Genetic diversity assessment of Sorghum [*Sorghum bicolor* (L.) Moench] and Pearl millet (*Pennisetum glaucum* L.) landraces collected from Zimbabwe's communal areas, based on microsatellites and isozymes

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ABSTRACT

Landraces of sorghum [*Sorghum bicolor* (L.) Moench] and pearl millet (*Pennisetum glaucum* L.) are grown throughout Zimbabwe and frequently given names describing important traits they possess or their origin. Little is known about the consistency of the relationships between names and genetic identity, and thereby the usefulness of names is developing strategies for germplam conservation and use. This study analysed genetic diversity of 52 sorghum and 47 pearl millet landraces from two districts of Zimbabwe, Nyanga North and Tshlotsho, using isozyme and microsatellite techniques. Six enzymes: alcohol dehydrogenase (ADH), peroxidase (POD), α-esterase (α-EST), β-glucosidase (β-GLU), acid phosphatase (ACID-P) and malate dehydrogenase (MDH), were tested by isoelectric focusing (IEF) and histochemical staining of polyacrylamide gels. Microsatellite analysis was done using polyacrylamide gels stained with ethidium bromide. Genetic similarities (simple matching coefficients) were calculated and dendrograms were used to compare relationships based on indigenous local names and the origin of the accessions.

Out of seven sorghum microsatellite primers tested, 20 polymorphic bands were scored with the number of bands ranging from two to five per genotype. The dendrogram revealed that the genotypes could be grouped into seven clusters at 62 per cent similarity level. Some genotypes clustered by indigenous local names into *Musoswe* (1355, 1441 and 1460), *Sorghum* (1459 and 1480), *Shodhani* (1450 and 1430) and *Ipwa* (1346 and 1496). Other groups identifiable by their villages of origin included Renzva (1459 and 1480), Kamunhukamwe (1430 and 1450), Siyazama (1572, 1592, 1593,1557 and 1555), Samakande (1343 and 1345) and Mangezi (1401 and 1412). Some genotypes from Nyanga North were closely related to those from Tsholotsho. These included genotypes 1483 (*Nyamuwaya-waya*) and 1487 (*Sorghum*) from Nyanga North which were 100 per cent similar to 1523 (*Isigobane*) and 1537 (*Tsweta khaki*) respectively from Tsholotsho district. Based on the markers used, seven of the 15 accessions collected from Tsholotsho were judged to be duplicates of material also found in Nyanga North.

At 68 per cent similarity level, all the 47 pearl millet genotypes grouped into four clusters. There was a strong association between microsatellite data and indigenous local names in some genotypes. Groups identifiable by local names included *Halale* (1596 and 1571), *Tsholotsho-bearded* (1644 and 1643), *Mudhambure* (1408, 1382, 1418, 1435 and 1396), *Isigumu* (1570 and 1581), *Isifumbata* (1564 and 1650) and *Nyagushe* (1386 and 1375). Some landraces clustered by villages into Sizanani (1544, 1562 and 1548), Phakamani (1644 and 1643), Siyazama (1579 and 1624), Renzva (1462 and 1466), Mangezi (1408, 1382, 1418, 1386 and 1396) and Kamunhukamwe (1440 and 1443). There were some pearl millet genotypes that clustered with those from a different village and district altogether. Genotypes 1466 (*Mudhambure*) and 1447 (*Nyagushe*) from Nyanga North which were 100 per cent similar to 1570 (*Isigumu*) and 1524 (*PMV-3*) respectively, from Tsholotsho. Despite being simple and rapid, isozyme analysis could not distinguish between genotypes in both crops. Only ACID-P and POD produced visible and well-resolved bands in sorghum and pearl millet respectively. The rest of the enzymes had either no, too low or over activity henece not conclussive.

Microsatellite markers showed that even when local names were similar, the genotypes those names represented were rarely similar genetically. The results of this study indicate that farmer-given names are neither the best guide to the presence of diversity nor a good unit of sampling. This underscores the need for further studies on the best sampling method.

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ABBREVATIONS

ACID-P	acid phosphatase
ADH	alcohol dehydrogenase
DNA	deoxyribonucleic acid
DNTP	deoxynucleotide triphosphate
DR&SS	Department of Research and Specialist Services
EDTA	ethylenediaminetetra-acetic acid
EST	esterases
FAO	Food and Agriculture Organization
GC	guanine and cytosine
GD	genetic distance
GLU	glucosidase
ICRISAT	International Crops Research Institute for Semi-Arid Tropics
IEF	isoelectric focusing
IPGRI	International Plant Genetic Resources Institute
MA	malic acid
MDH	malate dehydrogenase
MTT	tetrazolium thiazolyl blue
NA	napthyl acetate
NAD	nicotinamide adenine dinucleotide
NGO	non-governmental organization
PAA	polyacrylamide
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction

PMS	phenazine methosulfate
PMV	pearl millet variety
POD	peroxidases
PVP	polyvinylpyrrolidone
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
SDS	sodium dodecyl sulphate
SMC	simple matching coefficient
SSLP	simple sequence length polymorphism
SSR	simple sequence repeat
STMS	sequence tagged Microsatellite site
STR	short tandem repeats
TBE	Tris-borate EDTA
TE	Tris-EDTA
TEMED	tetramethylethylenediamine
UPGMA	unweighted pair-group average
VNTR	variable number tandem repeats

INTRODUCTION

Sorghum [*Sorghum bicolor* (L). Moench] and pearl millet (*Pennisetum glaucum* L.) are called *mapfunde* and *mhunga* in Shona, *amabele* and *nyauthi* in Ndebele respectively. They are ranked third and fourth most important cereal crops in Zimbabwe, after maize and wheat (FAO, 1996). Their socioeconomic importance is seen on the joint involvement of the government and non-governmental organisations in breeding and production research. Sorghum and pearl millet are essentially produced for fodder, construction material, brooms, syrup and most importantly grain for flour and brewing traditional opaque beer. The cereals make the staple food for many Zimbabweans, particularly those situated in semi-arid areas. Given enough research, sorghum and pearl millet have the potential of playing a leading role in hunger alleviation programmes in Zimbabwe (Dhlamini, 1996).

Zimbabwe's sorghum and pearl millet yields are relatively low at about 500 kg per hectare (FAO, 1996). This yield is far lower than the global average of 1310 kg/ha (FAO, 2002). Several factors contribute to the low production figures. About 82 per cent of the national produce comes from communal farmers. An estimated 77 per cent of communal areas are located in agro-ecological regions III, IV and V (Manyowa, 1993), which mostly have infertile soils, low and erratic rainfall coupled with high temperatures. Farmers mostly depend on low yielding but well-adapted landraces. It is estimated that each growing area has about ten landraces grown as mixtures, with two to four landraces per individual farmer (van Oosterhout, 1992). Despite their low yields, other factors such as prevalence of indigenous knowledge and perceptions about storability, nutritional value and resistance to biotic and abiotic stress make landraces very suitable for communal agriculture. However, relatively little attention has been accorded to the study and conservation of the diversity of this important biological resource.

Farmers influence the extent and distribution of genetic diversity in crop germplasm either directly, through selection or indirectly through management of agroecosystem components. Thus the genetic diversity in an area represents the outcomes of management processes and indigenous knowledge guiding farmer practices. One might therefore infer that any missing or insufficiently represented diversity constitutes a gap in knowledge that can only be understood after thorough studies. There is a growing realisation that information on genetic diversity is important to farmers, curators, breeders and germplasm conservationists, for example, when making decisions about planning collections, screening duplicate samples and germplasm exchange strategies. However, the major challenges lie in understanding how farmers describe and utilise available agrobiodiversity to overcome adverse agroecologoical factors. Ideally, an easy approach would be to study the processes that are used to maintain germplasm. The method involves collecting the material and identifying key persons or groups who are responsible for maintaining the germplasm. In Zimbabwe, the National Genebank holds bulks of partially or completely uncharacterized sorghum and pearl millet landraces collected from communal areas in the last thirty years (Mafa, 1999). Most of the material came from a joint germplasm collection mission between Department of Research and Specialist Services (DR & SS) in the Zimbabwean Ministry of Lands, Agriculture and Rural Resettlement and the International Plant Genetic Resources Institute (IPGRI).

In Zimbabwean communal agriculture, variety names play a major role in recognizing and distinguishing varieties. No literature is available on describing community based variety recognition and naming. However, it is generally known that indigenous local variety names are based on ethnic grouping, comparative morphology and geographical origin of the material. For example, some variety names refer to origin of material (such as *Malawi* for germplasm from Malawi), while some are more general like sweet Sorghum or *Ipwa* (sorghum mainly for chewing) (Mafa, curator National Genebank, DR & SS). Thus a name can give an indication of traits such as stress and disease resistance, panicle shape, response to bird attack, and use, among others. However, several questions remain unanswered about the identity of the germplasm in the National Genebank. Whether variety names are unique and consistent (within and between villages) needs a lot of investigation. In addition, it is not clear whether farmers recognize a local variety with the same name using the same traits. Studies are also needed to show whether there is more diversity between farmer-named varieties as compared to within varieties. Another key question is whether all varieties contain the same amount of diversity or only a few varieties represent the majority of the diversity within the community. Recently, a project was launched to address some of the concerns above and this study was part of this initiative to assess genetic diversity in some of the germplasm. However, the absence of a universally suitable genetic marker technique for germplasm evaluation has given rise to several alternatives.

Agromorphological traits are the oldest and most widely used in germplasm characterization, even in communal areas. Typically, this approach uses morphological and botanical characters to describe varieties. The major advantage of agromorphological characterisation is that it is relatively easy and cheap even with herbarium and dead specimens. However, it has its own weaknesses. Most plant characters are often subject to environmental variation and thus difficult to measure. Additionally, many polygenic traits are difficult to identify because of complex gene interactions (Ayad, Hodgkin, Jaradat and Rao, 1997).

Another approach to characterization of crop plants is the use of isozyme analysis (Hunter and Market, 1957). Classically, mutations on DNA may result in slightly different amino acids and overall protein charge and sometimes size. This results in proteins that show different mobility when separated by gel electrophoresis in an electric field. Thus, band profiles can effectively show genotype differences. Isozyme analysis is technically simple even with large sample sizes and it is reproducible between laboratories. It is also a codominant marker, meaning it can discriminate between homozygotes and heterozygotes (Simpson and Withers, 1986). For example, with monomeric isozymes homozygous plants will produce one band, whereas heterozygotes produce two. Consequently, the technique can be used in population genetics. Disadvantages of this method are that some enzymes are selectively neutral and thus electrophoresis will show only a portion of the actual variation present in amino acids. There is also a problem of co-migrating proteins and that isozymes are affected by environmental variation.

The introduction of the polymerase chain reaction (PCR) (Saiki, Gelfand, Stoffel, Scharfs, Higuchi, Horn, Mullis and Elrich, 1988) resulted in powerful tools for germplasm evaluation such as random amplified polymorphic DNA (RAPDs) and microsatellites. Microsatellites are genomic regions that comprise of highly polymorphic simple repetitive nucleotide motifs (two to six) that are frequently conserved between related species. Genotype differences are shown by differences in length of the repeats as revealed by PCR. Information transfer is easy with microsatellites because instead of transferring probes, primer sequences can be communicated. Microsatellites are also codominant markers and have been effectively used in studying genetic diversity and redundancy in sorghum (Dean, Dahlberg, Hopkins, Mitchell and Kresovich, 1999). However, their application is limited in less studied crops. For diversity studies in sorghum and pearl millet, a review of literature shows that isozymes and microsatellites are unsurpassed in applicability and on that basis were chosen for this study.

Overall objective

The overall objective was to assess the range of genetic diversity among sorghum and pearl millet landraces collected from communal areas of Zimbabwe.

Specific objective

1. To study genetic diversity of landraces of sorghum and pearl millet using microsatellite and isozyme markers.
2. To investigate the consistency of farmer variety names from a molecular perspective

Hypothesis

1. Landraces of sorghum and pearl millet collected from communal areas of Zimbabwe show no molecular diversity
2. Landraces of sorghum and pearl millet sharing the same local names and collected from the same source show no molecular diversity

LITERATURE REVIEW

2.1 Definitions

Landrace

Landraces are distinctive crop varieties that have adapted to the local conditions by developing a balanced population structure through accumulating genes for resistance/tolerance to both biotic and abiotic production constraints (Frankel and Soule, 1981).

Genetic Diversity

The term genetic diversity has sometimes been interchangeably used with species diversity and genetic resources, hence the need for an objective definition of the terms. Genetic diversity or genetic variability simply refers to variety in the genes in a given species (Horden, Peacock and Williams, 1993). Species diversity refers to the variety of species within a population (Prescott-Allen and Prescott-Allen, 1988). Thus genetic diversity is normally used to cover diversity within species while species diversity for diversity among species.

Genetic Resources

Several related definitions have been proposed for the term genetic resources. According to Horden *et al*. (1993), the genetic resources of a crop consist of the total genetic variations that exist in the crop species. This includes; genes from cultivated, weedy and related species that are sufficiently close to the cultivated forms for gene exchange to be possible by natural, manual or manipulation techniques. Prescott-Allen and Prescott-Allen (1988) defined genetic resources as the potentially useful characteristics of plants that are transmitted genetically. Thus genetic resource is taken as a category within species that has been or is likely to be used in the selection or improvement of domesticates or manipulation of wild stocks. Hawkes (1985) defined genetic resources as the total genetic diversity of any biological entity and in a narrower sense as comprising of the genetic diversity of plants useful to man. That was the definition that was adopted for this study. Genetic resources can be divided into currently grown commercial varieties (cultivars), obsolete commercial varieties, breeding lines and stocks and induced or naturally occurring mutants. Also included are old landraces that are normally populations that have not been scientifically bred or selected, primitive forms of crop plants of great genetic diversity (sometimes not distinguished from landraces). Other components include weedy races of various types and modes of origin related both to the crops and to the wild species from which they are derived or to which they are related. This category also comprises of related wild species including both presumed ancestors of the crops and wild species not so closely related. Genetic resources can be considered to be potential sources of valuable genetic diversity for crop improvement and germplasm conservation.

Molecular markers

A molecular marker is a sequence of DNA or a protein that is readily detectable and whose inheritance and polymorphism can be monitored (Ford-Lloyd and Painting, 1996). Polymorphisms are variations in DNA sequences that can occur on average 300-500 base pairs and can lead to observable phenotypic changes if located on the exon sequences (coding region).

2.2 Crop germplasm in Zimbabwe

2.2.1 Sorghum

Zimbabwe has a very rich source of sorghum germplasm which can be used in the national breeding programmes. Its primary gene pool, both cultivated and wild relatives, has been studied intensively. Sorghum was observed to be one of the cereals with the widest variety of cultivated landraces in Zimbabwe (Rao and Mushonga, 1987). For example, communal areas have on average ten landraces in any growing area, with an individual farmer growing two to four landraces each (van Oosterhout, 1992). Farmers often grow mixtures of landraces, maturing at different times to meet their immediate food requirements (Rao and Mushonga, 1987). However, most of the germplasm has not been very useful in the national breeding programmes because of poor documentation and characterisation data.

Currently, there are about 1378 Zimbabwean sorghum accessions at the International Crops Research Institute for Semi-arid Tropics (ICRISAT) (IBPGR/ICRISAT, 1984). Out of the 1378 lines at ICRISAT only 830 accessions have been characterised and described using morphological and agronomic traits. Rao and Mushonga (1987) presented a detailed catalogue of 285 accessions of the lines collected in the 1982 project. Considerable variation in race distribution and morphological traits such as plant height ranging from 60 to 300 cm, time to maturity ranging from early maturing (50 days to 50 per cent heading) to very late (160 days to 50 per cent heading) was observed during this study. Panicle length varied from small to very large and loose to compact panicles. Grain colour varied from white pearly to dark blue or black with or without subcoat (Rao and Mushonga, 1987). During collection, variation in race distribution was observed across the whole country with the race bicolor (sweet sorghum) being extensively distributed for chewing. Some wild relatives of the cultivated sorghums were found in some areas, for example, *Sorghum halepense* was found in Manicaland and Mashonaland while *Sorghum verticilliflorum* was found in the drier areas of the country (Rao and Mushonga, 1987).

2.2.2 Pearl millet

In Zimbabwe pearl millet is cultivated in the drought prone areas of Natural Region IV and V (Rao and Mushonga, 1987). In 1982, 145 samples were collected from communal areas and subsequently characterised using agromorphological descriptors (Rao and Mushonga, 1987). Considerable variation was observed within single fields for plant height, maturity, spikelet shape, size and presence or absence of bristles. Generally, farmers cultivated tall, late flowering and primitive cultivars with large spikelets. The germplasm collected, showed evidence of local adaptation, disease and pest resistance (Rao and Mushonga, 1987).

2.3 Genetic diversity studies

2.3.1 Sorghum

Until recently, information concerning genetic diversity in sorghum and relationships among intraspecific taxa came primarily from studies of comparative morphology and biogeography (Harlan and de Wet, 1972). However, this approach has its own inherent limitations. Interpretation of variety morphological divergence from its predecessor can pose problems because man has effectively selected crops for agronomically important traits. As a result phylogenetic relationships between crops and their progenitors are often not obvious (Doebley, 1989). This led to the exploration of other methods of assessing genetic diversity, to compliment the morphological markers.

