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Genetic diversity of *Annona senegalensis* Pers. populations as revealed by simple sequence repeats (SSRs)

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***Annona senegalensis* Pers. is one of the wild fruit tree for domestication in southern Africa. An assessment of the genetic diversity in *A. senegalensis* would assist in planning for future germplasm collection, conservation and fruit domestication programmes. During 2004 to 2006 nine populations were collected from different locations in Malawi and genetic diversity was evaluated using microsatellites or simple sequence repeats (SSRs) developed in *Annona cherimola.* In total 23 alleles were detected in the populations studied and genetic diversity parameters revealed high levels of heterozygosity with 4.0 to 14 alleles per locus and the populations were genetically different by 19% as given by the value of theta. Results demonstrated association between genetic and geographical distance in the species indicating that large-scale geographical and ecotypic differentiation was reflected by the SSR markers. The high genetic diversity is attributed to biological characteristics of the tree species and habitat heterogeneity. The study has revealed evidence of application of SSR markers from *A. cherimola* towards genetic fingerprinting of *A. senegalensis.* Implications of the SSR marker data for optimizing genetic management of the species are discussed.**

Key words: *Annona*, conservation, genetic diversity, heterozygosity, microsatellites, SSRs, population.

INTRODUCTION

Annona senegalensis Pers. is one of the most important wild indigenous fruit trees in southern Africa. It is a diploid member of Annonaceae family which is one of the largest tropical and subtropical plant families with about 2300 species of trees, shrubs and lianas. *A. senegalensis* is native and common in savannas throughout tropical Africa, from the Cape Verde Islands, the Nile and Upper Guinea to Transvaal and Zululand (Ahmed, 1986). The fruits of *A. senegalensis* form an important part of diet for most indigenous populations in southern Africa. The fruits are liked because of good flavour, sweetness and high nutrition. The food value varies considerably, but most

forms have an abundance of carbohydrates, proteins, calcium, phosphorus, iron, thiamine, niacin and riboflavin; while some are rich in magnesium, ascorbic acid and carotenes (Yoa and Wickramaratne, 1995). *A. senegalensis* has several medicinal purposes including use of parched green fruits to relieve diarrhoea and dysentery. The bark is chewed to relieve stomachache, while young boiled leaves, leafy twigs and roots are taken to alleviate pulmonary complaints. Dried, powdered leaves are regarded as purgative and as a remedy for mucous diarrhoea. Venereal diseases and intestinal dis- orders are treated with preparations of the roots (Sofowora, 1993). Unopened flower buds are used both in soup and seasoning of native dishes (Williamson, 1975). If domesticated *A. senegalensis* could therefore considerably improve nutrition, health and incomes of many rural people and thereby contributing to the improv-

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ement of people's livelihoods in southern Africa.

In Malawi high anthropogenic pressure on land has resulted in severe deforestation and rapid erosion of genetic material of *Annona* species, unless conservation efforts are made, the species is likely to disappear from most forests (Campbell and Popenoe, 1988). In this regard knowledge of genetic diversity of different populations is important to form a basis for conservation, genetic tree improvement and promotion of domestication of populations with desirable traits. Since the extent of genetic diversity is not known, it is imperative to have an elaborate strategy aimed at evaluating genetic diversity of Malawian populations of *A. senegalensis.* Studies on use of morphological traits and isozyme markers have been reported on members of Annonaceae especially *Annona cherimola* Mill. (Perfectti and Pascual, 2004; Cautin and Agusti*,* 2005). However, most morphological traits are highly influenced by environmental conditions or vary with development stage of plant and isozymes are limiting due to low levels of polymorphisms. Consequently DNA based techniques such as microsatellites or simple sequence repeats (SSRs) are effective in assessing genetic diversity of plant species because they provide unlimited potential markers to reveal differences at molecular level.

Simple sequence repeats or microsatellites consist of variable numbers of tandemly repeated units each of 1 to 6 bp and are abundant throughout eukaryotic genomes (Kijas et al., 1995; Kahl, 2001). Microsatellites are amplified using a polymerase chain reaction and they are later detected using various methods such as fluorescent dye labeling and silver staining. At present microsatellites are the most preferred marker types because they are highly polymorphic even between closely related lines, require low amounts of DNA and can be easily automated, can be exchanged between laboratories and are highly transferable between populations (Gupta et al., 1999). Another advantage is that the PCR products of different markers can be run on the same gel, saving time, labour and money. Compared to other classes of markers SSRs often carry high numbers of alleles at very low frequencies or private alleles present in only one or few populations. This greatly contributes to the assessment of genetic relationships among and within populations. One disadvantage of microsatellites is the presence of null alleles and these alleles are not amplified, hence they can not be scored. These can therefore lead to an underestimation of heterozygosity. The major disadvantage in the use of microsatellites markers is the considerable initial investment needed to develop and map them.

