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Ecological characterization of an *ex situ* conservation plantation in south-eastern Mozambique

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Abstract

Mozambican forests are exposed to risks that contribute to the loss of biodiversity and associated ecosystem services. Thus, *ex situ* conservation represents a key strategy to reduce genetic erosion. In this study, we evaluated the ecological status of the *ex situ* conservation plantation in Michafutene, Maputo province, one of the most important repositories of forest genetic resources in the country. Thirty plots were established in which all trees, shrubs and grass species were identified. A total of 2092 individuals spanning 39 species were scored. *Afzelia quanzensis* was the most important species (Importance Value Index – IVI = 203), but with a low silvicultural performance. Other important trees were *Albizia adianthifolia* (IVI = 32), *Albizia versicolor* (IVI = 16) and *Pterocarpus angolensis* (IVI = 12). A complementary genotyping analysis of *A. quanzensis* was conducted by intersimple sequence repeats, indicating that the germplasm collection has different provenances and represents a wide genetic pool. Thus, despite the poor management, there is a considerable potential for the conservation of *A. quanzensis* provided immediate and appropriate management activities are implemented to improve its ecological performance.

Key words: Afzelia quanzensis, conservation, ecology, ISSR markers, Mozambique

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Résumé

Mozambican forests are exposed to risks that contribute to the loss of biodiversity and associated ecosystem services. Thus, *ex situ* conservation represents a key strategy to reduce genetic erosion. In this study, we evaluated the ecological status of the *ex situ* conservation plantation in Michafutene, Maputo province, one of the most important repositories of forest genetic resources in the country. Thirty plots were established in which all trees, shrubs and grass species were identified. A total of 2092 individuals spanning 39 species were scored. Afzelia quanzensis was the most important species (Importance Value Index – IVI = 203), but with a low silvicultural performance. Other important trees were *Albizia adianthifolia* (IVI = 32), *Albizia versicolor* (IVI = 16) and *Pterocarpus angolensis* (IVI = 12). A complementary genotyping analysis of *A. quanzensis* was conducted by Inter-Simple Sequence Repeats, indicating that the germplasm collection has different provenances and represents a wide genetic pool. Thus, despite the poor management there is a considerable potential for the conservation of *A. quanzensis* provided immediate and appropriate management activities are implemented to improve its ecological performance. Les forêts mozambicaines sont exposées à des risques qui peuvent contribuer à la perte de leur biodiversité et des services écosystémiques qui y sont associés. La conservation *ex situ* constitue donc une stratégie clé pour réduire cette érosion génétique. Dans cette étude, nous avons évalué le statut écologique de la plantation *ex situ* de Michafutene, dans la province de Maputo, qui compte parmi les plus importants dépositaires des ressources

génétiques forestières du pays. Trente parcelles ont été établies, où toutes les espèces d'arbres, arbustes et herbes ont été identifiées. Au total, 2092 individus appartenant à 39 espèces ont été dénombrés. L'espèce la plus importante était *Afzelia quanzensis* (Indice de valeur d'importance – IVI = 32), mais avec une faible performance sylvicole. Parmi les autres arbres importants, citons *Albizia adianthifolia* (IVI = 32), *Albizia versicolor* (IVI = 16) et *Pterocarpus angolensis* (IVI = 12). Une analyse génotypique complémentaire d'*A. quanzensis* a été réalisée au niveau des microsatellites (Inter-Simple Sequence Repeats – ISSR), qui indique que la collection de germoplasmes vient de différentes provenances et représente un large pool génétique. Donc, malgré une gestion médiocre, la conservation d'*A. quanzensis* garde un potentiel considérable pour autant que des activités de gestion appropriées soient réalisées immédiatement pour en améliorer les performances écologiques. .

Introduction

Forest resources are often the backbone of human well-being and the economy of developing countries. However, over-exploitation of these resources and the corresponding impacts on biodiversity pose a risk to the preservation of its availability and accessibility. It is estimated that 50% of the world's wild vegetation is threatened or endangered (Thuiller *et al.*, 2005; Bramwell, 2007). Thus, conservation and sustainable use of genetic resources are crucial for ecosystem stability and their continuous provision of resources to rural and urban communities (CBD, 2006; Fraleigh, 2006). *Ex situ* conservation represents an important strategy and in many cases is the only possible way to minimize genetic erosion, while contributing to reduce species vulnerability to environmental pressures (Amaral, Thomson & Yanchuk, 2004; Fraleigh, 2006). Comparing to *in situ* conservation strategies, its main advantages are as follows: i) the possibility to establish rather big (and diverse) collections; ii) quick access to samples for evaluation, characterization, distribution and use; and iii) better safety against biotic and abiotic threats. However, underfunding and limited knowledge of *ex situ* collections imposes major drawbacks to this strategy.

