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Full Length Research Paper

Molecular genetic variation in the African wild rice *Oryza longistaminata* A. Chev. et Roehr. and its association with environmental variables

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Molecular markers, complemented by appropriate Geographical Information System (GIS) software packages are powerful tools in mapping the geographical distribution of genetic variation and assessing its relationship with environmental variables. The objective of the study was therefore to investigate the relationship between genetic diversity and eco-geographic variables using *Oryza longistaminata* as a case study. The methodology used was a novel technique that combined hierarchical cluster analysis of both molecular diversity generated using Amplified Fragment Length Polymorphism (AFLP) and climate data available in a GIS software. The study clearly established that there is a close relationship between genetic diversity and eco-geographic variables. The study also revealed that genetic diversity is a function of annual rainfall, and peak diversity occurs in intermediate rainfall areas reflecting the 'curvilinear theory' of clinal relationship between the level of genetic diversity and rainfall. The clear association of genetic diversity with rainfall allows the extrapolation of the potential impacts of global warming on diversity when empirical data on predicted climate models, particularly rainfall, are available. This knowledge would therefore be useful in the development of conservation measures to mitigate the effects of genetic erosion through climate change.

Key words: Genetic variation, molecular diversity, AFLP, GIS, eco-geographical distribution, environmental variables.

INTRODUCTION

Plant genetic resources are a key component of global biodiversity from which humanity derives most of its food and other essential goods and services. The conservation and sustainable utilization of this vital biological heritage is therefore imperative and paramount. It involves not only the management of natural habitats and wild plants but also safeguarding the genetic diversity of cultivated and domesticated species and their wild relatives (Kiambi and Atta-Krah, 2002; Nnadozie et al., 2003). This diversity is currently facing eminent threat and heavy pressures from both environmental and socio-economic factors. For instance, in recent years, there has been an increased emergence of empirical evidence and awareness on the implications that global warming might have for climate patterns and the resulting changes in not only the composition of ecosystems worldwide but also the patterns of genetic diversity, resilience and distribution of species (Parmesan and Yohe, 2003; Jutro, 1991). Climate change is now a global issue and raises serious conservation concerns, as it has led to numerous shifts in the distribution, abundance and extinction of species (Thomas et al., 2004; Williams et al., 2003; Pounds et al., 1999). This calls for an in-depth understanding of not only the patterns of distribution of intra-specific genetic variation but also its relationship with the ever changing environmental factors, as this is critical in increasing sampling efficiency, resulting in a wider representation of diversity conserved *ex situ* in gene banks and *in situ* as genetic reserves (Schoen and Brown, 1993).

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One of the main prerequisites in conservation and sustainable utilization of genetic resources is the detailed understanding of the extent and distribution of the genetic diversity available in a given taxon, as this ensures that maximum variation is captured during sampling (Dawson et al., 1995). With the rapid generation and availability of environmental and ecological databases, predictive models for climate change and patterns of genetic variation in natural populations according to their geographic origin have emerged (Pearson and Dawson 2003; Ferguson et al., 1998). The models have proved to be even more powerful in development of conservation strategies when molecular markers are used in combination with Geographical Information Systems tools to map the spatial distribution of genetic variation. Ferguson et al. (1998) used RAPD, complemented with GIS, to map the geographical distribution of genetic variation in four wild relatives of *Lens*. In their study, areas with high and unique diversity were identified and conservation measures proposed. Using AFLP and GIS, Sawkins et al. (1999) mapped the geographic distribution of intra-specific variation in two *Stylosanthes* species in South America. Parsons et al. (1999) have used hierarchical cluster analysis of RAPD markers in combination with GIS to visualize the spatial distribution of rice genetic diversity across Bangladesh.

The notion that the distribution of genetic variation is influenced by or associated with environmental variables including climate (particularly rainfall and temperature), soils, vegetation and elevation has been well established by Nevo et al. (1979) and Parsons et al. (1999). Using isozymes and Rapid Amplified Polymorphic DNA (RAPD) respectively, they reported the distribution of diversity along a rainfall gradient while Kark et al. (1999) evaluated genetic diversity across an ecological gradient. Patterns of phenotypic diversity in barley have been reported to be closely associated with climatic and other ecogeographic variables including altitude, temperature and rainfall (Negassa, 1985; Demissie and Bjornstad, 1997).

Oryza longistaminata belongs to the AA genome group of the sativa complex and hybridizes easily with the cultivated species, *Oryza glaberrima* and *O. sativa* (Bezançon et al., 1977; Vaughan, 1989). The species is allogamous, has a high rate of self-incompatibility and is thus predominantly outcrossing (Ghesquiere, 1987; Jones et al., 1996). *O. longistaminata* is widely distributed in the study area and is normally found in a wide range of wetland habitats including flood plains, riverbanks and shallow waters in pans, mopane woodlands and savanna wooded grasslands. It is also a common weed in cultivated rice fields and water canals (Vaughan, 1994). The objective of the present study is to develop a methodology for determining and prioritizing areas of high intra-specific diversity that could be targeted for *ex situ* and *in situ* conservation, by mapping spatial distribution of molecular diversity in *O. longistaminata* and investigating its relationship with environmental variables.

MATERIALS AND METHODS

Plant material

Following ecogeographical surveys, seed was collected from Kenya, Zimbabwe, Mozambique and Tanzania in collaboration with the national programs. The collecting missions were undertaken under the auspices of a collaborative programme involving the national PGR programmes of the region, the International Rice Research Institute (IRRI), SADC Plant Genetic Resources Centre (SPGRC), and the International Plant Genetic Resources Centre (IPGRI). The national programmes collaborators included the National Gene Bank of Kenya (NGBK), the Department of Research and Seed Services (DRSS, Zimbabwe), Instituto Nacional de Investigação Agrição (INIA, Mozambique), National Plant Genetic Resources Centre (NPGRC, Zambia), National Botanical Institute (NBI, Namibia) and the National Plant Genetic Resources Centre (NPGRC, Tanzania). Further seed and leaf samples originating from Botswana, Namibia, Mozambique, Madagascar and Zambia were obtained from the Gene Bank of the International Rice Research Institute. The full passport data for seed collections are available from the respective national programs and at the International Rice Research Institute, where safety duplicates have been conserved. A summary of the passport data is provided in Table 1.

Leaf material was harvested fresh, frozen and stored at -70^{o}C. 48 populations of *O. longistaminata* were selected for analysis to represent the geographical range of the species. For material received as seeds, young leaves were harvested from 2 week-old seedlings of each accession for DNA extraction.

DNA extraction and AFLP analysis

The protocol for isolation of genomic DNA developed by Gawel and Jarret (1991) was followed with modifications, using 20 mg of leaf material from 5 - 10 seedlings. AFLP analysis was carried out using the methodology of Virk et al. (2000), which is based on the protocol described by Vos et al. (1995). *Eco*RI from Pharmacia and *Mse*I from New England Biolabs, Inc. were used to restrict the genomic DNA by incubating the mix for 1 h at 37^{0}C. The genomic DNA was then ligated to *Eco*RI and *Mse*I adaptors by incubating the mix at 37^{0}C for 3 h. Pre-amplification was carried out using *Eco*RI-(EP; 5'-GACTGCGTACCAATTCA-3') and *Mse*I-(MP; 5'-GATGAGTCCTGAGTAAC-3') adapter-directed primers each having a single selective base (Vos et al., 1995). Pre-amplification was run for 2 h in a Hybaid Omnigene Thermocycler and the products then diluted 50 times. Amplification was carried out using four primers from Operon Technologies Inc. (Table 2). The *Eco*RI primers possessed three selective bases at the 3' end and were end-labeled with [γ-^{33}P] ATP using T4 polynucleotide kinase (TPNK–Pharmacia). The samples were denatured at 90^{o}C for 1 min and 2 μL of the mix was loaded on to a 5% denaturing polyacrylamide gel. The gel was cast in a Sequigen 38 x 50 cm gel apparatus (Bio-Rad) and electrophoresis run for 2 h at a constant temperature of 50^{0}C. It was then dried and exposed to a Kodak Biomax film for 4 - 5 days.