This study is the first attempt to study the genetic diversity of *A. senegalensis* from Malawi using simple sequence repeats. The objective of the study was to map out bio-geographical distribution of *A. senegalensis* in Malawi and determine genetic diversity among the populations using simple sequence repeats. The results could be useful in guiding future conservation and tree improvement programme of this species.

MATERIALS AND METHODS

Study area

The study was conducted in Malawi during 2004 to 2006. Field plots of 32 m radius were established at nine different locations throughout the country (Figure 1) . The identification of the sites was done at random, but taking into consideration geographical set-up of country, whereby all three regions were each represented by three sites. The names of populations are Mapapa-Karonga, Mphopha-Rumphi, Kaning'ina-NkhataBay in the Northern region; Bunda-Lilongwe, Chimaliro-Kasungu and Chibothera-Nkhotakota in Central Malawi and Mkulumadzi-Neno, Likhubula-Mulanje and Kuchawe-Zomba in Southern Malawi. Selected populations were heterogeneous for forest habitat type and altitude (Table 1).

Collection of leaf samples

A total of 135 leaf samples were collected from 9 localities with 15 individuals representing a population (Table 1) along the geographical range of natural distribution of *A. senegalensis* in Malawi. Young fresh leaves of 2 - 3 weeks old of approximately 4 x 2 cm in length and width respectively were collected from the nine locations. There were three plots per population and five individuals represented a plot thus giving a total of 135 samples for all populations. Leaves from individual trees were plucked off from the base of their peduncle and inserted into 8 cm plastic tubes and then placed in a cooler box containing super freeze coolant before being sent to Stellenbosch University in the Republic of South Africa for characterization.

DNA extraction

Total genomic DNA extraction was extracted from frozen leaf samples. About 200 mg leaf sample was used in DNA extraction using Nucleospin 8 Plant Extraction Protocol (Macherey–Nagel, 2004) following the manufacturer's instructions with minor modifications. The DNA sample was incubated at room temperature for 1 min and centrifuged at 13,000 rpm to recover pure DNA. Following precipitation of impurities and RNase digestion isolated DNA was resuspended in 200 l Tris-EDTA buffer and stored at -20° C until further analysis. DNA concentration was determined by both Nanodrop spectrophotometry at 260/280 nm and ethidium bromide staining on a 1% agarose gel electrophoresis (Sambrook et al., 1989)

Amplification and genotyping of microsatellites

The PCR reaction mixture contained 10 mM Tris-HCl, pH 9.5, 50 mM KCl, 200 µM dNTPs, 2 mM MgCl$_2$, 0.2 µM of each forward and reverse primer, 1.5 units *Taq* polymerase (Promega), and 2 ng DNA templates in 20 µl total volume. The amplifications were conducted with a Perkin- Elmer 9700 Thermal Cycler (Applied Biosytems, CA, USA). The programme consisted of an initial 5 min at 94°C that was followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C with a final extension period of 10 min at 72°C and lastly storage at 4°C until when the PCR products were required for use. A negative control with only the reaction mixture excluding DNA was also included in each experiment. Four *Annona*-specific primers with motif repeat rich in (CT)$_{20}$, (GA)$_{14}$, (CT) $_{14}$ and (CT)$_{10}$ were developed from *Annona cherimola* (Escribano et al., 2004) and used to detect the genetic diversity of *A. senegalensis* (Table 2). The SSRs were screened on Genetic Analyzer model 3730 XL automated DNA sequencer, G5 dye set running an altered genotyping module that increased the injection time to 30 s and injection voltage to 3 kV. About 1 µl of PCR product and 2µl of each of the
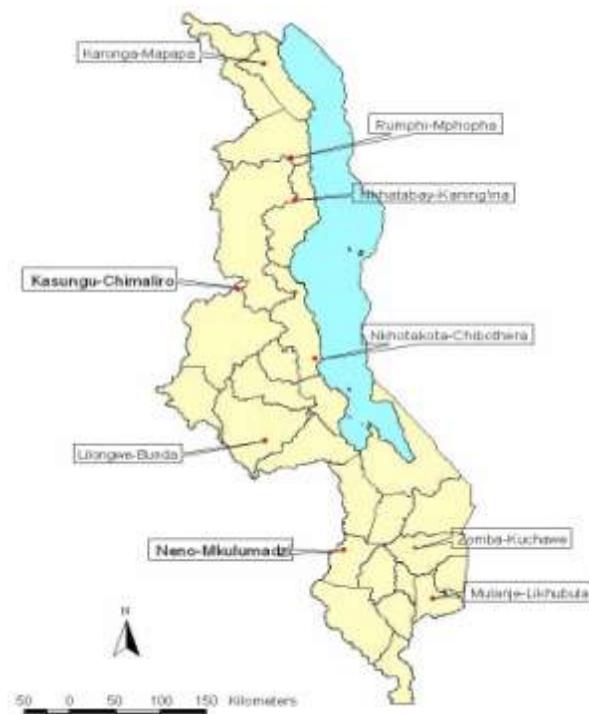
Figure 1. Map of Malawi showing the collection sites of
Annona senegalensis.