Mozambican forests represent an important repository of biodiversity, being a key provider of ecosystem services. However, the progressive exposure to logging and poor management (Uetimane, 2011), as well as the growing demand for timber, constitutes a serious threaten for forest resources in the country. Efforts have been made to counteract biodiversity loss, including the establishment and expansion of conservation areas, which at present represent almost 25% of the country's total area (MICOA, 2009). Incipient *ex situ* conservation strategies have been established including seed and germplasm collections maintained at the National Agricultural Research Institute (IIAM) and a number of plantations of native forest species across the country. One of the oldest forest plantations is located in Michafutene, district of Marracuene (Maputo province). It was established between 1930 and 1960 in an area of *ca.* 1000 ha, to test provenances and progenies of important timber species such as *Afzelia quanzensis* Welw. (pod mahogany), *Pterocarpus angolensis* DC. (umbila), *Millettia stuhlmannii* Taub. (panga-panga) and *Amblygonocarpus andongensis* (Welw. ex Oliv.) Exell & Tower (Scotsman's rattle) (IIAM, 2009). This long-standing conservation effort has been seriously compromised over the past 40 years, due to the civil war (1975–1992) and accelerated urbanization (since 1995). Besides the reduction in the conservation area (from 1000 ha to *ca.* 50 ha), information about species provenance and the ecology of the area has been lost. This information is highly relevant to define appropriate conservation strategies and actions.

The objectives of our study were as follows: (i) to evaluate the ecological status of the *ex situ* conservation area of Michafutene, with particular emphasis in *A. quanzensis*; (ii) to conduct a preliminary genotyping analysis of this species in order to assess its genetic diversity within the plantation. Two research questions were addressed: (i) what is the ecological status of *A. quanzensis* in the Michafutene stand? (ii) is the diversity of the *A. quanzensis* collection typical from an *ex situ* conservation zone?

Species selection was based on the timber value (first class timber according the Forestry and Wildlife Law), importance for rural communities (energy source and traditional medicine) as well as its vulnerability to disturbances (SANBI, 2006; MICOA, 2009). It was also selected because it dominates more than 70% of the area.

Materials and methods

Study site

The *ex situ* conservation area of Michafutene is located in the district of Marracuene, Maputo Province, 26° 50′ S and 32° 35′ E (Fig. 1a, b) (MAE, 2005). The plantation

covers an area of 49.74 ha, of which 29.44 ha are occupied by *Afzelia quanzensis*, 2.10 ha by *Millettia stuhlmannii*, 0.72 ha by *Amblygonocarpus andongensis* and 2.66 ha by wetlands. The remaining 14.82 ha contain infrastructures (IIAM, 2009).

The climate is classified as subtropical, with a mean annual temperature of 20°C and an annual temperature range of less than 10°C (MAE, 2005). The relative humidity varies between 55 and 75%, and the mean annual rainfall is 1000 mm. The rainy season occurs from October to April, with 60–80% of the rainfall concentrated between December and February (MAE, 2005).

Sampling and data collection

Data collection followed the protocols described by Kent (2012). Thirty randomized sampling plots of 50 × 20 m (0.1 ha) corresponding to 10% of the total area were distributed across the 29.44 ha area where *A. quanzensis* occurs and their geographic position was recorded with a Geographic Positioning System (GPS – Garmin 62S precision of 10 m). Species identification was carried out in the field and compared to botanical vouchers of specimens deposited at the Herbarium of the IIAM (LMU, Maputo, Mozambique). Diameter at Breast Height (DBH) and tree height (m) were measured for all woody species in the three stages: (i) nonestablished natural regeneration (DBH below 5 cm); (ii) established natural regeneration (DBH between 5 cm and 10 cm); and (iii) adults (DBH above 10 cm). Natural regeneration (DBH < 10 cm and height >1 m) was assessed in subplots of 25 × 20 m within a main plot. Abundance of grass/herbaceous components was assessed by allocating five 1 m^2 squares (four at the corner and one at the centre) within the main plots. Figure 1c shows the schematic representation of the sample plots.

To evaluate the diversity of *A. quanzensis* genotypes, thirty individual trees were randomly sampled within the 29.44 ha. Young healthy leaves were harvested from each individual and transported to the laboratory in silica gel rubin (Fluka). Once in the laboratory, they were frozen in liquid nitrogen and stored at −80°C until DNA extraction.

