Genetic diversity of local maize (*Zea mays L.*) germplasm from eight agro-ecological zones in Mozambique

Master thesis in Biology

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Abstract

The genetic diversity within and among 27 accessions of local maize maintained at the National Gene bank of Mozambique was studied. The accessions were originally collected from farmers' fields in 8 of the 10 agro-ecological zones of Mozambique. Eleven SSR markers were used. A total of 84 alleles were found with an average of 7.63 alleles per locus. The effective number of alleles per locus (Ne) ranged from 1.37 to 6.80 with an average of 3.47. The values for observed heterozygosity (Ho) ranged from 0.10 to 0.68 with an average of 0.36. Expected heterozygosity (He) ranged from 0.27 to 0.86 with an average of 0.67. Shannon's information index (I) ranged between 0.68 and 2.11 with an average of 1.40 per locus. Estimation of genetic diversity at each locus across all accessions (Nei) ranged from 0.27 to 0.85 with an average of 0.67. For the 27 accessions the effective number of alleles (Ne) ranged from 2.06 to 3.21 with an average of 2.56. The observed heterozygosity (Ho) across the eleven loci ranged from 0.25 to 0.48 with an average value of 0.35. Ten of the accessions scored less than the mean of the observed heterozygosity while four accessions recorded the same value as the mean observed heterozygosity of all accessions. AMOVA revealed significant differentiation among the ungrouped 27 maize accessions but most of the variation (88.28%) was found within accessions. There was no differentiation among accessions when they were grouped according to agro-ecological zones. Both the cluster analysis and the PCoA showed no clear grouping of accessions belonging to the same agro-ecological zones.

Keywords: Genetic diversity, maize, *Zea mays*, germplasm, Mozambique, Simple Sequence Repeat (SSR), AMOVA, PCA

List of abbreviations

CIMMYT - International Maize and Wheat Improvement Center.

DINA - National Department of Agriculture.

IIAM - Agricultural Research Institute of Mozambique.

IITA - International Institute of Tropical Agriculture.

IPGRI - International Plant Genetic Resources Institute.

NPGRC - National Plant Genetic Resources Centre.

PCR - Polymerase chain reaction.

QPM - Quality protein maize.

SADC – Southern African Development Community.

SPGRC - SADC Plant Genetic Resources Centre.

SSR - Simple sequence repeat.

UPGMA - Unweighted pair group method with arithmetic mean.

Dedication

In memory of my parents Virissimo Afonso and Cacilda Geral

Contents

1. Introduction

Maize (*Zea mays* L) is one of the most important cereal crops in the world and a strategic food crop for the majority of the developing countries (Lopes and Larkins, 1996).

1.1. Maize taxonomy and origin

Maize is a member of the grass family Poaceae (Gramineae), sub-family Paniccoideae. tribe Andropogeneae (Piperno and Flannery, 2001).
The genus *Zea* is a group of annual and perennial grasses native to Mexico and central America (Galinat, 1971).
The genus *Zea* includes the wild taxa, known collectively as teosinte (*Zea* ssp) and domesticated corn or maize (*Zea mays*). Based on the morphological characteristics and geographic delineations five species of *Zea* are currently recognized:

- *Zea diploperennis* Iltis, Doebley and Guzman, a perennial, diploid teosinte found in very limited regions of the highlands of Western Mexico (Buckler and Stevens, 2006).
- *Zea perennis* (Hitchcock) Reeves and Mangelsdorf, a perennial, tetraploid teosinte, with a very narrow distribution in the highlands of Western Mexico (Buckler and Stevens, 2006) .
- *Zea luxurians* (Durieu and Ascherson) Bird, an annual teosinte found in the more Equatorial regions of Southeastern Guatemala and Honduras (Buckler and Stevens, 2006)
- *Zea nicaraguensis* Iltis and Benz, closely related to *Zea luxurians* and found in Nicaragua (Orr and Sundberg, 2004).
- *Zea mays* L, a highly polymorphic, diploid annual species, including both wild teosinte and cultivated maize.

Maize was domesticated from teosinte, 6300 years ago in Mexico. After initial domestication, early farmers continued to select for advantageous morphological and biochemical traits in this important crop (Buckler and Stevens, 2006).
However, other sources suggest that maize was domesticated in central America about 9000 years ago (Matsuoka et al., 2002).
According to Staller (2010), the domestication of maize was a result of a hybridization event between an unknown pre-Columbian wild maize and a species of the related genus *Tripsacum*. Recently molecular data indicated that new maize was domesticated from annual Balsas teosinte (*Zea mays ssp. parviglumis*) in Southern Mexico 6,600 to 9,000 years ago. Selection for some favorable alleles at loci controlling plant morphology and kernel nutritional quality fixed those alleles, at least 4,400 years ago. Moreover, further selection by native Americans facilitated maize adaptation to various environments (GeoChemBio, 2012).

The chromosome number of *Zea mays* is 2n=20 (Fisk, 1925).

Maize is a cross pollinating, monoecious plant with separate male inflorescence (pollen-producing tassels) and female inflorescence (egg-producing ear) with extended silks (stigmas) on top and the mid of the same plant, respectively.

1.2. Maize status in Mozambique

In Mozambique, maize is the most important food crop, since more than 80% of the population living in rural areas use maize as dominant staple food. Cassava ranks as a distant second (IFAD, 2010). The demand for food is increasing worldwide due to population growth. The sustainable use of limited available natural resources is the crucial point to keep balance between population growth and food production (Bernardo et al., 2000). The production of cultivated maize was reduced from 1982 to 1997 in terms of both number of farmers and yield as Mozambique was facing problems of civil war (Tschirley et al., 1996). Moreover, the growth rate of maize production decreased by 20% in 2000 compared to 1999, because of floods followed by drought stress (FAO, 2000). However, the International Maize and Wheat Improvement Center (CIMMYT) reported an increase in maize production in Mozambique of about 28.5% from the end of the civil war in 2000. The increase in maize production is mainly attributed to the recovery of production from the previous years of drought which affected all provinces in Mozambique (Aquino et al., 2001).

Maize in Mozambique is a relevant food source since it contains nutritionally important grain constituents. It is important to develop new highly productive varieties to assure food security and safety. Evaluation of genetic diversity in maize germplasm is important for identifying genotypes that could be interesting in breeding programs (Berardo et al., 2009) Proper characterization of genetic resources is aimed to help the breeders to identify the most suitable material for the development of new varieties with high yield and high value in terms of oil and protein content. Maize can grow in light (sandy) medium or heavy (clay) soil with good drainage but it requires nitrogen, phosphorus and potassium to thrive (FAO, 2003) Mozambique has a moderate production of 18,780,000 metric tons compared to the major world producers of maize, USA, China, Brazil, Argentina, Mexico and France (FAO, 2010).

In Mozambique, drought is a factor that dramatically limits maize production in low-land areas of the country that are characterized by sandy soils (Verduijn, 2005). Small-holder farmers in Mozambique occupy more than 95% of the maize growing areas and together they produce more than 90% of the total annual production. In these small-holder production systems maize is grown mixed with other crops in small plots (0.3 to 1.3 ha) usually using traditional system and mostly these agro-systems depend on rainfall. The average yield of maize is very low (0.2 to 1.2 t/ha), because the farmers do not use improved seeds or fertilizer (DINA, 1995).

Since the population is constantly increasing we need to conserve genetic diversity for food and environmental security. The farmers have progressively abandoned their traditional varieties and landraces and shifted to more productive modern varieties because of higher

yield, and the number of landraces may decrease and thereby affect the genetic diversity of the crops (Irrondo and Maxted, 2008).

