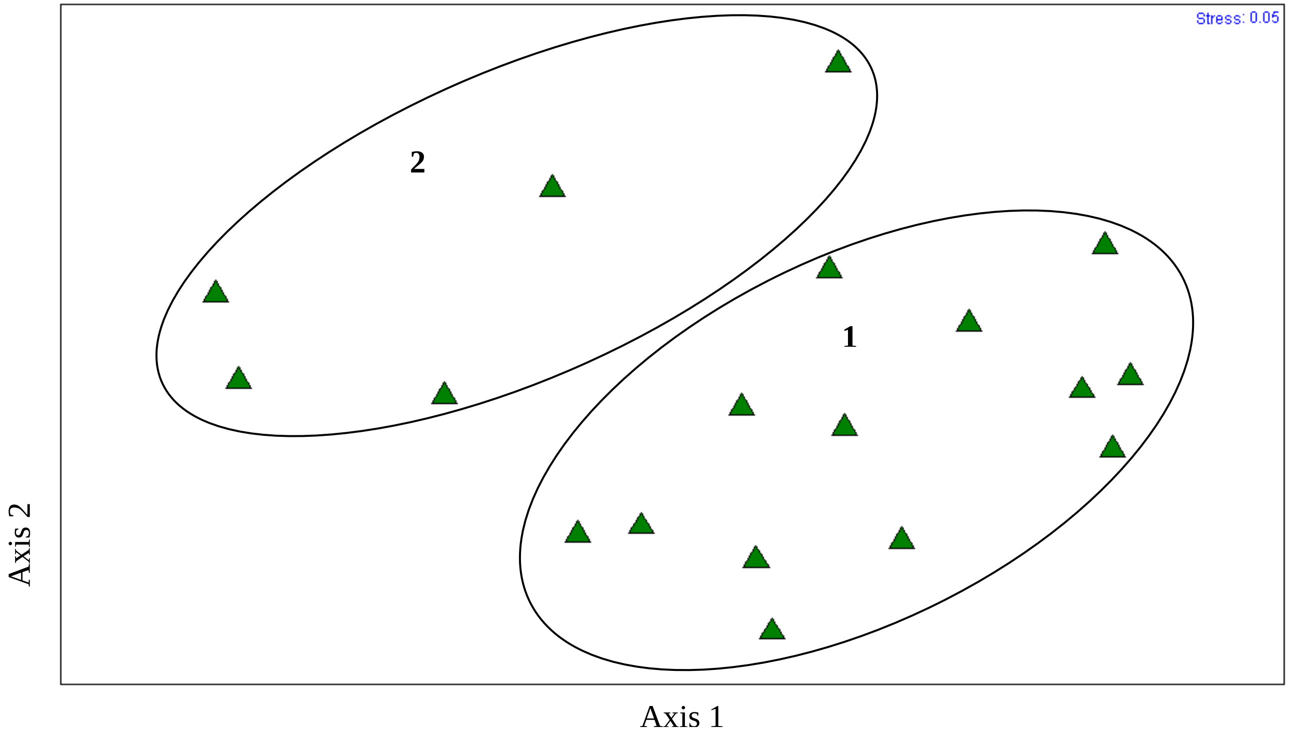

Figure 20: An ordination plot (non metric multidimensional scaling) generate by UPGMA based on Bray-Curtis similarities for SSR analyses at UNAM. The triangular symbols on the figure represent the accessions. Two major clusters 1 and 2 have been formed with a stress value of 0.05. The accessions in cluster 1 Omusati 930(7),Okavango 602(24), Omusati 894(24), Caprivi 66(10) and Caprivi 80(11). These accessions showed their uniqueness through population differentiation by appearing in different clusters.

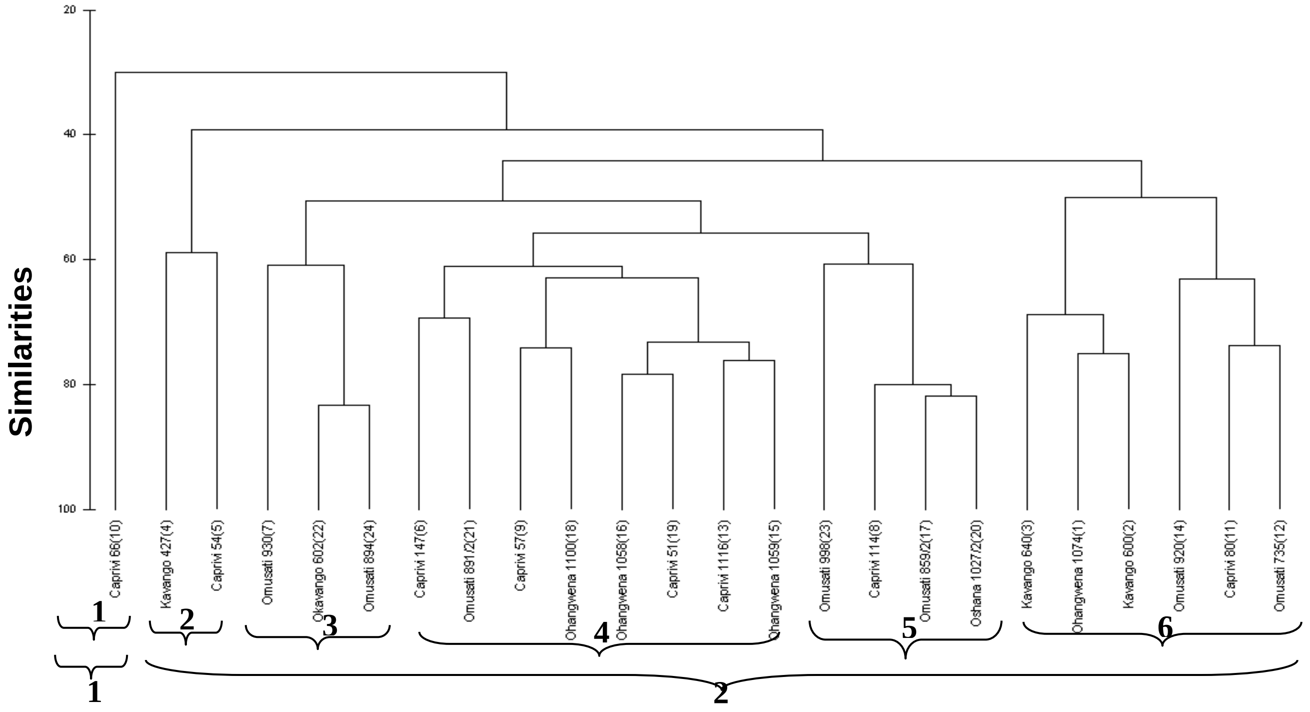

Figure 21: A dendrogram generated by UPGMA clustering showing the similarities among 24 sorghum accessions based on Bray-Curtis similarities. These data are the combinations of the RAPD and SSR amplification results at Potsdam presented in (Figure 17 and 19). Two major clusters and six sub clusters were formed. All accessions have been clustered in the second cluster, except accession Caprivi which is clustered single in cluster 1. The similarities among all the accessions in cluster 2 are about 99% and 30% similar to accession Caprivi 66(10) in cluster 1.