Horizontal and vertical structure

The horizontal structure (species abundance, dominance and frequency) was analysed through the relative

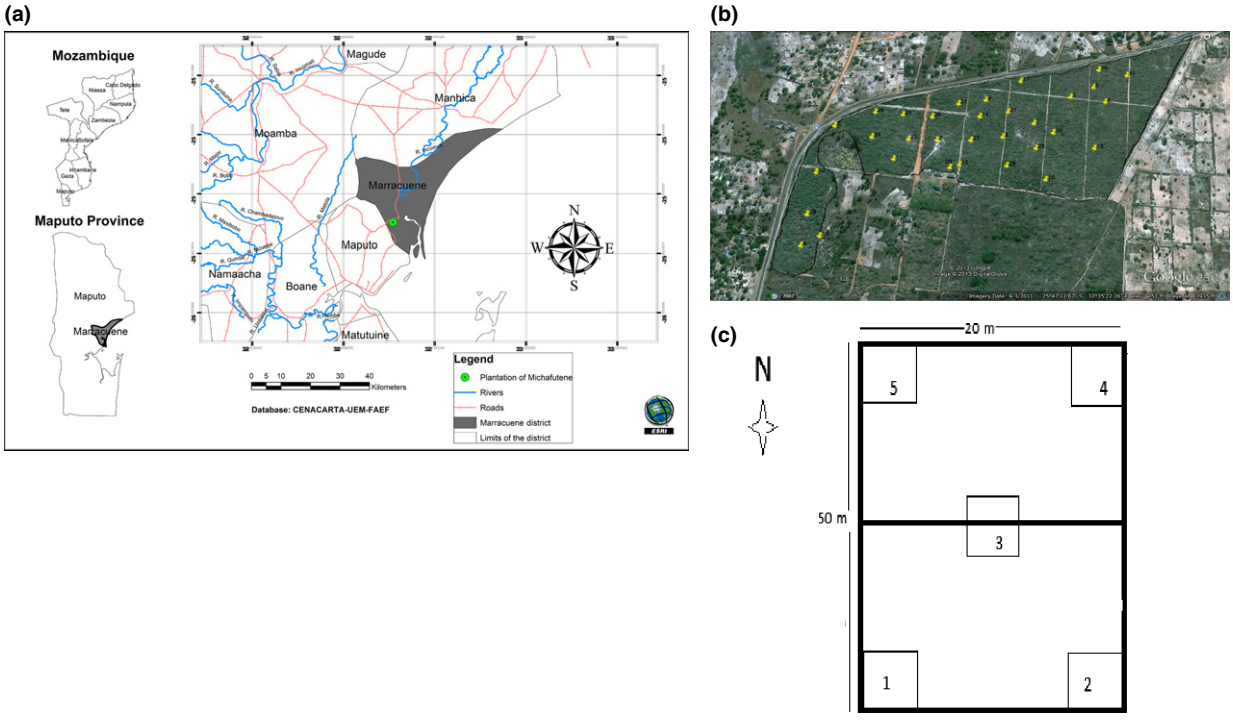

Fig 1 Map of the study site (a); map of the plantation (b); and map of the sampled plots (c)

ecological weight of a species in the stand and the diametric distribution of all individuals as described in Lamprecht (1990). Based on the data collected for each species, the following parameters were calculated: i) absolute abundance (number of individuals ha^{-1}); ii) relative abundance (%); iii) absolute dominance (basal area in $m^2\ ha^{-1}$); iv) relative dominance (%); v) absolute frequency (number of plots where at least one individual of one species occur); and vi) relative frequency (%). The relative abundance, dominance and frequency were then integrated to calculate the Importance Value Index (IVI = %Frequency + % Abundance + %Dominance).

To analyse the trend in overall diametric distribution, the trees were grouped by DBH class (5–10 cm, 11–15 cm, 16–20 cm, 21–25 cm, 26–30 cm, 31–35 cm, 36–40 cm and >40 cm). Established natural regeneration (DBH 5–10 cm) was identified at the species level, and the abundance per species was determined.

Vertical structure (vertical extracts) was determined by estimating the sociological position of trees in the stand, according to the formulas (1) and (2) (Finol, 1971):

$$\mathrm{SPabs}_i = \frac{[(n_{1i} * N_1) + (n_{2i} * N_2) + \cdots + (n_{ni} * N_j)]}{N} \qquad (1)$$

where SPabs_i = Absolute sociological position of species *i*.

$n_{1,2,i}$ = Number of trees of species i in the strata 1, 2, *j*.

$N_{1,2,i}$ = Total number of trees in the sampling area.

$$\mathrm{SP}\% = \frac{\mathrm{SPabs}_i}{\sum_{i=1}^{s} \mathrm{SPabs}_i} \times 100 \qquad (2)$$

where PS= Sociological Position of species i.

Plant association and distribution

Multivariate statistical analyses were conducted in the Multivariate Statistical Package (MVSP 3.1) developed by Kovach (2000) to assess plant association and distribution. The matrix for the analyses consisted of 30 rows and 49 columns. Rows corresponded to the number of plots and columns to tree species abundance. A cluster analysis of species abundance was performed to analyse particular associations between *A. quanzensis* and other species.