Numerical analysis results of sorghum enzyme diversity have been compared to the scheme based on morphology (Morden, Doebley and Schertz, 1989). No evidence of congruence was found between classical racial classification of Harlan and de Wet (1972) and the distribution of allele frequencies. At most, concordance between the enzymatic differentiation and the racial classification appeared in particular geographical areas. It was also reported that allozyme diversity in the cultivated sorghum was strictly less variable as compared to that of other cereals such as maize and barley (Aldrich, Doebley, Schertz and Stec, 1992). Most of the conclusions from the enzyme studies were also confirmed by investigations with other molecular markers such as restriction fragment length polymorphism (RFLPs) (Tao, Manners, Ludlow and Henzell, 1993) and microsatellites (Brown, Hopkins, Mitchell, Senior, Wang, Duncan, Gonzalez-Candelas and Kresovich, 1996; Taramino, Tarchini, Ferrario, Lee and Pe, 1997). Brown *et al*. (1996) in an exploratory study, surveyed diversity among 13 sorghum lines adapted to temperate conditions and for diverse genotypes using approximately 30 simple sequence repeats (SSRs). Taramino *et al*. (1997) used 13 SSR to reveal moderate to high levels of diversity among a group of sorghum lines of different racial classification and from different geographical origins. Dhlamini (1996) observed great diversity in anthocyanin content of 48 sorghum lines from Zimbabwe's communal areas using RAPDs. However, little work has been done to relate landrace local names and modern techniques of genetic diversity assessment especially with local materials in Zimbabwe.

2.3.2 Pearl millet

Information on pearl millet diversity is available in literature (Tostain, Riandey and Marchais, 1987; Tostain, 1992; Murty, Upadhyay and Manchanda, 1967). Murty *et al.* (1967) studied eight morphological characters and found low but distinct genetic diversity in African and Indian millets. In their study based on both botanical characters and environmentally dependent morphological characters, Upadhyay and Murty (1970) observed major variability in millets from the two regions. Bilquez and Sequier (cited in ICRISAT, 1987), found less distinctness between millets from Niger and Senegal, basing on agromorphological characters. Considering botanical characters only, Porteres (1976) found that African millets clustered into three groups according to geographical origin: West, East and Southern Africa. He also noticed an association between millet morphological groups and the main agrarian civilisations. Clegg, Rawson and Thomas (1984), studied a portion of chloroplast DNA, but found no polymorphism among several wild and cultivated millets. Gepts and Clegg (1989) observed greater diversity in wild than cultivated millets based on ribosomal DNA (genes coding for ribosomal RNA) analyses. Studies by Lagudah and Hanna (1989) showed differences in α-*Esterase* distribution with a high frequency of heterozygotes among the cultivated than wild millets. Gepts and Clegg (1989) split the Sahelian area into Western and Eastern regions basing on alcohol dehydrogenase analysis. Although many techniques have been used for diversity studies in millets, there is need for more research to include new collections. Furthermore, no molecular and isozyme diversity studies have been done with most pearl millet germplasm from Zimbabwe, especially landraces.

2.4 Genetic markers: Desirable properties of an ideal genetic marker

There are many different types of genetic markers that can be used in evaluation of germplasm for scientific research and crop improvement. However, none can suit all crops and all situations. Bretting and Widrlechner (1995) gave a description of the essential features of genetic markers. An ideal genetic marker should be polymorphic, thus it should possess many alleles per locus. The marker has to be highly heritable, that is, phenotypic expression should be relatively unaffected by environmental variability or by the interaction between genotype and environment. Inheritance should be simple, ideally Mendelian genes with codominant alleles detectable in diploid organisms. This allows the discrimination of homozygotes from heterozygotes. Various phenotypes of an ideal marker type should be governed by different loci that are well dispersed throughout the plant genome (Bretting and Widrlechner, 1995). Genetic markers should be fast, easy, inexpensive and reproducible within or between laboratories. Finally, an ideal marker should not differentially affect the plant's fitness and assays should not be lethal to man. Futhermore, the genetic marker should be tightly linked to the character of interest. The next section describes the different types of genetic markers used in diversity studies.

2.5 Morphological markers

This uses morphological and agronomic traits to characterise germplasm. For example, in Zimbabwe during the 1988/89 cropping season 545 sorghum accessions were evaluated using agromorphological descriptors detailed in the revised edition of sorghum descriptors (IBPGR/ICRISAT, 1984). This approach is technically simple and relatively cheap. However, it is time consuming. More often, insufficient characteristic agromorphological traits exist that can be used for effective germplasm evaluations. Furthermore, environmental fluctuations and management practices affect them. There can be an error associated with human judgment in the analysis of diagnostic characters because expression is often confined to a particular stage of development, such as flowering or fruit ripening (Morell *et al*., 1995). Agromorphological markers may be less useful in germplasm of limited diversity among varieties, for example, varieties with highly similar pedigrees. Genetic control of most morphological characters is so complex that using agromorphological markers in hybridisation detection and pedigree determination can be difficult. Despite these setbacks, agromorphological markers are still the most widely used for germplasm evaluations (Bretting and Widrlenchner, 1995).

2.6 Biochemical markers

Scientists have, over the years, developed and used biochemical methods to compliment morphological traits in germplasm evaluations. According to Bretting and Widrlechner (1995), biochemical markers can be grouped into low molecular weight markers (secondary metabolites), protein markers and DNA markers. Low molecular weight markers include anthocyanin and flavonoid pigments, non-protein amino acids, cyanogens, polyacetylenes, alkaloids and peptides. Secondary metabolites are not used for diversity studies because they may not be interpretable by allele/locus models. They are also not selectively neutral. Polymorphisms can be scored directly, or the pigments may be fractionated chromatographically. However, the latter may generate toxic wastes and more expensive than the former. Genetic basis of pigment polymorphism is well studied in only a few crops such as maize and it is still under study in many others (Coe, 1994).

2.6.1 Protein markers

Proteins are complex molecules, which are direct products of mRNA (direct gene products) (Pasteur, Pasteur, Bonhomme, Catalan, and Britton-Davidian, 1988). Mutations on the DNA may result in slightly different protein charges due to amino acid substitutions. This can result in different mobility when separated by gel electrophoresis. In polyacrylamide gel electrophoresis, separation of charged molecules such as proteins results from differences in comparison to charge and size. Highly charged proteins move rapidly in an electric field and the gel to a greater extent retards larger and less spherical proteins. These two effects may work together or in opposition. These mobilities have been used for diversity studies since they can directly reveal genetic polymorphism through demonstrating multiple forms of a specific enzyme (Wendel and Weeden, 1987). Basically, there are three different types of protein markers: seed proteins, isozymes and allozymes.

Seed storage proteins occur in sufficient quantities for analysis in the seed, which represents a well-defined stage in the plant's development and have been favourites for assessing diversity studies (Wendel and Weeden, 1987). However, there is a problem of non-homologous proteins co-migrating. Furthermore, there is usually high intraspecific variation among closely related species and this might need to be established before interspecific variation comparisons can be made. Band complexity is also a problem especially with greater than 20 bands (Ford-Lloyd and Painting, 1996).

Allozymes are different forms of an enzyme sharing the same catalytic activity but coded by different alleles on the same locus (Pasteur *et al*., 1988). Different forms of an enzyme sharing the same catalytic activity but coded by more than one gene locus are called isozymes (Ford-Lloyd and Painting, 1996). However, the term 'isozyme' is generally used to mean both classes. Briefly, the isozyme analysis procedure involves maceration of plant tissue in a specific buffer and separating the tissue extract using starch or polyacrylamide gel electrophoresis. Isozymes are then located by histochemical staining, that is, incubating the gel with an enzyme specific substrate (Pasteur *et al*., 1988). The substrate gives a coloured product when metabolised thereby revealing location of the isozyme on the gel. The rationale behind isozyme analysis is that each band in the profile represents a direct gene product and thus band profiles are a measure of genetic similarities/ differences among plant genotypes under study.

Isozymes are codominant markers thus they can discriminate between homozygotes and heterozygotes (Simpson and Withers, 1986). For example, with monomeric isozymes (that is, those consisting of a single polypeptide), homozygous plants for that locus will produce one band whereas the heterozygotes will produce two. For dimeric isozymes (that is, those consisting of two polypeptides) homozygous plants for that locus will produce one band whereas heterozygotes will produce three. Isozyme markers are robust and reproducible within and between laboratories. Assays can be from a variety of plant materials and analytical procedures are generally not complicated. Isozymes have been therefore a choice for germplasm evaluations (Crawford, 1990). However, some enzymes are selectively neutral and thus electrophoresis will detect only a portion of the actual variation present in amino acids (Koehn and Hilbish, 1987; Hillis, Moritz and Mable, 1996). Another limitation is the number of enzyme systems available as compared to the total variation in plants, that is, limited to coding regions of the genome.

2.6.2 DNA based markers

DNA based genetic markers are increasingly being utilised in cultivar development, quality control of seed production, measurement of genetic diversity for conservation management, varietal identification and intellectual property protection (IPP) (Angela and Edwards, 1995). Basically, DNA-based markers can be categorised into target-PCR and sequencing, hybridisation and amplification based technologies (Hillis *et al.*, 1996; Angela and Edwards, 1995). Target-PCR and sequencing involve designing primers to target specific regions of the genome. The target-amplified products can then be compared on agarose gel to the corresponding product from other individuals. Despite the accuracy and informativeness of these techniques, they are relatively expensive and difficult to do thus require a lot of experience (Weising, Nybom, Wolff and Meyer, 1995; Hillis *et al.*, 1996; Angela and Edwards, 1995). Hybridisation and amplification techniques have been used in the determination of genetic relationships between plant species and populations (Bretting and Widrlechner, 1995; Nienhuis, Tivang, Skroch and dos Santos, 1994).

2.6.2.1 Restriction fragment length polymorphism technology

Restriction fragment length polymorphism (RFLP) analysis was the first technology developed which enabled the detection of polymorphisms at DNA sequence level (Southern, 1975). The approach involves digesting genomic DNA with restriction enzymes, separating the resultant DNA fragments by gel electrophoresis, blotting the fragments on nylon membrane and hybridising with a labeled probe for visualization of the genomic fingerprint. A probe is a short sequence of oligonucleotides which share homology and is thus able to hybridise with corresponding sequences in the genome DNA. Polymorphisms in plants result from a number of processes such as base sequence changes which add or eliminate restriction sites and rearrangements such as insertions and deletions within restriction enzyme recognition sequences. Unequal crossing over or replication slippage may create variation in a number of tandem DNA repeats at minisatellites or microsatellite loci detectable by RFLP analysis (Schlotterer and Tautz, 1992). It is most suited to studies at the intraspecific level or among closely related taxa. Presence and absence of fragments resulting from changes in recognition sites are used for identifying species or populations. Restriction fragment length polymorphism analysis is a codominant marker thus enabling identification of heterozygotes from homozygotes. It is also reproducible within and between laboratories. This technology was used to study genetic diversity in sorghum (Deu, Gonzalez-de-Leon, Glaszmann, Degremont, Chantereau, Lanaud, and Hamon, 1994; Oh, Fredericksen and Magill, 1994) and also in tagging resistance genes for diseases such as downy mildew in lettuce (Landry, Kesseli, Leung and Michelmore, 1987). The RFLP technique is however labour intensive, tedious and requires large quantities of high quality DNA that is often difficult to get (Schlotterer and Tautz, 1992).

2.6.2.2 Random Amplified Polymorphic DNA (RAPD)

The Random amplified polymorphic DNA (RAPD) is a PCR-based technique where there is amplification of multiple segments of the genome using random short DNA sequences. The length of the primers is usually between eight to twenty base pairs with guanine to cytosine (GC) content greater than 50 per cent. In order to obtain amplification products there should be two identical or similar target sequences in close proximity to each other in the genome (that is, an amplifiable distance of 200-2000 base pairs) (Newberry and Ford-Lloyd, 1993). Whilst the investigator knows the primer sequence, they have no idea as to which, if any, gene or repeated sequence in the plant genome the primer is homologous (Newberry and Lloyd, 1993). Polymorphism is observed and scored by the presence and absence of a fragment and relates to sequence variation due to nucleotide insertion, deletion or substitution at annealing sites of primers. The RAPD technique has been shown to have a high power of resolution, especially in separating species complexes and sibling species. It has also been employed in fingerprinting (Milbourne, Meyer, Bradshaw, Baird, Bonar, Provan, Powell, and Waugh, 1997), genetic diversity and generation of linkage maps (Tao *et al*., 1993; Tingey and del Tufo, 1993). The technique is quick, easy and does not require prior sequence information. However, the homozygous presence of a fragment is not distinguishable from its heterozygote, and as such RAPDs are dominant markers (Welsh and McClelland, 1990). This means that a particular fragment can either be present (allele A) or absent (allele a) and hence the homozygotes (AA) cannot be distinguished from heterozygotes (Aa). This limits the use of RAPDs in population genetics. There is also the incidence of non-inherited bands that are probably PCR artifacts (Welsh and McClelland, 1990).

2.6.2.3 Microsatellites

Microsatellites are genomic regions which are comprised of highly polymorphic regions of DNA containing simple repetitive motifs of two to six nucleotides repeated up to a usual maximum of 60 (Golgstein and Pollock, 1997). These regions have also been termed simple sequence repeats (SSR) (Jacob, Lindpainter, Lincoln, Kusumi, Bunker, Mao, Ganten, Dzau and Lander, 1991), or short tandem repeats (STR) (Edwards, Cevitello, Hammond and Caskey, 1991), or sequence tagged microsatellite site (STMS) or simple sequence length polymorphism (SSLP) (Cregan, 1992). The DNA flanking repeat sequences is highly conserved hence primers can be constructed that are complimentary to the short unique sequences. Repeated regions have been found throughout genomes of many eukaryotes (Tautz and Renz, 1984; Wang, Weber, Zhon and Tanksley, 1994) and constitute an abundant source of DNA markers. Variation in tandem repeat length accumulates in the population more rapidly than point mutations and large insertions or deletion events responsible for most RFLPs and RAPDs.

Microsatellites are useful for a number of analyses. They were originally utilised for genetic mapping (Weissenbach, Gyapay, Dib, Vignal, Morissette, Millasseau, Vaysseix and Lathrop, 1992) and have been extensively used for linkage analyses in the association with disease susceptibility genes. In addition they have proved to be useful in the analysis of paternity and kinship and in the probability of sample identity at both the individual (Edwards, Hammond, Jin, Caskey, Chakraborty, 1992) and population levels. Microsatellite variation has been used to study the amount of hybridisation between closely related species in grape vine (Thomas and Schott, 1993). Comparison of levels of variation between species and populations has also proved useful in the assessment of overall genetic variation in soya beans (Akkaya, Bhagwat, and Cregan, 1992). They can be used to estimate effective population size (Senior and Heum, 1993) and to gain insight into the degree of population substructure including the amount of migration between subpopulations (Tao *et al*., 1997).

Mechanisms responsible for microsatellite hypervariability

Molecular basis of microsatellite variability is a subject of intense investigation and debate. However, experimental approaches have been established to determine mechanisms by which repeat units are added and/or subtracted from SSR loci. Several lines of arguments have been proposed such as replication slippage, transposition, recombinational events and /or unequal exchange between sister chromatids at meiosis or mitosis or between homologous chromosomes at meiosis, and the gene conversion hypotheses (Jarman and Wells, 1989; Jeffreys, Moncklon, Tamaki, Neil, Armour MaCleod, Collick, Allen and Jobling, 1993; Richards and Sutherland, 1992; Wolff, Nakamura, Odelberg, Shiang and White, 1991). Other mechanisms still under study include the case of 'explosive' amplification of trinucleotides based on microsatellites associated with human genetic diseases (Caskey, Pizzuti, Fu, Fenwick and Nelson, 1992; Richards and Sutherlands, 1992).

The slippage hypothesis implicates slipped-strand mispairing of newly replicated strands during the replication process (Kevinson and Gutman, 1987a,b). Strand, Prolla, Liskay and Petes (1993), in their study of strains of yeast carrying SSR loci cloned in plasmids, showed that mutations affecting mismatch repair caused 100 to 700-fold increase in mutations of SSR loci. They also observed that mutations in the DNA polymerase proofreading did not affect the stability of the loci. *In vitro* experiments by Schloretterer and Tautz (1992), confirmed that replication slippage may actually result in considerable amplification of a given simple sequence repeat.

Several studies have supported the recombination hypothesis: (1) a variety of minisatellite core sequences share analogue to the bacterial recombination signal 'chi' (Jeffreys, Wilson and Thein, 1985a,b), (2) minisatellite like sequences have been found at sites of meiotic crossing over (Chadley and Mitchell, 1988), and that both mini and microsatellites behave as recombinational spots in transfected mammalian cells. However, Strand *et al*. (1993) observed that the frequency of SSR mutations was similar for both meiosis and mitosis and implied that recombination was not an important factor. They also suggested that mutations at SSR loci were due to strand slippage during replication and failure to repair mismatches, rather than unequal crossing over. Henderson and Petes (1992) documented similar results on their studies with *E. coli.* No exchange of flanking markers was observed in newly created alleles in the study of variable human minisatellites (Wolff, Nakamura and White, 1988; Wolff, Plactke, Jeffreys and White, 1989; Wolff *et al*., 1991). This also ruled out unequal exchange between homologous chromosomes as a mutational mechanism. Nevertheless, the recombinational processes and replication slippage taken together may certainly contribute to the creation of microsatellites (Weising *et al*., 1995).