Conserving germplasm in a gene bank is an effective way of preserving large amounts of crop germplasm that may be used by future generations and for future plant breeding. This activity necessarily involves the protection of habitat and ecosystems (Irrondo and Maxted, 2008).

Shifting cultivation (bush fallow) is the traditional land use system in Mozambique. The increasing population is causing a substantial pressure on arable land by reducing the fertility of soils and reducing tree cover in some areas. This has affected the natural resources, because the population encroachment resulted in degazetting of forests.

Private commercial farmers contribute 25% of the marketed maize production. These include capital intensive farms of less than 50 ha (Tschirley et al., 1996).

Farms of 50 to 1000 ha are less capital intensive and produce cereals, meat and fruits for the commercial market. Farms of more than 1000 ha are found in high potential areas and produce industrial and export crops such as cotton, tea, tobacco and sugarcane (Tschirley et al., 1996).

The last category of farmers includes joint venture farms and state farms of up to 40000 ha for export crops (tea, cotton, copra and sugarcane) (da Silva et al., 1996).

1.3. Maize pests and diseases

These problems appear always in Northern Mozambique because of higher rainfall. Also at low-land soil or in areas with periodic drought the farmers can get problems with pests and diseases such as stem borer (*Chilo patellus*), grain weevils (*Sitophilus zea mays* Motsch), termites *(Microtermes* spp), *Fusarium, Diplodia* ear rots, *Helminthosporuim* ssp, downy mildew (*Pernosclerospora sorghi*) and maize streak virus (MSV) (Mariote, 2007).

1.4. Breeding strategy of maize in Mozambique

The maize breeding program of the Agricultural Research Institute of Mozambique (IIAM) is focused on the improvement of maize regarding drought stress, low soil nitrogen uptake, downy mildew, maize streak virus, stem borers and grain weevils in order to obtain more varieties adapted to the conditions in Mozambique. The maize breeding program of IIAM was focused on developing open pollinated varieties until 2000. However, a new activity started in 2002/2003 in order to produce hybrids that are especially adapted to the low-land areas, tolerant to drought and resistant to downy mildew (Fato et al., 2004; Denic et al., 2007; Fato, 2010). Groups of maize populations with different traits as well as quality protein maize (QPM) germplasm were obtained from CIMMYT and the International Institute of Tropical Agriculture (IITA). Maize plants were subjected to different drought conditions. Two different levels of soil fertility were used in two agro-ecological zones; the mid altitude and

low-land (Chauque et al., 2004 and Denic et al., 2012). The achievements up to date are the release of two hybrids in 2008 of which one hybrid was developed by a South African breeder based on a South African inbred line and two inbred lines from Ghana. The second hybrid was developed by the Mozambiqan plant breeders using locally selected material from populations sourced from International Institute of Tropical Agriculture (IITA). A third hybrid from the CIMMYT regional network was released in 2011. Two more hybrids from the same CIMMYT regional network were submitted for release in 2013. A total of twelve open pollinated maize varieties were released by IIAM during the last twelve years (Pedro Fato, personal communication, March 15, 2013).

IIAM has experimental stations in different provinces of Mozambique: the three main experimental sites situated in the Lichinga province in the high lands, in Chokwe in the South, and in Manica in the center of the country.

The IIAM section of technology transfer works with the smallholder farmers sector and cooperative farms in cooperation with the Rural Development Department of the Ministry of Agriculture in transfer of new technologies. The small holder farmers produce 50% of marketed maize in Mozambique. There are problems of extension services and there is a limited distribution of seed, pesticides, and farming tools and these services may even be unavailable some years (Nunes et al., 1986).

1.5. Aim of the study

It has been claimed and shown that Mozambique is a rich country in terms of genetic resources (da Silva et al., 1996). The aim of the study was to:

- Collect maize accessions in the field of farmers in Mozambique for evaluation of genetic diversity.
- Use SSR markers to characterize the accessions.
- Identify duplicates that will allow the gene bank manager to reduce the number of accessions kept in the gene bank.
- Help in the formulation of a new national maize breeding programme for Mozambique in which local germplasm kept at the gene bank or farmers' fields are utilized through pre-breeding activities.

2. Material and methods

2.1. Plant material

Maize seeds used in the study were originally collected from farmers' fields in eight out of the ten agro-ecological zones of Mozambique (Table 1, Figs. 1 and 2). The germplasm was collected according to the procedure of International Plant Genetic Resources Institute (IBPGR, 1991). The representative samples were then processed, assembled and conserved as numbered accessions at the National Gene Bank in Maputo.

Table 1. Main features of the ten agro-ecological zones of Mozambique.

Agro-ecological zones	Province	Altitude (m)	Temperature (°C)	Rainfall (mm)	Humidity Index[1]	Predominant Soils[2]	Production system
R1	Inland Maputo & South Gaza	Major part under 200	20-25	800-1000	Dry Semi-arid, with small humid semi-arid spots in the Libombos heights	Arenosols and Nitosols	Rain-fed cowpea, cassava and maize.
R2	Southern Maputo to Northern Inhambane	0-200	20-25	800-1000	Humid Semi-arid, with some sub-humid spots in the littoral	Arenosols, Fluvisols and Manangas	Mixed cereals, cassava and cashew. Maize, rice, vegetables, banana and sugar-cane.
R3	Centre and North of Gaza and West of Inhambane	100-200	22-26	400-600	Semi-arid and arid	Manangas and Arensols	Maize, sorghum, millet, rice and beans. However, rice and beans grown under irrigation.
R4	Sofala and Manica	200-1000	17.5-22.5	1000-1200	Sub-humid with humid semi-arid	Ferralsols and Luvisols	Maize, sorghum, cassava and cowpea are dominant while sweet potato and rice are cultivated in more moist areas.
R5	Sofala and Zambezia	0-200	24-28	1000-1400	Humid semi-arid	Fluvisol and Arenosols	Rice, cassava, maize and sorghum.

R6	Zambezia valley and Southern Tete	0-200	20-25	Mostly 500-800 with one area 1200 – 1400 and another with water deficit	Dry Semi -arid	Lixisolls and Fluvisols	Sorghum and millet.
R7	Zambezia, Nampula, Tete, Niassa and Cabo Delgado	200-1000	20-25	1000-1400	Humid Semi-arid , with sub-humid.	Ferralsols, Luvisols and Acrisols.	Cassava, maize, cowpea, pigeon pea and sorghum.
R8	Coastal Littoral of Zambezia, Nampula and Cabo Delgado	0-200	Above 25	800-1200	Humid Semi-arid with spots of sub- humid and an extensive dry and semi-arid area.	Lixisols, Leptosols and Arenosols	Cassava and millet. Rain-feed cultivation in low areas, cashew an important crop.
R9	North of Cabo Delgado	200-1500	20-26	1000-1200	Humid and semi-arid.	Nitosols	Maize is the dominant crop, sorghum, cowpea, cassava and sesame are also cultivated. Cashew is an important crop.
R10	Zambezia, Niassa,Tete and Manica	Above 1000	15-22.5	Above 1200	Sub-humid and humid.	Ferrasols and Leptosols	Maize is the dominant crop, commune beans and potatoes, finger millet cultivated.

Source: Programa de investimento em extensao agrarian/Documento de trabalho n°2/B 1ª versao-15 de Junho de 1996(DNER, 1996)

[1]Humidity index: Arid: < 500 mm of precipitation; Dry semi-arid: 500-800 mm, Humid semi-arid 800-1000 mm, Sub-humid, 1000-1400 mm.
[2]Based on FAO soil classification.

Table 2. Maize accessions analyzed in this study.