ISSR Amplification

Genomic DNA was extracted from 50 to 100 mg of ground leaves according to the protocol of Doyle & Doyle (1987). After ribonuclease treatment (Thermo Scientific, Oporto, Portugal), the average yield and purity were assessed spectrophotometrically (Lambda EZ201; Perkin Elmer, Lisbon, Portugal) by OD230, OD260 and OD280 readings and visually after 1% agarose electrophoresis. Twenty-seven primers were initially screened using four randomly selected accessions according to the protocol described in Maquia *et al.* (2013). Each set of reactions was performed in triplicates and included a negative control, in which the DNA has been replaced with sterile water. Thermal cycling was conducted in an iCycler (Bio-Rad, Amadora, Portugal) using the program: 1 cycle at 95°C for 3 min followed by 35 cycles at 95°C for 90 s, 42°C for 90 s, 72°C for 90 s and a final extension step at 72°C for 10 min. Four primers (Table 1) were labelled with WellRed-D3 fluorescent dye (Sigma Aldrich, Madrid, Spain) for subsequent capillary electrophoresis using the CEQ 8000 Genetic Analysis System (Beckman Coulter, Carnaxide, Portugal) and used to analyse the entire sample set, using the conditions described above. Two µl of each PCR product denatured in 24 µl of deionized formamide was used. The size of the fragments was estimated using a standard internal marker (D1 MapMarker 1000; BioVentures, Murfreesboro, TN, USA) with DNA fragments ranging from 50 to 1000 bp, added to each sample prior to electrophoresis. For all primers, loci smaller than 100 bp and greater than 1000 bp were excluded from the analysis because they were outside of the standard fragments' range.

Phenetic analysis

The AFLP Fragment Analysis Module of the Genetic Analysis System Software (Beckman Coulter), which is also appropriate for ISSR data, was employed to produce a binary matrix based on the presence (1) or absence (0) of amplification peaks. Data analysis was carried out using the software NTSYSpc version 2.01b (Applied Biostatistics Inc., Port Jefferson, NY, USA). The unweighted pair group method using arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested (SAHN) routines were performed based on the JACCARD's similarity coefficient for cluster analysis. The genetic similarity data matrix was used to perform Principal Coordinate Analysis

Table 1 Primer sequence, total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (P %), average number of bands, average and number of polymorphic bands

Primer	Sequence (5′–3′)	TNB	NPB	P%
ISSR 6	VHV(GT)7	45	45	100.00
ISSR 18	BDB(CA)7	34	33	97.06
ISSR 23	(AC)8YG	30	29	96.67
ISSR 24	(GA)8YC	30	27	90.00
Total		**139**	**134**	
Average		**34.75**	**33.5**	**95.93**

Bold values correspond to the total and average values.

(PCoA). Nei's genetic diversity, global genetic diversity, coefficient of gene differentiation, gene flow and Shannon's gene diversity index were calculated with POPGENE32 (Yeh *et al.*, 1997) assuming the Hardy–Weinberg equilibrium.

Results

Forest composition and structure

A total of 2092 individuals were recorded in the sampled area. These corresponded to 39 species spanning 23 families and 37 genera. *Afzelia quanzensis* was the most abundant species with 1025 individuals (*ca.* 49% of the total), followed by *Strychnos madagascariensis* Poir. (297 individuals or *ca.* 14%), *Albizia adianthifolia* W. Wight (143 individuals or *ca.* 7%), *Psydrax locuples* (K. Schum.) Bridson (110 individuals or *ca.* 5%) and *Garcinia livingstonei* T. Anderson (91 individuals or *ca.* 4%) (Fig. 2).

The Importance Value Index (IVI) revealed that *A. quanzensis* is ecologically the most important species in this forest (IVI = 203/300), reinforcing its importance for the ecology of the stand. Other ecologically important species in this area are *A. adianthifolia* (IVI = 32), *A. versicolor* (IVI = 16) and *Pterocarpus angolensis* (IVI = 12). Together, these four species represent 88% of the total IVI.

The uniform distribution of *A. quanzensis*, revealed by the dendrogram in Figure 3, clearly indicates that the species does not present any particular association. A cluster composed by *Terminalia sericea* Burch. ex DC., *Vangueria infausta* Burch., *Trichilia emetica* Vahl and *Vernonia colorata* Drake was observed in places where *A. quanzensis* was absent.

A. quanzensis was also dominant at young stages, with 707 individuals/ha or 51% of the total regeneration

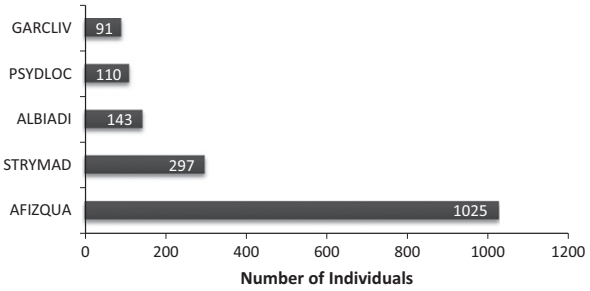

Fig 2 Most abundant species in the Michafutene plantation. GARCLIV: *Garcinia livingstonei*, PSYDLOC: *Psydrax locuples*, ALBIADI: *Albizia adianthifolia*, STRYMAD: *Strychnus madagascarensis*; AFZLQUA: *Afzelia quanzensis*

abundance. Other important species in the sapling layer include *S. madagascarensis* with 297 individuals ha^{-1}, *P. locuples* with 109 individuals ha^{-1}, *A. adianthifolia* with 102 individuals ha^{-1}, *G. livingstonei* with 91 individuals ha^{-1} and *Strychnos spinosa* Lam. with 90 individuals ha^{-1}.