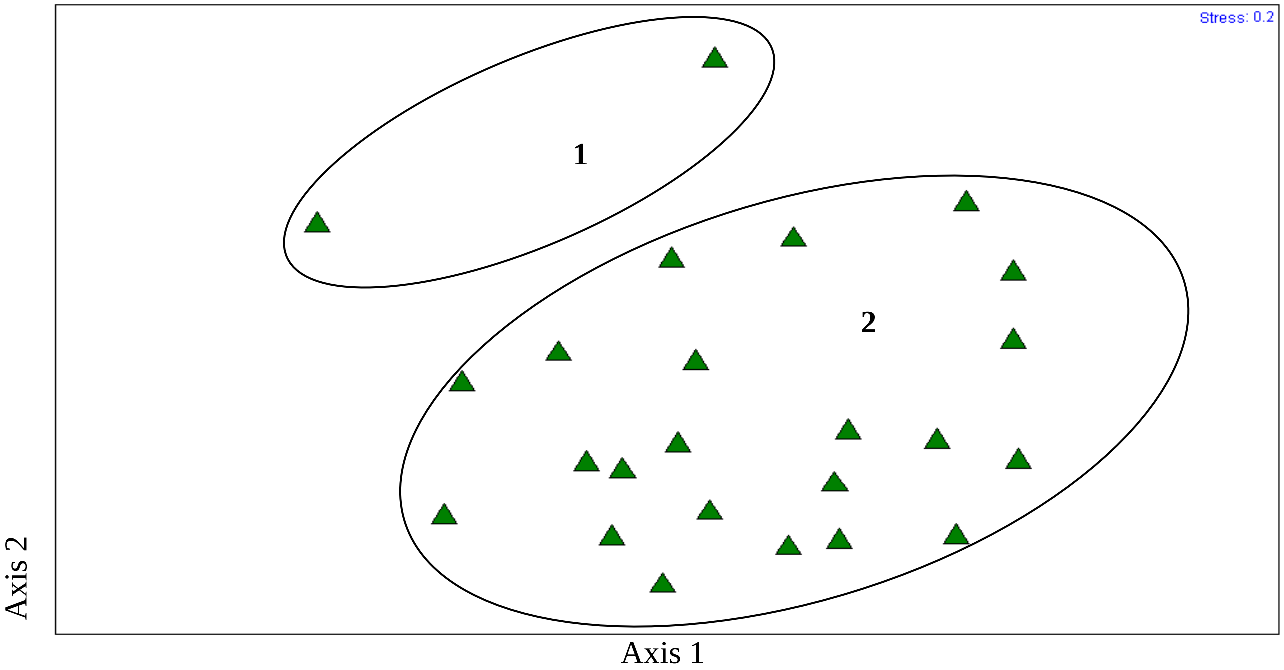

Figure 22: An ordination plot (non metric multidimensional scaling) generate by UPGMA based on Bray-Curtis similarities for SSR and RAPD analyses at Potsdam. The triangular symbols on the figure represent the accessions. Two major clusters 1 and 2 have been formed with a stress value of 0.2. Cluster 1 represent accessions Caprivi 66(10), Kavango 427(4) and Caprivi 54(5). These accessions showed their uniqueness through population differentiation by appearing in different clusters.

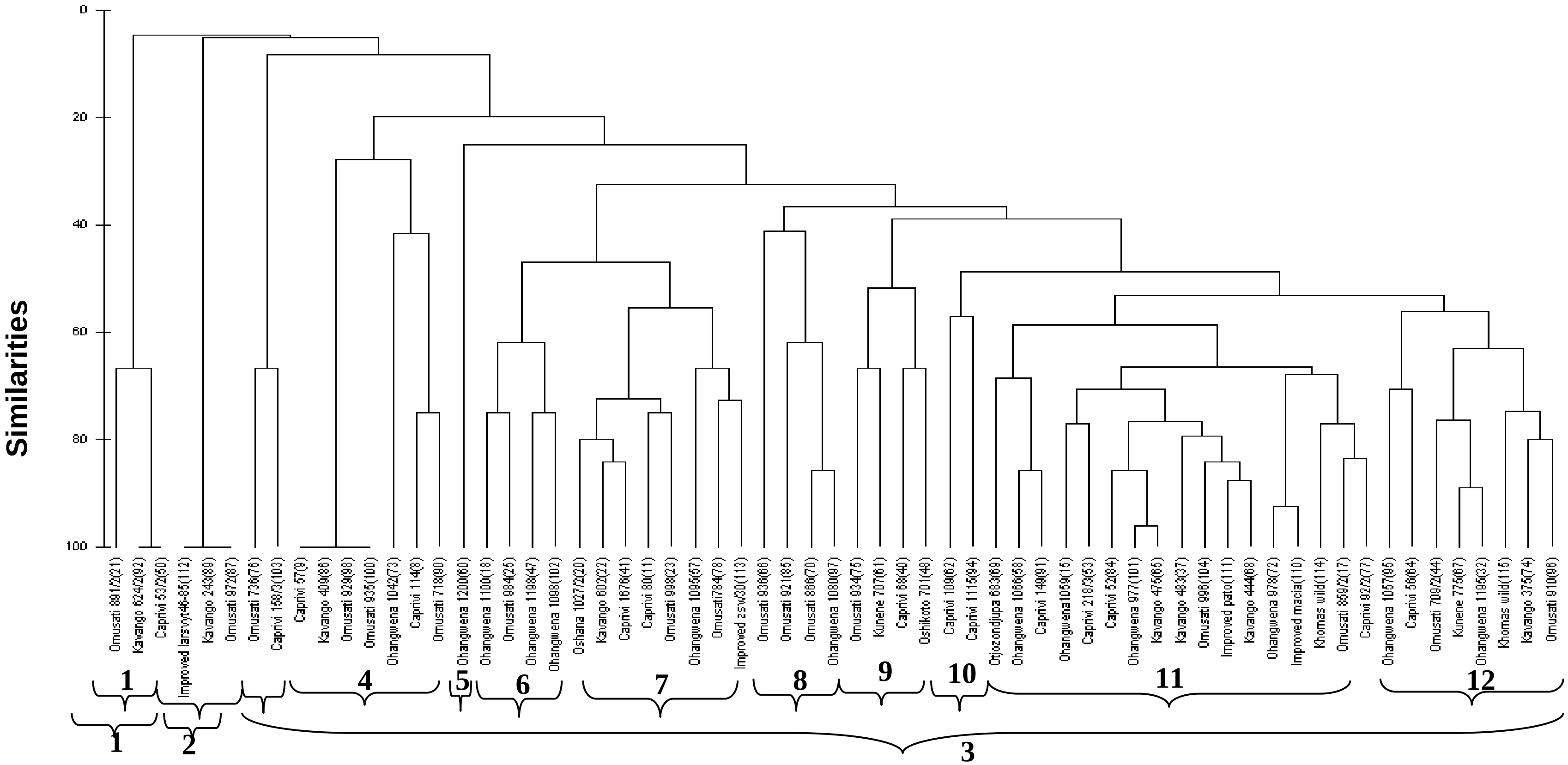

Figure 23: A dendrogram generated by UPGMA clustering showing the similarities among 63 sorghum accessions based on Bray-Curtis similarities. These accessions were amplified with SSR primers at UNAM. The numbers on the braces indicates the number of clusters formed. Three major clusters and twelve sub-clusters were formed. In the major cluster 1, accessions Kavango 624/2(92) and Caprivi 53/2(50) showed 100% similarities. In major cluster 2, accessions Kavango 243(89), Improved Larsvyt 48-85(112) and Omusati 972(87) showed 100% similarities. In major cluster 3, accessions Caprivi 57(9), Kavango 409(86), Omusati 929(98(and Omusati 935(100) showed 100% similarities. The similarities among accessions range between 5%- 15%.