Accessions number	Name of accession	Agro-ecological zones	Province	Village	Latitude degrees	Longitude degrees	Altitude, m above sea level
Acc1749	Kandjerendgere	R2	Inhambane	Muchai-2	23° 05'	034°22'	67
Acc1750	Gremo	R2	Inhambane	Mabote	23° 26'	034°07'	157
Acc1929	Kenya	R2	Inhambane	Mabote	22°09'	034°07'	157
Acc2274	Chibubane	R2	Inhambane	Mabote	22° 09'	034°07'	157
Acc2324	Mukhambe	R2	Inhambane	Muchai-2	23°05'	034°22'	77
Acc2608	Maguere	R2	Inhambane	Mabote	22° 09'	034°07'	157
Acc1772	Mangunda-2	R3	Gaza	Massingir	23°54'	031°57'	157
Acc2078	Chimwambane	R3	Gaza	Chicualacuala	23° 26'	031°46'	185
Acc2079	Chitsonga	R3	Gaza	Chicualacuala	23° 26'	031°46'	185
Acc1748	Mbuangafe	R4	Manica	Guro	17° 25'	034°21'	709
Acc1792	Munguenda	R4	Manica	Guro	17° 25'	033°21'	709
Acc1809	Munguenda	R4	Manica	Catandica	18° 01'	033°45'	611
Acc1810	Kandjerendgere	R4	Manica	Catandica	18° 01'	033°45'	611
Acc1817	Kandjerendgere	R4	Manica	Barue	16°58'	034°14'	802
Acc2121	Chibubane	R4	Manica	Catandica	18° 01'	033°45'	611
Acc1494	Makolo	R5	Sofala	Buzi	19° 53'	034°35'	7
Acc1939	Xigoia	R5	Sofala	Buzi	19° 53'	034°35'	7
Acc1657	Bantamo	R6	Tete	Moatize	16° 07'	033°44'	370
Acc2201	Xidiwane	R7	Niassa	Mavago	12° 31'	036°18'	720
Acc2513	Metho	R7	Cabo Delgado	Balama	13° 19'	038°35'	499
Acc1269	Kanhangulo	R8	Nampula	Monapo	14° 55'	040°18'	137
Acc2146	Sacana	R8	Cabo Delgado	Muidumbe	11° 49'	039°49'	512
Acc2165	Serena	R8	Cabo Delgado	Macomia	12° 14'	040°14'	330
Acc2482	Kalombe kusho	R8	Cabo Delgado	Mueda	11° 38'	039°37'	618
Acc2497	Lidjele	R8	Cabo Delgado	Muidumbe	11° 49'	039°49'	596
Acc2529	Nthale-wa Rame	R8	Cabo Delgado	Chiure	13° 25'	040°13'	299
Acc1685	Ngogodo	R10	Tete	Ulongwe	14° 42'	034°21'	1300

Table 3. SSR loci used for analysis of genetic diversity.

Primers	Primers sequences (5'-3')	Repeat motif	Alleles range (bp)	Na
Phi041	**F**:TTGGCTCGAAGCGCCGCAAA **R**:GCTTCTGATCCAGAGCGATTTGACGGCA	AGCC	201-221	5
Phi085	**F**:AGCAGAACGGCAAGGGCTAT **R**:GCTTCTTTTGGCACACCACGACGA	AACGC	237-264	9
Phi061	**F**:GACGTAAGCCTAGCTCTGCAT **R**:GCTTCTAAACAAGAACGGCGGTGCTGA	TTCTGTAT	134-190	12
Phi056	**F**:ACTTGCTTGCCTGCGTTAC **R**:GCTTCTCGCACACCACTTCCCAGAA	CCG	237-264	9
Bnlg1194	**F**:GCGTTATTAAGGCAAGCTGC **R**:GCTTCTACGTGAAGCAGAGGATCCAT	AG	161-180	8
Phi127	**F**:ATATGCATTGCCTGGAACTGGAAGGA **R**GCTTCTAATTCAAACACGCCTCCCGAGTGT	AGAC	114-129	4
Bnlg1520	**F**:TCCTCTTGCTCTCCATGTCC **R**:GCTTCTACAGCTGCGTAGCTTCTTCC	AGCT	172-205	9
Phi073	**F**:GTGCGAGAGGCTTGACCAA **R**:GCTTCTAAGGGTTGAGGGCGAGGAA	AGC	189-215	7
Bnlg1523	**F**:GAGCACAGCTAGGCAAAGG **R**:GCTTCTCTCGCACGCTCTCTCTTTCTTT	AG	189-201	6
Phi116	**F**:GCATACGGCCATGGATGGGA **R**:GCTTCTTCCCTGCCGGGACTCCTG	ACTG	148-178	6
Bnlg1484	**F:**GTAAAAGACGACGACATTCG **R**:GCTTCTGACGTGCACTCCGTTTAACA	AG	120-157	9
mean				7.63
St.dev				2.29

Source of primers: Loáisiga et al. (2012)