Table 1. Nine *Annona senegalensis* populations collected in Malawi with climatic data for localities.

Population	Region	Temperature (^{o}C)	Mean annual rainfall (mm)	Altitude (m)	Soil pH
Mapapa, Karonga	Northern	20	1025	561	6.3
Mphopa, Rumphi	Northern	20	890	1434	5.7
Kaning'ina, Nkhatabay	Northern	24	1009	700	5.3
Chimaliro, Kasungu	Central	22	762	1354	6.4
Bunda, Lilongwe	Central	22	925	1100	5.8
Chibothera,Nkhotakota	Central	25	1440	503	4.7
Kuchawe, Zomba	Southern	24	1000	1112	5.4
Likhubula, Mulanje	Southern	20	2300	1400	5.6
Mkulumadzi, Neno	Southern	25	950	990	5.4

Table 2. Locus name, primer sequence, repeat motif and type (I, imperfect; P, perfect) of the microsatellites analyzed.

| Oligo-name	Locus	Sequence (5'-3')	Repeat	No of bases	Type	G5 Dye
Annona1 F	M01	CTCTTCAAAGGTACGACTTC		20	I	black
Annona 1 R		TTGAGAAAAGGATAAGGATT	(CT)$_{20}$	20		
Annona 4 F	M04	ATTAGAACAAGGACGAGAAT		20	P	green
Annona4 R		CCTGTGTCTTTCATGGAC	(GA)$_{14}$	18		
Annona 6 F	M06	GGCATCCTATATTCAGGTTT		20	P	blue
Annona6 R		TTAAACATTTTGGACAGACC	(CT)$_{14}$	20		
Annona11F	M11	TACCTCTCGCTTCTCTTCCT		20	I	red
Annona11R		GATGATTAGACACAAGTGGATG	(CT)$_{10}$	22		
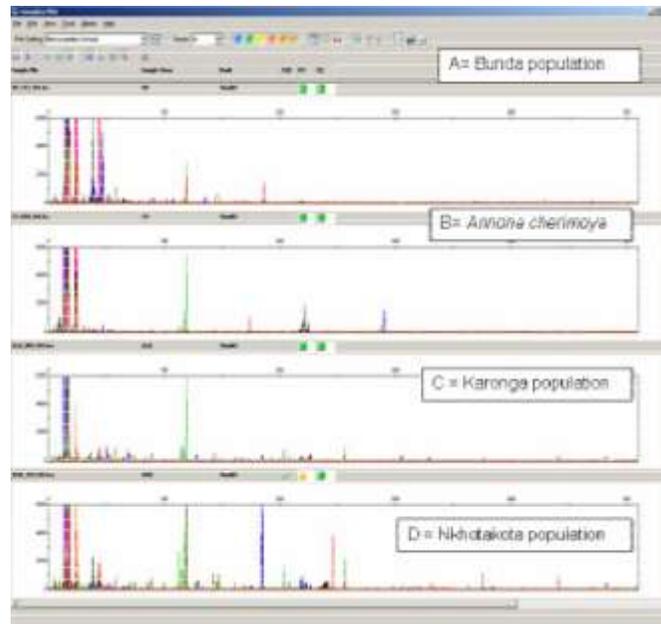
Figure 2. Amplification of four primers at three loci in *Annona senegalensis* and *A. cherimoya.*

four primers were loaded on 10 µl of HiDi Formamide and 1.5 µl of Gene Scan–500 LIZ size standards (Applied Biosystems) marker with an orange dye.