Statistical analysis

Polymorphic bands (176) for the 48 populations were scored as present (1) or absent (0). An assumption was made that the co-migrating fragments in different populations are allelic and map to similar genomic regions (Rouppe van der Voort et al., 1997; Waugh et al., 1997; Virk et al., 2000; Kiambi et al., 2005).

A dendrogram revealing the cluster analysis was generated using Jaccard's Coefficient and Unweighted Paired Group Method with Arithmetic mean (UPGMA) (NTSYS-pc Version 2.0; Rolf, 1993).

Table 1. Passport data of populations used in the study.

Population	Accession No.	Country of origin	Source	Latitude	Longitude	Rainfall (mm) at collecting site
104100BOT	104100	Botswana	IRRI	-19.967	23.417	454
020KEN	GBK0020	Kenya	NGBK	-4.459	39.478	1365
027KEN	GBK0027	Kenya	NGBK	-4.441	39.607	1240
028KEN	GBK0028	Kenya	NGBK	-4.535	39.379	1370
060KEN	GBK0060	Kenya	NGBK	-2.431	40.202	878
063KEN	GBK0063	Kenya	NGBK	-2.458	40.192	969
065KEN	GBK0065	Kenya	NGBK	-2.485	40.228	845
071KEN	GBK0071	Kenya	NGBK	-2.510	40.296	1140
105262KEN	105262	Kenya	IRRI	-2.500	40.283	940
104976KEN	104976	Kenya	IRRI	-2.517	40.467	849
104977KEN	104977	Kenya	IRRI	-2.517	40.450	802
T5021MAD	T5021	Madagascar	IRRI	-17.833	48.167	1226
T5022MAD	T5022	Madagascar	IRRI	-17.750	48.583	1240
101436MAD	101436	Madagascar	IRRI	-16.800	47.250	1757
T7736MOZ	MZ7736	Mozambique	INIA	-14.898	40.905	874
T7737MOZ	MZ7737	Mozambique	INIA	-14.918	40.392	1025
T7738MOZ	MZ7738	Mozambique	INIA	-15.003	40.510	1005
T7744MOZ	MZ7744	Mozambique	INIA	-16.204	39.915	1058
T7745MOZ	MZ7745	Mozambique	INIA	-16.522	39.337	1140
T7746MOZ	MZ7746	Mozambique	INIA	-17.687	36.884	1192
T7747MOZ	MZ7747	Mozambique	INIA	-17.644	36.846	1199
T7748MOZ	MZ7748	Mozambique	INIA	-17.627	37.258	1304
T7749MOZ	MZ7749	Mozambique	INIA	-17.475	37.308	1311
T7750MOZ	MZ7750	Mozambique	INIA	-17.633	37.233	1307
T7347NAM	7347NM	Namibia	NBI	-17.974	21.304	532
T7349NAM	7349NM	Namibia	NBI	-17.950	21.017	529
T7352NAM	7352NM	Namibia	NBI	-17.884	20.504	537
T7353NAM	7353NM	Namibia	NBI	-17.836	19.107	534
T7355NAM	7355NM	Namibia	NBI	-18.052	21.439	533
NL3TZA	NL003TZ	Tanzania	NPGRC	-5.883	35.183	587
NL21TZA	NL021TZ	Tanzania	NPGRC	-8.683	34.283	715
NL25TZA	NL025TZ	Tanzania	NPGRC	-8.700	34.383	690
NL27TZA	NL027TZ	Tanzania	NPGRC	-8.183	34.783	762
101431TZA	101431	Tanzania	IRRI	-7.000	37.000	1015
103886TZA	103886	Tanzania	IRRI	-2.550	32.550	1048
103902TZA	103902	Tanzania	IRRI	-5.550	35.233	600
103913TZA	103913	Tanzania	IRRI	-8.167	36.683	1394
103915TZA	103915	Tanzania	IRRI	-7.783	35.167	752
103916TZA	103916	Tanzania	IRRI	-7.300	35.533	676
104904TZA	104904	Tanzania	IRRI	-5.100	30.833	1056
NPL002ZAM	NPL002	Zambia	NPGRC	-13.133	31.833	920
NN009ZAM	NN009	Zambia	NPGRC	-17.483	24.467	693
104298ZAM	104298	Zambia	IRRI	-15.617	27.600	734
NN008ZAM	NN008	Zambia	NPGRC	-17.500	24.800	676
NPL12ZAM	NPL12	Zambia	NPGRC	-13.117	31.817	910
KM04ZIM	KM04ZM	Zimbabwe	DRSS	-16.083	30.750	810
KM06ZIM	KM06ZM	Zimbabwe	DRSS	-16.100	30.783	811
KM03ZIM	KM03ZM	Zimbabwe	DRSS	-16.200	30.583	812

Table 2. Primers used in the AFLP analysis.

Prema	Sequence
*Eco*RI (E1)	5'-GACTGCGTACCAATTCACC3'
*Eco*RI (E2)	5'-GACTGCGTACCAATTCAGC3'
*Mse*I (M1)	5'-GATGAGTCCTGAGTAACAC3'
*Mse*I (M2)	5'-GATGAGTCCTGAGTAACAT3'

The genetic diversity in the sub-regions was determined using Shannon Information (Maughan et al., 1996) diversity indices. Cluster analysis was done and a dendrogram to show the relation-ship between sub-regions was generated using the UPGMA algorithm of POPGENE Version 1.31 (Yeh et al., 1997).

Mapping the geographical variation

A method described by Rapoport (1982), and elaborated further by Nabhan (1990) and Ferguson et al. (1998) was used to plot areas of diversity based on AFLP data. A cluster analysis of the popula-tions was first performed using the UPGMA method of NTSYS-pc. Six clusters were designated in the resulting dendrogram using a cut-off point of 48% similarity. Populations designated as belonging to each cluster were then plotted on a map using ArchView® and assigned to 19 sub-regions of 2^o grid squares using AutoCAD Map 2000 Release 4 (AutoDesk® Inc., 2000). Genetic diversity values for each sub-region were then calculated using POPGENE Version 3.1 and the spatial distribution of this diversity was then plotted using ArchView® on the 19 sub-regions. Additionally, number of clusters per sub-region (NCSR), Shannon's Information Index and band frequency were also used to determine the distribution of diversity. Though NCSR provides an indication of diversity, it does not take into consideration within-cluster diversity. As demonstrated by Brown and Weir (1983), Nei's diversity on the other hand is influenced by the frequency of amplification products giving greater emphasis to evenly distributed alleles (frequency near to 0.5). Shannon's Information Index (Jana and Pietrzak, 1988) was therefore used as it has been widely applied in ecological and genetic diversity studies using RAPD markers (Dawson et al., 1995; Sawkins et al., 1999).

Generation and analysis of climate and vegetation data

Cluster analysis of climate data in each collecting locality and the means of climate variables in the sub-regions is based on the methodology in FloraMap (Jones and Gladkov, 1999). Each set of climate 'surfaces' consists of the monthly rainfall totals, monthly average temperatures and monthly average diurnal temperature range, to make 36 climate variates in three groups of 12 each. Using a method developed by Jones (1991), the temperature of each location was adjusted to take into account the elevation of the point of collection. A 12-point Fourier Transformation of the original climate data before rotation was undertaken (Jones et al., 1997).

Principal Component Analysis (PCA) was then used for climate data summarization. Cluster analysis was carried out using UPGMA with coefficient values for sequential, agglomerative, hierarchic and non-overlapping (SAHN) matrix updating algorithms after Jain and Dubes (1988). A dendrogram was then constructed to show the relationship between clusters at each level of clustering and between levels.

Vegetation mosaics were obtained from the Vegetation Map of Africa (UNESCO, 1981; White, 1993). The vegetation map of Flora Zambesiaca (Wild and Fernandes, 1968) was used for the analysis of the vegetation mosaics in the Southern Africa region. The geo-referenced co-ordinates of collecting localities were used to determine the vegetation mosaic(s) predominant in the different sub-regions in which cluster members (populations) are found.