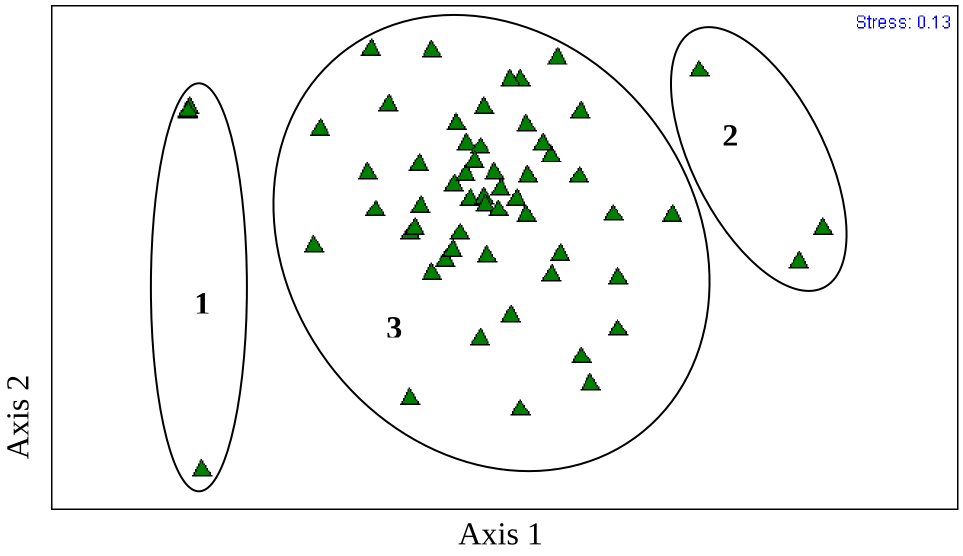

Figure 24: An ordination plot (non metric multidimensional scaling) generate by UPGMA based on Bray-Curtis similarities for SSR amplification at Potsdam. The triangular symbols on the figure represent the accessions. Three clusters 1, 2 and 3 have been formed with a stress value of 0.13. Cluster 1 represent accessions such as Omusati 891/2(21) and Kavango 624/2(92) while cluster 2 represent accessions Improved Larsvyt 46-85(112), Kavango 243(89) and Omusati 972(87). These accessions showed their uniqueness through population differentiation by appearing in different clusters.

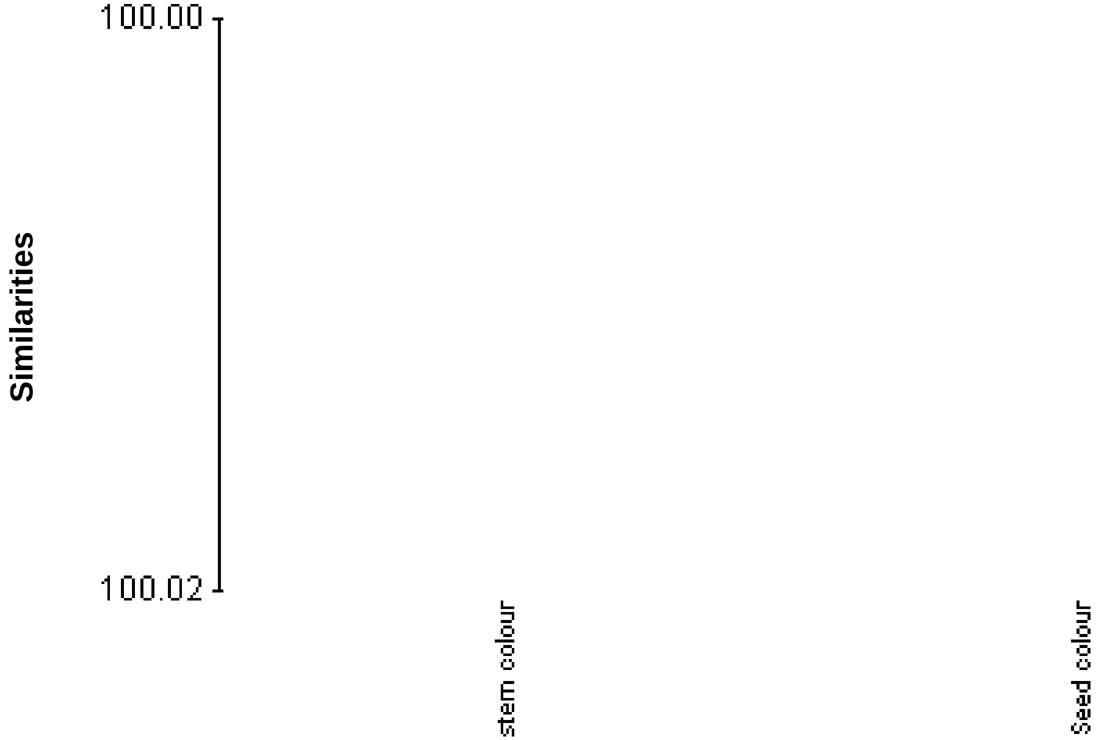

Figure 25: A Dendrogram generated by UPGMA clustering showing the similarities between seed and stem color among all *Sorghum bicolor* accessions used in this study. The similarities are based on Bray-Curtis similarities. Seed color and stem color showed 100.02 % similarity.

4.2.2 Diversity Indices

4.2.2.1 Shannon Wiener Index of Diversity H′ computed for SSR analysis at UNAM

The index ($H' = -\sum (Pi)(\ln Pi)$) where p_i is proportion of the *i*th type in the population, *ln* is the natural logarithm of *Pi*. Shannon Wiener index is calculated was H′=3.844 (Appendix D, table c).

4.2.2.2 Shannon Wiener Index of Diversity H′ computed for SSR and RAPD analysis Potsdam

The index ($H' = -\sum (Pi)(\ln Pi)$) where p_i is proportion of the *i*th type in the population, *ln* is the natural logarithm of *Pi*. Shannon Wiener index calculated was H′=3.152 (Appendix D, table d).

4.2.3 Simpson’s Index of Diversity

4.2.3.1 Simpson’s Index of Diversity computed for SSR analysis at UNAM

Simpson’s diversity (D) is calculated as: D = ∑ ni (ni-1)/N (N-1), where N = Total number of organisms in the survey, *ni* = the number of individuals for each species. Simpson’s Index of Diversity calculated was 1-D= 0.98 (Appendix D, table e).

4.2.3.2 Simpson’s Index of Diversity computed for SSR and RAPD analysis at Potsdam

Simpson’s diversity (D) is calculated as: D = ∑ ni (ni-1)/N (N-1), where N = Total number of organisms in the survey, *ni* = the number of individuals for each species. Simpson’s Index of Diversity calculated was 1-D= 0.96 (Appendix D, table f).

CHAPTER 5

5 DISCUSSION

Traditionally, genetic diversity studies in sorghum and other crops are based upon its phenotypic characteristics. However, this approach did not take into account the genetic diversity at the molecular level. In Namibia, up to date, the genetic diversity of sorghum has never been evaluated and that has hampered the utilization of this crops genetic resources. In this study, genetic diversity among *Sorghum bicolor* landraces, improved varieties and *Sorghum halepense* (wild) relatives was conducted in a PCR analysis using four RAPD and four SSR primers, all of which created amplification products.

5.1 DNA extraction from seed and leaves

Genomic DNA was extracted from seeds and leaves of sorghum plants. Despite the presence of metabolites that interfere with DNA isolation restriction and amplification (Bhoora, 2003; Sharma *et al.,* 2002), genomic DNA isolation from sorghum seed and leaves was carried out successfully in this experiment. This could be attributed to the use of young leaf tissues in the study. Generally, even though DNA isolation was relatively easy in this study, both from the seed and leaves, this does not negate the fact that it can be difficult in the case of plants having high concentrations of phenolic compounds like cactus or sorghum for that matter (Bhoora, 2003). Nevertheless, problems were experienced with loosing the DNA quality after prolonged storage of DNA in the deep freezer at -20˚C. This problem may be associated with the phenolic compound content in sorghum that could cause DNA degradation. Phenolic compounds are suspected to have an influence on DNA degradation, since *Sorghum bicolor* is reported to have phenolic compounds. (allelopathic effect) that are also released through their roots to into the soil (Sene *et al.,* 2004) to limit the growth of competitive plants growing within close proximity to sorghum. This allelopathic effect was reported to have negative effects on the crops growing near the sorghum (Sene *et al.,* 2004). Higher content of phenolic compounds (Proanthocyanidins (Pas), 3-deoxyanthocyanidins (3-DAs) and flavan-4-ols was detected in *Sorghum bicolor* seed before germinating those (Dicko *et al.,* 2005). It is however not clear in the literature on how the allelopathic could influence the quality and quantity of DNA materials extracted. Wajant (2004) also reported the enzyme (DNAses) heterotetrameric enzyme and hydroxynitritelyses (HNL) that were found in young sorghum seedlings. These DNAses are involved in catabolism of the cyanogenic glycoside, dhurrin. It suggested that these enzymes could also lead to DNA degradation if stored for a longer period of time (Wajant, 2004).