Data analysis

Automatic genotyping and scoring of allele size were performed by the GeneMapper® version 3.5 software. The FSTAT software version 2.9.3 (Goudet, 2001) was used to test genetic diversity parameters such as Hardy-Weinberg Equilibrium test, heterozygote deficit, population differentiation, allelic and genotype frequencies. Statistical analyses were done using GENEPOP 3.1 (Raymond and Rousset, 1995) and FSTAT. The extent of genetic differentiation between populations was estimated from the theta estimator of F_{ST} (Weir and Cockerham, 1984). Nei's genetic distance (Nei, 1972) matrices were computed to determine the genetic distance between genotypes. This method is appropriate for populations shaped by diverse evolutionary forces. The formula used to compute Nei's distance (D) is:

$$D = \ln I$$

$$I = \sum (p_{xi}p_{xj}) / \left[\left(\sum p_{xi}^2 \right) \times \left(\sum p_{xj}^2 \right) \right]^{1/2},$$

Where p_{xi} = frequency of allele x in population i, and p_{xj} = the frequency of allele x in population j. The genetic distances were then clustered using unweighted pair-group method with arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

Microsatellite amplification

Figure 2 shows typical electrophoregrams obtained with multiple sample loading of the four primers that were test-ed on (A) Bunda population, (B) *A. cherimoya* from which these primers were adopted, (C) Karonga population and (D) Nkhotakota population. Three loci were examined and the pooled analysis of the primers showed that the green primer was the best followed by the blue and then red. The black primer did not amplify in *A. senegalensis* but only in *A. cherimoya* from which the primers were developed from.

Gene and genotype frequencies

The total number of alleles detected across all the loci was 23 (Table 3). Gene and genotype frequencies evaluated at the three loci revealed that locus M06 had fourteen alleles and was the most polymorphic while M11 was the least polymorphic locus.

All the three primer pairs amplified multiple fragments in the nine populations used indicating high polymor-phism with putative alleles ranging from 4 to 14 per locus. The highest allelic diversity of 14 alleles was observed for the perfect binucleotide SSR locus *MO6* and the lowest allelic diversity for the imperfect binucleotide SSR locus *MO11.* Except for locus *M04* the other two loci had higher numbers of observed alleles than reported in the original publication (Escribano et al., 2004). This could be attributed to differences in phylogenetic distances between the two species *A. cherimola* and *A. senegalensis.* The SSR markers described herein had twice as many alleles as isozyme markers suggesting that SSR loci would better detect fine-scale genetic differentiation. The average number of alleles detected was 5.7 per polymorphic locus

Table 3. Distribution and frequency of alleles for three micro-satellite loci in A. senegalensis.

Locus	Allele	Frequency	Number of alleles
M04	As109	0.01	
	As113	0.02	
	As115	0.45	
	As119	0.49	5
	As121	0.03	
M06	As211	0.01	
	As217	0.19	
	As218	O.02	
	As219	0.42	
	As221	0.04	
	As223	0.12	14
	As225	0.05	
	As227	0.02	
	As231	0.01	
	As232	0.01	
	As233	0.01	
	As235	0.02	
	As237	0.07	
	As241	0.01	
M11	As169	0.39	
	As171	0.02	4
	As173	0.58	
	As175	0.01	
Total			**23**

which is higher than 2.05 ± 0.25, the range reported in *A. cherimola* through use of isozymes (Perfectti and Pascual, 2004) and other woody long-lived perennial plants (2.19 ± 0.09; Hamrick and Godt, 1990). The higher allelic richness in SSRs constitutes an advantage of SSR over other markers such as isozymes, random amplified polymorphic DNA (RAPDs) and amplified fragment length polymorphism (AFLP). Several studies have shown that SSR markers detect higher levels of polymorphism, providing a higher level of information per single marker (Powell et al., 1996; Pejic et al., 1998) . Our data on *A. senegalensis* is in agreement with these findings.

The most frequent allele was As173 which had a frequency of 58% (Table 3). This was the only allele that was present in all the nine provenances. The presence of allele As173 in all nine populations is interesting. A relationship between region of origin and genotypic or allelic frequencies has been observed in a wide range of plants, both cultivated and wild, for example, in sorghum

(Morden et al., 1990), trees such as chestnut (Huang et al., 1994). The causes of these relationships are subject of debate, Nevo and Beiles (1989) found that environ-mental and ecological factors are more important than geographic distances while Li and Rutger (2000) found that epistasis and selection of multiple gene complexes was responsible for macro-geographic differentiation. The other alleles that were found in high frequencies were alleles As119, As115 and As219 which had frequ-encies of 49, 45 and 42% respectively.