RESULTS

The primers used (E1 + M2 and E2 + M1) in AFLP analysis revealed a total of 184 bands that were 95.6% polymorphic. The 176 polymorphic bands ranged in size from 24 to 726 bp. Only polymorphic bands were scored as these are the indicators of genetic differences between the populations studied. The genetic diversity for all the 176 loci in the 48 populations was high, with a mean Shannon's Information Index of 0.460. The diversity ranged from 0.268 to 0.100 in different sub-regions.

An analysis of the UPGMA dendrogram reveals that the populations mostly clustered on the basis of geographic origin as shown in Figure 1. Genetic diversity cluster 1 comprises populations from Mozambique and Namibia. The cluster has a wide longitudinal and narrow latitudinal range of 21.40 and -3.16, respectively. The second cluster comprises two similar Madagascar populations occupying very narrow longitudinal and latitudinal ranges of $< 1^o$. Cluster 3 comprises three Tanzanian populations, which while having a very narrow geographic spread of $< 1^0$ have relatively low similarity. Cluster 4 has a wide geographical spread and is found in South and North Eastern Coasts in Kenya, the South West and North East of Zambia, Northern Zimbabwe and Central parts of Tan-zania. It has a latitudinal range of -5.03 and longitudinal range of 16.02. A closer examination of this cluster revealed sub-clusters that grouped populations from diffe-rent geographic locations together. Cluster 5 is a single population from Zambia found at latitude -15.61 and longitude 27.60. Cluster 6 has the widest geographical spread. It comprises populations from Kenya, Madagas-car, Zambia, Zimbabwe, Botswana and Tanzania, and has a latitudinal range of -17.40 and a longitudinal range of 23.01. This is the only cluster that does not show a clear grouping of populations on a geographical basis.

The geographical area over which *O. longistaminata* is distributed was divided into 2^o grid square sub-regions. The geo-referenced collecting sites of the 48 populations were found in 19 of these sub-regions. The number of clusters and the amount of genetic diversity found in each sub-region are shown in Figures 2 and 3.

Distribution of variation in the sub-regions

The dendrogram from the cluster analysis showing the relatedness of the 19 sub-regions on genetic distance is provided in Figure 4. The cluster analysis of the 19 sub-regions revealed 5 groups with similarities ranging from 47 to 83%. The clusters reflect the geographic origin of the populations. Cluster A comprises sub-regions 13 to 19, all from Kenya and Tanzania. Cluster B represents sub-region 5, which is unique and is found in Zambia. Cluster C comprises sub-region 11, which is also unique and found in Madagascar. Cluster D comprises sub-regions 3 from Botswana, 4 and 7 from Zambia, 6 from Zimbabwe and 12 from Tanzania. Cluster E comprises sub-regions 1 and 2 from Namibia and 8, 9 and 10 from Mozambique with clear separation of the two sub-groups at 73%.

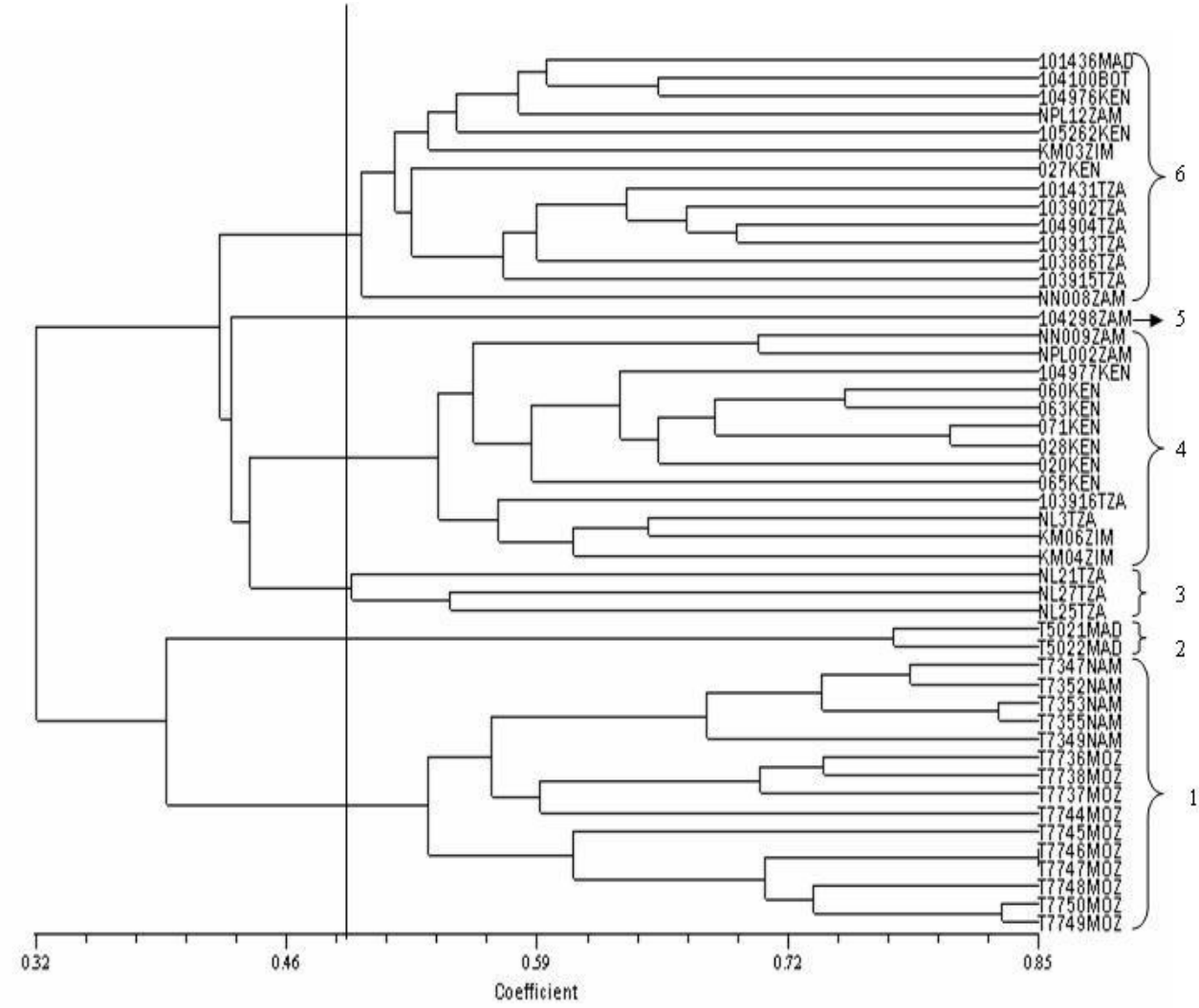

Figure 1. Clustering of 48 populations using 176 polymorphic AFLPs, based on NTSYs-pc UPGMA cluster analysis (Rohlf, 1993) and Jaccard's coefficient.

Genetic diversity within sub-regions

According to Shannon's Information Index, sub-region 14 in Iringa and Mbeya districts in South Western Tanzania, is the most diverse, with a mean genetic diversity of 0.268. It has five populations and three genetic diversity clusters including one which is unique. This is followed by sub-region 11, located in the northeastern part of Mada-gascar, with a mean genetic diversity index of 0.265. The sub-region has three populations and two clusters, one of which is unique. Other sub-regions with relatively high diversity are 19, (southwestern part of Coast province in Kenya) and sub-region 6 (Northern Province in Zimbabwe), with genetic diversity indices of 0.257 and 0.241 respectively.

The three sub-regions that have the lowest levels of diversity are in Mozambique. These are sub-regions 8 in Nicoadala and Maganja da Costa districts in Zambezia province, sub-region 9 in Angoche and Moha districts in Nampula province and 10 in Buzi district in Sofala. Using NCSR, sub-region 14 was the most diverse as it had the highest number of genetic diversity clusters (3), with one of them being unique. Though sub-regions 9 and 2 had a relatively high number of populations (5 and 4 respectively), the diversity was low using the three measures.