Na-Number of alleles

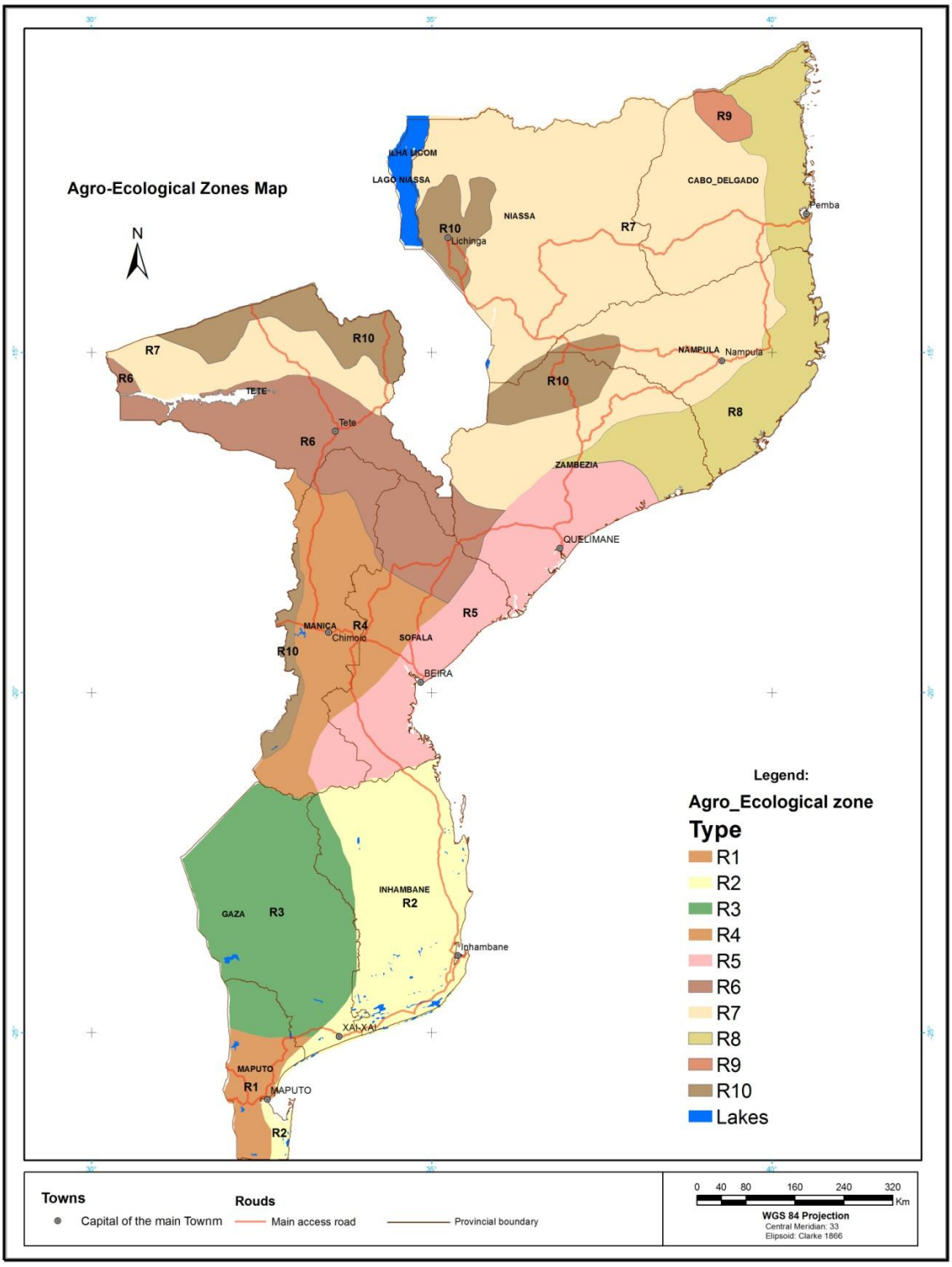


Figure1. Map of the agro-ecological zones of Mozambique

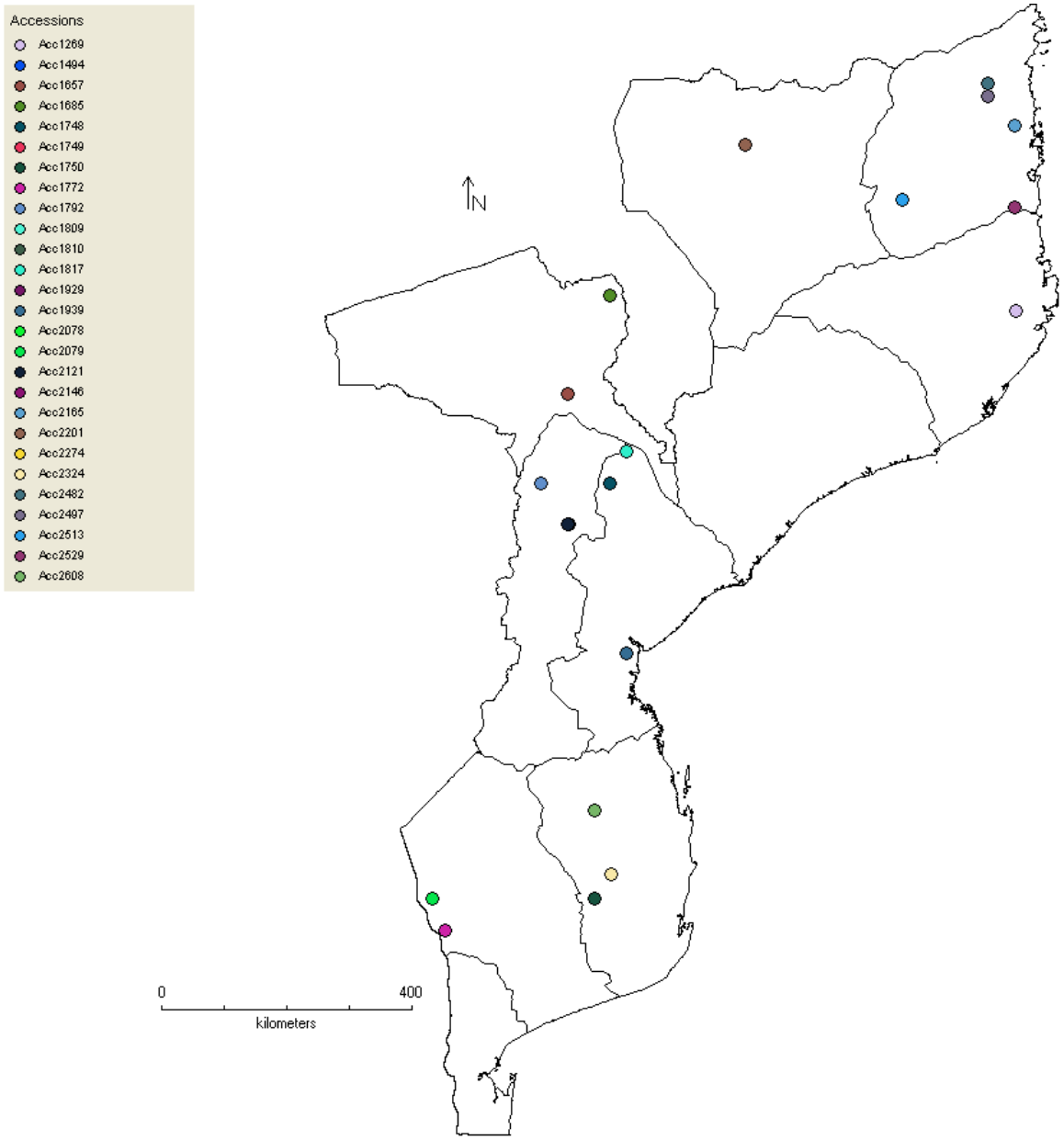


Figure 2. Map of Mozambique showing collection sites of the maize accessions

2.2. DNA extraction

Twenty seeds of each accession were sown in seedling trays in a greenhouse at SLU-Alnarp under a temperature of 25°C. When plants were two weeks old, two leaves per plant were collected from a total of 12 individuals per accession. The leaves were then kept at -80°C until DNA extraction.

DNA was extracted employing a modified CTAB procedure (Doyle and Doyle, 1987). Two leaves were put in a 2 ml Eppendorf tube and frozed in liquid nitrogen. Two metal balls (approximately 4 mm in diameter) were added to each Eppendorf tube. Plant material was ground in a mixer mill (frequency $30s^{-1}$ for 3 min). The finer the grind, the larger the yield. 1 ml of CTAB buffer was pipetted into each tube. The samples were shortly vortexed and then incubated for 1h at 60°C. After incubation, the tubes were put on ice for 5 minutes, and then 1 volume of chloroform/isoamyl alcohol (24:1) was added. The tubes were put into a rotating shaker for 10 minutes. The samples were then centrifuged for 10 minutes at 13 200 rpm in a fume hood. The top aqueous layer was carefully pipetted into new Eppendorf tubes. 1 volume of cold isopropanol (-20°C) was added and gently mixed with the sample. The tubes were placed on ice for 10 minutes and then centrifuged for 10 minutes at 13 200 rpm. The supernatant was removed and 1 ml ice cold (-20°C) 75% ethanol was added to the pellet. Tubes were put on a rotating shaker for 5 minutes and then centrifuged for 3 minutes at 13 200 rpm. The ethanol was removed using a pipette. Again, 1 ml ice-cold 75% ethanol was added and the tubes were put on a rotating shaker for 5 minutes and then centrifuged for 3 minutes at 13 200 rpm. Ethanol was removed and the pellet was left to air dry for about 15 minutes. The pellet was re-suspended in 100 µl of TE buffer and then 4 µl of RNAse (1mg/ml) was added. The samples were incubated in a water bath at 37°C for 30 minutes. In total 27x12=324 samples were extracted. Samples were stored in -20°C until SSR analysis.

2.3. PCR protocol for SSR primers

PCR was performed in a 20 µl reaction volume containing 1x PCR buffer, 0.2 mM dNTP mix, 1.25 mM $MgCl_2$, 0.1 µM primer pairs, 0.75 units Taq polymerase and 15 ng DNA for all DNA samples used in this study. Primers are listed in Table 3.