The percentage of unique and localized alleles across the populations was 15%. This means that 15% of the alleles detected across loci are private. In the study priv-ate alleles are in low frequency which may reflect new mutations in isolated populations. A number of popula-tions for instance Nkhotakota, Mulanje and Rumphi have loci fixed for single alleles and in some cases this may be apparent rather than real. Populations displaying unique alleles may represent wild relatives suggesting that the ancestral genotypes containing these alleles are not represented in the other collection of populations. Althou-gh populations displaying unique alleles may represent wild germplasm, it is also possible that the population specific alleles were derived from a mutation event since SSRs loci are known to have a high rate of mutation per locus per generation of 25×10^{-5} to 1×10^{-2} (Weber and Wong, 1993). The distribution of these private alleles also suggests a rare mutation, low frequency allele from *A. senegalensis* or mating with a close relative. Both of the two private alleles As241 and As232 are in very low frequency of only 1%. In this research work, smaller samples were used, private allelic richness is affected by size of the samples whereby large samples are expected to have more private alleles than small ones, on the other hand, intensive sampling of genetically similar popula-tions may reduce the number of alleles private to any population (Kalinowski, 2004). The two private alleles were geographically restricted hence the microsatellite loci presented could be used to investigate the evolu-tionary history or phylogeography of *A. senegalensis.*

There were a total of 34 genotypes detected across all three loci. Loci *M06* was the most genotypically rich loci with 22 genotypes, followed by loci *M04* and finally loci M11 with 7 and 5 genotypes respectively. The most freq-uent genotype was As 169/173 which had the frequency of 53%. This genotype is found in all populations except for Neno. This genotype is found in the heterozygous state and it could be responsible for a very important trait that is probably why it is more abundant in most popu-lations.

Microsatellite amplification across species would allow the detection of infra-specific taxa (Alvarez et al., 2001), the separation of morphologically similar taxa or an elucidation of the nature of morphologically distinct, yet genetically close, taxa. It has been shown that micro-satellite primers developed for a distinct species such as *A. cherimoya* can be useful for genetic analysis in related

Table 4. Genetic diversity indices and Hardy-Weinberg equilibrium P-values in nine
A. senegalensis populations.

Population	Average gene diversity (H)	Average difference	HWE p-value
Karonga, Mapapa	0.48	50.88	0.990
Rumphi, Mphopa	0.58	60.32	0.860
Mulanje, Likhubula	0.53	56.18	0.770
Neno, Mkulumadzi	0.39	41.34	0.440
Nkhata-Bay, Kaning'ina	0.62	66.36	0.440
Kasungu, Chimaliro	0.61	64.95	0.200
Lilongwe, Bunda	0.52	54.96	0.180
Nkhotakota, Chibothera	0.57	60.9	0.110
Zomba, KuChawe	0.22	23.49	0.007*
Means	0.50 ± 0.22	53.26 ± 11.72	

Means are followed by ± standard errors of the mean.
*The population was not in Hardy-Weinberg Equilibrium at $p < 0.05$.

species (Davis and Strobeck, 1998), but successful transferability depends upon the evolutionary distance between source and target species (Peakall et al., 1998; Roa et al., 2000; Rosetto, 2001). However, successful cross-amplification simply indicates that the flanking regions are conserved, but it does not tell anything about the character and structure of the fragment.

Hardy-Weinberg equilibrium test and genetic diversity

The results for test of Hardy-Weinberg equilibrium (HWE) were not significant for all the populations except for Kuchawe Zomba population which had statistically significant heterozygote deficiency from the HWE. The genetic diversity analysis of *A. senegalensis* at the three loci (*M04, M06* and *M11*), revealed that Nkhatabay and Kasungu were the populations with the highest genetic diversity of H=0.62 and H= 0.61 respectively (Table 4).

A specific U test (Rousset and Raymond, 1995) for heterozygote deficiency indicated statistically significant deficit for the Zomba population. No loci exhibited deviation resulting from heterozygote excess. The most common cause for heterozygote deficiency in SSR marker studies is the presence of null alleles, Null alleles occur when there is a mutation within the DNA sequence complementary to one or both primers, preventing PCR amplification of the SSR sequence (Callen et al., 1993). Other causes of heterozygote deficiency include selection or hitchhiking on a linked locus under selection, deviation from panmixis, or population admixture. Of the three, selection seemed to be a likely cause of HWE deviation as the Zomba population is under farmers' fields as opposed to other populations occurring in natural forests. The genetic diversity analysis of A. senegalensis at three loci (*M04, M06* and *M11*), revealed that Nkhatabay and Kasungu were the populations with the highest