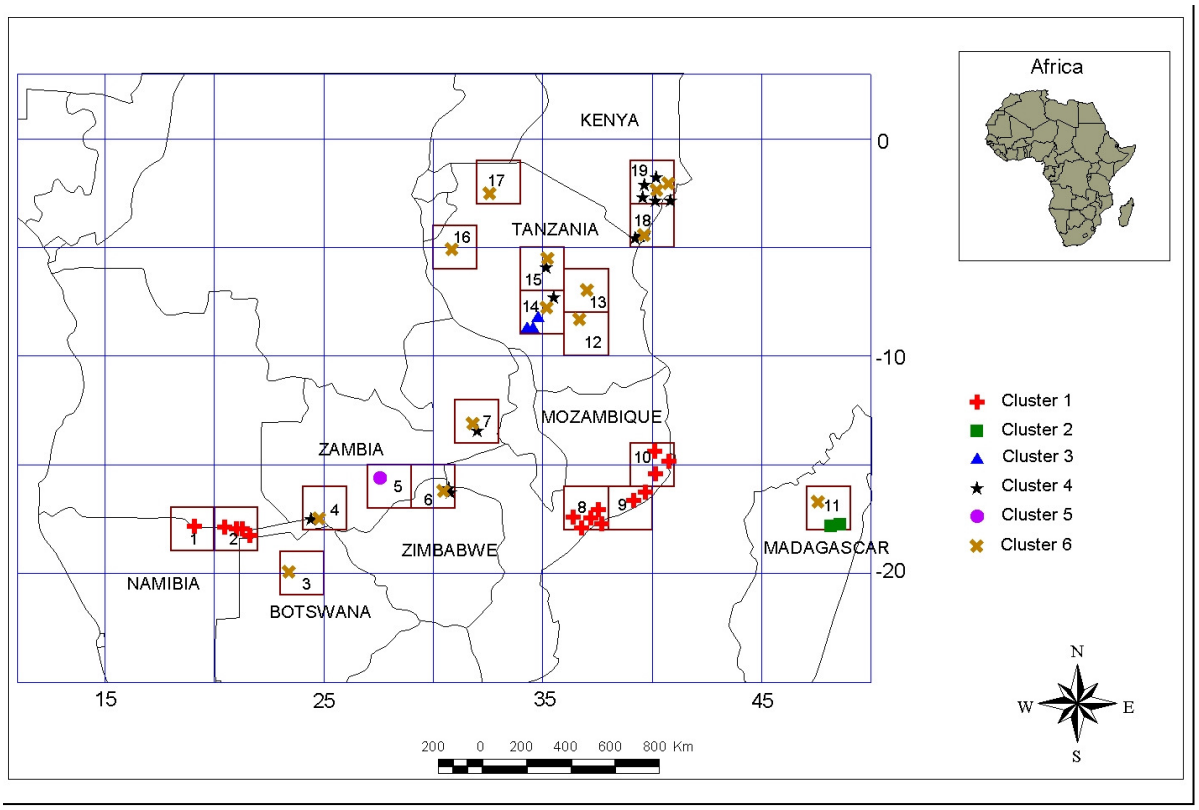

Figure 2. Geographical distribution of AFLP variation among populations of *O.longistaminata* using genetic diversity clusters.

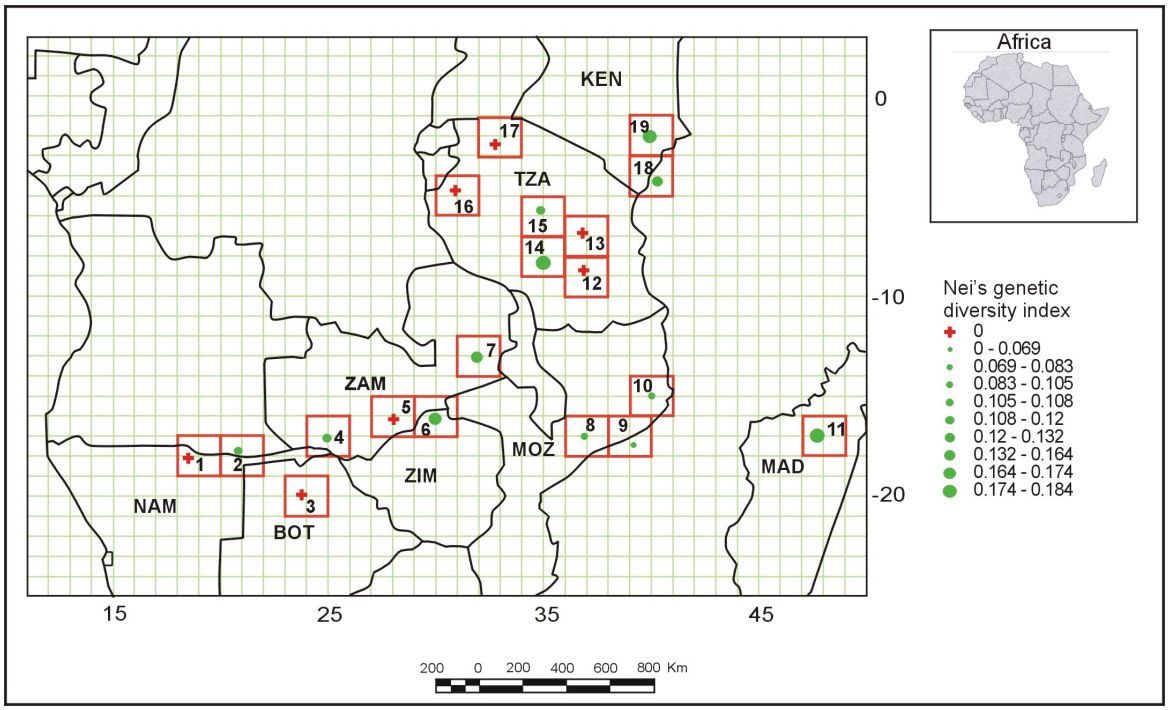

Figure 3. The geographical distribution of AFLP variation among populations of *O. longistaminata* in 19 sub-regions using Nei's genetic diversity indices.

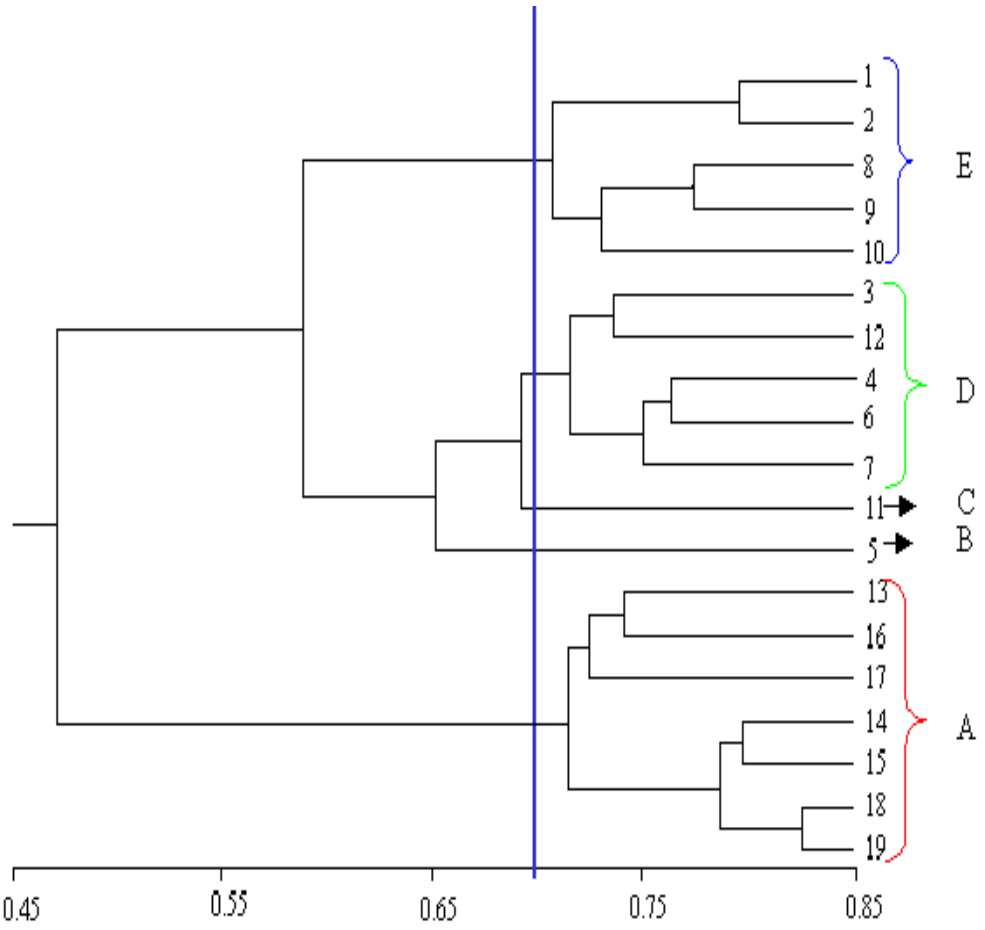

Figure 4. The relationship of 19 sub-regions based on 176 AFLPs generated from 48 populations using the UPGMA algorithm method of POPGENE.