A Touch-Down PCR amplification program (BioRad S1000 Thermal Cycler) was used:

95°C for 3 min, followed by 10 cycles of 94°C for 30 s, 68°C decreased by 1°C per cycle for 30 s and 72°C for 45 s. This was followed by 40 cycles of 94°C for 45 s, 58°C for 45 s and 72°C for 1 min and a final elongation at 72°C for 8 minutes.

2.4. Statistical analysis

PopGene version 1.31(Yeh and Boyle, 1999) was used to calculate number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), Shannon's information index (I), Nei's estimation of genetic diversity and unbiased heterozygosity (UHe).

The Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) package version 2.1.11 (Rohlf, 2000) was used for cluster analysis and PCoA. Arlequin version 3.5 (Excoffier and Lischer, 2010) was used for analysis of molecular variance (AMOVA). Calculation of Nei's genetic distance was performed using the FreeTree program (Pavlicek et al., 1999).

3. Results

3.1. Molecular analysis

The 11 microsatellite primer pairs used to analyze genetic variation in 27 accessions of maize from 8 agro-ecological zones revealed a total of 84 alleles with an average of 7.63 alleles per locus. The size of the alleles ranged from 114 to 264 base pairs (Table 3). The number of alleles per locus ranged from 4 for Phi127 to 12 for Phi061.

Table 4. Genetic diversity estimators for 11 microsatellite loci across 27 local maize accessions

Locus	Ne	Ho	He	I	Nei	UHe
Phi041	3.57	0.20	0.72	1.38	0.72	0.52
Phi085	2.96	0.34	0.66	1.34	0.66	0.54
Phi061	6.80	0.24	0.86	2.11	0.85	0.67
Phi056	4.12	0.52	0.76	1.66	0.76	0.63
Bnlg1194	3.86	0.68	0.74	1.51	0.74	0.68
Phi127	2.72	0.17	0.63	1.07	0.63	0.48
Bnlg1520	2.69	0.34	0.62	1.46	0.63	0.55
Phi073	3.53	0.57	0.72	1.47	0.72	0.62
Bnlg1523	3.14	0.13	0.68	1.33	0.68	0.55
Phi116	3.36	0.64	0.70	1.39	0.70	0.60
Bnlg1484	1.37	0.10	0.27	0.68	0.27	0.22
Mean	**3.47**	**0.36**	**0.67**	**1.40**	**0.67**	**0.55**
St.dev	1.33	0.21	0.15	0.35	0.15	0.13

Ne-Effective number of alleles
Ho-Observed heterozygosity
He-Expected heterozygosity
I-Shannon information index
Nei-Estimation of genetic diversity
UHe- Unbiased heterozygosity

The effective number of alleles (Ne) ranged from 1.37 (Bnlg1484) to 6.80 (Phi061) with an average of 3.47 across the 11 primers (Table 4). The values for observed heterozygosity (Ho) ranged from 0.10 (Bnlg1484) to 0.68 (Bnlg1194) with an average of 0.36. Expected heterozygosity (He) ranged from 0.27 (Bnlg1484) to 0.86 (Phi 061) with an average of 0.67. Shannon's information index (I) ranged between 0.68 (Bnlg1484) and 2.11 (Phi061) with an average of 1.40. Estimation of genetic diversity (Nei) ranged from 0.27 (Bnlg1484) to 0.85 (Phi061) with an average of 0.67. Unbiased heterozygosity (UHe) ranged from 0.22 (Bnlg1484) to 0.68 (Bnlg1194) with an average of 0.55.

Table 5. Genetic diversity in 27 accessions of maize from 8 agro-ecological zones.

Accession number	Name of accession	Ecological zone	Ne	Ho	He	I	Nei	%P
Acc1749	Kandjerendgere	R2	2.87	0.36	0.65	1.15	0.62	100
Acc1750	Gremo	R2	2.15	0.36	0.49	0.85	0.46	100
Acc1929	Kenya	R2	2.33	0.41	0.52	0.82	0.50	90.91
Acc2274	Chibubane	R2	2.57	0.30	0.58	0.98	0.55	90.91
Acc2324	Mukhambe	R2	2.80	0.33	0.63	1.12	0.59	100
Acc2608	Maguere	R2	2.86	0.36	0.64	1.15	0.61	100
Acc1772	Mangunda-2	R3	2.95	0.35	0.63	1.17	0.60	100
Acc2078	Chimwambane	R3	2.32	0.25	0.52	0.89	0.49	90.91
Acc2079	Chitsonga	R3	2.45	0.36	0.56	0.96	0.59	100
Acc1748	Mbuangafe	R4	2.29	0.37	0.49	0.89	0.47	100
Acc1792	Munguenda	R4	2.46	0.40	0.57	0.96	0.54	100
Acc1809	Munguenda	R4	2.91	0.42	0.64	1.10	0.60	100
Acc1810	Kandjerendgere	R4	3.21	0.40	0.69	1.23	0.66	100
Acc1817	Kandjerendgere	R4	2.09	0.32	0.52	2.09	0.49	100
Acc2121	Chibubane	R4	2.83	0.40	0.64	1.14	0.61	100
Acc1494	Makolo	R5	2.63	0.32	0.58	0.97	0.54	100
Acc1939	Xigoia	R5	2.15	0.30	0.46	0.78	0.44	90.91
Acc1657	Bantamo	R6	2.67	0.48	0.61	1.07	0.58	100
Acc2201	Xidiwane	R7	2.63	0.33	0.59	1.02	0.55	100
Acc2513	Metho	R7	2.22	0.26	0.52	0.89	0.50	100
Acc1269	Kanhangulo	R8	2.48	0.42	0.60	0.99	0.58	100
Acc2497	Lidjele	R8	2.96	0.35	0.64	1.15	0.61	100
Acc2146	Sacana	R8	2.70	0.31	0.64	1.10	0.61	100
Acc2165	Serena	R8	2.06	0.32	0.48	0.78	0.45	90.91
Acc2482	Kalombe kusho	R8	2.87	0.35	0.66	1.16	0.63	100
Acc2529	Nthale-wa Rame	R8	2.48	0.38	0.53	0.94	0.50	90.91
Acc1685	Ngogodo	R10	2.48	0.35	0.57	0.95	0.54	100
Mean			**2.56**	**0.35**	**0.58**	**1.04**	**0.55**	**97.98**
St. Dev.			0.29	0.04	0.06	0.23	0.06	3.71

Ne-Effective number of alleles

Ho-Observed heterozygosity

He-Expected heterozygosity

I-Shannon information index

Nei-Estimation of genetic diversity

%P-Percentage of polymorphic loci

3.2. Genetic structure

For the 27 accessions the effective number of alleles (Ne) ranged from 2.06 (Acc2165) to 3.21 (Acc1810) with an average of 2.56 (Table 5). The observed heterozygosity (Ho) across the eleven loci ranged from 0.25 (Acc2078) to 0.48 (Acc1657) with an average value of 0.35. Ten of the accessions scored less than the mean of observed heterozygosity, while accessions 1685, 2482, 2497 and 1772 recorded the same observed heterozygosity as the mean of all accessions.

The highest value for expected heterozygosity (He) was found in accession number 1810 (0.69) and the lowest one was recorded for accession 1939 (0.46). The average value was 0.58.

The lowest Shannon's information index (I), 0.78 was recorded for both accession 2165 and accession 1939 and the highest, 2.09 was found in accession 1817.

The highest value for estimation of genetic diversity (Nei's) was shown by accession 1810 and the lowest was detected in accession number 1939.

The percentage of polymorphic loci (%P) ranged from 90.91 to 100 with accessions 2529, 2165, 1939, 2274, 2078 and 1929 recording the lowest percentage thereby lowering the mean for all accessions to 97.98 %.

