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Population genetic responses of wild forage species to grazing along a rainfall gradient in the Sahel: A study combining phenotypic and molecular analyses

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Abstract Genetic diversity was studied in wild forage species subjected to grazing along a rainfall gradient in West Niger, within the Sahel with aim of identifying adapted genotypes, which could be used to reclaim degraded land. Two legumes (*Alysicarpus ovalifolius and Zornia glochidiata*) and two grasses (*Brachiaria xantholeuca* and *Cenchrus biflorus*) were selected to relate phenotypic adaptation to genetic diversity in response to grazing and rainfall. Populations of each species were sampled from both heavily grazed and ungrazed sites along a rainfall gradient, approximating 200 mm yr^{-1} to 800 mm yr^{-1} rainfall isohyets. The adaptative phenotypic expressions to aridity and grazing of the populations from each of the species were characterised by morphological measures performed on the plants sampled in the field. These analyses were then compared with the results from genetic analyses using the PCR-based techniques of amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD). Analyses of molecular data using cluster analysis (UPGMA), principal coordinates analysis (PCO), Mantel tests and an Analysis of Molecular Variance (AMOVA), revealed genotypic distinction between populations subjected to both differing aridity and grazing. The majority of the total genotypic variation sampled in all species occurred among individuals within a population. The significant morphological differentiation found among populations subjected to varying grazing and aridity stresses, determined through linear regression analyses, did not correlate significantly with the genotypic differentiation, as revealed by Mantel tests. The results suggest that grazing does not cause a loss of genetic diversity in the wild forage species studied, although with increasing aridity the impact of grazing on the genetic diversity of populations may increase.

Keywords Adaptive traits · AFLP · Population genetics · Sahel · Wild forages

Introduction

The Sahel extends along the southern edge of the Sahara Desert, encompassing 10 African countries that are among the poorest with rapidly growing populations close to 100 million (Snrech, 1994). With a single short rainy season of 2–4 months (600 mm yr^{-1}–100 mm yr^{-1} isohyets), livestock rearing and dry–land cropping are among the principal fundamentals

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of food security for the region. However, with high demographic increase and rapid urbanisation, pressure on Sahelian natural resources has increased dramatically to the point where land degradation (clearance for cropping, overgrazing, forestry and soil erosion) threatens the sustainability of rural livelihood. More specifically, the impact of livestock on rangeland vegetation and soils is often cited as one of the main degradation processes leading to a decline of biodiversity of the region (Hiernaux, 1996). This hypothesis has been supported by studies assessing the impact of such environmental changes on parameters such as plant species composition, nutrient concentration and standing mass of vegetation (Hiernaux, 1998).

Wild rangeland forages also form an important component of the Sahelian biodiversity. Due to imminent threats to forage species, this study was undertaken to assess the impact of grazing and aridity on genetic diversity of selected wild forage species. Populations of two annual grasses (*Cenchrus biflorus* Roxb. and *Brachiaria xantholeuca* (Hack. Ex Schinz) Stapf) and two legumes (*Alysicarpus ovalifolius* (Schum. & Thonn.) J. Léonard and *Zornia glochidiata* Reichb. Ex DC.) were selected based on good forage quality, adaptation to climate aridity and resistance to grazing stresses. Notably, the traits by which these four species are adapted to aridity and grazing stress differ. *Cenchrus biflorus* is one of the dominant outcrossing annual grass species in the northern Sahel. This species is resilient to grazing due to its fast growth and ability to tiller in response to defoliation and trampling. *Brachiaria xantholeuca,* one of the more common among several out-crossing annual *Brachiaria* species present in the Sahel, resists heavy grazing pressure by increasing the number of tillers that grow almost horizontally near the soil surface, reducing the risks of further defoliation. *Alysicarpus ovalifolius* is an in-breeding legume very variable in size (from 2 to up to 60 cm in height) and shape. The adaptation of *A. ovalifolius* to aridity and grazing is based on the ability of the plant to flower and set seeds in less than a month, and then flowering and fruiting are continuous processes spread over the growing season (Grouzis, 1988). *Zornia glochidiata* is a small size in-breeding legume (20 to 40 cm high), commonly found throughout the Sahel. The adaptation to aridity is based on its ability to stand dry spells and the staggered germination of *Z. glochidiata* seed stock allowing new waves of germination when a previous one failed.

Molecular techniques offer opportunities to directly study variation controlled at the genetic level avoiding many of the complications of environmental effects acting upon morphological characters. The genetic diversity of the wild forage species was characterised using two PCR-based techniques, AFLPs (Vos et al., 1995) and RAPDs (Williams et al., 1990). There are numerous examples of the application of the RAPD (Heaton et al., 1999; Li et al., 1998) and AFLP (Escaravage et al., 1998; O'Hanlon et al., 1999) in ecological investigations.

To date, several molecular studies have been carried out to investigate forage genetic diversity (Huff et al., 1993; Gunter et al., 1996; Li et al., 1998; Kölliker et al., 1999), however only one study, utilising the RAPD technique has been applied to the four selected forage species (Kußerow, 1997). Our study is the first to apply molecular techniques to relate genetic diversity to the impact of grazing and aridity stresses on the adaptive traits of Sahelian rangeland vegetation, specifically wild forages. In addition, the relationship between the molecular data and phenotypic data may also allow us to address issues surrounding the direction of molecular evolution, i.e. whether natural selection is a central mechanism directing molecular evolution or whether stochastic or neutral changes are responsible. Recent studies (Owuor et al., 1997; Li et al., 1999; Li et al., 2000) have suggested that natural selection overrides gene flow and genetic drift, thus providing support for the environmental theory of genetic diversity (Nevo, 1988), which emphasises the possible role of natural selection in diversifying and maintaining adaptive polymorphism in nature, thereby directing Darwinian evolution at the molecular and organism levels (Owuor et al., 1997). However, evidence against the environmental theory of genetic diversity has also been presented. Heaton et al. (1999) found that extreme ecological and phenotypic differences in distinct populations of chicozapote, a tropical tree, in Mexico were not matched by genetic divergence. Our study, also examining DNA polymorphism in sharply contrasting ecological conditions, contributes to the evidence for or against natural selection as the major evolutionary cause of molecular polymorphism.