genetic diversity of H= 0.62 and H= 0.61 respectively (Table 4). Populations of *A. senegalensis* from Nkhatabay and Kasungu were from protected forests which are contiguous as compared to woodlands on farm land. Distribution range and population size have been identified as major correlates of within population genetic variation in tropical tree species with restricted populations showing significantly less variation than those with a broader distribution. Forest fragmentation affects allelic diversity through structure of populations and gene flow. Aldrich and Hamrick (1998) found that gene flow from outside the sampling area was three-times greater in contiguous than remnant forests as such large contiguous forest populations will usually have a greater diversity of alleles compared to small fragmented populations. The high genetic variation in *A. senegalensis* is attributed to its wide geographic distribution and population size. Karron et al. (1988) suggested that not only do the geographic distribution and population size influence level of genetic variation, but habitat heterogeneity and historical distribution. The high levels of allelic diversity, along with the high levels of heterozygosity can mainly be attributed to biological characteristics such as predominantly outcrossing, anemophily, and being a perennial woody plant, all of which contribute to accumulation and retention of genetic variation (Hamrick and Godt, 1989).

Zomba had the lowest genetic diversity (H= 0.22) and this low level of genetic diversity suggests that there is a lot of inbreeding due to its small population size. This is also causing their population to experience genetic drift. Stochastic forces such as small dispersed populations may face genetic drifts and bottlenecks which are important in driving their population structure and evolution of *Annona* lineages. Apart from genetic drift there is also evidence to suggest that this population is probably the only one apart from Neno that is undergoing considerable degree of selection. This is evident by the small size of the population with low genetic variation and it is
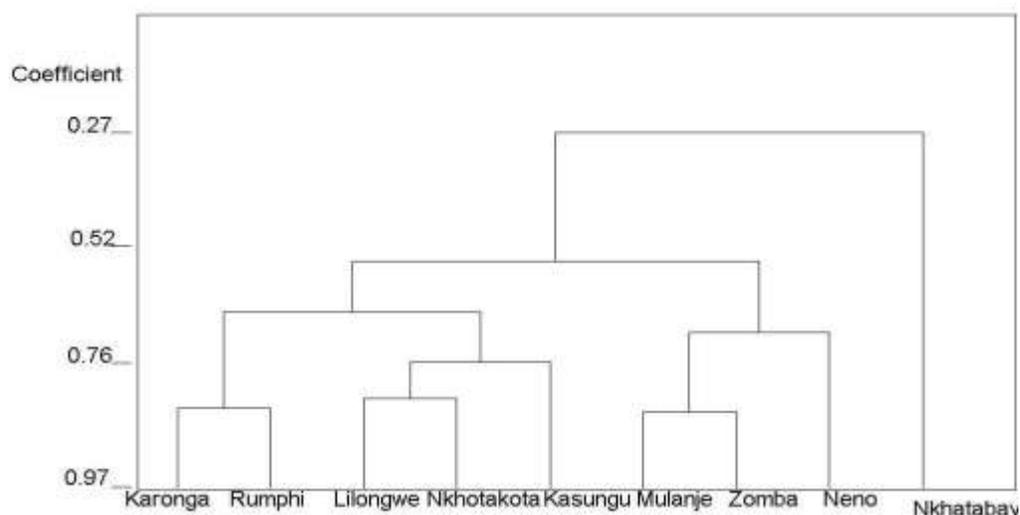
Figure 3. Dendrogram of nine *A. senegalensis* obtained with three *A. cherimola* SSR markers.

on a managed farmland. There is evidence to suggest that the small population size of Zomba is not as a result of genetic failure on the part of the population but rather as a result of a population bottleneck that must have occurred in site.

The analysis for the overall diversity of *A. senegalensis* across loci and population as given by Nei's estimate of heterozygosity is 62%. The analysis further revealed that diversity within populations (50%) is greater than across population (11%). The gene flow across population of all three loci as estimated from F_{ST} was 0.025. For such highly polymorphic loci, any apparent population structure based on finite samples must be interpreted cautiously due to stochastic sampling errors in allele frequency estimates and the strong likelihood that some rare alleles may be missed. This low genetic differentiation among population suggests that there was more gene flow for sites within agroecological region because of their close proximity. This high level of gene flow indicates that most populations are randomly mating, with very little selection. Avearge heterozygosity of 62% suggests that *A. senegalensis* can respond favorably to selection providing room for species conservation, crop improvement through breeding and other biotechnology techniques. The low level of differentiation across *A. senegalensis* populations warrants conservation of this species to maintain its genetic richness. The high genetic diversity within populations suggests that a large number of individuals have to be sampled from fewer populations for conservation purposes. The advantage of species with great genetic diversity like *A. senegalensis* is that it can easily adapt and conform to a wide range of environmental conditions as opposed to non-genetically diverse species such as those of the domesticated crops, which can not survive in a wide range of environments without human supervision. The practical usefulness of this information is that it will help in making rational decision as to

which populations to prioritize in terms of conservation and domestication. The populations with the highest genetic diversity, namely Kaning'ina-Nkhatabay and Chimaliro-Kasungu need to be prioritized in terms of in situ conservation of *A. senegalensis*.