The analysis of molecular variance (AMOVA) revealed significant differentiation among the ungrouped 27 maize accessions (P=0.00; Table 6), however, most of the variation (88.28%) was found within accessions. There was no differentiation among accessions when they were grouped according to agro-ecological zones.

Nei's genetic distance between pairs of the 27 accessions ranged from 0.124 between accessions 1772 and 2324 to 0.646 between accession 1929 and accession 1269 (Table 7).

Table 6. Analysis of molecular variance (AMOVA) for 27 maize accessions from Mozambique based on SSR data (A) without grouping the accessions (B) by grouping accessions based on eight agro-ecological zones.

Groups	Source of variation	d.f.	Variance component	% of variation	Fixation index	p-value
(A) **ungrouped**	Among accessions	26	Va = 0.332	11.72	FST = 0.1172	0.00
	Within accessions	537	Vb = 2.503	88.28		
	Total	**563**	**2.836**			
(B) **agro zones**	Among groups	7	Va = – 0.001	– 0.04	FST = 0.1171	0.00
	Among accessions within groups	19	Vb = 0.333	11.76	FSC = 0.1175	0.00
	Within accessions	537	Vc = 2.503	88.28	FCT = – 0.0003	0.49
	Total	**563**	**2.836**			

FST = the degree of gene differentiation among accessions in terms of allele frequencies

FSC = the deficiency or excess of average heterozygotes in each accessions

FCT = the deficiency or excess of average heterozygotes in a group of accessions

d.f.= degrees of freedom

Table 7. Nei's genetic distance between the 27 maize accessions from Mozambique.

	Acc2146	Acc1750	Acc1939	Acc1810	Acc2497	Acc2274	Acc2078	Acc1749	Acc1657	Acc2482	Acc1809	Acc1772	Acc2165	Acc1494	Acc1269	Acc1817	Acc1685	Acc2324	Acc2079	Acc2513	Acc1748	Acc2608	Acc2529	Acc2201	Acc2121	Acc1792	Acc1929
Acc2146																											
Acc1750	0.314																										
Acc1939	0.341	0.422																									
Acc1810	0.294	0.294	0.414																								
Acc2497	0.181	0.249	0.376	0.227																							
Acc2274	0.316	0.440	0.372	0.336	0.277																						
Acc2078	0.248	0.544	0.331	0.416	0.359	0.230																					
Acc1749	0.254	0.302	0.387	0.319	0.264	0.384	0.443																				
Acc1657	0.205	0.262	0.447	0.307	0.207	0.298	0.350	0.302																			
Acc2482	0.361	0.480	0.586	0.367	0.351	0.617	0.632	0.430	0.290																		
Acc1809	0.177	0.397	0.410	0.367	0.243	0.341	0.412	0.388	0.307	0.475																	
Acc1772	0.198	0.205	0.294	0.218	0.192	0.296	0.342	0.284	0.202	0.409	0.250																
Acc2165	0.288	0.371	0.381	0.310	0.259	0.440	0.421	0.343	0.237	0.474	0.281	0.146															
Acc1494	0.160	0.292	0.321	0.256	0.214	0.408	0.351	0.289	0.193	0.345	0.341	0.190	0.302														
Acc1269	0.315	0.279	0.437	0.321	0.219	0.404	0.575	0.343	0.280	0.414	0.301	0.232	0.218	0.383													
Acc1817	0.466	0.419	0.377	0.479	0.447	0.449	0.337	0.472	0.300	0.632	0.593	0.334	0.389	0.480	0.494												
Acc1685	0.224	0.423	0.424	0.319	0.222	0.383	0.332	0.417	0.248	0.545	0.202	0.174	0.140	0.268	0.316	0.397											
Acc2324	0.195	0.262	0.273	0.273	0.193	0.254	0.292	0.251	0.207	0.420	0.217	**0.124**	0.157	0.190	0.228	0.392	0.174										
Acc2079	0.151	0.392	0.493	0.379	0.291	0.279	0.331	0.344	0.194	0.563	0.291	0.315	0.551	0.273	0.474	0.495	0.306	0.353									
Acc2513	0.254	0.399	0.381	0.389	0.321	0.242	0.221	0.311	0.223	0.575	0.349	0.295	0.276	0.291	0.413	0.380	0.319	0.199	0.355								
Acc1748	0.309	0.409	0.399	0.317	0.367	0.576	0.550	0.232	0.315	0.518	0.609	0.357	0.399	0.155	0.451	0.550	0.474	0.354	0.349	0.384							
Acc2608	0.227	0.241	0.385	0.267	0.181	0.406	0.313	0.308	0.336	0.422	0.440	0.221	0.407	0.246	0.328	0.519	0.351	0.265	0.397	0.376	0.363						
Acc2529	0.257	0.439	0.511	0.297	0.220	0.321	0.521	0.291	0.331	0.469	0.272	0.284	0.206	0.390	0.193	0.624	0.285	0.280	0.394	0.393	0.421	0.460					
Acc2201	0.182	0.341	0.428	0.342	0.225	0.233	0.274	0.344	0.200	0.514	0.203	0.299	0.388	0.292	0.281	0.443	0.289	0.205	0.162	0.256	0.464	0.392	0.277				
Acc2121	0.277	0.286	0.464	0.311	0.155	0.208	0.351	0.265	0.341	0.563	0.294	0.304	0.516	0.407	0.351	0.543	0.357	0.298	0.284	0.370	0.583	0.264	0.367	0.272			
Acc1792	0.278	0.299	0.446	0.241	0.202	0.317	0.285	0.264	0.342	0.447	0.258	0.254	0.380	0.363	0.332	0.426	0.260	0.270	0.339	0.347	0.532	0.221	0.338	0.264	0.195		
Acc1929	0.279	0.509	0.470	0.571	0.439	0.544	0.339	0.325	0.339	0.450	0.493	0.330	0.460	0.296	**0.646**	0.554	0.514	0.407	0.459	0.454	0.519	0.447	0.643	0.498	0.512	0.558	

Table 8. Comparison of the alleles range (bp) found in this study with previous studies.

SSR locus	[1]Hoxha et al. (2004)	[2]Yuan et al. (2004)	[3]Zhang et al. (2003)	[4]Senior et al. (1998)	[5]Wang et al. (2011)	[6]This study
Phi041			195-213		296-334	201-221
Phi085	233-260	70-95		70-95		237-264
Phi061				80-88		134-190
Phi056		84-93		84-93		237-264
Bnlg1194	137-217					161-180
Phi127		112-128	111-126	112-138		114-129
Bnlg1520					156-204	172-205
Phi073		90-99		90-99		189-215
Bnlg1523					183-263	189-201
Phi116	148-174		150-168	154-173	240-262	148-178
Bnlg1484						120-157

[1]Used 20 Albanian local open pollinated varieties.

[2]Used 15 inbred lines.

[3]Used 45 public inbred lines.

[4]Used 94 inbred lines.

[5]Used 231 inbred lines.

[6]Used 27 accessions of farmers' varieties.

Table 9. Comparison of the number of alleles found in this study with previous studies.

SSR locus	[1]Enoki et al. (2002)	[2]Jambrovic et al. (2008)	[3]Senior et al. (1998)	[4]Xu et al. (2004)	[5]Wang et al. (2011)	[6]This study
Phi041					6	5
Phi085			5			9
Phi061		2	3			12
Phi056	4		4	3		9
Bnlg1194		5				8
Phi127	4		5	4		4
Bnlg1520	9	3			6	9
Phi073			4			7
Bnlg1523	8	4			17	6
Phi116			7		5	6
Bnlg1484				3		9

[1]Used 65 inbred lines.

[2]Used 15 inbred lines.