Despite all these factors, DNA extraction from the sorghum seed was rather difficult, despite the good DNA yield obtained. It was labor intensive and time consuming to ground the seeds and reduce them to a fine powder. This was not the case with the leaves that was easy and fast to work with. This was probably due to the fact that the seed were ground dry and not soaked in water prior to grinding. The seeds could not be soaked as this would activate many enzymes and other phenolic compounds in the seed that could interfere with effective DNA isolation (Dicko *et al.*, 2005).

Despite the outlined problems, both of the protocols used for DNA extraction either from the seeds or the leaves worked well in this study. This is probably because the methods have been used before and proven successful. Many authors have written on many different methods of DNA extraction. These include the standard methods as outlined by Abu Assar *et al. (*2005) and Ghebru *et al*. (2002). The protocol used for extraction of DNA from *Sorghum bicolor,* improved varieties and its wild relatives is effective as many researchers have used the same method successfully in the past (Anas, 2004). The DNA purification kit (see appendix 2) gave a high DNA yield of 10ng/µl as indicated by clean bands.

5.2 RAPD analysis

DNA amplification using 4 RAPD primers (45R, 51R, 88R and 99R) worked well on 24 *Sorghum bicolor* accessions analyzed at Potsdam-Germany. A high level of genetic diversity among the genotypes was revealed by RAPDs. A total number of 205 bands or loci were detected from 24 sorghum genotypes using 4 RAPD primers with primer 51R giving the highest band count of 77 and 88R with the lowest band count of 27. The number of amplification products (bands) per primer varied from 2 to 6, with the mean of 4. This is different from the mean of 6.66 observed by Agrama and Tuinstra (2000). The results would be expected to be different since the primers used were from different manufacturers and the sorghum population is different. Polymorphic bands were detected in this study, of which out of the total amount of bands (205), 31.2% of were polymorphic. This is not surprising since it was the case in a similar study where many polymorphisms were detected per primer (Ayana *et al.,* 2000). RAPD primers amplify the genome randomly leading to many sites amplified and hence polymorphisms (Ayana *et al.,* 2000). High DNA polymorphism with RAPD primers was also experienced in the study on banana and sugarcane varieties (Iqbal *et al.,* 1995). It was also observed that each RAPD primer amplified the accessions differently and this is revealed by different banding patterns obtained. This is true as it has been confirmed by (Tao *et al.*, 1993) that individual primers differed significantly in their ability to detect genetic polymorphism in the species. Despite the high number of bands obtained using RAPDs primers it does not rule out the advantage that SSRs has over RAPD that SSRs uses specific primers (Agrama and Tuinstra, 2004). This study also revealed that RAPDs can be used as a tool in the study of genetic diversity among sorghum germplasm as reported previously by many researchers including Agrama and Tuinstra (2004); Cregan *et al*. (1999); Ayana *et al. (*2000). RAPD primers are known for their ability to randomly amplify the genomes (Cregan *et al*., 1999) and therefore many bands were usually detected. This was also observed in many studies on the genetic diversity of *Sorghum bicolor* (Cregan *et al*., 1999; Nkongolo and Nsapato, 2004) where RAPD primers produced multiple numbers of amplifications of all 24 accessions analyzed in that study. The study also revealed that about 9 different bands were observed at different loci. The observed bands were between ±100 and 800 bp when compared with the 100bp DNA ladder (Cregan *et al*., 1999; Nkongolo and Nsapato, 2004). Our results are also in line with the results of Agrama and Tuinstra (2004) who reported the banding patterns in sorghum using RAPD primers. In this study, the highest number of bands scored was (A) observed at 800bp with 26% and the lowest were scores (E and H) observed at ± 100bp with 2% as presented in (Table 5).

5.3 SSR analysis

The 24 sorghum genotypes evaluated at Potsdam University and 63 genotypes evaluated at UNAM were differentiated from each other by producing polymorphic bands using two (Sb6-84 and Sb6-34) SSR primer pairs obtained from the literature (Anas, 2004) and two (CD230935 and CD231028) newly designed primer pairs. A high level of genetic diversity among the genotypes was revealed by SSRs. This was revealed by the level of polymorphism detected with each primer between 165-218 bp. These results agreed with those of Folkertsma *et al.* (2005) who reported from their study that when SSR primers were used, a total amount of 123 bands were obtained from 21 SSR primer pair. SSR primers amplified 3-5 bands per genotype and few multi amplifications were observed per genotype in that study. These results are also in agreement with the findings of Ghebru *et al*. (2002) and Anas (2004). This was not surprising as SSRs have been used in a similar study and have revealed high polymorphism on polyacrylamide gel (Taramino *et al.,* 1997; Bhattramakki *et al.,* 2000). Taramino *et al.* (1997) observed that one locus in particular allowed the identification of all other inbred lines used in that study. This scenario is similar in this study in which accessions 24, 27 and 28 showed a different banding pattern when screened with Sb6-342 primer which was not the case with RAPD primers. These results gave the impression that polyacrylamide gives a high resolution power than superfine agarose gel does (Taramino *et al.,* 1997). The SSRs are favored over RAPDs and have been used in identification of P54 loci in sorghum in some studies (Taramino *et al.,* 1995). The study found that the SSRs were the effective molecular markers in *Sorghum bicolor* studies (Taramino *et al.,* 1995).

The SSRs also revealed a clear level of genetic polymorphism with 28% polymorphic bands obtained with 2 SSR primer pairs on 24 accessions than the 31.2% polymorphic bands obtained with 4 RAPD primers. This is because SSR primers are site specific and can amplify the recognized site (Folkertsma, 2005). The high level of polymorphism associated with SSR primer may be a function of unique replication slippage mechanism responsible for generating SSR allelic diversity (Agrama and Tuinstra, 2003). These results give the same impression as those from similar experiments (Ghebru *et al.*, 2002; Abu Assar *et al*., 2005; Pejic *et al*., 1998) in which different alleles where detected on polyacrylamide gel. The differences in allelic detection between SSR and RAPD in this study may also be attributed to the use of polyacrylamide gel for allele detection at Potsdam University where polyacrylamide gel was used with the SSR amplification product analyses and not with RAPD amplification products.

Polyacrylamide gels have greater resolving power than the superfine agarose gel (Anas and Yoshinda, 2003) that was used for SSR detection at UNAM and RAPD detection at Potsdam. According to Agrama and Tuinstra (2004), the increased resolution of polyacrylamide gel over that of superfine agarose gel separation usually results in the detection of larger number of alleles per locus. In the case where SSR loci containing dinucleotide repeats whose amplification products are in the 130 to 200 bp range cannot be detected on agarose gel (Agrama and Tuinstra, 2004). This may have contributed to the low number of polymorphic bands observed when agarose gel was used to analyze the SSR amplification at UNAM in this study. This statement is also supported by Anas (2004), who reported that polyacrylamide gels provide precise estimation of fragment size and identify small size differences between fragments. The 87 sorghum accessions analyzed had unique alleles since the fragments observed were found at different sizes of the 100bp DNA ladder. This indicates that there is variation among the sorghum accessions (land races) analyzed in this study. High level of genetic diversity among sorghum landraces was also reported by (Ghebru *et al.,* 2002) who indicated that farmer varieties or landraces are known for their diverse characteristics and have been used for diverse breeding materials. A similar study by Agrama and Tuinstra (2003) also indicated that sorghum landraces consisted of high genetic variations in their study on the phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPD. In addition to the results obtained, Smith *et al.* (2000) also reported that SSR were the best primers used in that experiment and could have great potential in selection among sorghum inbreds.