Geographical and genetic distance among populations

The analysis of genetic distance has revealed some genetic structure of *A. senegalensis* populations in Malawi. A mantel matrix correspondence test conducted to assess the relationship between geographical distance and genetic distance (data not shown) demonstrated that there was a significant correlation between the two distance matrices (r = 0.48, p < 0.05). The genetic population of *A. senegalensis* has three main clusters (Figure 3).

Most of the populations from northern and central Malawi formed one cluster while southern Malawi populations formed the second cluster. Each cluster represents populations that are congenial. Cluster 1 consists of Karonga, Rumphi, Lilongwe, Nkhotakota and Kasungu, while cluster 2 consists of Mulanje, Zomba and Neno. Nkhatabay population from the lake shore area forms its own cluster. This pattern shows that distance has a role to play in the clustering of the provenances. As it is shown populations that are close to each other in distance share the same or almost similar genotypes as opposed to populations that are further apart. It is likely that the uniquely different environment of the lakeshore area could have contributed to diversity of the population hence clustering away from geographically closer populations. All the populations from southern Malawi from the same agroecological zone and geographically closer to each other form one cluster. The reason for this observation is that populations that are closer in terms of physi-

cal distance may be closely related because of high chances of gene flow. Connectivity between populations as a result of gene flow between populations in the same cluster is likely to be important in maintaining genetic diversity and minimizing genetic drift. Apart from distance, the determination of clustering provenances together could also be attributed to environmental similarities. For example Mulanje and Zomba have similar environmental conditions in the sense that they are both highland populations. The close relationship bet-ween central and Northern Malawi populations might be explained by either historical relationship in probably in sharing recent common ancestry or more likely geogra-phical proximity and large population sizes which favour genetic interchanges. This information help in making a rational decision regarding prioritizing populations which require conservation. This implies that populations of *A. senegalensis* in Malawi can not be considered a single panmictic unit although the genetic differentiation is low. For purpose of conservation of genetic resources seed collection need to be done across different populations to ensure a more representative sample of genetic variation.

Conclusion

The results have demonstrated that there are significant differences in the genetic diversity of populations of *A. senegalensis* and SSR have proved to be suitable for characterizing *A. senegalensis*. The higher level of polymorphism revealed by SSR indicated that populations could be identified and information on genetic diversity obtained from relatively few loci. The high level of genetic diversity is due to out breeding nature of *A. senegalensis* and this is a typical characteristic of wild tree species. The immense pool of genetic richness exhibited by *A. senegalensis* provides an opportunity for potential tree improvement, as well as a genetic conservation programmme. The study also shows some evidence of high cross-species transferability of *A. cherimola* SSRs in *A. senegalensis* for detection of genetic diversity. For conservation purpose priority should be given to populations that are more genetically diverse and these include Nkhatabay, Kasungu, Rumphi and Nkhotakota.

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REFERENCES

Aldrich P, Hamrick J (1998). Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. Science 281:103–105.

Alvarez AE, van de Wiel CCM, Smulders MJM, Vosman B (2001). Use of microsatellites to evaluate genetic diversity and species relationships in the genus Lycopersicon. Theor. Appl. Genet. 103: 1283–1292.

Ahmed MS (1986). Pollination and selection in *Annona squamosa* and *Annona cherimola*. Ministry of Agric. Egypt Bull., p. 157.

Callen DF, Thompson AD, Shenn Y, Phillips HA, Richards RI, Mulley JC (1993). Incidence and origin of 'null' alleles in the $(AC)_n$ microsatellite markers. Am. J. Hum. Genet. 52: 922-927.

Campbell CW, Popenoe J (1988). Effect of gibberellic acid on seed dormancy of *Annona diversifolia* Saff. Proc. Trop. Reg. Am. Soc. Hort. Sci. I 1: 31-36.

Cautin R, Agusti M (2005). Phenological growth stages of the cherimoya tree (*Annona cherimola* Mill.) Scientia Hort 105: 491-497.

Davis CS, Strobeck C (1998). Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Mol. Ecol. 7: 1776–1778.

Escribano P, Viruel MA, Hormaza JI (2004). Characterization and cross-species amplification of microsatellite markers in cherimoya (*Annona cherimola* Mill. Annonaceae). Mol. Ecol. Notes 4: 746-748.