The forest contained a significant herbaceous and grass component of 606 individuals distributed over 46 species. The most abundant species in the area were as follows: *Aristida adscensionis* L., *Brachiaria deflexa* (Schumach.) C.E. Hubb. ex Robyns, *Cynodon dactylon* (L.) Pers., *Digitaria ciliaris* (Retz.) Koeler, *Enneapogon cenchroides* (Licht. ex Roem. & Schult.) C.E. Hubb., *Eragrostis chapelieri* Peters, *Bulbostylis barbata* (Rottb.) C.B. Clarke, *Cyperus amabilis* Vahl, *Cyperus sp., Aneilema indehiscens* Faden, *Coleotrype* sp., *Commelina africana* L. and *Commelina benghalensis* L.

The Michafutene forest was composed of three vertical strata defined according to the sociological position of woody vegetation. The dominant height found in this forest was 7.8 m and was composed of *A. quanzensis*. The upper stratum presented an average height of 5.3 m and was represented by 82.5% of the total number of individuals. The middle stratum comprised 17% of the total number of individuals and reached an average height of 2.6 to 5.2 m. The lower stratum (<2.5 m) was represented by 0.48% of the total number of trees. This stratum was also characterized by the presence of grass and herbaceous species. *A. quanzensis* was well represented in all three strata indicating its representation in different developmental stages. Thirty-five per cent of the species were only present in the upper stratum but with very low abundances. These were as follows: *Commiphora africana* (A. Rich.) Engl., *Eucalyptus camaldulensis* Dehn., *P. locuples* and *V. colorata*, all with only one individual, *Olax dissitiflora* Oliv. with two