5.4 Cluster analysis and genetic diversity analysis

The UPGMA dendrograms constructed in this study are based on the genetic relationship among the sorghum accessions and also on the relationship of geographical (Regional Government Areas) background from where they were collected. The genetic relationship was estimated based on the number of alleles detected per accession analyzed in this study. The same procedure was also followed in the study carried out by Zhang *et al.* (2002) looking at the genetic diversity of cereal crops. Cluster analysis for both SSR and RAPD data showed that Namibian-cultivated sorghum are similar and are closely related genetically with % similarities ranging between 15-100%. This is because the accessions from different regions were found to cluster and scattered randomly all over the dendrogram, as was with the case in the study by (Tanto and Demissie, 2005). Moreover, it was surprising to learn that different lines from different regions tended to cluster together despite their differences in the Regional Government Areas from which they were collected. According to Anas *et al.* (2003) clustering together is an indication of a close relationship while different accessions would cluster separately. These results are not unique as the same scenario was experienced by Anas *et al.* (2003). A narrower genetic background was observed between the improved varieties, wild relatives and landraces and could not be distinctly separated from each other through clustering.

Cluster analysis failed to group accessions of the same region together as one would anticipate. Wild relatives and improved varieties of sorghum were found to have grouped with the landraces from the regions. These results assume that there is an intense seed exchange among farmers between different regions that caused similarities among accessions based on geographical localization (Tanto and Demissie, 2005; Ayan *et al.,* 2000). Wild relatives did not show an outstanding difference from the landraces and improved varieties. This closer relationship between wild relatives and landraces as well as improved varieties might also be as a result of gene flow from wild sorghum relatives (Tanto and Demissie, 2005). This is due to sorghum being a wind-pollinated crop (Djé *et al*., 2004) and the pollen could be carried in the air between varieties. Another reason could be the breeding materials used in bringing up the improved varieties that might have the same genetic make up (Uptmoor *et al.*, 2003). A third factor that could contribute to the low genetic variation between the landraces and wild relatives may be as a result of the reduced population size (Szabó, 1999) of the wild sorghum due to habitat change. Habitat change could be the result of over-stocking and town expansion of cities and towns at the expense of wild species habitat (Kurtto and Helynranta 1998; Szabó 1999). Ayana *et al.* (2000) reported the same conclusions from results obtained through a study on Ethiopian sorghums.

In Namibia the situation of similarities among accessions used at different regions could also be attributed to the seed producing company that lies in the north central region of Omusati, which all the seed buyers go to get their seed from during the cultivation seasons (MAWRD, 2002).

According to Tanto and Demissie, (2005) it is possible that these seed purchasers collect seeds from farmers in that region and perhaps, transfer these new seed varieties to their own regions. This situation was experienced by (Tanto and Demissie, 2005) in the study of genetic diversity among major crops in Ethiopia where it was found that farmers usually pocket small samples of seeds from other farmers.

In Namibia people own pieces of farm land wherever they wish to have it which make it possible for the farmers who live for example in Oshana and own a piece of farm land in

Oshikoto to take the seed from Oshana to plant at the field in Oshikoto (MAWRD, 2002). This was experienced in the study by Tanto and Demissie (2005), where genetic diversity was measured by number of farmers' varieties grown for each crop. It was found that farmers usually exchanged the seeds and therefore genetic diversity was low based upon geographical distribution of originations of the crops grown (Tanto and Demissie, 2005).

The 100% similarities of the sorghum accessions on the basis stem and seed color indicates that these morphological characteristics are not influenced by environmental factors. These accessions were collected from different geographical localities. Despite the high similarity rates of 100% on the bases of seed color and stem color of the accessions used in this study, the genetic diversity calculated was not 100%.

The results revealed by clustering in this study were true as confirmed by ordination plots (2D) that gave a good degree of fitness. Ordination plots give clear information as to which sample is more similar with the other (Jongman *et al.,* 1995).The ordination plots also showed similar clustering among the accessions by randomly clustering the accessions across clusters as it was found when dendrograms were constructed.

Despite the low genetic diversity revealed by cluster analysis, Shannon Wiener and Simpson diversity indices calculation indicated high genetic diversity among the accessions. This could be that the accessions used across the regions are different on the genetic level but the same accessions are shared widely among the regions. Despite the congregation, of accessions based on the regional background, diversity indices calculation using Shannon Wiener and Simpson diversity indices, confirmed high variations among the Namibian sorghum landraces, improved varieties and wild relatives.

Shannon Wiener diversity calculated was $H' = 3.844$ and $H'=3.152$ for SSR analyzed at UNAM and SSR and RAPD analyses at Potsdam respectively. The Simpson diversity calculated was D= 0.98 and D= 0.96 for SSR analyzed at UNAM and SSR and RAPD analyses at Potsdam respectively. This high variability could be attributed to the different morphological groups in this study covered as was also experienced by Assar, (2005) that different morphological groups of accessions stand a chance of high genetic variations. The same results were reported by Djé *et al.* (2000) in his study on genetic diversity among *Sorghum bicolor* found high genetic diversities in accessions with same morphological characteristics belonging to the race bicolor.

CHAPTER 6

6 CONCLUSIONS AND RECOMMENDATIONS

To the best of our knowledge, this is the first report on the genetic relationship among the Namibian sorghum genotypes at NBRI. This study provides a detailed analysis and quantification of the genetic diversity among Namibian sorghum germplasm at NBRI. Namibian cultivated sorghums that included improved genotypes from SADC countries showed similarities indicating a low genetic diversity. Genotypes from the same place of collection and those from closely situated regions showed a closer genetic relationship.

The genetic analysis results also revealed that there were no genetic differences between the wild relatives, improved varieties and the cultivated varieties based on the Regional Governmental; however, the result indicated that Namibian sorghum genotypes are diverse at molecular level. This was also found in the study of genetic diversity among *Sorghum bicolor* from different regions including southern Africa. The study revealed that the regional levels of genetic diversity in cultivars were greater in northern and central Africa compared to southern Africa (Aldrich *et al.*, 1992). Accessions from Southern Africa were found to be closely related. It is, therefore, concluded that the germplasm screened in this study are different at the molecular level and therefore constitutes different genetic make up. However, the same accessions are interchangeably shared among farmers across the regions and thus caused the clustering based on regional background to be random. The results have indicated that it is possible to use SSR and RAPD primers for studying genetic diversity in sorghum. Both SSR and RAPD primers can be efficiently used by researchers in developing countries where the technology is low because neither requires radioactive isotopes. However, SSR primers were found to be more effective for identifying genetic relationship among a diverse collection of accessions than RAPD primers. This was concluded following the differences in banding patterns obtained using SSR and RAPD. SSR bands were more clear and usually 1-5 in total whereas RAPD band were multiple and difficult to count. Despite the high number of bands obtained using RAPD primers it does not rule out the advantage that SSR have over RAPD that SSR are specific primers. In addition the newly designed primers (CD230935 and CD231028) worked well and amplified mostly all accessions analyzed.

It was also concluded that it is possible to improve the efficiency of using germplasm as a genetic resource and its use could be improved only if the information on the genetic diversity is available. It is concluded from the results that sorghum accessions at NBRI are closely related based on their geographical origins but diverse at the molecular level. This suggests that sorghum genotypes introduced or collected from the same place tend to have a close genetic background. This is true as supported by Anas (2004), that the same or limited genetic source of one’s country or research can cause low genetic diversity among breeding lines.

It could be clearly seen when the accessions are similar or different simply by comparing the levels at which the bands were detected. In this case, it would be better to sequence the DNA to give the sequences of the bands obtained in order to distinguish these accessions. Therefore, it is concluded that SSR profiling is useful and works well in sorghum diversity studies. The SSR method of genetic diversity could be better improved in Namibia for the identification of sorghum inbred lines and hybrids that could be used in many applications of research in Namibia. This would eventually contribute to food security, conservation of Namibia genetic resources and the alleviation of poverty amongst farmers, whose resources are in a dire need to be enriched.