The highest number of populations in one sub-region (19) was 7, found in Tana River Delta and Garsen in Kenya. This sub-region however, had only 2 diversity clusters, neither of them being unique. The level of diversity was low considering that 7 populations were represented, suggesting no correlation between the amount of diversity and the number of populations. An Analysis of Variance (ANOVA) was done to test the relationship between the number of populations and genetic diversity. The multiple regression analysis revealed no correlation ($r = 0.357$, $p < 0.008$) between the number of populations and the amount of genetic diversity (Figure 5) as demonstrated by the equation $y = 0.0134 x + 0.1382$.

Association of genetic variation with environmental and climatic factors relationship with climatic factors

A Principal Component Analysis was performed on the transformed climate data to assess the grouping of the climatic types. Three principal components accounted for 88.1% of the variation. The 1st principal component accounted for 52.6% of the variation, the 2nd for 26.7% and the 3rd for 8.8%. A cluster analysis of the climatic data for the 48 populations was then performed in order to understand the grouping on the basis of climatic variables using the Sequential Agglomerate and Hierarchical Numerical matrix of the UPGMA algorithm method in FloraMap. Due to similarities of environmental conditions in some of the collecting localities, only 32 out of the 48 populations had distinct corresponding climate pixels and these were used to generate the dendrogram shown in Figure 6. The analysis of population-climate clusters revealed a classification with a strong geographic orientation almost entirely on a country basis. The cluster analysis revealed 6 climate 'envelopes' which are distinguished by both rainfall and temperature regimes and bear resemblance to the genetic diversity clusters in Figure 1.

Population-climate cluster 1 is found in sub-regions 6, 7 and 12. It comprises the TZA (Tanzania), ZAM (Zambia), ZIM (Zimbabwe) and BOT (Botswana) populations and closely resembles genetic diversity cluster 6 with the exception of the KEN (Kenya) populations. Population-

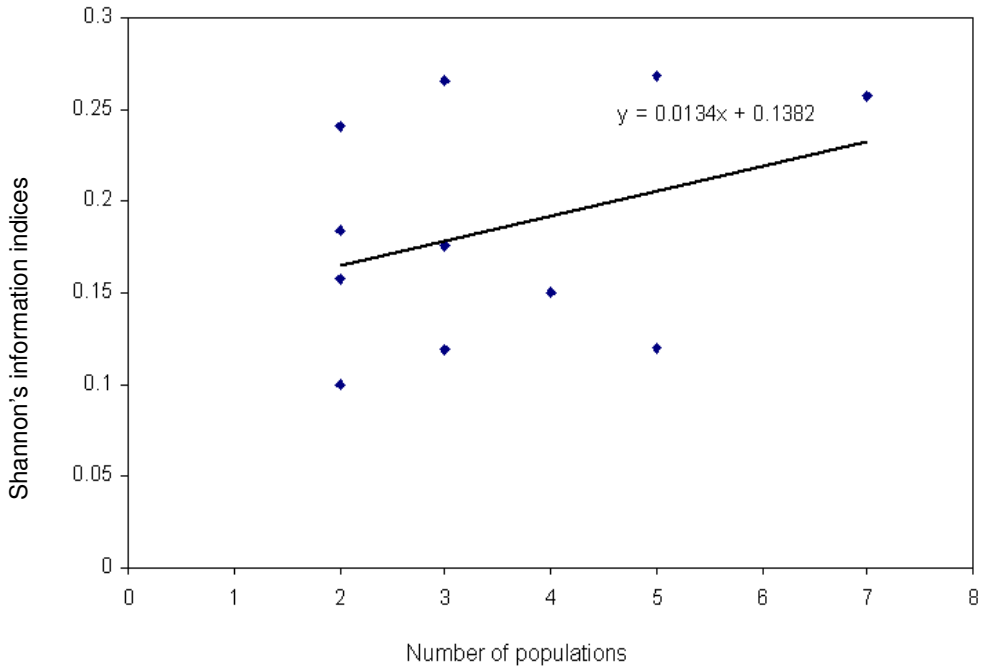

Figure 5. The relationship between number of populations and genetic diversity.

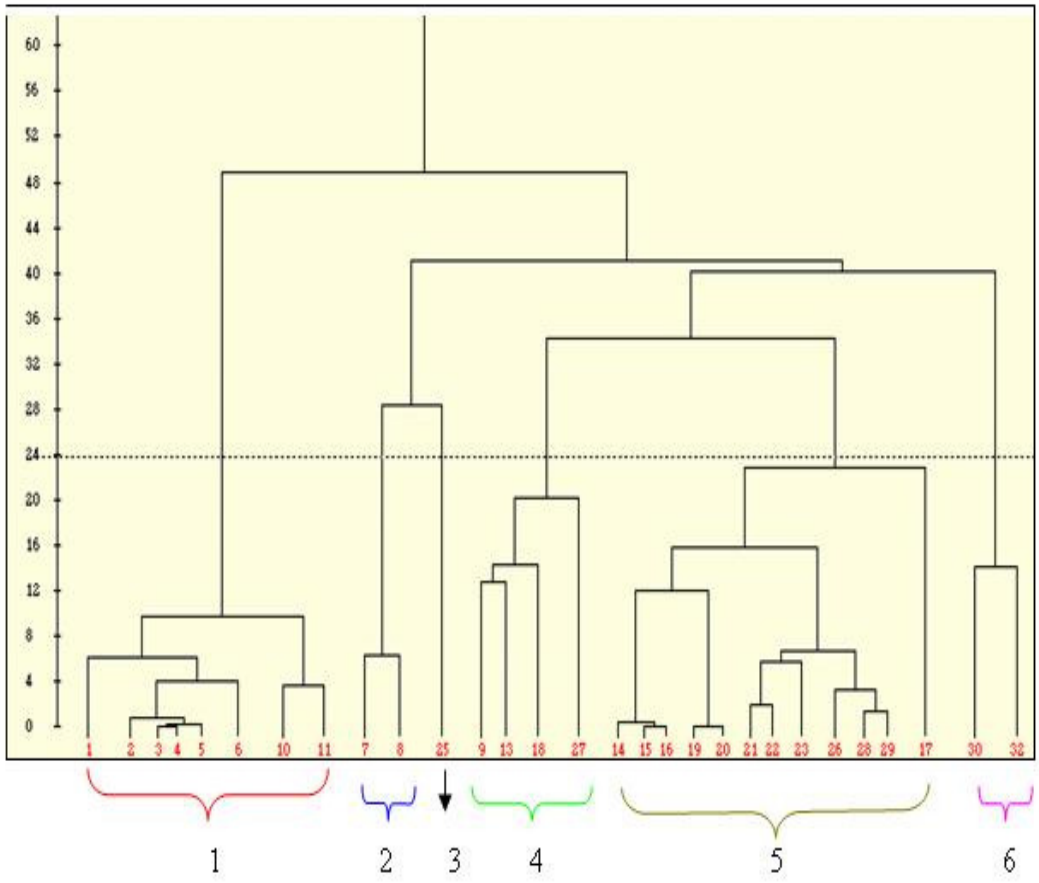

Figure 6. The clustering of 32 populations using UPGMA method in Flora Map (Jones and Gladkov, 1999).