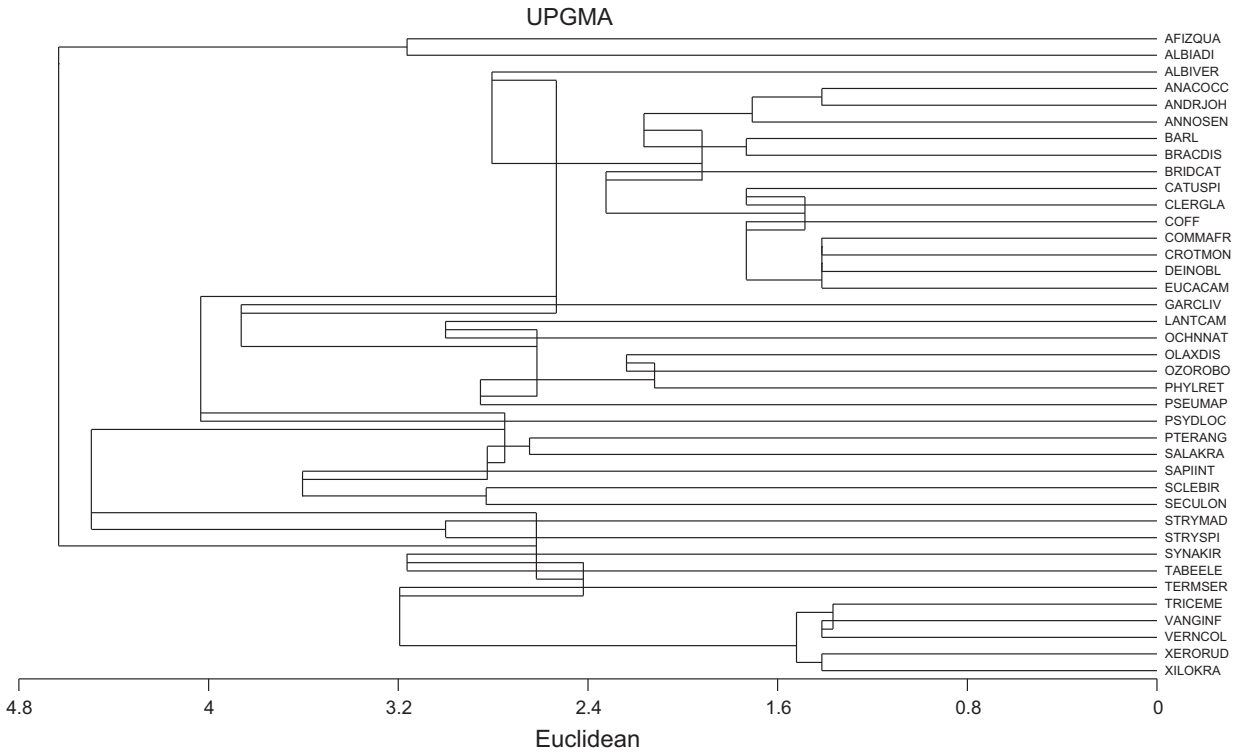

Fig 3 Dendrogram of tree species association in the Michafutene Forest. AFIZQUA: *Azelia quanzensis*; ALBIADI: *Albizia adianthifolia*; ALBIVER: A*lbizia versicolor*; ANAOCC: *Anacardium occidentale*; ANDRJOH: *Androstachys johnsonii*; ANNOSE: *Annona senegalensis*; BARL: *Barleria sp.*; BRACDIS: *Brachylaena discolor*; BRIDCAT: *Bridelia cathartica*; CATUSPI: *Catunaregum spinosa*; CLERGLA: *Clerodendrum glabrum*; COFF: *Coffea* sp.; COMMAF: *Commiphora africana*; CROTMO: *Crotolaria monteiroi*; DEINOBL: *Deinbollia oblongifolia*; EUCACAM: *Eucalyptus camaldulensis*; GARCLIV: *Garcinia livingstonei*; LANTCAM: *Lantana camara*; OCHNNAT: *Ochna natalitia*; OLAXDIS: *Olax dissitiflora*; OZOROB: *Ozoroa obovata*; PHYLRET: *Phyllanthus reticulatus*; PSEUMA: *Pseudolachnostylis maprouneifolia*; PSYDLOC: *Psydrax locuples*; PTERANG: *Pterocarpus angolensis*; SALAKRA: *Salacia kraussii*; SAPIINT: *Sapium integerrimum*; SCLERBIR: *Sclerocarya birrea*; SECULON: *Securidaca longipedunculata*; STRYMA: *Strychnos madagascariensis*; STRYSPI: *Strychnos spinosa*; SYNAKIR: *Synaptolepis kirkii*; TABEELE: *Tabernaemontana elegans*; TERMSE: *Terminalia sericea*; TRICEME: *Trichilia emetica*; VANGINF: *Vangueria infausta*; VERNCOL: *Vernonia colorata*; XEROEUI: *Xeromphis rudis*; XILOCRA: *Xilotheca kraussiana*

individuals and *Sapium integerrimum* (Hochst. ex Krauss) J. Léonard with five individuals. Species represented only in the middle stratum were as follows: *Coffea* sp., *S. spinosa*, *Tabernaemontana elegans* Stapf and *T. emetica*, which by nature will never reach the upper level.

Silviculturally, *A. quanzensis* showed low performance as compared to its range in natural habitats (average DBH = 16 cm, total height = 5 m and commercial height = 2 m). However, the DBH class size distribution for this species presented an inverted J-shaped curve, with most of the trees falling within the range of 5 and 15 cm, that is young classes, with only 1% of the trees belonging to the adult class (Fig. 4a). The same result was found for the all stand (considering all tree species together, including *A. quanzensis*) (Fig. 4b), but trees with DBH above 40 cm were represented only by *A. quanzensis*.

Genetic analysis

In total, the four primers generated 139 bands, from which 134 were polymorphic. The number of polymorphic bands ranged between 27 (90%) for primer ISSR 24 and 45 (100%) for primer ISSR 6, with an average of 33.5 (95.93%) (Table 1).

A dendrogram based on UPGMA analysis was generated using the Jaccard's coefficient showing the clustering pattern between the individuals (Fig. 5a). Four main clusters (I, II, III and IV) were formed. Cluster I grouped

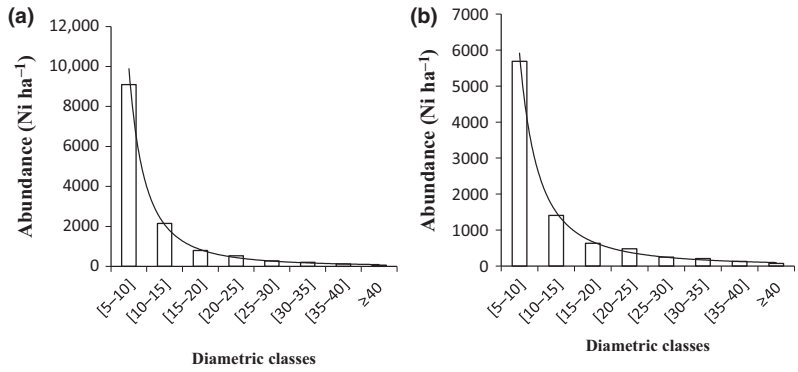

Fig 4 DBH class size distribution (in cm) of the all stand (a) and of *A. quanzensis* (b) in the Michafutene plantation