CHAPTER 7

7 REFERENCES

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APPENDIX A

Table a: List of sorghum accessions used in this study

ID	Accession Number	Origin	Collecting Institute	Supplying Source	Genetic status	Scientific Name	Region	Village	Stem colour	Seed Colour
1	1074	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohang-Wean	Eenana	White	White
2	600	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Oka-Vango	Kankudi	White	White
3	640	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Oka-Vango	Sapirama	Red	Pink
4	427	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Oka-Vango	Mutjokotjo	White	White
5	54	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Mukasa	White	Red
6	147	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Makalongo	Red	Red
7	930	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Okalongo	Red	Yellow
8	114	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Mazamvani	Red	Red
9	57	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Masikili	Red	Yellow

10	66	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Malimi	Purple	Red
11	80	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Muaniko	Red	Red
12	735	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Etunda	Red	Yellow
13	1116	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Singalamwe	Whit	Red
14	920	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Okathitu	Red	Red
15	1059	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Endola	White	Red
16	1058	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Endola	Red	White
17	859/2	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Enongo	Red	White
18	1100	Namibia	ICRISAT	NBRI	Landrace	*Sorghum*	Ohangwena	Ohanjekeve	Red	Red
19	51	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Mukasa	White	White
20	1027/2	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Oshana	Ongwediva	Red	Red
21	891/2	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Okathitu	Red	Red
22	602	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Okavango	Kankudi	Purple	Pink
23	998	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Makongo	White	White
24	894	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Omuthitu gwonyama	Red	Red
25	984	Namibia	ICRISAT	NBRI	Landrace	*Sorghum*	Omusati	Okafuku	Red	yellow

						bicolor				
32	1195	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Enyana	Red	Red
37	483	Namibia	ICRISAT	NBRI	Landrace	*Sorghum*	Okavango	Kabaro	White	White
40	68	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Malimi	Red	Yellow
41	1676	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Andara	Red	Yellow
44	709/2	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Ogongo	White	White
47	1198	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Enyana	Red	Yellow
48	701	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Oshikoto	Veterinary codon	Red	Red
50	53/2	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Mukassa	Red	Red
53	218/3	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Sesheke	Red	Red
57	1095	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Oshikunde	White	Red
58	1066	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Endola	Red	Brown
60	1200	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Ekoka	Red	White
61	707	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Kunene	Rwacana	Red	White
62	109	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Ikava		Brown
64	56	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Masikili	Red	Red

65	475	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Okavango	Sumi	Red	Red
66	936	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Omutundungu	Red	Red
67	775	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Kunene	Eunda	Red	Red
68	444	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Okavango	Desi	Red	Red
69	683	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Otjozondjupa	Tsumkwe	Red	Yellow
70	866	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Enongo	Red	White
72	978	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Oshuulo	White	Yellow
73	1042	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Onuumba	Red	Brown
74	375	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Okavango	Sisungu	Red	Red
75	934	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Omutundungu	Red	Red
76	736	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Etunda	Red	Red
77	92/2	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Tolodi	Red	Red
78	784	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Onesi	Red	Yellow
84	52	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Mukassa	Red	Cream
85	921	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Okathitu	Red	Cream

86	409	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Okavango	Tjoha	Red	White
87	972	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Elim	Red	Red
89	243	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Okavango	Mabuse	White	Red
90	718	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Ogongo	Red	White
91	149	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Makolongo	Red	Red
92	624/2	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Okavango	Mpoto	Red	Red
94	1115	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Mukabu	Red	Red
95	1057	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Omatunda	Red	White
96	910	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Oshitalamesho	Red	Cream
97	1080	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Okongo	White	White
98	929	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Okalongo	Red	Red
100	935	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Omutundungu	Red	Brown
101	977	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Oshuulo	Red	Cream
102	1098	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Ohangwena	Oshikunde	Red	Red
103	158/3	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Caprivi	Sabelo	Red	White

104	996	Namibia	ICRISAT	NBRI	Landrace	*Sorghum bicolor*	Omusati	Makongo	Red	Brown
110	Macia	Mozambique	ICRISAT	MAWF	Improved	Improved	SADC	N/A	White	White
111	Pato	Tanzania	ICRISAT	MAWF	Improved	*Sorghum bicolor*	SADC	N/A	Red	Yellow
112	Larsvyt 46-85	Latin-America	ICRISAT	MAWF	Improved	*Sorghum bicolor*	SADC	N/A	Red	Yellow
113	ZSV/30	Zimbabwe	ICRISAT	MAWF	Improved	*Sorghum bicolor*	SADC	N/A	red	Yellow
114	Wild	Namibia	collected	Student	Wild	*Sorghum halepense*	Khomas	N/A	Red	Red
115	Wild	Namibia	collected	Student	Wild	*Sorghum halepense*	Khomas	N/A	Red	Red

Table b: Identification codes for the accessions that are on the agarose gels for SSR UNAM. The numbers in bold are the order of the wells as it is on the gels while the regular number is for the accession number (ID) as it appear on the entry table (a) (Apendix A).

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
8	9	11	13	14	15	16	17	18	20	21	22	23	25	26	31	40	41	47
20	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
56	74	62	75	84	101	70	97	114	96	95	76	109	88	99	94	64	115	61
39	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**
92	49	54	53	105	78	113	103	79	52	85	58	91	67	60	66	81	57	73
58	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**	**75**	**76**
106	77	89	37	87	111	48	112	102	72	110	55	50	65	68	90	69	104	44
77	**78**	**79**	**80**	**81**														
32	86	67	98	100														

APPENDIX B

DNA purification

The following steps were followed to purify the DNA samples from *Sorghum bicolor* seeds at Potsdam.

- **Adjusting DNA binding conditions**

Two volumes (200µl) of buffer NT was mixed with one volume (100µl) of PCR reaction mix.

- **Binding DNA**

In this experiment all DNA samples from the seeds in the experiment carried out at Potsdam were purified by placing the nucleoSpin Extract 2 columns into 2 ml collecting tubes and then the samples loaded. This was done for each sample in separate nucleoSpin Extract 2 columns into 2 ml collecting tubes. The samples were then centrifuged for 1 minute at 11000 rpm. The flow through was discarded and the nucleoSpin Extract 2 column placed back into the collecting tube.

- **Silica membrane washing**

Buffer NT3 (600µl) was added to the nucleoSpin Extract 2 column and then centrifuged for 1 minute at 11000 rpm. The flow-through was again discarded and the nucleoSpin Extract 2 column placed back into the collecting tube.

- **Silica membrane drying**

The nucleoSpin Extract 2 column placed back into the collecting tube was further centrifuged for 2 minute at 11000 rpm to remove buffer NT3 quantitatively. This is because the buffer contains some ethanol and the residual ethanol from the buffer NT3 would inhibit subsequent reactions and has to be removed by further centrifuge. In addition to centrifugation, total removal can be achieved by incubation of nucleoSpin Extract 2 column for 2-5 minutes at 70°C prior to elution. Care is taken that the spin column doesn't come in contact with the flow-through while removing it from the centrifuge and the collection tube.

- **DNA elution**

The nucleoSpin Extract 2 column was placed into a clean 1.5 ml micro centrifuge tube and then 30µl elution buffer NE added, and incubated for 1 minute at room temperature. Incubation helps to increase the yield of eluted DNA. Then centrifuge the nucleoSpin Extract 2 column for 1 minute at 11000 rpm. ET1 (60µl) buffer was then added to increase the quantity of DNA product. Note that the yield of large fragments can be increased by using pre-warmed elution buffer 70°C and incubate at room temperature for 1 min before collecting elute by centrifugation. After purification and collection of the flow-through, PCR reaction was run.

Chemical components used in DNA extraction

- **EB**

10 mM Tris (pH 8,0)

500 mM NaCl

50 mM EDTA

- **SDS**

10% SDS (w/v)

Im Wasser aufgelöst

- **Na-Acetate**

3M Na-Acetate pH 5,2

- **K-Acetate**

3M K-Acetate pH 4,8

- **Proteinase K**

10 mM Tris-HCl pH:8

10 mM EDTA

1 mM NaCl

0,5% SDS (w/v)

100 μg/ml Proteinase K

- **RNAse**

10 mg/ml RNAse A
10 mM Tris-HCl, pH: 7,5
15 mM NaCl

APPENDIX C

Agarose gel preparation

A 1% agarose gel is prepared to run electrophoresis by taking 1g/100ml 1XTAE buffer. The solution is placed in the microwave at high temperature for 10 minutes until well dissolved dividing the time into portions by allowing it to run for 5 minutes and steer it and put it back again, just to make sure that no residues on the bottom. After cooking and the solution well dissolved, the solution is taken to water bath to bring it to 60°C. After cooling down, an amount of 5µl ethidium bromide is added to the solution in order to get DNA bands easily visible after electrophoresis and this mark the final step in the preparation of agarose gel and it was ready to use.