Merits and limitations of Microsatellites

Microsatellite analysis provides more information more easily than other DNA based markers such as RAPDs and RFLPs (Akkaya *et al*., 1992). Valuable attributes include high informativeness because of codominance. Technically, the technique is simple and the marker can rapidly be regenerated without the use of radioactivity. Only a small quantity of DNA is required since the technique is very sensitive. Data can be scored easily and unambiguously. Polymorphism is high and they are reproducible between and within laboratories (Tautz and Renz, 1984). Loci are frequently conserved between related species and sometimes genera, thus the markers are broadly applicable. Information transfer is easy with SSRs because there is no need of physically transferring the probes between laboratories rather only simple sequences of primer pairs can just be communicated. Finally, microsatellites are very flexible since they can be used as sequence-tagged-sites to provide anchors between genetic linkage maps and physical chromosome locations (Tautz and Renz, 1984). Significant limitation of microsatellite analysis may be the initial investment and the technical expertise required to clone and sequence the loci (Wolff *et al*., 1991). Sequence information for DNA flanking the repeated segment is required and some of it is available in databases for well-studied crop species. Otherwise it is necessary to produce genomic libraries enriched in microsatellites, select potentially useful clones and sequence the DNA so as to design suitable primers. All this requires considerable amount of time and other resources. The widespread application of this technology has been greatly hampered by the relatively few sequences known. Nevertheless, this technique is a particular favourite in population genetics and this growing interest is likely to result in a large increase in the number of microsatellites known in major crops.

2.7 Similarity measures

Relationships between varieties, landraces and populations can be expressed in terms of similarity coefficients. The similarity coefficient is a quantification of elements in the columns of a data matrix representing the character states of two individuals under study. Basically, the estimation of similarity coefficients involves scoring gel profiles, calculating genetic distance values and phenetic analysis. Gel profile photographs are scored into ones and zeros (for example 10111101) representing band presence (1) and absence (0) respectively. This matrix is called a vector. Thus for binary data when comparing two individuals i and j there are four possible outcomes (Table 1).

Table 1. Possible outcomes of comparing genotypes i and j using gel profiles

		Genotype j	
		1	0
Genotype i	1	1,1 (a)	0,1 (b)
	0	1,0 (c)	0,0 (d)

By comparing the outcomes, various possible formulae for calculating similarity coefficients have been proposed (Everitt, 1993). The most common are:

Nei and Li (1979) which is defined as the number of bands present in both genotypes divided by the number of bands present in the two genotypes

$$NL = 2ab / (a + b)$$

Where 2ab is the number of shared bands between i and j, a and b are the number of bands observed in individuals i and j respectively (Table 1), Jaccard's coefficient (J) (Gower, 1985; Dudley, 1994)

$$J = a / (a + b + c)$$

Where in all cases a, b and c represent the bands observed in individuals i and j from Table 1.

Simple matching coefficient (SMC) is defined as the number of bands present in both genotypes and the number of bands absent in both genotypes divided by the total number of polymorphic bands in the study

$$SMC = a + d / a + b + c + d$$

Where in all cases a, b, c, d represent the bands observed in individuals i and j from Table 1.

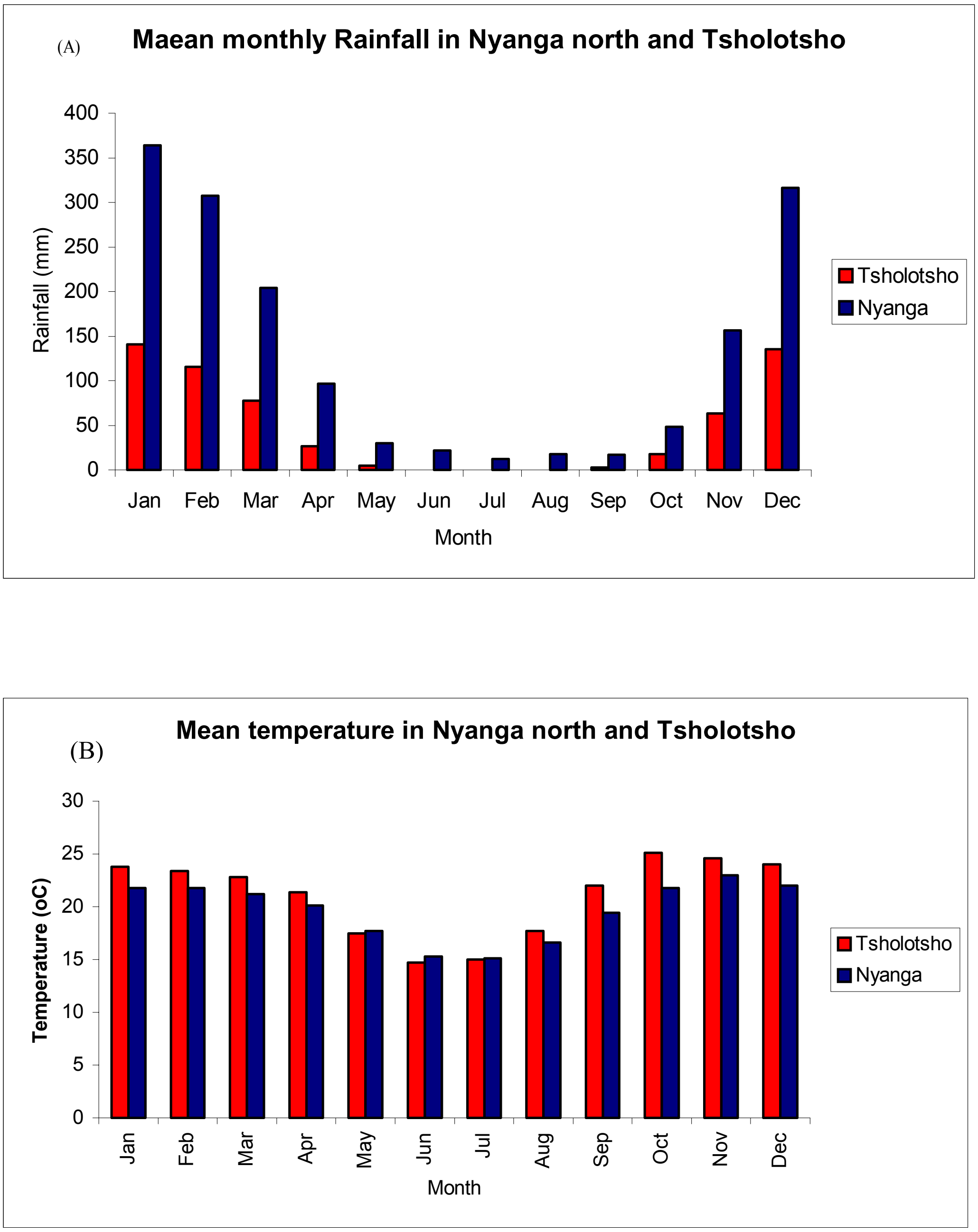
The upper limit of the similarity measurements is one and the lower limit is zero (Everitt, 1993). In such cases where zero and one band similarity measure there is always dissimilarity, which is the genetic distance (GD_{ij}). Just like similarity (S_{ij}), dissimilarity is symmetric ($S_{ij} = S_{ji}$) and non-negative. The degree of similarity increases with increase with S_{ij} and decreases with increasing GD_{ij}. Since by nature an organism has maximal similarity to itself, thus $S_{ii} = 1$. A similarity value of one means no genetic difference, while zero means completely different genotypes respectively.

There is, however, some ambiguity in the definition of shared bands as some workers exclude shared absence of a band (0,0 comparisons), while others count it as equivalent to shared presence (1,1 comparison). Whether one includes negative matches depends with the data being used. For binary data (dichotomous) with presence and absence being used, considering two individuals very similar simply because they lack a large number of qualities may be unreasonable. Thus, this may cause one to disregard the SMC as a valuable measure of similarity compared to the other two above. However, many genetic studies using the three similarity measures have found no major differences in the values (Stiles, Lemme, Sondur, Morshidi and Manshardt 1993; Virk, Ford-Lloyd, Jackson and Newbury, 1995).

MATERIALS AND METHODS

3.1 Germplasm

The study was carried using 52 landraces of sorghum and 47 landraces of pearl millet (Appendix 3 and 4 respectively). Briefly, the National Genebank collected germplasm from Tsholotsho and Nyanga North districts (27°45'E,19°50'S) and Nyanga North (32°45'E,18°15'S) in the 1998-1999 season. Site selection was based on possible collaboration among partners, presence of non-governmental organisations (NGO) already working with the communities in related projects and semi-arid conditions (Natural regions IV and V) (Table 1 and figure 1). Germplasm collection strategy was to have at least 30 samples randomly drawn from a population to try and capture as much variation as possible (Mafa, 1999). Collections were made from three districts, three villages per district, and three farmers per village. In this case a district was defined as an area with defined geographical composition and farmers have a certain way of naming and conserving the genetic diversity. A village was defined as a group of about 50 households under one social administrative authority and has a common obligation to conserve and manage genetic resources of sorghum and pearl millet. A sample was a single plant.


(A) Maean monthly Rainfall in Nyanga north and Tsholotsho

(B) Mean temperature in Nyanga north and Tsholotsho

Figure 1. Comparison of mean monthly rainfall (Fig 1a) and mean temparture (Fig 1b) in Nyanga North and Tsholotsho.

Figure 2. Map showing the location of the two study sites Nyanga North and Tsholotsho, each represented in the study by three villages.

3.2 DNA Extraction

Preparation of DNA was done at the Crop Science Department, University of Zimbabwe. For each landrace collection, six seeds were germinated in a greenhouse and seedlings allowed to grow for two weeks. For both crops DNA extraction was as described by Picknett, Sanders, Ford and Holt (1987). All centrifugation was done in an Eppendorf Centrifuge 5415C unless stated otherwise. Leaf material weighing about 0.5 grams was ground in liquid nitrogen in a mortar. Sodium dodecyl sulphate (SDS) (500 µl) extraction buffer [1 M Tris-HCl pH 8.0, 0.5 M EDTA (pH 8.0), 3 M NaCl, PVP-40, 10 per cent SDS] was added to the powder and incubated for 10 minutes at 65^{o}C. Thereafter, 160 µl of Potassium acetate was added and mixed by inverting several times. An equal volume of chloroform was added, followed by centrifugation for 5 minutes at 10 000 revolutions per minute (rpm). The aqueous phase was collected and the extraction procedure repeated once starting from the chloroform stage. An equal volume of isopropanol was added to the supernatant and mixed gently by inverting several times followed by centrifugation at 10 000 rpm for 5 minutes. The resulting DNA pellet was blotted using 3MM Whatman filter for 15 minutes to dry. Dried pellets were dissolved in 400 µl TE-RNAase (10 µg/ml) and incubated for 30 minutes at room temperature. Proteinase K (0.1 mg/ml) was added and samples incubated for 30 minutes at room temperature. The tube was inverted for 5 minutes after adding an equal volume of phenol followed by centrifugation for 5 minutes at 10 000 rpm. An equal volume of phenol: chloroform (50:50) mixture was added to the supernatant and mixed by inverting the tube for 5 minutes. After centrifugation for 5 minutes at 10 000 rpm, the supernatant was collected and an equal volume of chloroform added and mixed gently. Centrifugation was done for 5 minutes at 10 000 rpm. To the supernatant, a 1/10 volume of 3 M Sodium acetate (pH 5.2) and 2.5x volume chilled absolute ethanol were added and mixed by inverting the tubes several times. The DNA was incubated at -20°C for 12 hours followed by centrifugation at 10 000 rpm for 10 minutes. DNA was washed in 70 per cent ethanol and re-suspended in 150 µl sterile water.

DNA was quantified using an Ultraviolet Spectrophotometer (Shimadzu Biospec-1601) in 1-centimeter quartz glass cuvettes. One millilitre of Tris-EDTA [TE: 10 mM Tris.HCI pH 7.4, 1 mM EDTA pH 8.0] was used to calibrate the spectrophotometer at 260 nm absorbance. Five microlitres of sample DNA were mixed with 995 µl Tris-EDTA and absorbance at 260 nm wavelength was recorded. DNA concentration (µg/µl) was calculated as the product of the absorbance at 260 nm wavelength (A_{260}), the dilution factor (200) and a constant (47.5). For PCR, the DNA was then diluted using filter sterilized distilled water to the required concentrations.

3.3 Microsatellite analysis

3.3.1 Sorghum amplification protocol

Primer sets (Table 2) were provided by Texas A&M University, Texas, USA. The PCR reactions for both primer screening and final data collection experiments were performed in 25.0 µl-volumes (Mr Charles Mutengwa, Crop Science Department, University of Zimbabwe) containing 30 ng template DNA, 1x PCR Buffer (50 mM KCl, 10 mM Tris-HCl, pH (8.3), 1.5 mM $MgCl_2$), 0.25mM deoxynucleotide triphosphates, 4.13 to 5.68 pmoles (30 ng) of each primer and 1.2 units *Taq polymerase* (Table 5 below shows the volumes in µl of the PCR reagents). Temperature cycling was performed on a GeneAmp PCR system 9700 (Perkin-Elmer) using 0.5 seconds ramp times. The amplification profile consisted of initial denaturation of template DNA at 94°C for 4 minutes, followed by 35 cycles of 94°C for 60 seconds, 60 seconds at annealing temperature (Table 2), and 60 seconds elongation at 72°C. The final cycle was identical to the above but had a final elongation of 10 minute at 72°C. Primer screening, electrophoresis and detection procedures were as described in section 3.3.3 of this thesis.

Table 2. Characteristics of *Sorghum bicolor* microsatellite primers

Marker	Linkage group	Primer sequence	Annealing Temperature
Xtxp8	B	F: ATA TGG AAG GAA GAA GCC GG R: AAC ACA ACA TGC ACG CAG G	60
Xtxp9	Unknown	F: AAT AGC ACC GCC GCG CG R: CAT TGT GGA GTC CCT GAT AC	55
Xtxp10	F	F: ATA CTA TCA AGA GGG GAG C R: AGT ACT AGC CAC ACG TCA C	50
Xtxp16	Unknown	F: TAG GGA AGA GCA AGT GCA GAC R: AAG AAA GGG CCC AGA GTT TC	60
Xtxp17	I	F: CGG ACC AAC GAC GAT TAT C R: ACT CGT CTC ACT GCA ATA CTG	55
Xtxp19	B	F: CTT TCA ATC GGT TCC AGA C R: CTT CCA CCT CCG TAC TC	55
Xtxp21	D	F: GAG CTG CCA TAG ATT TGG TCG R: ACC TCG TCC CAC CTT TGT TG	60

Table 3. Volumes of PCR Reagents (in assuming 10 per cent pipetting loss) for sorghum microsatellite reactions

Reaction Number	Water	10X Buffer	dNTPs	Primer (Forward)	Primer (Reverse)	*Taq Polymerase*	Template DNA
8	108.33	22.0	11.0	5.28	5.28	2.11	66.0
12	162.49	33.0	16.5	7.92	7.92	3.17	99.0
24	324.98	66.0	33.0	15.84	15.84	6.34	198.0
48	649.97	132.0	66.0	31.68	31.68	12.67	396.0

3.3.2 Pearl millet amplification protocol

The PCR reaction mixtures were prepared as described by John Innes Centre (John Innes Centre, personal communication). All PCR reactions were performed in 20.0 µl volumes containing 50 ng pearl millet genomic DNA, 30 ng of each primer (Table 4), 10x Roche Diagnostic PCR buffer [100 mM Tris-HCI pH 8.3, 15 mM $MgCl_2$, 500 mM KCl], 0.2 mM dNTPs, 0.5 units Roche Diagnostic *Taq polymerase*. Temperature cycling was done on GeneAmp PCR system 9700 (Perkin Elmer) using 0.5 seconds ramp times. The amplification profile consisted of initial denaturation of the template DNA at 94°C for 3 minutes, followed by 35 cycles of 94^oC for 60 seconds, annealing temperature (Table 4) for 60 seconds and 72°C for 60 seconds. The final PCR cycle was identical to the above cycles except that the extension time at 72°C was increased to 4 minutes. Soaking was at 4°C.

Table 4. Sizes, linkage groups and annealing temperatures of pearl millet microsatellite primers

Marker	Size (based on 80 base pair genotype)	Linkage Group	Annealing Temperature
PSMP2001	304	5	Ramp 61
PSMP2006	256	3	Ramp 52
PSMP2008	238	4	Ramp 61
PSMP2013	153	7	Ramp 61
PSMP2018	203	6	Ramp 61
PSMP2019	260	7	Ramp 61
PSMP2056	213	3	Ramp 61
PSMP2059	119	2	Ramp 61
PSMP2066	267	2	Ramp 61
PSMP2069	225	1	Ramp 61

3.3.3 PCR Components

Eight genotypes representing the sampled geographical areas were randomly selected from the 47 genotypes to be evaluated. They were used to screen the ten primers (Table 4) for polymorphism as described by Dhlamini (1996). A cocktail (125.84 µl) was prepared for 8 reactions (Table 5) excluding template DNA. From the cocktail, 15.0 µl were pipetted into 200 µl thin-walled PCR tubes containing 5.0 µl of pearl millet genomic DNA. The tubes were inverted several times, and followed by a quick spin in a centrifuge (Eppendorf Centrifuge 5415C) for 60 seconds at 10 000 rpm. The PCR amplification products were separated on 8 per cent non-denaturing polyacrylamide gels and stained with ethidium bromide and viewed over UV light (see section 3.3.4 below). Gel profiles were used to select the polymorphic primers to be used in the final evaluation with all 47 pearl millet genotypes.