eight accessions (2, 4, 5, 13, 14, 34, 60, 80), with a maximum similarity coefficient of 0.696 (between individuals 13 and 60) and a minimum of 0.329 (between 2 and 80). Cluster II comprised 18 accessions (11, 20, 27, 31, 35, 37, 39, 41, 42, 43, 49, 55, 58, 59, 63, 66, 67, 79) with similarity coefficients ranging from 0.833 (between accessions 37 and 79) to 0.331 (between accession 11 and the remaining accessions). Two accessions were grouped in Clusters III (24, 33) and IV (50, 56), presenting a similarity coefficient of 0.303 and 0.500, respectively. The results of the PCoA were comparable to the cluster analysis (Fig. 5b). The first three most informative PCo explained 94.45% of the total variation (62.15%, 17.94% and 14.36% for PCo1, PCo2 and PCo3, respectively).

Nei's genetic diversity (h), global genetic diversity (Ht), coefficient of gene differentiation (Gst), estimation of gene flow (Nm) and Shannon's gene diversity index (I) were 0.2736, 0.1745, 0.5683, 0.3799 and 0.4289, respectively. When Jaccard pair-wise genetic distances were correlated with physical distances of the different accessions, determined from the GPS coordinates, very low Pearson ($r = 0.137$; $P = 0.598$) and Spearman ($r = 0.337$; $P = 0.055$) correlation were observed (data not shown).

Discussion

The Michafutene plantation represents one of the few *ex situ* conservation areas of *Afzelia quanzensis* and one of the last remnants of this plant species in Mozambique. It also represents an important forest fragment in the Maputo province, which is growing at accelerate levels. According to Kowero, Njuki & Nair (2008), urban growth in developing countries represents a huge challenge because people living in cities with rural habits increase the demand for energy, food and shelter. For instance, in Tanzania, Hosier, Mwandosya & Luhanga (1993) reported that for each 1% increase in urban areas there is an increase in 14% demand for charcoal. Thus, the maintenance of forest fragments in an urbanized environment is crucial as they may represent important source of goods and services, alleviating the pressure over natural forests.

Afzelia quanzensis dominates the Michafutene plantation and is uniformly distributed across the area as demonstrated by the dendrogram analysis and the J-shaped diametric distribution. Other tree timber species such as *Albizia adianthifolia*, *A. versicolor* and *Pterocarpus angolensis* are also ecologically important in this forest fragment. Besides that, fruit tree species, including *Strychnus spinosa*, *S. madagascariensis*, *Garcinia livingstonei*, and a variety of grass species are also abundant. This indicates that natural ecological processes have shaped the area, which is expected after more than 50 years without proper management. It also reveals that this plantation may represent an important spot for biodiversity conservation and provision of goods and services for the communities around it, but proper management activities are needed. The mean DBH (16 cm) and dominant height (5 m) of *A. quanzensis* indicate that its silvicultural performance is very low if compared to natural habitats (DBH up to 1 m and height = 15 m; Orwa *et al.*, 2009). According to Mligo *et al.* (2009), *A. quanzensis* in general performs very well in its natural environment and in managed plantations. The authors report that in Tanzania *A. quanzensis* along with other timber species are underrepresented in large DBH classes in areas that have been heavily logged. However, after years of logging abandonment, the species tends to recover relatively fast. In fact, in our study, we found that *A. quanzensis* is well represented in the lower