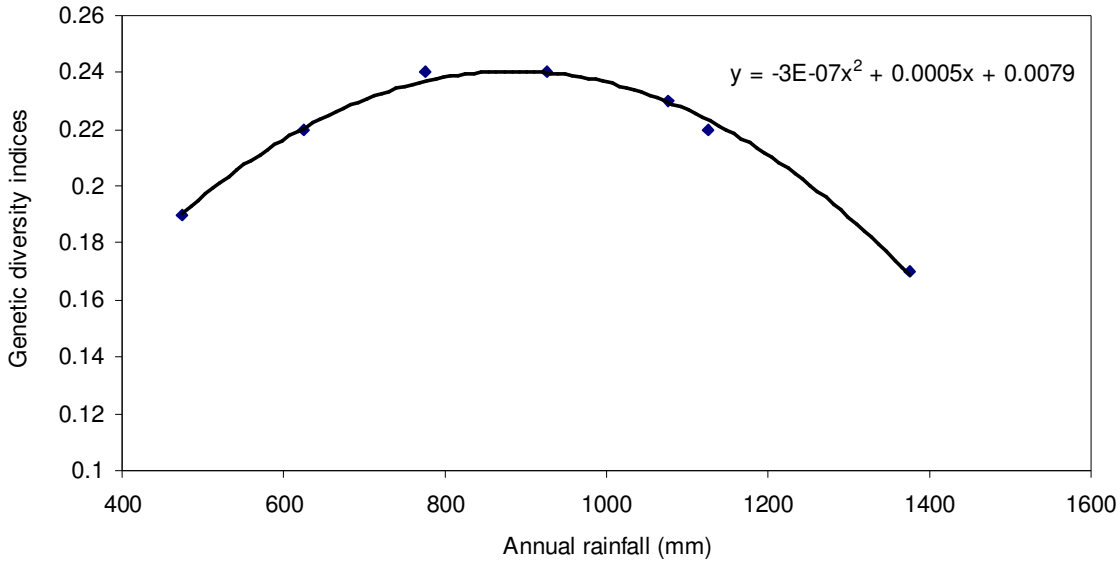

Figure 7. The relationship between genetic diversity and annual rainfall.

climate cluster 2 is unique comprising two Madagascar populations, T5022MAD and T5021MAD, found in sub-region 11. It corresponds to genetic diversity cluster 2 and is characterized by very high rainfall, relatively low

dry and wet season temperatures and is the only one with a winter season, though mild. It is the only one of its type in this study. Population-climate cluster 3 is unique and found in sub-region 5, comprising one Zambia population (104298ZAM). Its uniqueness is attributed to 4 months of continuous dry spell with no rain. It corresponds to genetic diversity cluster 5.

Population-climate cluster 4 is found in sub-regions 18, 19 and 11 and comprises TZA and KEN populations. It is characterized by high rainfall, low and very uniform monthly temperatures, with a range of only 0.2°C between the dry and wet season. Population-climate cluster 5 comprises all the NAM (Namibia) and 7 MOZ (Mozambique) populations and is found in sub-regions 1 and 2 in Namibia, and 8, 9 and 10 in Mozambique and corresponds to molecular diversity cluster 1. The Namibia populations are found in drier climates than the Mozambique populations. Population climate cluster 6 is found in sub-region 14, comprising two Tanzania populations (NL25TZA and NL21TZA). It corresponds to genetic diversity cluster 3 in which there is another population from Tanzania also.

Relationship with rainfall and vegetation mosaics

A non-linear regression statistical analysis revealed a 'curvilinear' relationship between genetic diversity and annual rainfall along a gradient running from 400 mm to 1400 mm (Figure 7). Using ANOVA and a non-linear regression quadric equation of ($y = a + bx + cx^2$), the co-efficients were determined as $y = -3E - 07x^2 + 0.0005x + 0.0079$. The co-efficient of multiple determination was $r = 0.962$ with a high level of significance ($p < 0.0014$). Along this rainfall gradient, genetic diversity is highest in the mid-ranges between 800 – 1000 mm and lowest in the extremes; 400 – 600 mm and 1200 – 1400 mm.

The analysis of vegetation mosaics using the White (1993) classification method and UNESCO (1981) World Vegetation Map revealed 12 distinct vegetation mosaics (Table 3) that were closely associated with genetic diversity clusters. The mosaics fall under seven main vegetation types (phytochoria). The first is II, which is the 'Zambezian regional center of endemism'. In this are sub-regions 1 - 7, part of 8, 12, 13 and 14 and all the genetic diversity clusters, except 3, are represented also. The 'Somalia-Masai regional center of endemism' (IV), has only one sub-region (15) in which the genetic diversity clusters 4 and 6 are represented. The 'Afromontane archipelago-like regional center of endemism' (VIII) com-prises sub-region 14, in which genetic diversity clusters 3, 4 and 6 are represented. The 'Guinea-Congolia/Zambezia regional transition zone' (X), where sub-regions 16 and 17 are found has only the genetic diversity cluster 6 represented. The 'Zanzibar-Inhambane regional mosaic' (XIII) contains sub-regions 8, 9, 10, 18 and 19 and three genetic diversity clusters (1, 4 and 6). The 'East Malagasy regional center of endemism' (XIV) and 'West Malagasy regional center of endemism' (XX) both contain sub-region 11, and comprise genetic diversity clusters 2 and 6.

Table 4 summarizes the ecogeographic distribution of the diversity clusters and associated environmental attributes. The genetic diversity cluster 1 is associated with three different vegetation mosaics. These are 22a in Namibia and 16a and 26 in Mozambique. This is the

Table 3. Vegetation mosaics with associated sub-regions and genetic diversity clusters.

Vegetation mosaic	No. of populations	Phytochoria	Sub-regions	Diversity clusters
22a	5	II	1, 2	1
28	3	II	3, 4	4, 6
28, 42	9	II, IV	6, 7, 14, 15	3, 4, 6
64	1	II	5	5
19a	2	VIII	14	3
26	1	VIII	14	4
18	2	XIV	11	2
35a, 26	2	II, IV	16, 17	6
16a, 26	10	II, XIII	8, 9, 10	1
16a	2	II	12, 13	6
22b	1	XX	11	6
16a, 16b, 77	10	XIII	18, 19	4, 6

Key to vegetation mosaics (Wild and Fernandes, 1968; UNESCO, 1981)

16a - East Africa coastal mosaic (Zanzibar-Inhambane)
16b - Forest transition/mosaics: East Africa coastal mosaic – forest patches
18 - Forest transition/mosaics: cultivated and secondary grassland replacing upland and montane forest (Malagasy)

19a - Forest transition/mosaics: undifferentiated montane vegetation (Afromontane)
22a - Mosaic of dry deciduous forest and secondary grassland (a) Zambezian
22b - Forest transition/mosaics: mosaic of dry deciduous forest and secondary grassland (Malagasy)
26 - Drier Zambezia Miombo woodland (dominated by *Brachystegia* and *Julbernadia*)
28 - Colophospermum mopane woodland
35a - Woodland mosaics and transitions: transition from undifferentiated woodland to Acacia deciduous bushland and wooded grassland
42 - Somalia-Masai Acacia commiphora deciduous bushland and thicket
64 - Edaphic grassland mosaics: with semi aquatic vegetation
77 - Mangrove

cluster with the narrowest longitudinal range, with the excep-tion of the unique clusters, which have localized distribution. Diversity cluster 2 is unique to sub-region 11 in Madagascar and is characterized by a unique climate group (2) and unique vegetation mosaic (18). Cluster 3 is unique to sub-region 14 and its undifferentiated afromon-tane high altitude and vegetation mosaic (19a) makes it different from all others. Cluster 4 has a broad range of distribution and is predominantly found in mid-level rainfall ranges (between 700-915mm) in the *Aw* climate group of the Koppen (1918) classification. Cluster 5 is unique to sub-region 5 in Zambia and has a climatic 'envelope' characterized by climate group 3 and a unique vegetation mosaic (64). Cluster 6 is the most widely distributed and is found in almost all the sub-regions.