Table 5. Volumes of PCR Reagents (in µl assuming 10 per cent pipetting loss) for pearl millet microsatellite reactions

Reaction Number	Water	10X Buffer	dNTPs	Primer (Forward)	Primer (Reverse)	*Taq Polymerase*	Template DNA
8	89.76	17.6	7.04	5.28	5.28	0.88	44.0
12	134.64	26.4	10.56	7.92	7.92	1.32	66.0
24	269.28	52.8	21.12	15.84	15.84	2.64	132.0
48	538.56	105.6	42.24	31.68	31.68	5.28	264.0

3.3.4 Electrophoresis and detection

The PCR products were mixed with 5.0 µl of 6x loading buffer III [0.25 per cent Bromophenol blue, 0.25 per cent xylene cyanol FF, 30 per cent glycerol in water]. Samples containing 12.0 µl of PCR products were loaded on 8 per cent (w/v) non-denaturating polyacylamide [acrylamide: bisacrylamide (29:1), 1x TBE buffer (89 mM Tris, 89 mM borate, 2 mM EDTA pH 8.0), 10 per cent (w/v) Ammonium persulfate, N, N, N', N'-tetramethylethylenediamine (TEMED)]. The

DNA samples were electrophoresed in 1x TBE Buffer at 80 volts for 3 hours on a Mini Protean II Cell Vertical Electrophoresis System (BioRad). The gels were stained with ethidium bromide (1 µg/ml) and photographed on ultraviolet light translluminator with a Polaroid type 667 film. Band size was estimated with a 100 base pair ladder (Roche Diagnostics marker XIV).

3.4 Isozyme analysis

3.4.1 Sample preparation

Sample preparation for both crops was as described by Tostain *et al*. (1987). Seeds were disinfected with 5per cent Sodium hypochlorite, wrapped in filter paper towel. They were kept in the dark at room temperature until seven days after emergence. Thereafter, seedlings were immersed in water for 24 hours before grinding to enhance expression of anaerobic alcohol dehydrogenase (ADH). Coleoptiles, approximately 12 mm long were ground in liquid nitrogen in a mortar. The ground tissue was then transferred to 1.5 ml microcentrifuge tubes containing 300 µl of homogenising buffer solution [16.7 per cent (w/v) Sucrose and 8.3 per cent Sodium ascorbate pH 7.38]. This was followed by centrifugation (Eppendorf Centrifuge 5415C) for 10 minutes at 14 000 rpm.

3.4.2 Gel preparation and Electrophoresis

A modified method of Westermeier (1993) was used. Polyacrylamide gel (PAA) containing 5.3 ml of acrylamide stock, 13.4 ml water, 1.5 ml pharmalyte, 140 µl ammonium persulfate and 20 µl TEMED were prepared on Gel Bond® sheets. A 0.4 mm gel spacer was used. The PAA gel was placed on an isoelectric focus unit (IEF) (Pharmacia Biotechnology Multiphor 11) fixed with mineral oil. Pre-focusing of the gel was done at 600 V, 20 mA, 10 watts for 10 minutes at 4°C, and this was followed by addition of sample applicators to either positive or negative sides of the gel. Eighteen microlitres of the sample homogenate was added to applicators and the gel was pre-run for 20 minutes followed by removal of applicators and further electrophoresis for 45 minutes.

3.4.3 Staining

Staining reactions for alcohol dehydrogenase (ADH), acid phosphotase (ACID-P), peroxidases (POD), esterases (EST), β-glucosidase (GLU) and malate dehydrogenase (MDH) were prepared as described by Morden *et al*. (1989), and these are described below.

3.4.3.1 Alcohol dehydrogenase (ADH)

A solution containing 1ml 95 per cent ethanol, 20 mg nicotinamide adenine dinucleotide (NAD) (1 ml), 20 mg tetrazolium thiazolyl blue (MTT) (2 ml), 5 mg phenazine methosulfate (PMS) (1 ml) was mixed with 50ml 0.05 M Tris.HCI buffer pH (8.0) on an automatic stirrer. The mixture was poured onto the gel slice and incubated for 30 minutes at 37°C. After rinsing the gel in water, it was fixed in glacial acetic acid: methanol: water (1:5:5) (Fix 1).

3.4.3.2 α-Esterase (EST)

A solution of 2.5 ml N-propanol, 20 mg b-Napthyl acetate (1ml b-NA) and 25 mg Fast garnet GBC Salt (0.5 ml GBC) was mixed with 50 ml phosphate assay buffer pH (6.0). The mixture was poured onto the gel slice. After 5 minutes, 30 mg b-Napthyl acetate (1.5 ml b-NA) was added followed by incubation for 45 minutes at 37°C. The gel was fixed for 10 minutes in Fix 1 as described in 3.4.3.1.

3.4.3.3 Peroxidase (POD)

Solution one consisting of 97 ml sodium acetate buffer (pH 5.0) and 2 ml 0.1 M calcium chloride was prepared 5 minutes before staining. Using a separate beaker, 65 mg of 3-amino-9-ethylcarbazone was dissolved in 5 ml dimethylformamide to make solution two. Equal proportions of solutions one and two were mixed and 60 ml of water added, followed by stirring. The mixture was poured onto the gel until bands formed and the gel fixed in 50 per cent glycerol.

3.4.3.4 Malate Dehydrogenase (MDH)

A solution containing 20 mg NAD (1 ml), 10 mg NBT (1ml), neutral 100 mg D, L-malic acid (2 ml MA) and 1.25 mg PMS (0.25 ml) was mixed with 50 ml 0.1 M Tris.HCI pH 9.1 and, poured onto the gel slice. The gel was incubated for 60 minutes and fixed in Fix 1 as described in 3.4.3.1.

3.4.3.5 Acid phosphotase (ACID-P)

A solution containing 100 ml sodium acetate buffer, 100 mg Fast blue BB salt, 250 mg PVP-40, 1 g Sodium chloride, 10 drops of 10 per cent $MgCl_2$, 100 mg α-naphthyl-acid phosphate was poured on the gel slice. The gel was incubated for 60 minutes and fixed in a solution of 10 per cent glycerol in 8 per cent acetic acid.

3.4.3.6 β-Glucosidase

Solution 1 containing 50 ml 0.05 M Potassium phosphate buffer (pH 6.5), 1.0 g PVP-40 and 100 mg Fast blue BB salt (1 ml BB) was prepared just before staining. Fifty milligrams of 6-bromo-2-naphthyl-β-D-glucoside in N, N-dimethyl formamide to make solution 2. The two solutions were mixed and poured onto gel slice followed by incubation for 60 minutes. The gel was allowed to stand overnight and fixed in water.

3.5 Data collection and statistical analysis

Gels were scored manually with each polymorphic band being treated as a unit character and scoring was for the presence (1) or absence (0) of a band. Two criteria were used for scoring bands: the band being scored had to stain strongly and there had to be an unambiguous difference between the allelic states of the band being scored (i.e. presence or absence of a band). Each band was named by assigning a number that included its molecular weight in front of the primer name for microsatellites (Dhlamini, 1996). The degree of similarity between collections was calculated using simple matching coefficients. Similarities were graphically expressed using the unweighted pair-group method of arithmetic averages (UPGMA). Dendrograms were constructed using GENESTAT version 5 (1987).

RESULTS

4.1 Relationships among Sorghum landraces

4.1.1 Microsatellite analysis

Out of the seven-microsatellite primers tested, 20 polymorphic bands were scored. The number of bands and their corresponding sizes ranged from one to five and 140 to 280 base pairs respectively (Table 6). Figure 1 shows a typical banding profile of primer *Xtxp9.* A dendrogram of the 52 sorghum genotypes (figure 2 and see Appendix 5 for the similarity matrix) showed that the most dissimilar sorghum genotypes were 1482 and 1482 which were both locally called *Sorghum* and were from Renzva village in Nyanga North. They joined the rest of the genotypes at a similarity level of 52 per cent. This genotype was repeated to test the accuracy of the clustering method. At 62 per cent similarity level all, the 52 genotypes grouped into seven aggregate clusters designated A to G (figure 2). Seven of the 15 accessions fom Tsholotsho were identical to accessions from Nyanga North for all markers used. These are explained in the next sections.

Table 6. Identification and range of band sizes for microsatellite primers used in the analysis sorghum

Marker	Primer sequence	Band size range
Xtxp8	F: ATA TGG AAG GAA GAA GCC GG R: AAC ACA ACA TGC ACG CAG G	140 – 200
Xtxp9	F: AAT AGC ACC GCC GCG CG R: CAT TGT GGA GTC CCT GAT AC	150-160
Xtxp10	F: ATA CTA TCA AGA GGG GAG C R: AGT ACT AGC CAC ACG TCA C	150
Xtxp16	F: TAG GGA AGA GCA AGT GCA GAC R: AAG AAA GGG CCC AGA GTT TC	140 – 200
Xtxp17	F: CGG ACC AAC GAC GAT TAT C R: ACT CGT CTC ACT GCA ATA CTG	165 – 225
Xtxp19	F: CTT TCA ATC GGT TCC AGA C R: CTT CCA CCT CCG TAC TC	200 – 280
Xtxp21	F: GAG CTG CCA TAG ATT TGG TCG R: ACC TCG TCC CAC CTT TGT TG	160 – 180

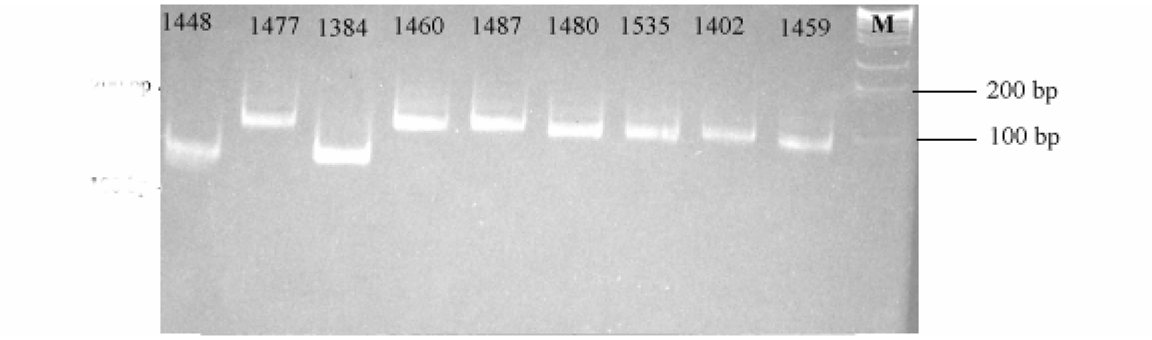


Figure 3. Banding patterns of microsatellite primer *Xtxp9* on sorghum genotypes 1448, 1477,1384, 1460, 1487, 1480, 1535, 1402 and 1459, separated on 8 per cent PAGE and stained with ethidium bromide. Lane M is the 100 base pair ladder

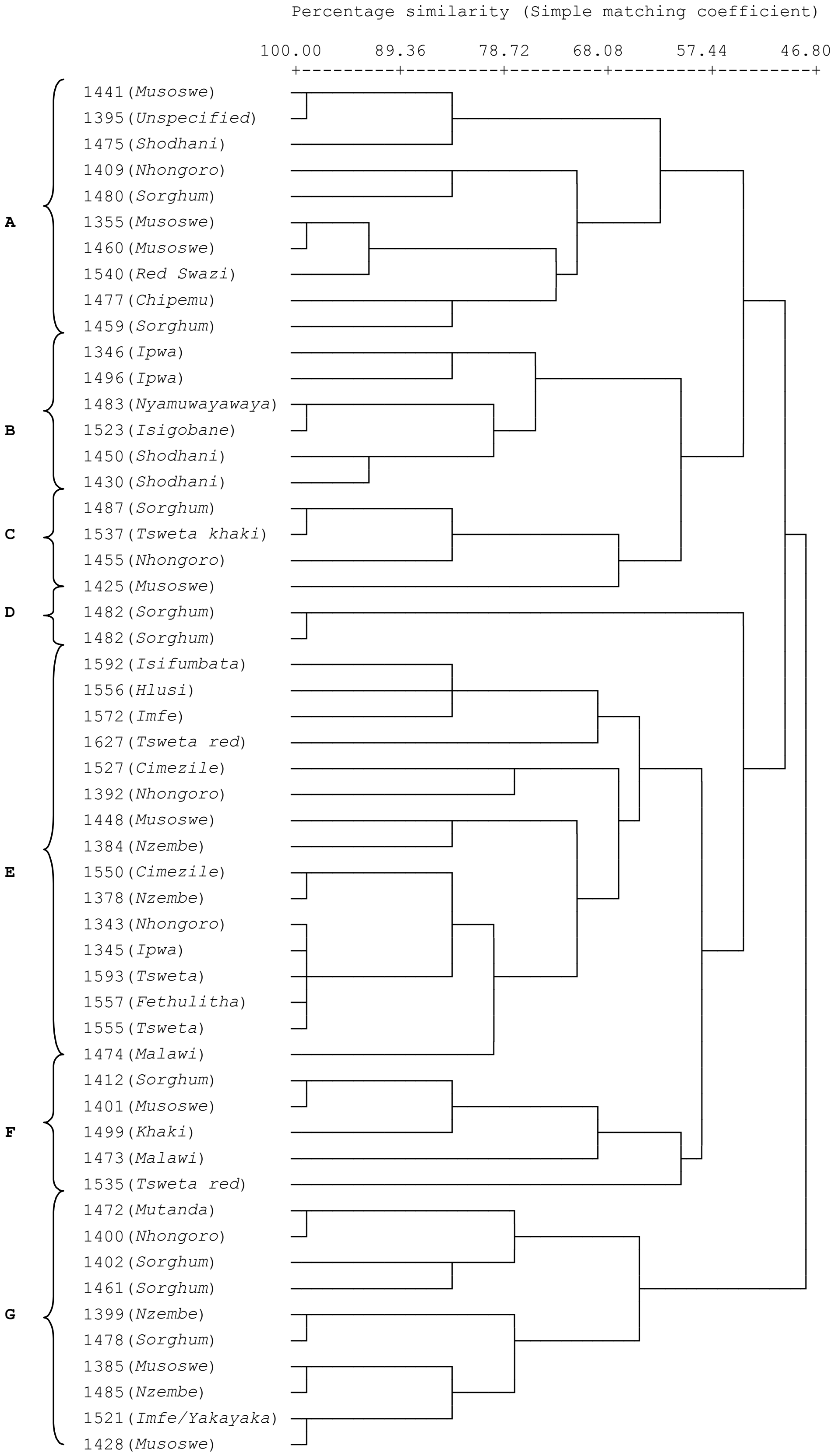


Figure 4. Dendrogram showing average linkage clustering of some sorghum lines collected in Zimbabwe, based on sorghum microsatellite data

Cluster A was predominantly genotypes collected from Nyanga North. Only one genotype (1540) in this cluster came from Sizanani village in Tsholotsho. The cluster contained five genotypes (1475, 1480, 1460, 1477 and 1549) collected from Renzva village in Nyanga North. Genotypes 1441, 1355 and 1460 were locally called *Musoswe*, and they came from Kamunhukamwe, Chibvende and Renzva villages respectively. Genotype 1355 was 100 per cent similar to 1460, but 62 per cent similar to 1441. However, 1441 and 1355 were essentially produced for chewing stalks and seed for flour, whilst 1460 was for chewing and beverages. Also included in cluster A were 1459 and 1480, locally called *Sorghum*. They were 72 per cent similar, both came from Renzva village and they were produced for flour. Genotypes 1395 (unspecified name) and 1409 (*Nhongoro*) were 62 per cent similar, had seed (flour) and beverage as major products. The last group in cluster A consisted of 1477 (*Chipemu*) and 1459 (*Sorghum*) which were 84 per cent similar, both came from Renzva village and were produced mainly for flour and beverages.

Cluster B comprised six genotypes of which only one (1523) was from Tsholotsho. Genotypes 1483 (*Nyamuwa-yawaya*) from Renzva village in Nyanga North, was 100 per cent similar to 1523 (*Isigobane*) a landrace from Siyabandela in Tsholotsho. However, 1483 was produced essentially for flour while both flour and stalks for chewing were major products from 1523. Also closely related were 1346 and 1496 at 84 per cent similarity level. These two landraces were produced mainly for stalk (chewing) and hence probably the name *Ipwa* (Sweet sorghum). Some of the genotypes that were grouped together in cluster B included 1430 and 1450. They were 92 per cent similar and shared the characteristics of being locally called *Shodhani*, came from Kamunhukamwe village in Nyanga North and were produced for flour and beverages.

Cluster C consisted of four genotypes, of which three (1487, 1409 and 1425) were from three different villages in Nyanga North, while 1537 was from Tsholotsho. Landrace 1487 (*Sorghum*) from Renzva village was 100 per cent similar to 1537 (*Tsweta khaki*) from Sizanani village in Tsholotsho. However, 1537 was a multi-purpose landrace produced for fodder (leaves), stalk for chewing and seed for flour, while 1487 was mainly produced for flour. Cluster D was made up of 'two genotypes' (1482) locally called *Sorghum*. These clustered at 100 per cent similarity.