Goudet J (2001). FSTAT a programme to estimate and test gene diversity and fixation indices (Version 2.9.1) Available from http://www.unil.ch/izea/fstat.html. Update from Goudet (1995).

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999). Molecular markers and their application in wheat breeding: a review. Plant Breed. 118: 369-390.

Hamrick JL, Godt MJW (1989). Allozyme diversity in plant species. In: Genetics and Conservation of Rare plants, Eds Falk DD, Holsinger KE. Oxford University Press, Oxford, pp. 75-86.

Hamrick JL, Godt MJW (1990). Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS Plant Population Genetic, Breeding and Genetic Resources Sinauer, Sunderland, MA, pp. 43-63.

Huang H, Dane F, Norton JD (1994). Allozyme diversity in Chinese, Seguin and American chestnut (*Castanea* spp). Theor. Appl. Genet. 88: 981–985.

Kahl G (2001). The Dictionary of Gene Technology. Wiley- VCH, Weinheim.

Kalinowski ST (2004). Counting alleles with rarefaction: Private alleles and hierachical sampling designs. Conserv. Genet. 5: 539-543.

Karron JD, Linhart YB, Chaulk CA, Robertson CA (1988). Genetic structure of populations of geographically restricted and widespread species of *Astragalus* (Fabaceae). Am. J. Bot. 75: 1114–1119.

Kijas JMH, Fowler JCS, Thomas MR (1995). An evaluation of sequence-tagged microsatellite markers for genetic analysis within *Citrus* and related species. Genome 38: 349-355.

Li Z, Rutger JN (2000). Geographic distribution and multilocus organization of isozyme variation of rice (*Oryza sativa* L.). Theor. Appl. Genet. 101: 379-387.

Macherey-Nagel (2004). Nucleospin Macherey-Nagel DNA Extraction kit. http://www.Macherey-Nagel.com

Morden CW, Doebly JF, Schertz KF (1990). Allozyme variation among the spontaneous species of sorghum (Poaceae). Theor. Appl. Genet. 80: 226-304.

Nei M (1972). Genetic distance between populations. Amer. Naturalist 106:283-92. [Div. Biological and Medical Sciences, Brown Univ., Providence, RI

Nevo E, Beiles A (1989). Genetic diversity of wild emmer wheat in Israel and Turkey. Theor. Appl. Genet. 77: 421 – 455.

Peakall R, Gilmore S, Keys W, Morgante M, Rafalski A (1998). Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implica-

tions for the transferability of SSRs in plants. Mol. Bio. Evol. 15: 1275–1287.

Pejic I, Ajmone- Marsan P, Morgante M, Kozumplik V, Castilioni P, Taramino G, Motto M (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDS, SSRs and AFLPs. Theor. Appl. Genet. 97: 1248 -1255.

Perfectti F, Pascual L (2004). Genetic diversity in a worldwide collection of cherimoya cultivars. Genet. Res. Crop Evol. 52: 959-966.

Powell W, Morgante M, Andre C Hanafey M, Vogel J, Tingey S, Rafalski A (1996). The comparison of RFLP, RAPD, AFLP and SSRs markers for germplasm analysis. Mol. Breed. 2: 225 - 238.

Raymond M, Rousset F (1995). GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. J. Hered. 86: 248–249.

Roa AC, Chavarriaga-Aguirre P, Duque MC, Maya MM, Bonierbale MW, Iglesias C, Tohme J (2000). Cross-species amplification of cassava *(Manihot esculenta)* (Euphorbiaceae) microsatellites: allelic polymorphism and degree of relationship. Am. J. Bot. 87: 1647–1655.

Rosetto M (2001). Sourcing of SSR markers from related plant species. In: Henry, R.J. (Ed.), Plant Genotyping: The DNA Fingerprinting of Plants. CABI Publishing, Wallingford, pp. 211– 224.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press, New York.

Sofowora EA (1993). Medicinal plants and traditional medicine in Africa, 2nd edition, P. (85) 174: 195-198.

Weber JL, Wong C (1993). Mutation of human short tandem repeats. Hum. Mol. Genet 2: 1123 -1128.

Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. Evolution 8: 1358-1370.

Williamson J (1975). Useful Plants of Malawi. University of Malawi, Government Printer, Zomba, Malawi.

Yoa M, Wickramaratne DBM (1995). Roemerine, an aporphine alkaloid from *Annona senegalensis* that reverses the multidrug-resistance phenotype with cultured cells. J. Nat. Prod. Lloydia 58(4): 598-604.