The summary of sub-regional attributes based on genetic diversity, NCSR, climatic groups and vegetation mosaics is shown in Table 5. An analysis of the sub-regions using genetic diversity and climatic parameters reveals that sub-region 14 is the most diverse. The sub-region has the highest genetic diversity indices, the highest number of clusters that include a unique one and the highest number of climate groups and vegetation mosaics. This sub-region is unique as it has an afromon-tane climate and vegetation. It is located in the Southwestern part of Tanzania in Iringa district. However it has a mid-range annual rainfall of 730 mm and a lower band frequency (63.5), compared with the other sub-regions. Sub-region 11 is the second most diverse, having two clusters with one of them being unique and two different vegetation mosaics. The least diverse sub-regions are 9 and 10 both in Mozambique. They have only one cluster, the lowest genetic diversity indices and each has one vegetation mosaic.

An analysis of variance and multiple regression revealed a highly significant relationship between genetic variation and environmental and climatic factors ($r = 0.922$, $p < 0.002$) as demonstrated by the linear regression equation $y = 0.0417x + 0.0507$ (Figure 8).

DISCUSSION

Understanding the geographic patterns of genetic diversity is highly relevant to conservation biology and especially to decision-making processes that allow sys-

Table 4. The diversity cluster attributes and geographical range.

Diversity cluster	No. of pops.	No. of climate groups	No. of vegetation mosaics	Sub-regions found	Geographical range	
					Latitude	Longitude
1	15	2	3	5	-14.89-18.05	19.10-40.90
2	2	1	1	1	-17.75-17.85	48.16-48.58
3	3	2	3	1	-8.18-8.70	34.28-34.78
4	13	4	6	7	-243-17.48	24.46-40.46
5	1	1	1	1	-15.61	27.06
6	14	6	8	13	-2.50-19.96	23.41-47.42

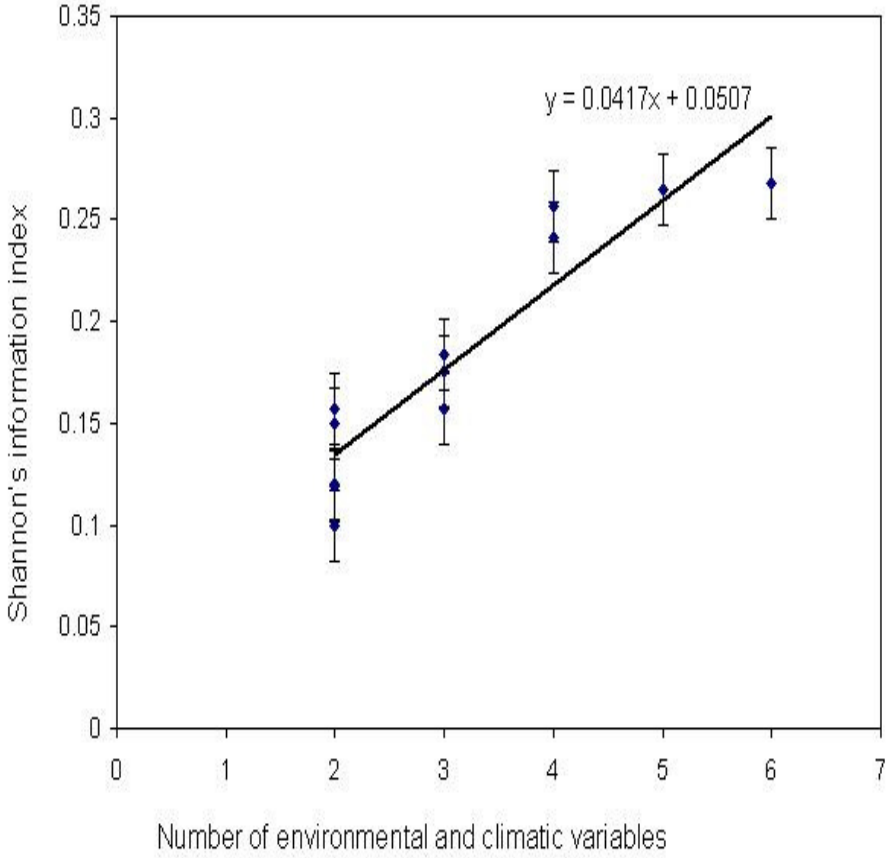

Figure 8. The relationship between number of environmental and climatic variables with genetic diversity.

tematic rather than opportunistic targeting of populations and areas for both *ex situ* and *in situ* conservation of genetic resources (Kark et al., 1999). Geographic patterns of variation may arise from the historical effects of variable local population sizes, migration and mutation rates (Nevo et al., 1979). Since these parameters are themselves affected by environmental (biotic and physical) variables present in different geographic localities, associations between environment and patterns of genetic variation may arise.

Diversity relationships in sub-regions and association with climate and vegetation

Though genetic diversity between the sub-regions studied differed, significant groupings that reflect geographic locations were observed. From the cluster analysis of diversity within sub-regions, five major genetically diverse groups with a strong underlying geographical basis and environmental associations have emerged. Sharp geographical differentiation is observed over short dis-

Table 5. Sub-regions and their related clusters and genetic diversity.

Sub region	No. of populations	No. of clusters	No. of unique clusters	Shannon's Information Index	Mean band frequency	Mean annual rainfall (mm)	No. of climate groups	No. of vegetation mosaics
1	1	1	0	0	75	534	1	1
2	4	1	0	0.150	78.6	473	1	1
3	1	1	0	0	63.8	404	1	1
4	2	2	0	0.157	64.9	593	1	1
5	1	1	1	0	63.8	723	1	1
6	2	2	0	0.241	68.6	811	1	1
7	2	2	0	0.184	75.8	915	1	1

8	5	1	0	0.120	66.5	1283	1	2
9	2	1	0	0.100	63.8	1356	1	1
10	3	1	0	0.119	78.5	1120	1	1
11	3	2	1	0.265	81	1400	2	2
12	1	1	0	0	59	1311	1	1
13	1	1	0	0	58	1032	1	1
14	5	3	1	0.268	63.5	830	3	3
15	2	2	0	0.157	65	588	1	1
16	1	1	0	0	58	1016	1	1
17	1	1	0	0	56	941	1	1
18	3	2	0	0.176	62.6	1370	1	1
19	7	2	0	0.257	67.4	937	1	2

tances (for example in the 3rd genetic diversity cluster, sub-region in Madagascar) and this pattern may partly be associated with restricted gene flow and selection at the micro-geographical level (Allard and Kahler, 1971; Nevo et al., 1979). This pattern is observed in other sub-regions, where there are up to three genetic diversity clusters that are geographically close, but each is distinguishable with a distinct climate group and vegetation mosaic. This diversity between populations that is unrelated to geographic distance, which has also been reported by Kark et al. (1999), rules out simple isolation-by-distance models and suggests intense local differentiation (Christiansen and Frydenberg, 1974).

Genetic diversity can also be found associated with specific vegetation mosaics, particularly the mopane woodland mosaic that is dominated by *Colophospermum mopane*, a woodland tree species. The strong association of *O. longistaminata* populations with this vegetation type is noteworthy. This woodland is found in the Okavango delta in Botswana, Zambezi, Luangwa, Limpopo and Shashi valleys in Zambia and northern Zimbabwe. Eight out of the nine populations from these countries were collected from *Colophospermum mopane* woodlands and vegetation types. This close association between *O. longistaminata* and the vegetation type has been noted during collection missions and its presence was used in many instances as a good indicator of possible occurrence of *O. longistaminata,* especially where there were wetlands.