Cluster E was made up of 16 genotypes, with almost half of them coming from each of the two districts. It contained five genotypes (1592, 1572, 1593, 1557 and 1555) from Siyazama village in Tsholotsho. Landrace 1592 (*Isifumbata*) and 1572 (*Imfe*) were 85 per cent similar and they were both produced for flour and stalks (chewing). This pair was also 100 per cent similar to genotype 1557 (*Fethulitha*) from the same village (Siyazama) and with the same products. Cluster E also contained genotypes 1556 (*Hlusi*) and 1550 (*Cimezile*) which were both from Sizanani village and joined at 65 per cent similarity. Genotype 1550 also shared a common local name with 1527 from Siyabandela, but they were 64 per cent similar. There were three genotypes (1384, 1392 and 1378) from Mangezi in Nyanga North. Genotypes 1384 and 1378 were 78 per cent similar, both genotypes were locally called *Nzembe* and also produced for seed (flour). Two landraces, 1343 (*Nhongoro*) and 1345 (*Ipwa*) were 100 per cent similar and were collected from the same village (Samakande). The pair was also 100 per cent similar to 1593, 1557 and 1555, which were all from Siyazama village in Tsholotsho. Genotypes 1593 and 1557 were locally called *Tsweta*.

Cluster F comprised five landraces, of which two (1412 and 1401) were 100 per cent similar. These genotypes were locally called *Sorghum* and *Musoswe* respectively and they had two common characteristics. They both came from Mangezi village and were produced mainly for seed. The other three landraces in this cluster came from different origins. Cluster G contained ten genotypes with nine of them coming from Nyanga North. Only genotype 1521 (*Imfe/yakayaya*) was from Siyabandela in Tsholotsho. Landraces 1400, 1402, 1399 and 1385 came from Mangezi, while 1472, 1461, 1478 and 1485 were from Renzva village. Genotypes 1385 and 1428 were 85 per cent similar, locally called *Musoswe* and they were produced for chewing stalks. However, the landraces came from two different villages, that is, Mangezi and Kamunhukamwe villages respectively. Genotype 1428 was 100 per cent similar to 1521 (*Imfe/yakayaka*) from Siyabandela, while 1385 was also 100 per cent similar to 1485 (*Nzembe*) from Renzva village. Landraces 1472 (*Mutanda*) and 1400 (*Nhongoro*) were 100 per cent similar but they came from Renzva and Mangezi villages respectively. Finally, there were three genotypes (1402, 1461 and 1478), which were locally called *Sorghum.* Genotype 1402 and 1461 were 82 per cent similar and 66 per cent similar to 1478. However, 1478 and 1461 were from Renzva while 1402 was collected from Mangezi village.

Within the district of Nyanga North, the village of Renzva had material from all six clusters reperesented in the district and this was followed closely by the village of Mangezi with five of the clusters and Kamunhukamwe with three clusters represented. In the district of Tsholotsho, the village of Siyabandela and Sizanani both had material from three clusters. A total of 22 local names were recorded for 28 of the 36 accessions collected. Thirteen of the names were unique, while the rest were used for more than one accession.

4.1.2 Isozyme analyses for sorghum

Active and well-resolved bands were produced in one (ACID-P) out of the six isoenzymes tested. However, the enzyme was weakly polymorphic producing predominantly two bands, one slow migrating (cathodal) and fast migrating (anodal). Consequently the enzyme could not distinguish between collections. The isoenzymes β-GLU, ADH, MDH, POD and α-EST either had too low, over or no activity such that they could not produce well-resolved bands. Figure 3 shows polyacrylamide gel electrophoresis of acid phosphotase. No further analyses were conducted on the isozymes.

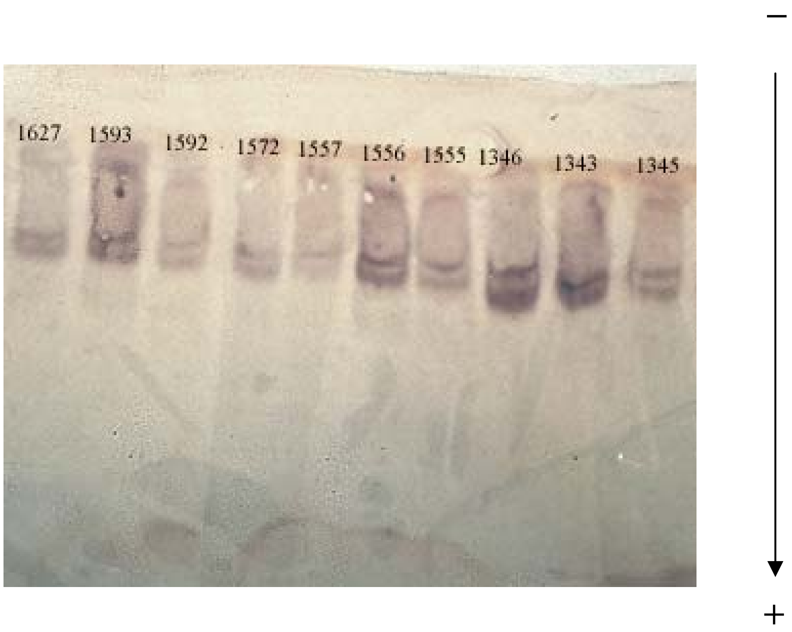


Figure 5 A polyacrylamide gel electrophoresis profile of acid phosphatase in sorghum genotypes: 1627, 1593, 1592, 1572, 1557, 1555, 1346, 1343 and 1445.

4.2 Relationship among Pearl millet landraces

4.2.1 Microsatellite analyses

The DNA extraction method used in this work gave good DNA yield and quality. Of the ten primers screened for the ability to detect polymorphism in the eight pearl millet genotypes, four primers (PSMP2001, PSMP2006, PSMP2018 and PSM2066) produced no amplification products. Four primers (PSMP2019, PSMP2059, PSMP2056 and PSMP2069) produced a single monomorphic band, while the remaining two (PSMP2008, PSMP2013) had multiple banding profiles. Polymorphic primers were used to evaluate the 47 landraces. The number of bands ranged from two to four, with band sizes ranging from 150 to 220 base pairs. Figure 4 shows a typical example of a microsatellite banding pattern obtained with primer *PSM2008*.

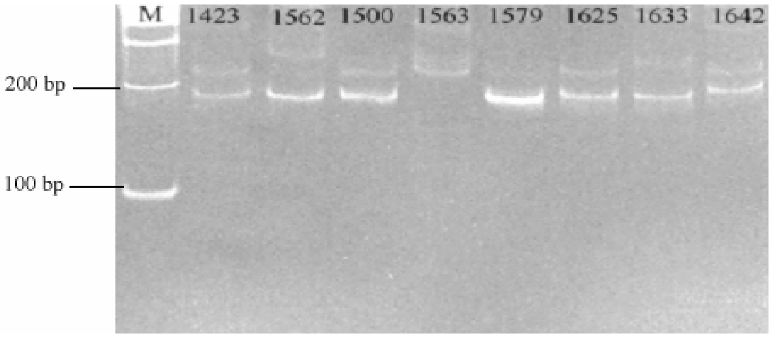


Figure 6. Banding patterns of microsatellite primer PSMP2008 pearl millet genotypes: 1423, 1562, 1500, 1563, 15791625, 1633 and 1642 separated on 8 per cent PAGE and stained with ethidium bromide. Lane M is the 100 base pair ladder

Cluster analysis of the 47 pearl millet genotypes (figure 5; see also Appendix 6 for the similarity matrix) showed that the landraces could be grouped into four clusters at 68 per cent similarity level. The clusters were designated A to D. Cluster A comprised 17 genotypes of which about 70 per cent of them came from Tsholotsho district. Five genotypes (1584, 1596, 1571, 1563 and 1606) were collected from Siyazama village in Tsholotsho. Landraces 1596 and 1571 were locally called *Halale* and were 82 per cent similar. Genotypes 1544, 1562 and 1548 from Sizanani were also grouped together. Landraces 1443 and 1440 from Kamunhukamwe village were 82 per cent similar and locally called *Mudhambure*. Genotypes 1500 (*PMV-3*) and 1501 (*Halale*) were 100 per cent similar and both were from Siyabandela village. Another interesting pair was 1644 and 1643. These were 100 per cent similar, both locally called *Tsholotsho-bearded* and also came from the same village (Phakamani).

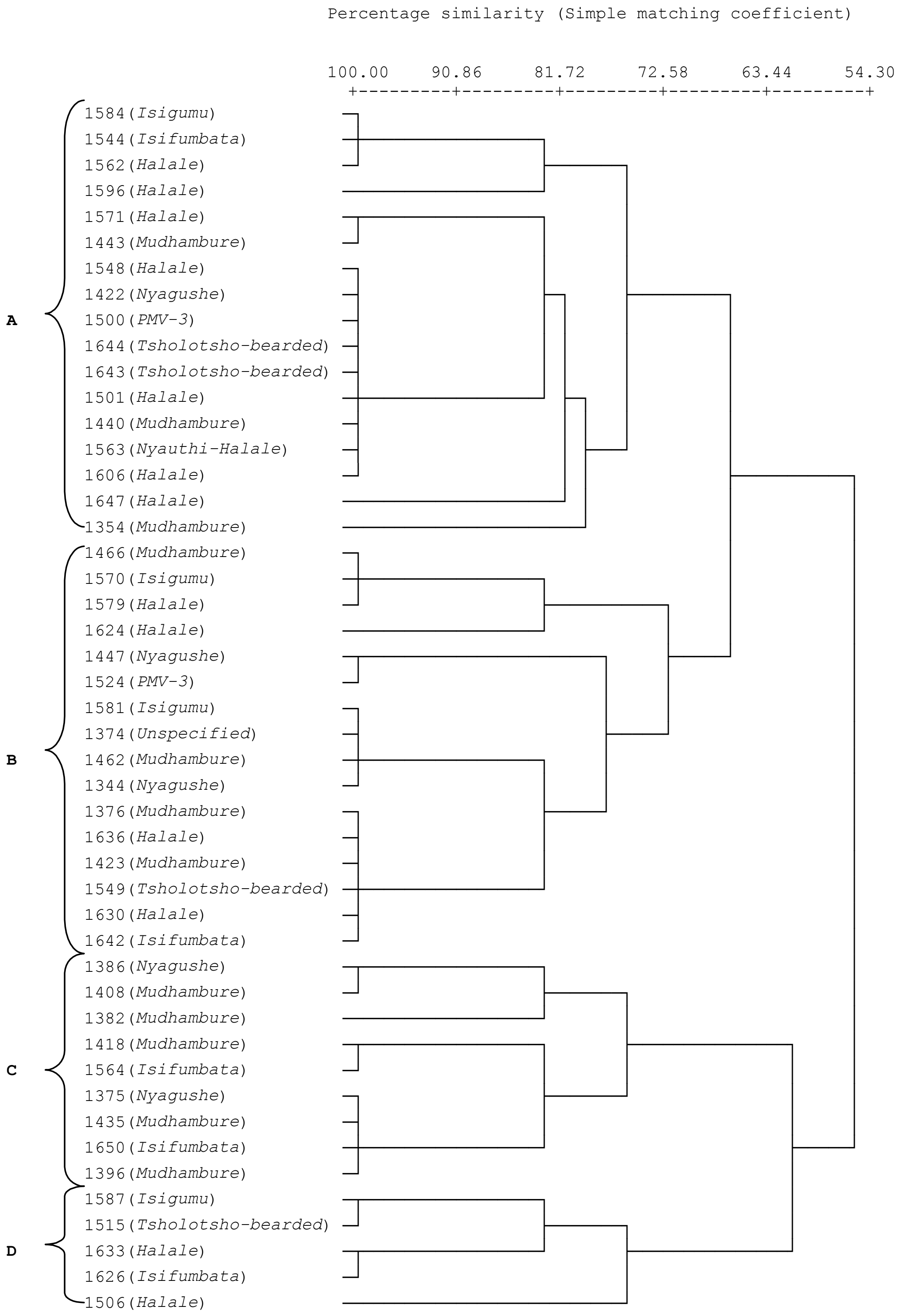


Figure 7. Dendrograms showing average linkage clustering for some Zimbabwean collection of pearl millet based on microsatellite data

Cluster B grouped nine genotypes from Tsholotsho and seven from Nyanga North. It contained a group of four landraces (1579, 1624, 1636 and 1630) that were locally called *Halale*. Genotype 1579 and 1624 were 82 per cent similar and they came from Siyazama and Phakamani villages respectively. Landrace 1579 was 100 per cent similar to 1570 (*Isigumu*) which was also from Siyazama village. Two genotypes (1466 and 1462) from Renzva village in Nyanga North were also grouped together in this cluster. They were 70 per cent similar and both locally called *Mudhambure*. Other genotypes grouped by village included 1624, 1636, 1630 and 1642 from Phakamani village while genotypes 1374 (unspecified name) and 1376 (*Mudhambure*) were from Karikoga village. Genotype 1642 (*Isifumbata*) was 100 per cent similar to 1376, 1630, 1649, 1423 and 1636. Sharing the same local name (*Isigumu*) and the same village (Siyazama) were landraces 1570 and 1581. They had 70 per cent similarity. Genotype 1447 from Kamunhukamwe and 1344 from Samakande were 78 per cent similar and both locally called *Nyagushe*. Interestingly some genotypes from Nyanga North were 100 per cent similar to some from Tsholotsho. For example, genotype 1466 (*Mudhambure*) from Renzva was 100 per cent similar to 1570 (*Isigumu*) and 1579 (*Halale*) which came from Siyazama village in Tsholotsho. Genotype 1447 (*Nyagushe*) from Kamunhukamwe was also 100 per cent similar to *PMV-3* (1524) from Siyabandela. Other genotypes which were 100 per cent similar included 1581 (*Isigumu*) from Siyazama and 1462 (*Mudhambure*) from Renzva village.

Cluster C comprised nine genotypes; seven of them were from Nyanga North. There were five genotypes (1408, 1382, 1418, 1386 and 1396) which were collected from Mangezi village. Genotypes 1386 and 1375 were 82 per cent similar and were both locally called *Nyagushe*. Five genotypes (1408, 1382, 1418, 1435 and 1396) were locally called *Mudhambure*. Genotypes 1408, 1382 and 1418 joined at about 72 per cent similarity level.

Also included in cluster C were two genotypes, 1564 from Siyazama and 1650 from Phakamani villages respectively. They were 75 per cent similar and locally called *Isifumbata*. Genotypes 1386 and 1408 were 100 per cent similar. Landraces 1418 and 1564 were 100 per cent similar but they were from Nyanga North and Tsholotsho respectively. Landraces 1375, 1435, 1650 and 1636 were 100 per cent similar, but they were from four different villages altogether. Genotype 1650 (*Isifumbata*) was from Phakamani village in Tsholotsho while the other three were from villages in Nyanga North.

All the five genotypes in cluster D were collected from Tsholotsho district. Genotypes 1515 (*Tsholotsho-bearded*) and 1506 (*Halale*) were from Siyabandela village, while 1633 (*Halale*) and 1626 (*Isifumbata*) were 100 per cent similar and both coming from Phakamani village. Genotypes 1506 and 1633 could also be grouped according to their common local name (*Halale*).

4.2.2 Isozyme analyses

Visible and well-resolved bands were observed in one (POD) out of six enzymes tested in pearl millet collections. Figure 6 shows a typical polyacrylamide gel profile of POD. The enzyme had a minimum of one and a maximum of three visible bands in the gel. Bands were either most cathodal (slowest migrating), intermediate migrating and most anodal (fastest mobility). The enzymes ADH, MDH, β-Glu, ACID-P and α-EST had either too low or no activity and thus did not produce well-resolved bands. No further analyses were done on the isozymes.

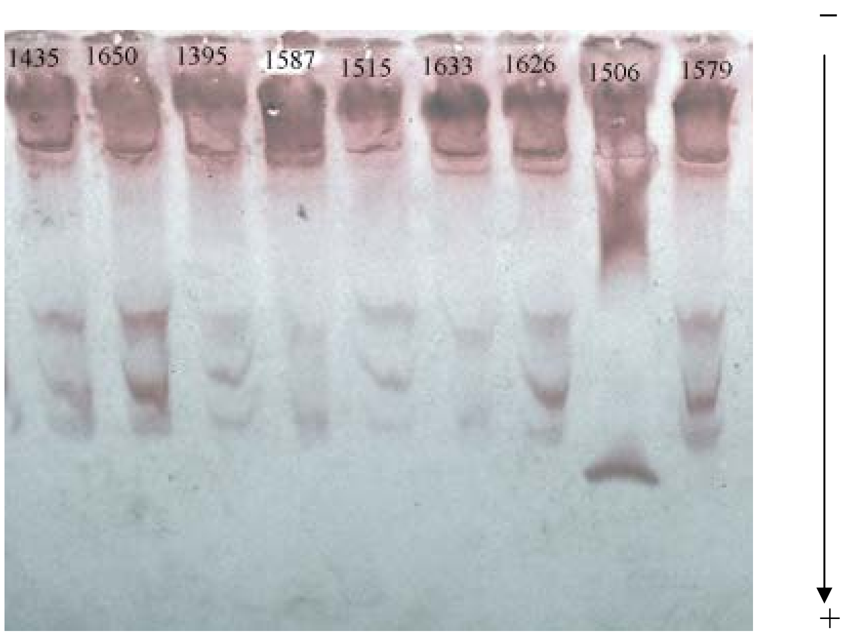


Figure 8. A polyacrylamide gel profile for peroxidase (POD) on pearl millet genotypes: 1435, 1650, 1395, 1587, 1515, 1633, 1626, 1506 and 1579.