Preparation of the polyacrylamide gel for the Lycor machine

KB plus 6.5% gel matrix (20ml) was transferred to a beaker and placed at room temperature for 30 minutes before use. APS 10% (150µl) was added and then 15µl TEMED was added and then the solution was stirred to mix. The mixture is poured into a syringe plugging the thumb in the opening so that the mixture cannot be wasted while pouring it in. after placing the stopper back, place a filter on the outlet of the syringe to filter any residues. The mixture is then forced out of the syringe by squeezing it between two glass plates fixed together. A fined comp is fixed between the plates in order to make slots for the samples. The glass plates are left at room temperature for 2hours for the gel to solidify. Care should be taken when handling the gel matrix because it is toxic and give unpleasant smell.

Loading the samples onto the Lycor machine

Once the gel has solidified, the comp is removed and the glass plates are fixed onto the Lycor machine to load the samples. These slots are small and the machine detection is very accurate and very sensitive, that is why a minimum amount of sample is required. An amount of 0.5µl was taken from PCR product into new flat cap strips, 3µl loading buffer was added on each sample and loaded on the Lycor machine. Special tips that are sharp were used to load the samples. These tips can be used to load more than one sample if no stain is seen on them. Once all the samples have been loaded, the machine is closed and the computer fixed to it is switched on. Once the program is in place, then a start run button is pressed for the machine to run.

APPENDIX D

Diversity Indices

Shannon Wiener Diversity Index

Shannon Wiener is calculated as: The index (H′= -∑ (Pi) (lnPi)) where p_i is proportion of the *i*th type in the population, *ln* is the natural logarithm of *Pi.*

Table c: Shannon Wiener Index of Diversity H′ computed for SSR analysis at UNAM

Accession	Number of bands	Proportion(Pi)	ln(pi)	PilnPi
Caprivi 114(8)	3	0.01	-4.828	-0.039
Caprivi 57(9)	1	0.00	-5.809	-0.017
Caprivi 80(11)	9	0.03	-3.688	-0.092
Ohangwena1059(15)	12	0.03	-3.381	-0.115
Omusati 859/2(17)	6	0.02	-4.074	-0.069
Ohangwena 1100(18)	5	0.01	-4.268	-0.059
Oshana 1027/2(20)	8	0.02	-3.772	-0.087
Omusati 891/2(21)	2	0.01	-5.115	-0.031
Kavango 602(22)	9	0.03	-3.688	-0.092
Omusati 998(23)	7	0.02	-3.912	-0.078
Omusati 984(25)	3	0.01	-4.828	-0.039
Caprivi 68(40)	3	0.01	-4.828	-0.039
Caprivi 1676(41)	10	0.03	-3.575	-0.100
Ohangwena 1198(47)	4	0.01	-4.509	-0.050
Kavango 375(74)	6	0.02	-4.074	-0.069
Caprivi 109(62)	7	0.02	-3.912	-0.078
Omusati 934(75)	4	0.01	-4.509	-0.050

Caprivi 52(84)	12	0.03	-3.381	-0.017
Ohangwena 977(101)	13	0.04	-3.296	-0.122
Omusati 866(70)	3	0.01	-4.828	-0.039
Ohangwena 1080(97)	4	0.01	-4.509	-0.050
Khomas wild(114)	7	0.02	-3.912	-0.078
Omusati 910(96)	4	0.01	-4.509	-0.050
Ohangwena 1057(95)	8	0.02	-3.772	-0.087
Omusati 736(76)	1	0.00	-5.809	-0.017
Caprivi 1115(94)	7	0.02	-3.912	-0.078
Caprivi 56(64)	9	0.03	-3.688	-0.092
Khomas wild(115)	7	0.02	-3.912	-0.078
Kunene 707(61)	2	0.01	-5.115	-0.031
Kavango 624/2(92)	1	0.00	-5.809	-0.017
Caprivi 218/3(53)	14	0.04	-3.244	-0.127
Omusati784(78)	4	0.01	-4.509	-0.050
Improved zsv/30(113)	7	0.02	-3.912	-0.078
Caprivi 158/3(103)	2	0.01	-5.115	-0.031
Omusati 921(85)	3	0.01	-4.828	-0.039
Ohangwena 1066(58)	6	0.02	-4.074	-0.069
Caprivi 149(91)	8	0.02	-3.772	-0.087
Kunene 775(67)	5	0.01	-4.268	-0.060
Ohangwena 1200(60)	14	0.04	-3.244	-0.127
Omusati 936(66)	3	0.01	-4.828	-0.039
Ohangwena 1095(57)	5	0.01	-4.268	-0.060
Ohangwena 1042(73)	1	0.00	-5.809	-0.031
Caprivi 92/2(77)	6	0.02	-4.074	-0.069
Kavango 243(89)	1	0.00	-5.809	-0.031
Kavango 483(37)	7	0.02	-3.912	-0.078
Omusati 972(87)	1	0.00	-5.809	-0.031
Improved pato(111)	8	0.02	-3.772	-0.031
Oshikoto 701(48)	6	0.02	-4.074	-0.069
Improved larsvyt46-85(112)	1	0.00	-5.809	-0.039

Ohangwena 1098(102)	4	0.01	-4.509	-0.050
Ohangwena 978(72)	6	0.02	-4.074	-0.069
Improved macia(110)	7	0.02	-3.912	-0.078
Omusati 996(104)	11	0.03	-3.473	-0.108
Caprivi 53/2(50)	1	0.00	-5.809	-0.031
Kavango 475(65)	12	0.03	-3.381	-0.115
Kavango 444(68)	8	0.02	-3.772	-0.031
Omusati 718(90)	5	0.01	-4.268	-0.060
Otjozondjupa 683(69)	9	0.03	-3.688	-0.092
Omusati 709/2(44)	6	0.02	-4.074	-0.069
Ohangwena 1195(32)	4	0.01	-4.509	-0.050
Kavango 409(86)	1	0.00	-5.809	-0.031
Omusati 929(98)	1	0.00	-5.809	-0.031
Omusati 935(100)	1	0.00	-5.809	-0.031
Total	355	1.00	H′= -∑PilnPi=3.844	

Table d: Shannon Wiener Index of Diversity H′ computed for SSR and RAPD analysis Potsdam

Accessions	Number of bands	Proportion(Pi)	ln(pi)	PilnPi
Ohangwena 1074(1)	8	0.03	-3.507	-0.105
Kavango 600(2)	8	0.03	-3.507	-0.105
Kavango 640(3)	8	0.03	-3.507	-0.105
Kavango 427(4)	10	0.04	-3.219	-0.129
Caprivi 54(5)	7	0.03	-3.507	-0.105
Caprivi 147(6)	12	0.05	-2.996	-0.150
Omusati 930(7)	11	0.04	-3.219	-0.129
Caprivi 114(8)	14	0.05	-2.996	-0.150
Caprivi 57(9)	15	0.06	-2.813	-0.169
Caprivi 66(10)	13	0.05	-2.996	-0.150
Caprivi 80(11)	10	0.04	-3.219	-0.129
Omusati 735(12)	9	0.03	-3.507	-0.105
Caprivi 1116(13)	12	0.05	-2.996	-0.150
Omusati 920(14)	8	0.03	-3.507	-0.105
Ohangwena 1059(15)	9	0.03	-3.507	-0.105
Ohangwena 1058(16)	14	0.05	-2.996	-0.150
Omusati 859/2(17)	11	0.04	-3.219	-0.129
Ohangwena 1100(18)	12	0.05	-2.996	-0.150
Caprivi 51(19)	9	0.03	-3.507	-0.105
Oshana 1027/2(20)	11	0.04	-3.219	-0.129
Omusati 891/2(21)	14	0.05	-2.996	-0.150
Okavango 602(22)	12	0.05	-2.996	-0.150
Omusati 998(23)	12	0.05	-2.996	-0.150
Omusati 894(24)	12	0.05	-2.996	-0.150

Total	261	1.00	-76.916	-3.152
			H’= -∑PilnPi=3.152	

Simpson’s Index of Diversity

Simpson’s diversity (D) is calculated as: D = ∑ ni(ni-1)/N(N-1), where N = Total number of organisms in the survey, *ni* = the number of individuals for each species.