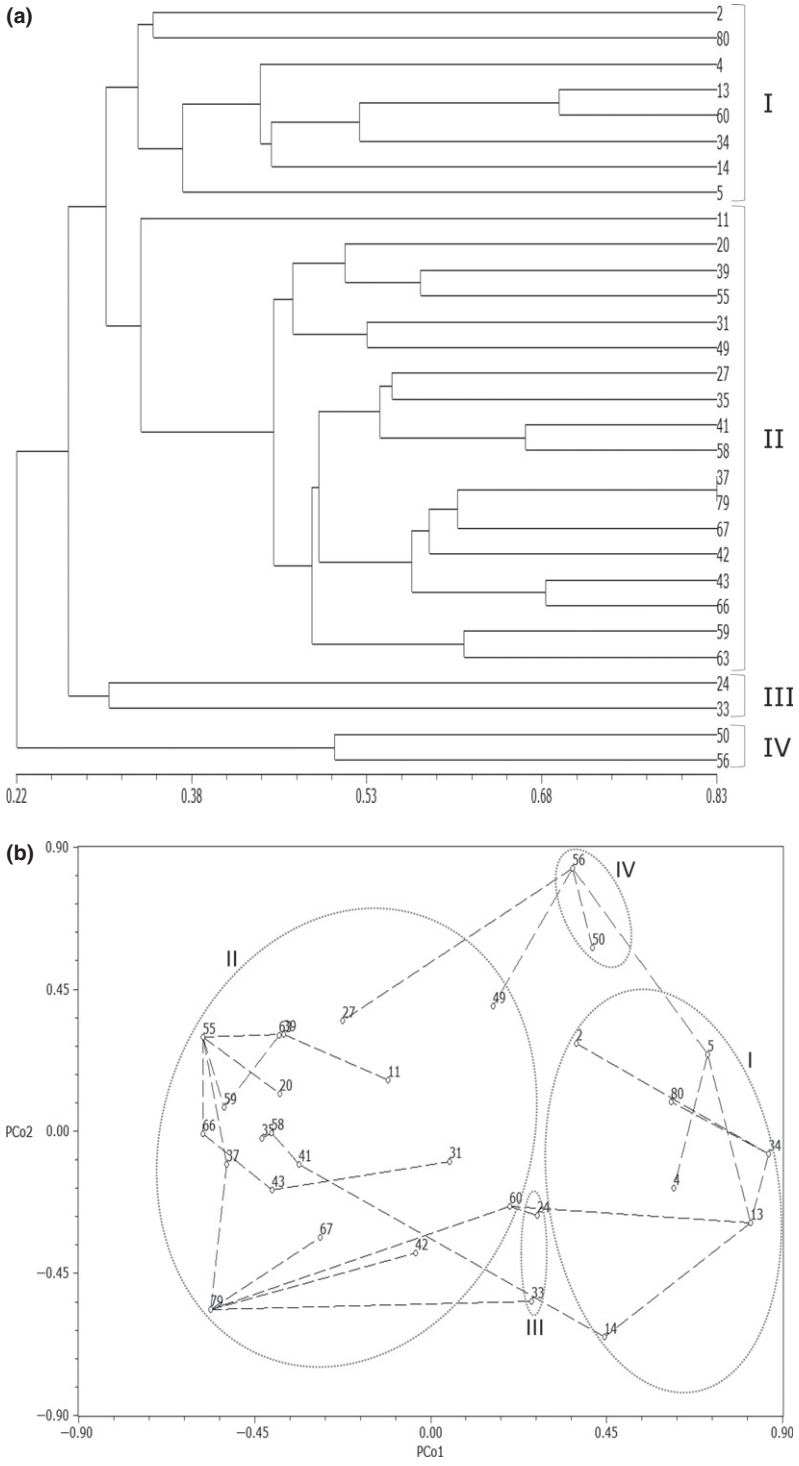

Fig 5 Dendrogram based on UPGMA analysis generated using the Nei and Li's coefficient (a) and Principal Coordinate Analysis (PCoA) (b) representing the phenetic relationships among the sampled accessions

stratum and presents relatively good performance. These results indicate that in order to develop this area to accomplish the objective of conserving *A. quanzensis* biodiversity it is crucial to establish a forest management plan for the area. Key management activities should involve i) elimination of competition by grass and shrub species at the young stages to enhance *A. quanzensis* growth and silvicultural performance; ii) removal of adult individuals especially those with low performance to allow young individuals to thrive in the ecosystem.

ISSR markers were successful in generating a high level of polymorphic banding patterns (95.93%) in *A. quanzensis*, which is consistent with previous observations from other species of the Fabaceae family, for example 95.6% in *Brachystegia boehmii* Taub. and 89.4% in *Burkea africana* Hook. (Maquia *et al.*, 2013), 99.38% in *Astragalus rhizanthus* Royle ex Benth. (Anand *et al.*, 2010) and 92.2% in *Glycyrrhiza uralensis* Fisch. ex D.C. (Yao *et al.*, 2008). Cluster and PCoA analyses grouped the individuals into four groups, with a degree of genetic diversity ranging from 16.67 to 94.29%. Allelic diversity analysis, that is Nei's genetic diversity (h), total genetic diversity (Ht), genetic differentiation (Gst), genetic flux (Nm) and Shannon Index (I), revealed a considerably high degree of genetic diversity among the sampled individuals. These findings are in line with similar studies conducted in other conservation areas (Maquia *et al.*, 2013; Pakkad, Ueno & Yoshimaru, 2008, suggesting that this species can propagate sexually in a wide variety of environments, thus preserving a wide genetic pool. Most importantly, the distribution of the accessions among the clusters was not correlated with their geographic location within the plantation, indicating that the selection of material for the plantation (between 1930 and 1960) was rigorous, probably representing genotypes from different geographic locations within the country and eventually the region.

Conclusion

The Michafutene plantation has a great potential for the conservation of *A. quanzensis* provided immediate and appropriate management activities are implemented. Besides the improvement of silvicultural practices, the establishment of a seed bank would be of utmost importance for preserving biodiversity for future plantations and/or rehabilitation programmes in the region. We also believe that this work may constitute an incentive for the preservation of this genetic heritage, which is being increasingly exposed to human pressure, as well as for the intensification of similar research initiatives in Mozambique and in the region.

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