DISCUSSION

5.1 Sorghum

The results from microsatellite analysis showed that the 52 sorghum genotypes could be grouped into seven clusters (figure 2). In cluster A, two groups were distinguishable by villages, that is, Renzva (1475, 1480, 1460, 1477 and 1549) and Mangezi (1395 and 1409). Genotypes 1441, 1355 and 1460 were locally called *Musoswe*, but they were from Kamunhukamwe, Chibvende and Renzva villages respectively. Besides having the same local name (*Musoswe*), genotypes 1441 and 1460 were also 100 per cent similar and both produced for chewing (stalks). It could, therefore, be argued that the two genotypes were duplicates. If that assertion is correct, then that might be an indication of wide germplasm exchange within and between villages which agrees with the findings of Huvio (1999). She observed that farmers in the two districts under study added new varieties to compliment old ones and that 80 per cent of the seed came from neighbours. About 60 per cent of the farmers walked long distances to buy or exchange seed (Huvio, 1999). The spread of germplasm could, therefore, be by design (to improve local crop) or by accidental mixing of grains. Other groups in cluster A included genotypes 1459 and 1480. They were locally called *Sorghum*, both came from Renzva village and were essentially produced for flour. However, they joined at 72 per cent similarity level. *Sorghum* is a broad general name and it might suggest that the landraces did not have obvious basis for naming such as origin, agromorphological traits or uses. Genotypes 1477 (*Chipemu*) and 1459 (*Sorghum*) were collected from Renzva village and both were produced for flour and beverages. Although they were only 82 per cent similar on the dendrogram, the two common characteristics still support their grouping together in cluster A.

Cluster B comprised of landraces predominantly from Nyanga North. Genotype 1483 (*Nyamuwaya-waya*) from Nyanga North was 100 per cent similar to 1523 (*Isigobane*) from Tsholotsho. The former was used for flour while the latter was produced for both flour and stalks. Although the meaning of their indigenous local names was not available similarity at molecular level might be explained by the role of NGOs in the areas. The two areas lie more than 800 km apart. Some NGOs (such as Community Technology Development Trust (COMMUTECH) are actively involved in organizing seed fairs, community seedbanks and teaching local farmers on the storage and utilization of traditional varieties in both areas. It is possible that through these initiatives a lot of germplasm exchange occurs. Alternatively the two genotypes (1523 and 1483) might have originated from the same source and then spread by human migration.

Another interesting pair in cluster B comprised of 1450 and 1430. They were 92 per cent similar and clustered by local name (*Shodhani*), village (Kamunhukamwe) and uses (flour and beverage). It was not clear whether the two collections were from different farmers or not. However, one cannot assume that the high similarity was only by chance. Looking at it from this perspective, it could be said that a similarity coefficient might be only an indication of an association between genotypes. That is, the association is relative and not absolute. One could therefore make a deduction from the dendrograms and assume that any genotypes with 70 per cent similarity or more might have a strong association.

Sweet sorghums (1346 and 1496), locally called *Ipwa*, grouped together at 82 per cent similarity. They were both produced for chewing, but came from Samakande and Renzva villages respectively. *Ipwa* is a general name for sorghum that is produced for chewing (sweet sorghums). Since these two genotypes are both from Nyanga North and they have a high similarity, it could be possible that they were selected from the same parent. Based on farmers' interviews (Mafa, curator National Genebank of Zimbabwe) and experience in communal agriculture it is known that seed is generally saved from the previous harvest, and in cases of insufficiency, new seeds are obtained from neighbouring farmers or markets. Furthermore, individual farmers select plants that are 'appealing to them' and use their seed in the next season. It therefore follows that after generations of selections there will be a lot of variation even within varieties with the same name because the names do not change accordingly. The grouping of these two genotypes by local names and use agrees with findings from studies in Nepal (Bajracharya, Rijal, Khatiwada, Paudel, Uphadhyay, Pandey, Tiwari and Chaudhary, 2000). In these studies, high correlation between indigenous local variety names of crops such as barley, rice, taro, pigeon pea and sponge gourd and their agromorphological traits were observed. However, no molecular analysis was done on these studies. Despite having the same indigenous local name 1346 and 1496 appeared different at molecular level. This could be as a result of the high stepwise mutation rates of microsatellites (Djé, Forcioli, Ater, Lefébvre and Vekemans (1999). Although sorghum is predominantly inbreeding species, rates of outcrossing as high as 0.10 to 0.15% (Doggett, 1998) and spontaneous hybridization are believed to occur. This might also possibly explain the differences between the two genotypes and several other genotypes already mentioned here at molecular level. Furthermore, when assessing intraspecific variation in population genetic diversity, it should also be considered that it is larger in inbreeding species than outbreeders (Djé *et al*., 1999).

Cluster C had three genotypes (1487, 1409 and 1425) which grouped according to the district of origin (Nyanga North). Genotype 1487 (*Sorghum*) from Renzva was 100 per cent similar to 1537 (*Tsweta khaki*) from Sizanani in Tsholotsho. However, 1487 was produced for chewing and flour while 1537 was used for flour. It can be assumed that 1487 did not have obvious basis for naming, thus the general name. The strong association between the two genotypes basing on molecular data and the uses was quite interesting. It could suggest wide germplasm exchange even between districts through the iniatives of the local NGOs. The distance between the two districts is not so large to effectively prevent this exchange by distance isolation considering the transport revolution. However, the environment between these two districts is very unsuitable for sorghum production making it somewhat surprising to find almost half the genotypes found in Tshlotsho to be also in Nyanga North. Another possible explanation could be inconsistency in variety description and naming between the two districts. However, there is need for more supporting information, ideally from agromorphological data to validate this argument. Cluster D was made up of 'two genotypes' (1482) with the same code locally called *Sorghum*. Genotype 1482 was repeated to test the accuracy of the clustering method. The grouping of the two 'entries' within one cluster at 100 per cent similarity showed that the analysis was accurate, effective and reliable. However, it was by coincidence that this genotype appeared to be the most dissimilar to the rest of the genotypes. It was not clear why it was so different from the rest, but the local name (*Sorghum*) suggests that it did not have obvious distinguishing characters.

Cluster E contained genotypes 1592, 1572, 1593, 1557 and 1555 which grouped according to their village of origin (Siyazama). Among these were 1592 (*Isifumbata*) and 1572 (*Imfe*) that were 82 per cent similar and used for chewing. Genotypes 1593 and 1555 grouped according to local name (*Tsweta*), origin (Siyazama village) and were also 100 per cent similar and could be duplicates. These could be duplicates basing on the results from this analysis. Other genotypes that grouped according to their origin included 1343 and 1345 (Samakande) and 1550 and 1556 from Siyazama. In cluster E, 1412 and 1401 grouped according to origin (Mangezi), use (flour) and 100 per cent similar. However 1412 was locally called *Sorghum* which suggests that it could be related to any of the genotypes from the area or none. It was also interesting to note that genotypes 1473 and 1474 locally called *Malawi* and both from Renzva village were found in two different clusters, that is, E and F respectively. They were 57 per cent similar. The name probably suggests that the germplasm might have come from Malawi. Cluster G had 90 per cent of the genotypes coming from Nyanga North. Among these were four genotypes (1400, 1402, 1399 and 1385) that were from Mangezi village. Genotypes 1385 and 1428 grouped by origin (Mangezi village), use (chewing), local name (*Musoswe*), but they were 82 per cent similar.

Nyanga North was originally selected for this study for the large diversity of Soghum and Tsholotsho because it represented the more marginal areas. It is therefore perhaps not surprising to find Nyanga North more diverse than Tsholotsho. However, sorghum plays a much more crucial role in Tsholotsho than Nyanga North where maize is also common and one could therefore expect substantial diversity. Although some strong relationships were observed between the microsatellite data and landrace indigenous local names including origin and use, some discordance was also observed with other genotypes. Possible explanations for the discrepancies include the complexity of the whole process of farmer-based variety identification and naming. For example, some names were based on uses (for example sweet sorghum or *Imfe*) while some on origin. Germplasm exchange could also explain the observed similarities among the varieties (Huvio, 1999). Trading practices or population movements during early and present cultivation of sorghum could have spread the germplasm. Sorghum is a traditional crop that is oftenly exchanged in the areas where collection was done (Mafa, 1999).

5.2 Pearl millet

The results from the cluster analysis showed that the genotypes could be grouped into four clusters (figure 5). In cluster A, three groups could be identified by their villages of origin, that is, Siyazama (1584, 1596, 1571, 1563 and 1606), Kamunhukamwe (1443 and 1440) and Sizanani (1544, 1562 and 1548). There were two groups of genotypes clustered according to their local names: *Halale* (1596 and 1571) and *Mudhambure* (1443 and 1440). Genotypes 1644 and 1643 had three characteristics in common. They were locally called *Tsholotsho-bearded*, both came from Phakamani village in Tsholotsho and they were 100 per cent similar. *Tsholotsho-bearded* is a local awned variety. The name is derived from its origin (Tsholotsho) and the morphology of the panicle (awned). It was not clear whether the two genotypes were collected from the same farmer or not. Their association was very strong despite the limited number of primers used suggesting that they are duplicates. However, other genotypes with the same local name (1549 and 1515) from Siyazama and Sizanani villages were very dissimilar at 68 per cent and 54 per cent repectively. This might suggest that 1644 and 1643 were grown in close proximity to cross-pollinate, that is no isolation distance. COMMUTECH has been actively involved organizing seed fairs and *in situ* genetic resources conservation schemes (Mafa, 1999) and probably such germplasm has been exchanged that way. Pearl millet is naturally outcrossing and large phenotypic and genotypic diversity exists in open pollinated varieties and landraces because of heterogeneity. However, this diversity can be easily lost through inbreeding depression especially in a closed system, for example, during seed multiplication. This might explain the differences within all the genotypes locally called *Tsholotsho-bearded*.

Cluster B contained four groups that could be distinguished by their villages of origin, that is, Siyazama (1579 and 1624), Renzva (1466 and 1462), Phakamani (1624, 1636, 1630 and 1642) and Karikoga (1374 and 1376). Four groups clustered according to their local names: *Halale* (1579, 1624, 1636 and 1630), *Mudhambure* (1466 and 1462), *Isigumu* (1570 and 1581) and *Nyagushe* (1447). Some genotypes were very closely related based on molecular data but not so close basing on local names and villages. For example, 1447 (*Nyagushe*) from Kamunhukamwe in Nyanga North was 100 per cent similar to 1524 (*PMV-3*) from Siyabandela in Tsholotsho. Pearl millet variety (PMV-3) is an improved open-pollinated variety that was bred by Crop Breeding Institute of DR&SS and well distributed in most pearl millet growing areas. Therefore, being a product of a national programme it can be possible that genotype 1447 is a duplicate which was given a different name. Genotype 1581 (*Isigumu*) from Siyazama was also 100 per cent similar to 1462 (*Mudhambure*) from Renzva village in Nyanga North. Microsatellites polymorphisms can arise from chromosomal aberrations or stepwise mutations such that deletions or additions on different chromosomes might not be distinguished as long as they result in the same band size. This results in false positives a concept that might explain some of the surprise relationships.

In cluster C, there were two groups identifiable by villages: Mangezi (1408, 1382, 1418, 1386 and 1396) and Siyazama (1564 and 1650). Three groups could be distinguished by indigenous local names, that is, *Mudhambure* (1408, 1382, 1418, 1435 and 1396), *Nyagushe* (1386 and 1375) and *Isifumbata* (1564 and 1650). Genotypes 1386 and 1408 were 100 per cent similar. Other genotypes that were 100 per cent similar included 1375, 1435, 1650 and 1636. Cluster D comprised genotypes from Tsholotsho district. Genotypes 1515 and 1506 came from Siyabandela and the latter was also locally called *Halale* together with 1633.

Generally about 90 per cent of the germplasm had pair-wise similarities of 100 per cent which suggests low genetic diversity among the landraces included in this study. This agrees with literature. Clegg *et al.* (1984), in a study of a portion of chloroplast DNA, found no polymorphism among several wild and cultivated millet samples. Gepts and Clegg (1989) also observed greater diversity in wild millets than in cultivated for ribosomal DNA.

Some discrepancies were observed between the microsatellite dendrogram (figure 3) and variety local names. For example, *Tsholotsho-bearded* was found in three different clusters (A, B and D). Literature on variety description and naming at community level is limited. As a result, a possible explanation for the discordance could only be by speculation. Firstly, variety naming is a function of complex interacting factors encompassing ethnicity, agromorphological characters, uses and personal tastes (Mafa, 1999). This makes the approach subjective, ambiguous and less accurate for large-scale germplasm characterization. Additionally, as new varieties are introduced some germplasm is lost by substitution or genetic erosion. It is not clear how the system counters these forces. Secondly, some varieties have very general names that cover broad groups (for example *Isifumbata*) and most of these do not give any information about genetic diversity within the group. Contamination of the original material could also explain the dendrogram structure. This arises through independent selection of very different material or inbred lines from the originally diverse line, or by different materials receiving the same name (Morden *et al.*, 1989). Some names were found in almost all the clusters and very similar to material from a completely different locality. This might either be by chance or could suggest high-level germplasm exchange (Huvio, 1999). Additionally, the number of polymorphic primers used in this study was limited. Two out of ten primers were polymorphic. This might have missed some of the genetic variation and thus influencing the structure of the dendrogram. Although more studies are needed to validate the results, this study showed some relationships between microsatellite data and indigenous local names. Overally genetic diversity was very low with pearl millet in both districts and for any reasonable inferences on conservation further studies are needed As a result the following discussion will be focused on sorghum.

5.3 Implications on Conservation

Now that the diversity has been identified and mapped, the next step will be to preserve the diverse material and make it available to future breeding programs. The first consideration would be to decide whether the best place to preserve the genotypes would be on farmes where they are presently growing or in a new structure specifically designed for that purpose. Smale, Bellon, Jarvis and Sthapit (2002) suggest that on farm conservation of plant genetic resources is most rational where both the public value of diversity and private value are high, that is, areas where there is a lot of genetic diversity and where it makes a substantial contribution to farmer livelihoods. They also argue that marker diversity is likely to be a good indicator of the public value of the diversity. On the basis of the high levels of sorghum diversity that study found in Nyanga North relative to Tsholotsho, one can tentatively make the case that Nyanga North would be a better site for on-farm conservation than Tsholotsho. Further studies would be required to determine the private value, or the contribution of the diversity to farmer livelihoods, in order to determine the sustainability of on-farm conservation at the two sites. Even if conservation efforts were to focus on Nyanga North, it should be noted that Tsholotsho has diversity not found in Nyanga North.

Although there was a tendency for different accessions with the same name to be genetically related, this was not consistent across the two districts, across villages and among farmers. Even within farmes there were several examples of the same name being used for genetically unrelated accessions. Based on this study, names can therefore not be considered a good guide to the presence of diversity, nor do they appear to be a good unit of sampling.

CONCLUSION

1. Isozyme data was inconclusive in showing relationships among landraces from both crops. Only ACID-P and POD produced visible and well–resolved bands for pearl millet and sorghum respectively. The rest of the enzymes had either no, too low or over activity.
2. Some sorghum landraces with the same indigenous local names, source (village or district) or both, were highly similar when analysed using microsatellite data. At 62 per cent similarity all 50 sorghum genotypes could be grouped into seven clusters. However, high similarities were observed among genotypes with indigenous local names: *Musoswe* (1355, 1441 and 1460), *Sorghum* (1459 and 1480), *Shodhani* (1450 and 1430) and *Ipwa* (1346 and 1496). Other similar landraces grouped by villages included: Renzva (1459 and 1480), Kamunhukamwe (1430 and 1450), Siyazama (1572, 1592, 1593,1557 and 1555), Samakande (1343 and 1345) and Mangezi (1401 and 1412).
3. Some genotypes from Nyanga North were closely related to those from Tsholotsho. Genotypes 1483 (*Nyamuwaya-waya*) and 1487 (*Sorghum*) from Nyanga North were 100 per cent similar to 1523 (*Isigobane*) and 1537 (*Tsweta khaki*) respectively, from Tsholotsho.
4. Microsatellite data showed high similarities among some pearl millet landraces with the same indigenous local names and some with a common source. At 68 per cent similarity level, 47 pearl millet genotypes could be grouped into four clusters. Genotypes identifiable by local names: *Halale* (1596 and 1571), *Tsholotsho-bearded* (1644 and 1643), *Mudhambure* (1408, 1382, 1418, 1435 and 1396), *Isigumu* (1570 and 1581), *Isifumbata* (1564 and 1650) and *Nyagushe* (1386 and 1375) showed very

low molecular diversity. Some highly similar landraces grouped by villages: Sizanani (1544, 1562 and 1548), Phakamani (1644 and 1643), Siyazama (1579 and 1624), Renzva (1462 and 1466), Mangezi (1408, 1382, 1418, 1386 and 1396) and Kamunhukamwe (1440 and 1443).

5. Some pearl millet landraces from different districts were shown to have high similarities at molecular level. Genotypes 1466 (*Mudhambure*) and 1447 (*Nyagushe*) from Nyanga North were 100 per cent similar to 1570 (*Isigumu*) and 1524 (*PMV-3*) respectively, from Tsholotsho.
6. Conservation efforts should primarily focus on high diversity areas, a high diversity of areas, and recognize the importance of individual with special interest in diversity. On the basis of the high levels of sorghum diversity that was detected in this study in Nyanga North relative to Tsholotsho, one can tentatively make the case that Nyanga North would be a better site for on-farm conservation than Tsholotsho.

RECOMMENDATIONS

1. There is need for detailed agromorphological data for the germplasm used in this study to form the basis of molecular characterization and possibly to correlate these morphological traits to the molecular data.