Based on genetic diversity, number of diversity clusters per sub-region, climatic and vegetation variability, sub-region 14 was the most diverse and unique (Table 5). The diverse climate groups and vegetation mosaics ranging from Miombo woodlands to Somalia-Masai *Acacia Commiphora* deciduous forests and the predominant Afromontane type, clearly underpin the high level of genetic diversity found. In addition, the altitudinal range of 671 m between the lowest and highest elevation of collecting localities of the five populations represented in this sub-region may contribute to this high diversity, which is consistent with similar studies by Negassa (1985), Demissie and Bjornstad (1997), and Brown and Lomolino (1998). The environmental heterogeneity in sub-region 11 also gives rise to a high level of diversity. In contrast, sub-regions in Namibia and Mozambique have very low diversity, which would correspond with environmental homogeneity. This is consistent with Levin's (1968) environmental amplitude or niche-width variation hypothesis, and further expounded by Nevo (1978), who reported a correlation of within-species diversity and ecological heterogeneity.

The analysis of diversity over all the sub-regions clearly indicates that the total amount of genetic diversity in a given set of germplasm populations or geographic locations does not necessarily depend on the number of populations but more on the environmental conditions in which they are found or were collected. For example,

sub-regions 6 and 7 had 2 populations and 11 had three populations but all three had more diversity than sub-regions 5 and 4, which had 5 and 4 populations respectively.

The association of diversity with rainfall observed in this study has also been reported by Nevo et al. (1979) who made various inferences on patterns of genetic differentiation including single peaked clines in the overall level of diversity and simple clines along transects of increasing aridity. We have found that genetic diversity is a function of annual rainfall, and peak diversity occurs in inter-mediate rainfall areas reflecting the 'curvilinear theory' of clinal relationship between the level of genetic diversity and rainfall (Nevo et al., 1979). Parsons et al. (1999) studying rice diversity in Bangladesh reported similar results: genetic diversity was highest at the intermediate level of rainfall. Negassa (1985) also reported the association of barley phenotypic characters with rainfall, with peak diversity at intermediate levels. However, we are the first to report a "curvilinear" relationship between genetic diversity assessed using molecular markers, and rainfall on a wide geographical scale (eight countries) with a latitudinal range of 39.57 and longitudinal range of 37.9.

Implications for conservation

From prediction models and global biodiversity scenarios for the year 2100, it is quite clear that significant changes in the current composition of biomes and distribution of species are expected as a consequence of climate change (Sala et al., 2000; Parmesan and Yohe, 2003). This has obvious implications on conservation and may lead to unprecedented losses of biodiversity, particularly inherent species and the genetic diversity contained therein. For instance, on the basis of mid-range climate change scenarios for 2050, Thomas et al. (2004) have predicted that 15 - 37% of the species in the various regions studied, including Southern Africa are likely to become extinct.

When attempting to study the potential impact of climate change on genetic diversity, in the specific case of *O. longistaminata*, it would not be appropriate to make inferences based upon how ecosystems or vegetation types would be affected, as this species is observed to grow in a fairly wide range of vegetation types, where there is no clear correlation with diversity. The clear association of genetic diversity with rainfall would, however, allow us to consider the potential impacts of climate change on diversity if there was a clearer picture of how rainfall in Africa would be affected by global warming. While there is no means of definitive prediction, it seems likely from recent studies that across the range of this species in Africa, mean seasonal rainfall may both decrease significantly (15 - 25% for December-January-February (DJF) e.g. in Namibia) and increase significantly (> 50% for DJF e.g. in East equatorial Africa), the percentage depending upon the climate change scenario used (Hulme et al., 2001). Despite portraying considerable uncertainty over the likely changes (magnitude and direction) in rainfall expected with global warming in Africa, Hulme et al. (2001) suggest that climate change scenarios should be used to 'explore the sensitivity' of environmental systems and economically valuable assets in Africa. This is now possible with economically important resources such as wild rice species germplasm.

We can therefore infer from our results for instance that an increase in annual rainfall in Southern Tanzania from its optimal value for diversity (800 - 1000) to the maximum for the distribution of the species in Africa (1200 - 1400) would result in a decrease in diversity of 25% (equivalent to Shannon's index of 0.06). For regions where the annual rainfall might be reduced by 50%, we can predict a loss of diversity of at least 20% (Shannon's diversity index of 0.05). However, because of the degree of simplicity of this impact assessment, what we are unable to do is to predict the degree to which adaptation may take place i.e. how quickly can or does diversity change in response to a change in a key environmental variable over a period of decades? Whatever the answer is, and despite the uncertainty about the predictions of future climate change, the potential for effective conservation of these genetic resources *in situ* must be questioned, and the necessity for *ex situ* conservation becomes more urgent.

The study has also clearly demonstrated that there is a relationship between environmental heterogeneity and genetic diversity. Using genetic diversity and environmental parameters, sub-regions 11 and 14 demand consideration for locating *in situ* conservation sites. Their environmental heterogeneity is associated with the relatively high levels of diversity. Sub-region 11 is located in A'tondrazaka in Madagascar in the marshy areas surrounding Lake Alaotra. The sub-region has a unique major climatic group *Caw* (Koppen, 1918) that is the only one of its type in the area of study, indeed in the whole of East and Southern Africa region. Sub-region 14 is located in Iringa in Southern Tanzania near Mt. Rungwe and in close proximity to the convergence of Kipengere and Udzungwa ranges in a generally Afromontane vegetation and climate zone. It would seem prudent to carry out a gap analysis as a first step towards development of an integrated conservation strategy for this wild rice genetic diversity hot spots. The notion of gap analysis for genetic diversity has emerged due to the recent concerns for the on site or *in situ* conservation of biodiversity. This is the process by which the distribution of diversity in a given areas is compared with the geographic coverage of existing conservation programs, thereby enabling the identification of gaps (Scott et al., 1992). Gap analysis should be based on ecogeographic surveys but other surveys that relate genetic diversity to spatial, ecological and social conditions would also be necessary (Ingram, 1990). This is a suggested crucial first step in the designation of *in situ* conservation sites and development of *ex situ* conservation strategies for the diversity of wild rice in these priority areas. This study has narrowed the task of gap analysis for both *ex situ* and *in situ* conservation of the wild rice gene pool in the study area. However, more analysis needs to be undertaken in these areas before making specific recommendations on *in situ* and *ex situ* conservation measures.

Measures of genetic diversity based on allelic richness are considered particularly important for *ex situ* conservation in an effort to safeguard and make available to breeders the largest possible array of alleles (Marshall and Brown, 1975; Schoen and Brown, 1993). It is therefore also important to consider sub-regions 1, 2, 7, 10 and 11, all of which have a high mean number of bands, for further exploration and collection leading to *ex situ* conservation of germplasm. These proposals for in *situ* and *ex situ* conservation are not mutually exclusive or contradictory, but rather complementary. The difference in emphasis would give more weight to *ex situ* conservation for sub-regions with high genetic diversity using band frequency and more weight to *in situ* conservation for sub-regions with high genetic diversity using all the other parameter.

The results of this study have also reveal that genetic diversity is related to environmental diversity and that distance by itself does not determine the degree of affinity between two populations; widely separated geographic areas may share similar diversity whereas close ones may show marked differences. The clinal relationship observed between rainfall and diversity is consistent with the environmental principle of distribution of populations within a species in a gradient marked by a lower and upper limit of tolerance to biotic and/or physical factors, with an area of optimum range. The clear relationship between diversity and rainfall has allowed us to make predictions about the possible losses of diversity that might occur due to global warming, such losses being at the level of approximately 25% if genetic adaptation among populations is not able to keep pace with climate change.

We report a novel method that combines hierarchical analysis of both molecular diversity and climate data. These data were manipulated and spatially analyzed using GIS software packages to assess the association between molecular and climate data, while mapping the spatial distribution of diversity. Specifically we have (i) identified diverse clusters of populations based on molecular data (ii) analyzed climate data in collecting localities of the populations and sub-regions where molecular diversity clusters are distributed (iii) assessed associations of climate variables with genetic diversity (iv) mapped the spatial distribution of diversity in different geographic locations and (v) determined and prioritized areas of high intra-specific diversity for *ex situ* and and *in situ* conservation of genetic resources, particularly in the context of global warming.

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