[3]Used 94 inbred lines.

[4]Used 15 inbred lines.

[5]Used 231 inbred lines.

[6]Used 27 accessions of farmers' varieties.

Table 10. Comparison of the genetic diversity found in this study with previous studies.

SSR locus	[1]Kashiani et al. (2012)	[2]Rupp et al. (2009)	[3]Xiang et al. (2010)	[4]Qi-Lun et al. (2007)	[5]Camus-Kulandaivelu et al. (2006)	[6]This study
Ne	1.07	2.16	1.47	3.9		2.56
Ho	0.06	0.17		0.37		0.35
He	0.67	0.51		0.69		0.58
I	0.05		0.40			1.04
Nei's	0.04		0.27		0.62	0.55
%P		93.33	74.18			97.88

[1]Used 13 tropical near-homogenous lines and 99 SSR markers.

[2]Used 15 progenies and 113 SSR markers.

[3]Used 22 maize landraces and 44 SSR markers.

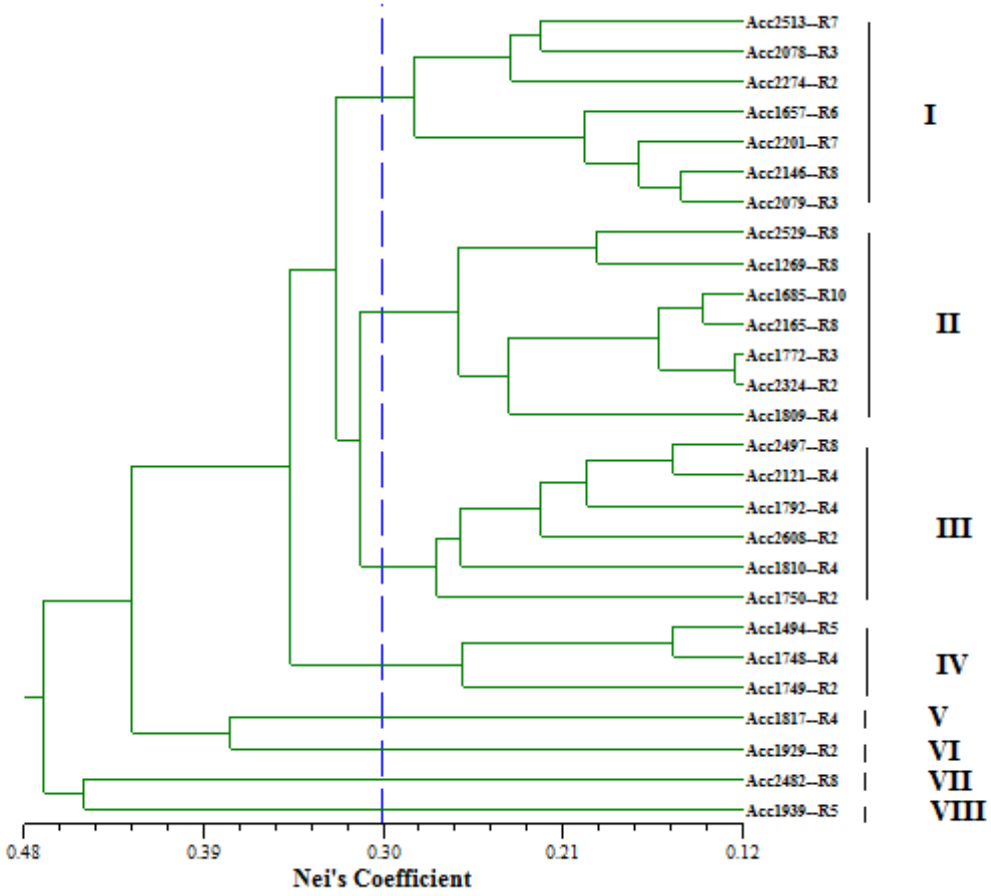
[4]Used 54 maize landraces and 42 SSR markers.

[5]Used 131 maize landraces and 18 SSR markers.

[6]Used 27 accessions of farmers' varieties and 11 SSR markers.

3.2.1. Cluster analysis

The UPGMA dendrogram of the 27 accessions of local maize from Mozambique based on Nei's genetic distance (Fig. 3) showed four clusters with multiple accessions and four single accession clusters at the 0.30 Nei's coefficient level. The first cluster was made up of two accessions from agro-ecological zone 7, two from agro-ecological zone 3 and one each from agro-ecological zone 2, 6 and 8, respectively. The second cluster was made up of 7 accessions of which three (Acc2529, Acc1269 and Acc2165) belonged to agro-ecological zone 8 and the remaining 4 were collected in zones 2, 3, 4 and 10, respectively. Accessions 2121, 1792 and 1810 belonging to agro-ecological zone 4 constituted half of the number of the accessions of the third cluster, whereas the remaining three accessions originated from zones 2 and 8. The 4th cluster was made up of accessions 1494, 1748 and 1749 belonging to agro-ecological zones 4, 5 and 2, respectively. The remaining four clusters were made up of single accessions 1817 from zone 4, 1929 from zone 2, 2482 from zone 8 and 1939 from zone 5. Accessions 2482 and 1939 were the most distantly related to the other accessions.



.

Figure 3. UPGMA dendrogram of 27 accessions of local maize from Mozambique based on Nei's genetic distance