2. More isozymes and microsatellite markers might need to be studied for both sorghum and pearl millet.

3. Further studies should be conducted to have a better understanding of community-based variety identification and naming

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APPENDIX 1

Stain solutions

Solution 1	Ingredient	Concentration (mg/ml)	Usual volume (ml)
BB	Fast Blue BB salt	100	30
GBC	Fast garnet GBC salt	50	40
$MgCl_2$	Magnesium chloride	100	100
MTT	MTT, tetrazolium thiazolyl blue	10	60
a-NA	a-Naphthyl acetate (in 1:1, water: acetone)	20	50
b-NA	b-Naphthyl acetate(in acetone)	20	50
NAD	b-Nicotinamide adenine dinucleotide	20	100
NBT	Nitro blue tetrazolium	10	100
PMS	Phenazine methosulfate	5	100
FIX1	Fixative solution (acetic acid, glacial: methanol : water; 1:5:5 by volume)	--	--
FIX2	Fixative solution (glycerol:acetic acid, glacial:water:ethanol; 1:2:4:5 by volume)	--	--

APPENDIX 2

a) Microsatellite scores for 52 Zimbabwean sorghum landraces. The banding patterns are either present (1) or absent (0). Genotype 1482 was repeated

Primer (band base pairs)	Sorghum genotypes (order Appendix 3)
Txp8(*140*)	00000000000000000000000000000000000000000000100000010
Txp8(*150*)	01001010110011111010011000000000000011100000000000001
Txp8*(160)*	00000000000010000001011000111000111110000001000001000
Txp8*(165)*	00000000000000000000000000011100000110000110011110100
Txp8*(190)*	10000001001000000000000100000000000000000000000000000
Txp9*(150)*	00000000000000000000000000001010001010000110000100100
Txp9*(160)*	11111111111111111111111111110101110101111001111011011
Txp10*(150)*	11111111111111111111111111111111111111111111111111111
Txp16*(140)*	00100000000000000000000000000000000000000000000000010
Txp16*(145)*	10000000000000000000000000000000000000000000000000000
Txp16*(150)*	00010000100000000100011000000010000000010011000000000
Txp16*(160)*	01001101110011011100011111101111111111101100111111101
Txp16*(165)*	00000000000110000000000000000000000000000000000000000
Txp17*(165)*	11111111111011110100000100001000111010011110011101100
Txp17*(200)*	00000000000100000000000010011101110001011000011000100
Txp17*(220)*	00000000000000000000000110000000000000000000000000000
Txp17*(225)*	00000000000000000010010010000000000000000000000000000
Txp21*(160)*	00000000100100011101100101011000100001000001000011001
Txp21*(180)*	11111110110110010110100110111101111101111101111100110

b) Microsatellite scores for two primers and 47 pearl millet landraces. Bands are either present (1) or absent (0)

Primer (band base pairs)	Pearl millet genotypes (order Appendix 4)
PSMP8(*200*)	11111111111111111110111111111111111101111111111
PSMP8(*250*)	00111010000101111000100111001100111000001110010
PSMP13(*150*)	00000100101000100000001001100000000000100110001
PSSMP13(*180*)	00100000110000000111011101001011110000000000100
PSMP13(*200*)	11111101011111111101110001011110101011011001011

APPENDIX 3

Account number, local names and villages of 50 sorghum genotypes collected from Zimbabwe's communal areas

Account Number	Local Name	Village	Locality
1499	Khaki	Siyabandela	Tsholotsho
1384	Nzembe	Mangezi	Nyanga North
1485	Nzembe	Renzva	Nyanga North
1392	Nhongoro	Mangezi	Nyanga North
1399	Nzembe	Mangezi	Nyanga North
1401	Musoswe	Mangezi	Nyanga North
1402	Sorghum	Mangezi	Nyanga North
1400	Nhongoro	Mangezi	Nyanga North
1409	Nhongoro	Mangezi	Nyanga North
1412	Sorghum	Mangezi	Nyanga North
1425	Musoswe	Kamunhukamwe	Nyanga North
1355	Musoswe	Chibvende	Nyanga North
1378	Nzembe	Mangezi	Nyanga North
1430	Shodhani	Kamunhukamwe	Nyanga North
1441	Musoswe	Kamunhukamwe	Nyanga North
1450	Shodhani	Kamunhukamwe	Nyanga North
1455	Ipwa	Kamunhukamwe	Nyanga North
1448	Musoswe	Kamunhukamwe	Nyanga North
1428	Musoswe	Kamunhukamwe	Nyanga North
1460	Musoswe	Renzva	Nyanga North
1459	Sorghum	Renzva	Nyanga North
1461	Sorghum	Renzva	Nyanga North
1474	Malawi	Renzva	Nyanga North
1483	Nyamuwaya-waya	Renzva	Nyanga North
1482	Sorghum	Renzva	Nyanga North
1472	Mutanda	Renzva	Nyanga North
1487	Sorghum	Renzva	Nyanga North
1480	Sorghum	Renzva	Nyanga North
1478	Sorghum	Renzva	Nyanga North
1484	Nzembe	Renzva	Nyanga North
1523	Isigobane	Siyabandela	Tsholotsho
1527	Cimezile	Siyabandela	Tsholotsho
1535	Tsweta Red	Sizanani	Tsholotsho
1537	Tsweta khaki	Sizanani	Tsholotsho
1540	Red Swazi	Sizanani	Tsholotsho
1555	Tsweta	Sizanani	Tsholotsho
1557	Fethulitha	Sizanani	Tsholotsho
1550	Cimezile	Sizanani	Tsholotsho
1475	Shodhani	Renzva	Nyanga North
1346	Ipwa	Samakande	Nyanga North
1343	Nhongoro	Samakande	Nyanga North

Account Number	Local Name	Village	Locality
1345	Ipwa	Samakande	Nyanga North
1592	Isifumbata	Siyazama	Tsholotsho
1572	Imfe	Siyazama	Tsholotsho
1593	Tsweta	Siyazama	Tsholotsho
1627	Tsweta red	Phakamani	Tsholotsho
1395	Unspecified	Mangezi	Nyanga North
1477	Chipemu	Renzva	Nyanga North
1496	Ipwa	Renzva	Nyanga North
1556	Hlusi	Sizanani	Tsholotsho
1473	Malawi	Renzva	Nyanga North
1385	Musoswe	Mangezi	Nyanga North
1521	Imfe/yakayaka	Siyabandela	Tsholotsho

APPENDIX 4

Account number, local names and villages of 48 Pearl millet genotypes collected from Zimbabwe's communal areas

Account Number	Local name	Village	Locality
1500	PMV-3	Siyabandela	Tsholotsho
1501	Halale	Siyabandela	Tsholotsho
1544	Isifumbata	Sizanani	Tsholotsho
1562	Halale	Sizanani	Tsholotsho
1596	Halale	Siyazama	Tsholotsho
1548	Halale	Sizanani	Tsholotsho
1422	Nyagushe	Mangezi	Nyanga North
1643	Tsholotsho-bearded	Phakamani	Tsholotsho
1644	Tsholotsho-bearded	Phakamani	Tsholotsho
1440	Mudhambure	Kamunhukamwe	Nyanga North
1563	Nyauthi-Halale	Siyazama	Tsholotsho
1606	Halale	Siyazama	Tsholotsho
1647	Halale	Phakamani	Tsholotsho
1354	Mudhambure	Chibvende	Nyanga North
1571	Halale	Siyazama	Tsholotsho
1443	Mudhambure	Kamunhukamwe	Nyanga North
1524	PMV-3	Siyabandela	Tsholotsho
1466	Mudhambure	Renzva	Nyanga North
1570	Isigumu	Siyazama	Tsholotsho
1579	Halale	Siyazama	Tsholotsho
1624	Halale	Phakamani	Tsholotsho
1581	Isigumu	Siyazama	Tsholotsho
1374	Unspecified	Karikoga	Nyanga North
1462	Mudhambure	Renzva	Nyanga North
1344	Nyagushe	Samakande	Nyanga North
1376	Mudhambure	Karikoga	Nyanga North
1636	Halale	Phakamani	Tsholotsho
1423	Mudhambure	Mangezi	Nyanga North
1549	Tsholotsho-bearded	Sizanani	Tsholotsho
1630	Halale	Phakamani	Nyanga North
1642	Isifumbata	Phakamani	Tsholotsho
1386	Nyagushe	Mangezi	Nyanga North
1382	Mudhambure	Mangezi	Nyanga North
1418	Mudhambure	Mangezi	Nyanga North
1564	Isifumbata	Siyazama	Tsholotsho
1375	Nyagushe	Karikoga	Nyanga North
1435	Mudhambure	Kamunhukamwe	Nyanga North
1650	Isifumbata	Phakamani	Tsholotsho
1587	Isigumu	Siyazama	Tsholotsho
1515	Tsholotsho-bearded	Siyabandela	Tsholotsho
1633	Halale	Phakamani	Tsholotsho
1626	Isifumbata	Phakamani	Tsholotsho
1506	Halale	Siyabandela	Tsholotsho

Account Number	Local name	Village	Locality
1396	Mudhambure	Mangezi	Nyanga North
1447	Nyagushe	Kamunhukamwe	Nyanga North
1584	Isigumu	Siyazama	Tsholotsho
1408	Mudhambure	Mangezi	Nyanga North

APPENDIX 5 SIMPLE MATCHING COEFFICIENTS FOR SORGHUM

SIMPLE MATCHING COEFFICIENT

	1627	1593	1592	1572	1557	1556	1555	1346	1343	1345	1450	1473	1474	1483	1523	1496	1550	1355	1527	1482(1	1482(2	1475	1378	1430	1392	1409	1455	1472	1521	1487	1535	1480	1477	1460	1459	1400	1399	1412	1441	1448	1384	1385	1428	1425	1401	1402	1461	1478	1537	1540	1485	1499	1395
1627		0.692	0.692	0.692	0.692	0.692	0.692	0.692	0.692	0.692	0.615	0.538	0.692	0.538	0.538	0.538	0.538	0.385	0.462	0.538	0.538	0.231	0.538	0.692	0.538	0.385	0.231	0.385	0.385	0.385	0.385	0.385	0.538	0.385	0.385	0.385	0.538	0.538	0.231	0.538	0.538	0.385	0.385	0.308	0.538	0.692	0.538	0.538	0.385	0.462	0.385	0.538	0.231
1593			0.692	0.692	1.000	0.846	1.000	0.538	1.000	1.000	0.615	0.538	0.846	0.846	0.846	0.692	0.846	0.538	0.769	0.538	0.538	0.538	0.846	0.692	0.692	0.538	0.385	0.538	0.385	0.538	0.538	0.538	0.692	0.538	0.538	0.538	0.692	0.692	0.538	0.692	0.846	0.538	0.385	0.308	0.692	0.846	0.692	0.692	0.538	0.615	0.538	0.538	0.538
1592				0.846	0.692	0.846	0.692	0.538	0.692	0.692	0.462	0.692	0.692	0.538	0.538	0.538	0.538	0.385	0.462	0.538	0.538	0.231	0.538	0.538	0.538	0.385	0.231	0.385	0.385	0.385	0.385	0.385	0.538	0.385	0.385	0.385	0.538	0.538	0.231	0.538	0.538	0.385	0.385	0.462	0.538	0.692	0.538	0.538	0.385	0.462	0.385	0.692	0.231
1572					0.692	0.846	0.692	0.692	0.692	0.692	0.462	0.692	0.692	0.538	0.538	0.692	0.538	0.385	0.462	0.692	0.692	0.231	0.538	0.538	0.538	0.385	0.231	0.385	0.538	0.385	0.385	0.385	0.538	0.385	0.385	0.385	0.538	0.538	0.231	0.692	0.538	0.385	0.538	0.615	0.538	0.692	0.538	0.538	0.385	0.462	0.385	0.538	0.231
1557						0.846	1.000	0.538	1.000	1.000	0.615	0.538	0.846	0.846	0.846	0.692	0.846	0.538	0.769	0.538	0.538	0.538	0.846	0.692	0.692	0.538	0.385	0.538	0.385	0.538	0.538	0.538	0.692	0.538	0.538	0.538	0.692	0.692	0.538	0.692	0.846	0.538	0.385	0.308	0.692	0.846	0.692	0.692	0.538	0.615	0.538	0.538	0.538
1556							0.846	0.538	0.846	0.846	0.615	0.692	0.692	0.692	0.692	0.538	0.692	0.538	0.615	0.538	0.538	0.385	0.692	0.692	0.692	0.538	0.385	0.538	0.385	0.538	0.538	0.538	0.692	0.538	0.538	0.538	0.692	0.692	0.385	0.538	0.692	0.538	0.385	0.462	0.692	0.846	0.692	0.692	0.538	0.615	0.538	0.538	0.385
1555								0.538	1.000	1.000	0.615	0.538	0.846	0.846	0.846	0.692	0.846	0.538	0.769	0.538	0.538	0.538	0.846	0.692	0.692	0.538	0.385	0.538	0.385	0.538	0.538	0.538	0.692	0.538	0.538	0.538	0.692	0.692	0.538	0.692	0.846	0.538	0.385	0.308	0.692	0.846	0.692	0.692	0.538	0.615	0.538	0.538	0.538
1346									0.538	0.538	0.769	0.385	0.538	0.692	0.692	0.846	0.385	0.538	0.462	0.538	0.538	0.385	0.385	0.846	0.385	0.538	0.385	0.231	0.385	0.538	0.231	0.231	0.385	0.538	0.231	0.231	0.385	0.385	0.385	0.538	0.385	0.231	0.385	0.615	0.385	0.538	0.385	0.385	0.538	0.615	0.231	0.385	0.385
1343										1.000	0.615	0.538	0.846	0.846	0.846	0.692	0.846	0.538	0.769	0.538	0.538	0.538	0.846	0.692	0.692	0.538	0.385	0.538	0.385	0.538	0.538	0.538	0.692	0.538	0.538	0.538	0.692	0.692	0.538	0.692	0.846	0.538	0.385	0.308	0.692	0.846	0.692	0.692	0.538	0.615	0.538	0.538	0.538
1345											0.615	0.538	0.846	0.846	0.846	0.692	0.846	0.538	0.769	0.538	0.538	0.538	0.846	0.692	0.692	0.538	0.385	0.538	0.385	0.538	0.538	0.538	0.692	0.538	0.538	0.538	0.692	0.692	0.538	0.692	0.846	0.538	0.385	0.308	0.692	0.846	0.692	0.692	0.538	0.615	0.538	0.538	0.538
1450												0.308	0.462	0.769	0.769	0.615	0.462	0.769	0.538	0.462	0.462	0.462	0.462	0.923	0.615	0.769	0.615	0.308	0.154	0.615	0.462	0.462	0.615	0.769	0.462	0.308	0.462	0.462	0.462	0.308	0.462	0.308	0.154	0.538	0.462	0.615	0.462	0.462	0.615	0.846	0.308	0.308	0.462
1473													0.692	0.385	0.385	0.385	0.692	0.231	0.462	0.538	0.538	0.231	0.692	0.385	0.538	0.538	0.385	0.538	0.231	0.538	0.538	0.538	0.385	0.231	0.231	0.538	0.385	0.692	0.385	0.385	0.385	0.231	0.231	0.615	0.692	0.538	0.385	0.385	0.538	0.308	0.231	0.692	0.385
1474														0.692	0.692	0.692	0.692	0.385	0.615	0.538	0.538	0.385	0.692	0.538	0.538	0.385	0.231	0.385	0.385	0.385	0.385	0.385	0.538	0.385	0.385	0.385	0.538	0.538	0.385	0.692	0.692	0.385	0.385	0.308	0.538	0.692	0.538	0.538	0.385	0.462	0.385	0.538	0.385
1483															1.000	0.846	0.692	0.692	0.769	0.385	0.385	0.692	0.692	0.846	0.538	0.692	0.538	0.385	0.231	0.692	0.385	0.385	0.538	0.692	0.385	0.385	0.538	0.538	0.692	0.538	0.692	0.385	0.231	0.462	0.538	0.692	0.538	0.538	0.692	0.769	0.385	0.385	0.692
1523																0.846	0.692	0.692	0.769	0.385	0.385	0.692	0.692	0.846	0.538	0.692	0.538	0.385	0.231	0.692	0.385	0.385	0.538	0.692	0.385	0.385	0.538	0.538	0.692	0.538	0.692	0.385	0.231	0.462	0.538	0.692	0.538	0.538	0.692	0.769	0.385	0.385	0.692
1496																	0.538	0.538	0.615	0.538	0.538	0.538	0.538	0.692	0.385	0.538	0.385	0.231	0.385	0.538	0.231	0.231	0.385	0.538	0.231	0.231	0.385	0.385	0.538	0.692	0.538	0.231	0.385	0.615	0.385	0.538	0.385	0.385	0.538	0.615	0.231	0.385	0.538
1550																		0.385	0.769	0.538	0.538	0.538	1.000	0.538	0.692	0.692	0.538	0.692	0.231	0.692	0.692	0.692	0.538	0.385	0.385	0.692	0.538	0.846	0.692	0.538	0.692	0.385	0.231	0.462	0.846	0.692	0.538	0.538	0.692	0.462	0.385	0.692	0.692
1355																			0.462	0.385	0.385	0.692	0.385	0.692	0.538	0.692	0.846	0.538	0.385	0.538	0.385	0.692	0.846	1.000	0.692	0.538	0.385	0.385	0.692	0.538	0.692	0.538	0.385	0.462	0.385	0.538	0.692	0.385	0.538	0.923	0.538	0.231	0.692
1527																				0.462	0.462	0.769	0.769	0.615	0.769	0.615	0.462	0.462	0.154	0.615	0.462	0.462	0.462	0.462	0.308	0.462	0.462	0.615	0.615	0.462	0.615	0.308	0.154	0.385	0.615	0.615	0.462	0.462	0.615	0.538	0.308	0.462	0.615
1482(1)																					1.000	0.231	0.538	0.385	0.692	0.538	0.385	0.385	0.385	0.385	0.538	0.538	0.538	0.385	0.385	0.385	0.385	0.538	0.231	0.538	0.385	0.231	0.385	0.615	0.538	0.538	0.385	0.385	0.385	0.462	0.231	0.538	0.231
1482(2)																						0.231	0.538	0.385	0.692	0.538	0.385	0.385	0.385	0.385	0.538	0.538	0.538	0.385	0.385	0.385	0.385	0.538	0.231	0.538	0.385	0.231	0.385	0.615	0.538	0.538	0.385	0.385	0.385	0.462	0.231	0.538	0.231
1475																							0.538	0.538	0.538	0.538	0.692	0.538	0.231	0.538	0.231	0.538	0.538	0.692	0.385	0.538	0.231	0.385	0.846	0.538	0.692	0.385	0.231	0.308	0.385	0.385	0.538	0.231	0.538	0.615	0.385	0.231	0.846
1378																								0.538	0.692	0.692	0.538	0.692	0.231	0.692	0.692	0.692	0.538	0.385	0.385	0.692	0.538	0.846	0.692	0.538	0.692	0.385	0.231	0.462	0.846	0.692	0.538	0.538	0.692	0.462	0.385	0.692	0.692
1430																									0.538	0.692	0.538	0.385	0.231	0.692	0.385	0.385	0.538	0.692	0.385	0.385	0.538	0.538	0.538	0.385	0.538	0.385	0.231	0.462	0.538	0.692	0.538	0.538	0.692	0.769	0.385	0.385	0.538
1392																										0.692	0.538	0.538	0.231	0.538	0.692	0.692	0.692	0.538	0.538	0.538	0.538	0.692	0.385	0.385	0.538	0.385	0.231	0.462	0.692	0.692	0.538	0.538	0.538	0.615	0.385	0.538	0.385
1409																											0.846	0.538	0.077	0.846	0.692	0.692	0.538	0.692	0.385	0.538	0.385	0.692	0.692	0.231	0.385	0.231	0.077	0.769	0.692	0.538	0.385	0.385	0.846	0.769	0.231	0.538	0.692
1455																												0.692	0.231	0.692	0.538	0.846	0.692	0.846	0.538	0.692	0.231	0.538	0.846	0.385	0.538	0.385	0.231	0.615	0.538	0.385	0.538	0.231	0.692	0.769	0.385	0.385	0.846
1472																													0.538	0.692	0.538	0.846	0.692	0.538	0.538	1.000	0.538	0.692	0.692	0.538	0.692	0.692	0.538	0.308	0.692	0.692	0.846	0.538	0.692	0.462	0.692	0.538	0.692
1521																														0.231	0.385	0.385	0.538	0.385	0.692	0.538	0.692	0.231	0.231	0.692	0.538	0.846	1.000	0.154	0.231	0.538	0.692	0.692	0.231	0.308	0.846	0.231	0.231
1487																															0.538	0.538	0.385	0.538	0.231	0.692	0.538	0.692	0.692	0.231	0.385	0.385	0.231	0.615	0.692	0.692	0.538	0.538	1.000	0.615	0.385	0.538	0.692
1535																																0.692	0.538	0.385	0.692	0.538	0.692	0.692	0.385	0.231	0.385	0.538	0.385	0.462	0.692	0.538	0.385	0.692	0.538	0.462	0.538	0.538	0.385
1480																																	0.846	0.692	0.692	0.846	0.385	0.692	0.692	0.538	0.692	0.538	0.385	0.462	0.692	0.538	0.692	0.385	0.538	0.615	0.538	0.538	0.692
1477																																		0.846	0.846	0.692	0.538	0.538	0.538	0.692	0.846	0.692	0.538	0.308	0.538	0.692	0.846	0.538	0.385	0.769	0.692	0.385	0.538
1460																																			0.692	0.538	0.385	0.385	0.692	0.538	0.692	0.538	0.385	0.462	0.385	0.538	0.692	0.385	0.538	0.923	0.538	0.231	0.692
1459																																				0.538	0.692	0.385	0.385	0.538	0.692	0.846	0.692	0.154	0.385	0.538	0.692	0.692	0.231	0.615	0.846	0.231	0.385
1400																																					0.538	0.692	0.692	0.538	0.692	0.692	0.538	0.308	0.692	0.692	0.846	0.538	0.692	0.462	0.692	0.538	0.692
1399																																						0.538	0.231	0.385	0.538	0.846	0.692	0.154	0.538	0.846	0.692	1.000	0.538	0.462	0.846	0.385	0.231
1412																																							0.538	0.385	0.538	0.385	0.231	0.462	1.000	0.692	0.538	0.538	0.692	0.462	0.385	0.846	0.538
1441																																								0.538	0.692	0.385	0.231	0.462	0.538	0.385	0.538	0.231	0.692	0.615	0.385	0.385	1.000
1448																																									0.846	0.538	0.692	0.308	0.385	0.538	0.692	0.385	0.231	0.462	0.538	0.385	0.538
1384																																										0.692	0.538	0.154	0.538	0.692	0.846	0.538	0.385	0.615	0.692	0.385	0.692
1385																																											0.846	0.000	0.385	0.692	0.846	0.846	0.385	0.462	1.000	0.231	0.385
1428																																												0.154	0.231	0.538	0.692	0.692	0.231	0.308	0.846	0.231	0.231
1425																																													0.462	0.308	0.154	0.154	0.615	0.538	0.000	0.462	0.462
1401																																														0.692	0.538	0.538	0.692	0.462	0.385	0.846	0.538
1402																																															0.846	0.846	0.692	0.615	0.692	0.538	0.385
1461																																																0.692	0.538	0.615	0.846	0.385	0.538
1478																																																	0.538	0.462	0.846	0.385	0.231
1537																																																		0.615	0.385	0.538	0.692
1540																																																			0.462	0.308	0.615
1485																																																				0.231	0.385
1499																																																					0.385