Table e: Simpson’s Index of Diversity computed for SSR analysis at UNAM

Accession	Number of bands(n)	n(n-1)
Caprivi 114(8)	3	6.00
Caprivi 57(9)	1	0.00
Caprivi 80(11)	9	72.00
Ohangwena1059(15)	12	132.00
Omusati 859/2(17)	6	30.00
Ohangwena 1100(18)	5	20.00
Oshana 1027/2(20)	8	56.00
Omusati 891/2(21)	2	2.00
Kavango 602(22)	9	72.00
Omusati 998(23)	7	42.00
Omusati 984(25)	3	6.00
Caprivi 68(40)	3	6.00
Caprivi 1676(41)	10	90.00
Ohangwena 1198(47)	4	12.00
Kavango 375(74)	6	30.00
Caprivi 109(62)	7	42.00
Omusati 934(75)	4	12.00
Caprivi 52(84)	12	132.00
Ohangwena 977(101)	13	156.00
Omusati 866(70)	3	6.00
Ohangwena 1080(97)	4	12.00
Khomas wild(114)	7	42.00
Omusati 910(96)	4	12.00
Ohangwena 1057(95)	8	56.00
Omusati 736(76)	1	0.00

Caprivi 1115(94)	7	42.00
Caprivi 56(64)	9	72.00
Khomas wild(115)	7	42.00
Kunene 707(61)	2	2.00
Kavango 624/2(92)	1	0.00
Caprivi 218/3(53)	14	182.00
Omusati784(78)	4	12.00
Improved zsv/30(113)	7	42.00
Caprivi 158/3(103)	2	2.00
Omusati 921(85)	3	6.00
Ohangwena 1066(58)	6	30.00
Caprivi 149(91)	8	56.00
Kunene 775(67)	5	20.00
Ohangwena 1200(60)	14	182.00
Omusati 936(66)	3	6.00
Ohangwena 1095(57)	5	20.00
Ohangwena 1042(73)	1	0.00
Caprivi 92/2(77)	6	30.00
Kavango 243(89)	1	0.00
Kavango 483(37)	7	42.00
Omusati 972(87)	1	0.00
Improved pato(111)	8	56.00
Oshikoto 701(48)	6	30.00
Improved larsvyt46-85(112)	1	0.00
Ohangwena 1098(102)	4	12.00
Ohangwena 978(72)	6	30.00
Improved macia(110)	7	42.00
Omusati 996(104)	11	110.00
Caprivi 53/2(50)	1	0.00
Kavango 475(65)	12	132.00
Kavango 444(68)	8	56.00
Omusati 718(90)	5	20.00
Otjozondjupa 683(69)	9	72.00
Omusati 709/2(44)	6	30.00
Ohangwena 1195(32)	4	12.00
Kavango 409(86)	1	0.00
Omusati 929(98)	1	0.00
Omusati 935(100)	1	0.00
Total	355	2436.00

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

$$D = \frac{2436}{355(354)}$$

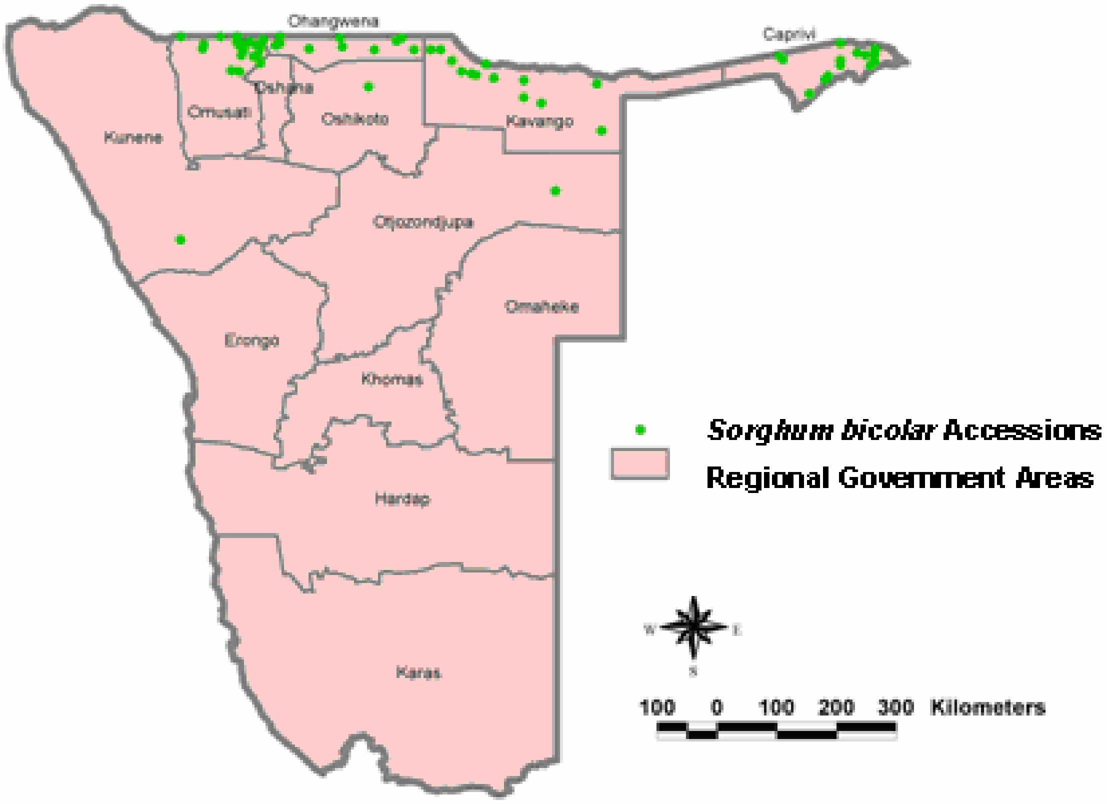
$$D = \frac{2436}{125670}$$

D= 0.02 (Simpson’s Index)

Simpson’s Index of Diversity for SSR data at UNAM is:1-D= 0.98

Table F: Simpson's Index of Diversity computed for SSR and RAPD analysis at Potsdam

Accessions	Number of bands(n)	n(n-1)
Ohangwena 1074(1)	8	56.00
Kavango 600(2)	8	56.00
Kavango 640(3)	8	56.00
Kavango 427(4)	10	90.00
Caprivi 54(5)	7	42.00
Caprivi 147(6)	12	132.00
Omusati 930(7)	11	110.00
Caprivi 114(8)	14	182.00
Caprivi 57(9)	15	210.00
Caprivi 66(10)	13	156.00
Caprivi 80(11)	10	90.00
Omusati 735(12)	9	72.00
Caprivi 1116(13)	12	132.00
Omusati 920(14)	8	56.00
Ohangwena 1059(15)	9	72.00
Ohangwena 1058(16)	14	182.00
Omusati 859/2(17)	11	110.00
Ohangwena 1100(18)	12	132.00
Caprivi 51(19)	9	72.00
Oshana 1027/2(20)	11	110.00
Omusati 891/2(21)	14	182.00
Okavango 602(22)	12	132.00
Omusati 998(23)	12	132.00
Omusati	12	132.00


Ohangwena
Caprivi
Oshana
Omusati
Oshikoto
Kavango
Kunene
Otjozondjupa
Omaheke
Erongo
Khomas
Hardap
Karas
● *Sorghum bicolar* Accessions
Regional Government Areas
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100 0 100 200 300 Kilometers