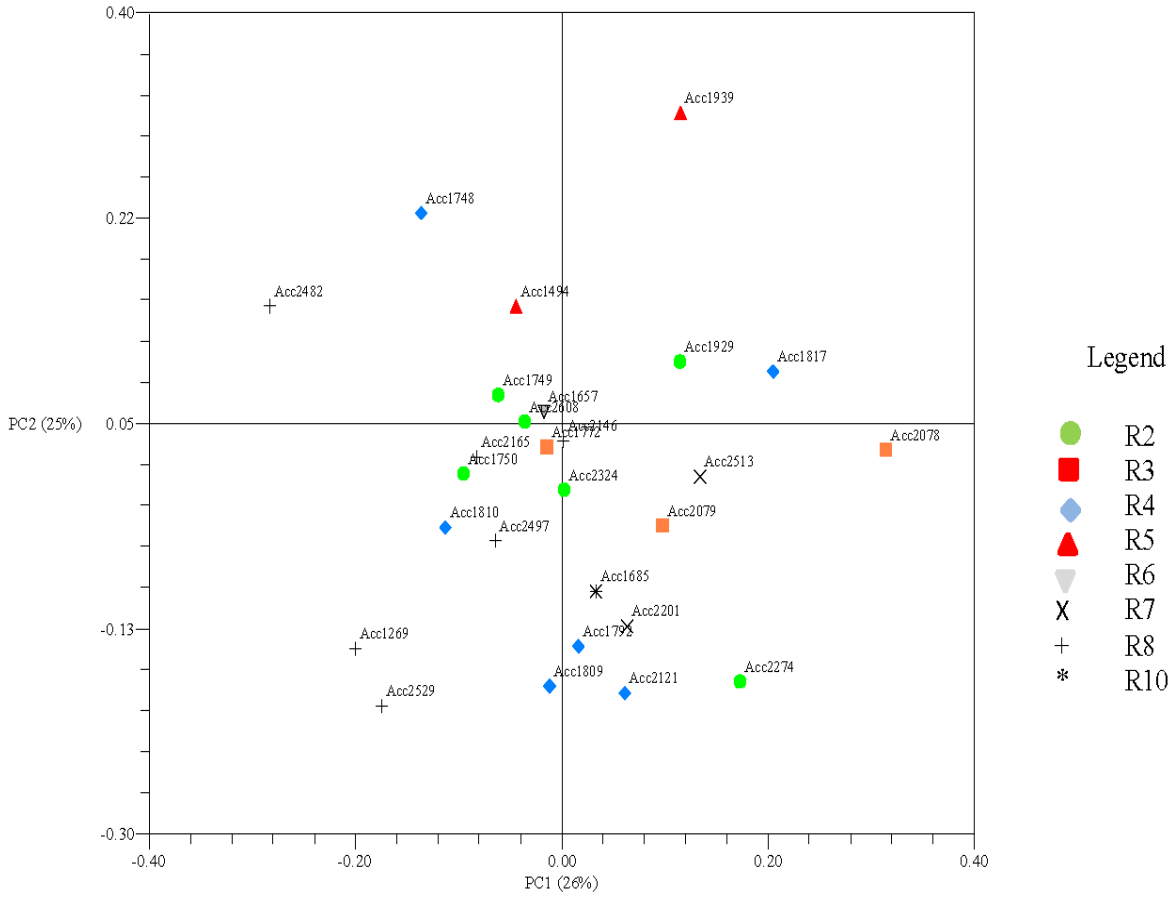


Figure 4. A two-dimensional plot of principal co-ordinate analysis of 27 accessions from 8 agro-ecological zones in Mozambique based on Nei's genetic distance

3.2.2. Principal co-ordinate analysis (PCoA)

Components PC1 and PC2 explained 26% and 25% of the total variation, respectively. Thus 51% of the total variation is explained by PC1 and PC2 together (Figure 4). The results indicated that most accessions lie near to the centre except accession 2078, 1939 and 2482. No clear grouping based on agro-ecological zones was found.

4. Discussion

The use of microsatellites for characterization of maize (*Zea mays* L.) and other cultivated crops is well documented (Enoki et al., 2002).

The eleven microsatellite markers used in this study were capable of differentiating among the twenty-seven accessions of the local maize originating from eight agro-ecological zones in Mozambique. The fragment sizes of the different alleles were smaller for Phi041 than what was reported by Wang et al. (2011), for Bnlg1194 than what was reported by Hoxha et al. (2004) and for Bnlg1523 than what was reported by Wang et al. (2011) (Table 8). The allele sizes detected for Phi085 were higher than what was reported by Yuan et al. (2004), Senior et al. (1998) and Hoxha et al. (2004). The range and allele size for locus Phi061 was larger than that reported by Senior et al. (1998). Although the allele size for locus Phi127 was smaller than that reported by Senior et al. (1998), it was higher than the results of Yuan et al.(2004), and Zhang et al. (2003). The range of allele size of locus Phi116 was lower than that reported by Wang et al. (2011) but higher than what was found by Zhang et al. (2003), Senior et al. (1998) and Hoxha et al. (2004). The number of alleles recorded for locus Phi041 was lower than that reported by Wang et al. (2011) (Table 9). The number of alleles for loci Phi085, Phi061, Phi056, Bnlg1194, Phi073 and Bnlg1484 detected in this study were higher than those reported by Senior et al.(1998), Jambrovic et al. (2008), Xu et al.(2004), Enoki et al. (2002) and Xu et al. (2004). The number of alleles found for Phi127 in this study was similar to previous results by Enoki et al. (2002) and Xu et al. (2004) but lower than those reported by Senior et al. (1998). The number of alleles for locus Bnlg1520 was similar to the results obtained by Enoki et al. (2002) but higher than those reported by Wang et al. (2011) and Jambrovic et al. (2008). The number of alleles detected in this study for locus Bnlg1523 was lower than those reported by Enoki et al. (2002), and Wang et al. (2011) but higher than what was found by Jambrovic et al. (2008). The detected number of alleles for Phi116 was lower than that reported by Senior et al. (1998) but higher than the results of Wang et al. (2011).

For the loci Phi041 and Phi127, the effective number of alleles (Ne), the observed heterozygosity (Ho) and the expected heterozygosity (He) were lower than those reported by Qi-Lun et al. (2007). In a study involving 124 maize landraces and 46 loci, Sharma et al. (2010) reported an effective number of alleles (Ne) of 2.69 and an expected heterozygosity (He) of 0.62 for locus Phi041, which are lower than our values. They also reported a Ne value of 3.22 and an expected heterozygosity (He) of 0.62 for locus Phi127. In the present study Ne, Ho, He, I and Nei of locus Bnlg1520 were all higher than those reported by Kashiani et al. (2012). This is probably due to the fact that they used 99 microsatellite markers for 13 tropical sweet corn inbred lines while we used 27 accessions of farmers' varieties and 11 SSR markers.

A comparison of genetic diversity estimates (Table 10) showed that the mean effective number of alleles (Ne) in this study was 2.56 and higher than what was reported by Kashiani et al. (2012) in tropical sweet corn lines (Ne=1.07), by Rupp et al. (2009) in 15 sweet corn varieties (Ne=2.16) and by Xiang et al. (2010) (Ne=1.47) but lower than the 3.9 reported by Qi-Lun et al. (2007).

The mean observed heterozygosity (Ho) in this study (0.35) was higher than the mean value reported by Kashiani et al. (2012) and by Rupp et al. (2009) but lower than the mean value of 0.37 reported by Qi-Lun et al. (2007). In the present study the expected heterozygosity (He) was lower than He=0.67 found by Kashiani et al. (2012) and He=0.69 reported by Qi-Lun et al. (2007), but higher than He=0.51 reported by Rupp et al. (2009). Since the observed heterozygosity (Ho) was lower than the expected heterozygosity (He) in all our analysed accessions (Table 5) this indicates that the populations from which the accessions were collected might have been more isolated and received less external gene flow (Relethford and Blangero, 1990).

The Shannon information index (I) and estimation of genetic diversity (Nei) in the present study were higher than the values found by Kashiani et al. (2012) and by Xiang et al. (2010). However, Camus-Kulandaivelu et al. (2006) reported of a somewhat higher genetic diversity value (Nei=0.62). The percentage of polymorphic loci (%P) in the present study was higher than that reported by Rupp et al. (2009) and Xiang et al. (2010).

The SSR analysis conducted in the present study showed a high degree of diversity of local maize populations. Although the variation within and among accessions was very high, the differentiation between accessions when grouped according to agro-ecological zones was insignificant and showed negative value. The high diversity within and among accessions is expected in open pollinating farmers' varieties that are distinct from each other. The negative value for differentiation between accessions when grouped under agro-ecological zones is an evidence of larger differences among and within accessions than between the groups of accessions. Louette et al. (1997) and Gómez et al. (2000) as quoted by Hoxha et al. (2004) argued that for open pollinated crops such as maize, diversity within a given variety is often the result of deliberate replacement, exchange or mixing of seed by farmers.

5. Conclusions

The present study showed that there is a high genetic diversity within and among local maize accessions originating from farmers' varieties in eight agro-ecological zones in Mozambique. However, the differentiation between agro-ecological zones was non-significant.

The SSR molecular markers used in this study were highly polymorphic and revealed differences among the maize accessions.

This is one of the first studies involving molecular markers analysis of farmers' varieties from Mozambique. Thus, similar studies would be valuable to characterize the collections kept at the National Gene Bank of Mozambique.

The detection of high genetic diversity in the twenty-seven accessions used in this study should be an incentive to plant breeders in Mozambique to include local farmers' varieties in the breeding programme of IIAM.

This study was very important, since existing genetic variability can be used for identification of new sources of germplasm with special traits, which could be crossed with the existing varieties to give rise to novel gene and trait combinations.

The absence of differentiation among groups of accessions originating from various agro-ecological zones indicate that there is a need of further investigation involving more accessions and molecular markers in combination with morphological characterization.

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