APPENDIX 6 SIMPLE MATCHING COEFFICIENTS FOR PEARL MILLET LANDRACES

Simple matching Measure

	1402	1423	1562	1500	1563	1579	1600	1642	1386	1462	1466	1606	1524	1501	1354	1440	1644	1650	1344	1581	1647	1395	1384	1418	1643	1382	1570	1549	1596	1571	1375	1374	1564	1584	1626	1630	1548	1506	1306	1422	1587	1515	1636	1435	1443	1624	1544
1402		0.833	0.500	0.667	0.667	0.667	0.500	0.833	0.333	0.667	0.667	0.667	1.000	0.667	0.500	0.667	0.667	0.500	0.667	0.667	0.500	0.500	0.333	0.333	0.667	0.167	0.667	0.833	0.667	0.833	0.500	0.667	0.333	0.500	0.500	0.833	0.667	0.167	0.833	0.667	0.333	0.333	0.833	0.500	0.833	0.500	0.500
1423			0.667	0.833	0.833	0.833	0.667	1.000	0.500	0.833	0.833	0.833	0.833	0.833	0.667	0.833	0.833	0.667	0.833	0.833	0.667	0.667	0.500	0.500	0.833	0.333	0.833	1.000	0.500	0.667	0.667	0.833	0.500	0.667	0.667	1.000	0.833	0.333	1.000	0.833	0.500	0.500	1.000	0.667	0.667	0.667	0.667
1562				0.833	0.833	0.500	0.667	0.667	0.500	0.833	0.500	0.833	0.500	0.833	0.667	0.833	0.833	0.667	0.833	0.833	0.667	0.667	0.500	0.833	0.833	0.667	0.500	0.667	0.833	0.667	0.667	0.833	0.833	1.000	0.667	0.667	0.833	0.333	0.667	0.833	0.500	0.500	0.667	0.667	0.667	0.333	1.000
1500					1.000	0.667	0.833	0.833	0.333	0.667	0.667	1.000	0.667	1.000	0.833	1.000	1.000	0.500	0.667	0.667	0.833	0.500	0.333	0.667	1.000	0.500	0.667	0.833	0.667	0.833	0.500	0.667	0.667	0.833	0.833	0.833	1.000	0.500	0.833	1.000	0.667	0.667	0.833	0.500	0.833	0.500	0.833
1563						0.667	0.833	0.833	0.333	0.667	0.667	1.000	0.667	1.000	0.833	1.000	1.000	0.500	0.667	0.667	0.833	0.500	0.333	0.667	1.000	0.500	0.667	0.833	0.667	0.833	0.500	0.667	0.667	0.833	0.833	0.833	1.000	0.500	0.833	1.000	0.667	0.667	0.833	0.500	0.833	0.500	0.833
1579							0.500	0.833	0.667	0.667	1.000	0.667	0.667	0.667	0.833	0.667	0.667	0.500	0.667	0.667	0.500	0.500	0.667	0.333	0.667	0.500	1.000	0.833	0.333	0.500	0.500	0.667	0.333	0.500	0.500	0.833	0.667	0.500	0.833	0.667	0.667	0.667	0.833	0.500	0.500	0.833	0.500
1600								0.667	0.500	0.500	0.500	0.833	0.500	0.833	0.667	0.833	0.833	0.667	0.500	0.500	0.667	0.667	0.500	0.833	0.833	0.667	0.500	0.667	0.500	0.667	0.667	0.500	0.833	0.667	1.000	0.667	0.833	0.667	0.667	0.833	0.833	0.833	0.667	0.667	0.667	0.667	0.667
1642									0.500	0.833	0.833	0.833	0.833	0.833	0.667	0.833	0.833	0.667	0.833	0.833	0.667	0.667	0.500	0.500	0.833	0.333	0.833	1.000	0.500	0.667	0.667	0.833	0.500	0.667	0.667	1.000	0.833	0.333	1.000	0.833	0.500	0.500	1.000	0.667	0.667	0.667	0.667
1386										0.667	0.667	0.333	0.333	0.333	0.500	0.333	0.333	0.833	0.667	0.667	0.167	0.833	1.000	0.667	0.333	0.833	0.667	0.500	0.333	0.167	0.833	0.667	0.667	0.500	0.500	0.500	0.333	0.500	0.500	0.333	0.667	0.667	0.500	0.833	0.167	0.833	0.500
1462											0.667	0.667	0.667	0.667	0.500	0.667	0.667	0.833	1.000	1.000	0.500	0.833	0.667	0.667	0.667	0.500	0.667	0.833	0.667	0.500	0.833	1.000	0.667	0.833	0.500	0.833	0.667	0.167	0.833	0.667	0.333	0.333	0.833	0.833	0.500	0.500	0.833
1466												0.667	0.667	0.667	0.833	0.667	0.667	0.500	0.667	0.667	0.500	0.500	0.667	0.333	0.667	0.500	1.000	0.833	0.333	0.500	0.500	0.667	0.333	0.500	0.500	0.833	0.667	0.500	0.833	0.667	0.667	0.667	0.833	0.500	0.500	0.833	0.500
1606													0.667	1.000	0.833	1.000	1.000	0.500	0.667	0.667	0.833	0.500	0.333	0.667	1.000	0.500	0.667	0.833	0.667	0.833	0.500	0.667	0.667	0.833	0.833	0.833	1.000	0.500	0.833	1.000	0.667	0.667	0.833	0.500	0.833	0.500	0.833
1524														0.667	0.500	0.667	0.667	0.500	0.667	0.667	0.500	0.500	0.333	0.333	0.667	0.167	0.667	0.833	0.667	0.833	0.500	0.667	0.333	0.500	0.500	0.833	0.667	0.167	0.833	0.667	0.333	0.333	0.833	0.500	0.833	0.500	0.500
1501															0.833	1.000	1.000	0.500	0.667	0.667	0.833	0.500	0.333	0.667	1.000	0.500	0.667	0.833	0.667	0.833	0.500	0.667	0.667	0.833	0.833	0.833	1.000	0.500	0.833	1.000	0.667	0.667	0.833	0.500	0.833	0.500	0.833
1354																0.833	0.833	0.333	0.500	0.500	0.667	0.333	0.500	0.500	0.833	0.667	0.833	0.667	0.500	0.667	0.333	0.500	0.500	0.667	0.667	0.667	0.833	0.667	0.667	0.833	0.833	0.833	0.667	0.333	0.667	0.667	0.667
1440																	1.000	0.500	0.667	0.667	0.833	0.500	0.333	0.667	1.000	0.500	0.667	0.833	0.667	0.833	0.500	0.667	0.667	0.833	0.833	0.833	1.000	0.500	0.833	1.000	0.667	0.667	0.833	0.500	0.833	0.500	0.833
1644																		0.500	0.667	0.667	0.833	0.500	0.333	0.667	1.000	0.500	0.667	0.833	0.667	0.833	0.500	0.667	0.667	0.833	0.833	0.833	1.000	0.500	0.833	1.000	0.667	0.667	0.833	0.500	0.833	0.500	0.833
1650																			0.833	0.833	0.333	1.000	0.833	0.833	0.500	0.667	0.500	0.667	0.500	0.333	1.000	0.833	0.833	0.667	0.667	0.667	0.500	0.333	0.667	0.500	0.500	0.500	0.667	1.000	0.333	0.667	0.667
1344																				1.000	0.500	0.833	0.667	0.667	0.667	0.500	0.667	0.833	0.667	0.500	0.833	1.000	0.667	0.833	0.500	0.833	0.667	0.167	0.833	0.667	0.333	0.333	0.833	0.833	0.500	0.500	0.833
1581																					0.500	0.833	0.667	0.667	0.667	0.500	0.667	0.833	0.667	0.500	0.833	1.000	0.667	0.833	0.500	0.833	0.667	0.167	0.833	0.667	0.333	0.333	0.833	0.833	0.500	0.500	0.833
1647																						0.333	0.167	0.500	0.833	0.333	0.500	0.667	0.500	0.667	0.333	0.500	0.500	0.667	0.667	0.667	0.833	0.667	0.667	0.833	0.500	0.500	0.667	0.333	0.667	0.333	0.667
1395																							0.833	0.833	0.500	0.667	0.500	0.667	0.500	0.333	1.000	0.833	0.833	0.667	0.667	0.667	0.500	0.333	0.667	0.500	0.500	0.500	0.667	1.000	0.333	0.667	0.667
1384																								0.667	0.333	0.833	0.667	0.500	0.333	0.167	0.833	0.667	0.667	0.500	0.500	0.500	0.333	0.500	0.500	0.333	0.667	0.667	0.500	0.833	0.167	0.833	0.500
1418																									0.667	0.833	0.333	0.500	0.667	0.500	0.833	0.667	1.000	0.833	0.833	0.500	0.667	0.500	0.500	0.667	0.667	0.667	0.500	0.833	0.500	0.500	0.833
1643																										0.500	0.667	0.833	0.667	0.833	0.500	0.667	0.667	0.833	0.833	0.833	1.000	0.500	0.833	1.000	0.667	0.667	0.833	0.500	0.833	0.500	0.833
1382																											0.500	0.333	0.500	0.333	0.667	0.500	0.833	0.667	0.667	0.333	0.500	0.667	0.333	0.500	0.833	0.833	0.333	0.667	0.333	0.667	0.667
1570																												0.833	0.333	0.500	0.500	0.667	0.333	0.500	0.500	0.833	0.667	0.500	0.833	0.667	0.667	0.667	0.833	0.500	0.500	0.833	0.500
1549																													0.500	0.667	0.667	0.833	0.500	0.667	0.667	1.000	0.833	0.333	1.000	0.833	0.500	0.500	1.000	0.667	0.667	0.667	0.667
1596																														0.833	0.500	0.667	0.667	0.833	0.500	0.500	0.667	0.167	0.500	0.667	0.333	0.333	0.500	0.500	0.833	0.167	0.833
1571																															0.333	0.500	0.500	0.667	0.667	0.667	0.833	0.333	0.667	0.833	0.500	0.500	0.667	0.333	1.000	0.333	0.667
1375																																0.833	0.833	0.667	0.667	0.667	0.500	0.333	0.667	0.500	0.500	0.500	0.667	1.000	0.333	0.667	0.667
1374																																	0.667	0.833	0.500	0.833	0.667	0.167	0.833	0.667	0.333	0.333	0.833	0.833	0.500	0.500	0.833
1564																																		0.833	0.833	0.500	0.667	0.500	0.500	0.667	0.667	0.667	0.500	0.833	0.500	0.500	0.833
1584																																			0.667	0.667	0.833	0.333	0.667	0.833	0.500	0.500	0.667	0.667	0.667	0.333	1.000
1626																																				0.667	0.833	0.667	0.667	0.833	0.833	0.833	0.667	0.667	0.667	0.667	0.667
1630																																					0.833	0.333	1.000	0.833	0.500	0.500	1.000	0.667	0.667	0.667	0.667
1548																																						0.500	0.833	1.000	0.667	0.667	0.833	0.500	0.833	0.500	0.833
1506																																							0.333	0.500	0.833	0.833	0.333	0.333	0.333	0.667	0.333
1306																																								0.833	0.500	0.500	1.000	0.667	0.667	0.667	0.667
1422																																									0.667	0.667	0.833	0.500	0.833	0.500	0.833
1587																																										1.000	0.500	0.500	0.500	0.833	0.500
1515																																											0.500	0.500	0.500	0.833	0.500
1636																																												0.667	0.667	0.667	0.667
1435																																													0.333	0.667	0.667
1443																																														0.333	0.667
1624																																															0.333