The objectives of the present study were to characterise the molecular genetic diversity of selected populations of four wild forage species of the Sahel and

to relate these analyses to the phenotypic expression of adaptive traits to aridity and grazing, and thus to determine the level of correlation among them and thereby shedding light on possible forces directing molecular evolution in these forages. The ultimate goal is to identify adapted genotypes, which could be used in reclaiming degraded land in semi-arid zones and provide high quality fodder and also provide information that may be useful for future conservation efforts.

Material and methods

Plant material

Plant collections were made in August/September 1998 from the north and south of Niamey (Fig. 1 and Table 1) in the Sahelian area along a rainfall gradient of $270.0 \pm 99.6\,\mathrm{mm\,yr^{-1}}$ in Menaka to $796.8 \pm 139.3\,\mathrm{mm\,yr^{-1}}$ in Gaya (mean and SD from 1961–1990; Sivakumar et al., 1984, 1993). Each location was selected to correspond to a rainfall gradient (Table 1). Grazed and ungrazed sites were targeted at each location for ease of results comparison. Six sites grouped in five locations were selected from rangeland that had been protected from grazing or subjected to light grazing pressure (due to the distance to water points from the livestock path) and from natural rangeland that had been subjected to heavy grazing over many years. Collections were also made from the ICRISAT research station at Sadoré, Niger, within the $600\,\mathrm{mm\,yr^{-1}}$ rainfall gradient. The station has a specially protected reserve, ungrazed for over 20 years and could be compared with nearby intensely grazed site. At each site, samples of *A. ovalifolius* (137), *Z. glochidiata* (110), *B. xantholeuca* (117) and *C. biflorus* (106) were taken from each corner of a 1 m quadrant, which was placed randomly at four points within the site. All the selected sites are on the same geological unit, 'Continental terminal', which consists of very sandy soils (more than 95% sand) that are acidic and poor in organic matter.

Genomic DNA isolation

High molecular weight genomic DNA samples were isolated from 200 mg of lyophilized leaf material following a modified CTAB procedure based on the protocols described by Saghai-Maroof et al. (1984), Rogers and Bendich (1985) and Doyle and Doyle (1987), as described in Weising et al. (1995). The quality of genomic DNA was examined by agarose gel electrophoresis and spectrophotometry. DNA samples were diluted to a final concentration of 100 ng/μl for the AFLP procedure and 5 ng/μl for the RAPD procedure, and stored at $-20\,^{\circ}\mathrm{C}$.

AFLP protocol

The AFLP procedure was carried out using the commercially available kit from Perkin-Elmer Biosystems for fluorescent fragment detection. *Eco*RI and *Mse*I were used for DNA digestion. Adapter ligation, preselective and selective amplification were carried out according to the AFLP™ Plant Mapping Kit protocol from Perkin Elmer. Four primer combinations were selected for use in this study: *Eco*RI + ACA/*Mse*I + CAC, *Eco*RI + AAC/*Mse*I + CAG, *Eco*RI + ACA/*Mse*I + CTC, *Eco*RI + ACC/*Mse*I + CAC, following an initial screening of twenty-two primer combinations in total. PCR amplifications were performed using a Perkin-Elmer 9600. AFLP fragments were separated on an ABI PRISM™ 377 DNA Sequencer (Perkin Elmer) in a 5% Long Ranger™ gel. Genescan-500 ROX-labelled size standard was loaded in each lane in order to facilitate sizing of the fragments. GeneScan and GenoTyper software captured the data automatically.

RAPD protocol

PCR amplifications were performed based on the standard protocol of Williams et al. (1990) in a 25 μl volume consisting of 20 ng of template DNA in the presence of 10*x* Promega Buffer and 1 U of Promega *Taq* polymerase, 1.9 mM $MgCl_2$, 100 μM dNTPs and 0.2 pM of random decamer primer. The amplification reactions were performed in PTC-100™ (MJ Research, Inc.) programmed for an initial denaturation at 94 °C for 3 min, followed by 44 consecutive cycles each consisting of 30 sec at 94 °C, 45 sec of annealing at 36 °C, 2 min of extension at 72 °C, and a final 4 min extension at 72 °C, followed by recovery at 4 °C. The amplification products were separated and visualised on a 1.8% agarose gel using 1xTBE buffer containing ethidium bromide, and were photographed using polaroid film. Of the sixteen RAPD primers used in initial screening, eight were selected (primer details available on request). Amplified bands were scored as present or absent for each DNA sample. All bands scored were

Table 1 Details of rainfall gradient, calculated from rainfall data from 1961–1990 (Sivakumar et al., 1993, 1984), along which the grass and legume populations were sampled

Site	Nearest weather station	Rainfall in mm yr^{-1}	Abbreviated code
Ekrafan	Menaka	270.0 ± 99.6	200
Toukounous	Toukounous	330.2 ± 105	300
Banizoumbou	Ouallam	396.7 ± 125.2	400
Kodey	Sadoré	574.9 ± 137.7	600
Sadoré	Sadoré	574.9 ± 137.7	600
Bengou	Gaya	796.8 ± 139.3	800

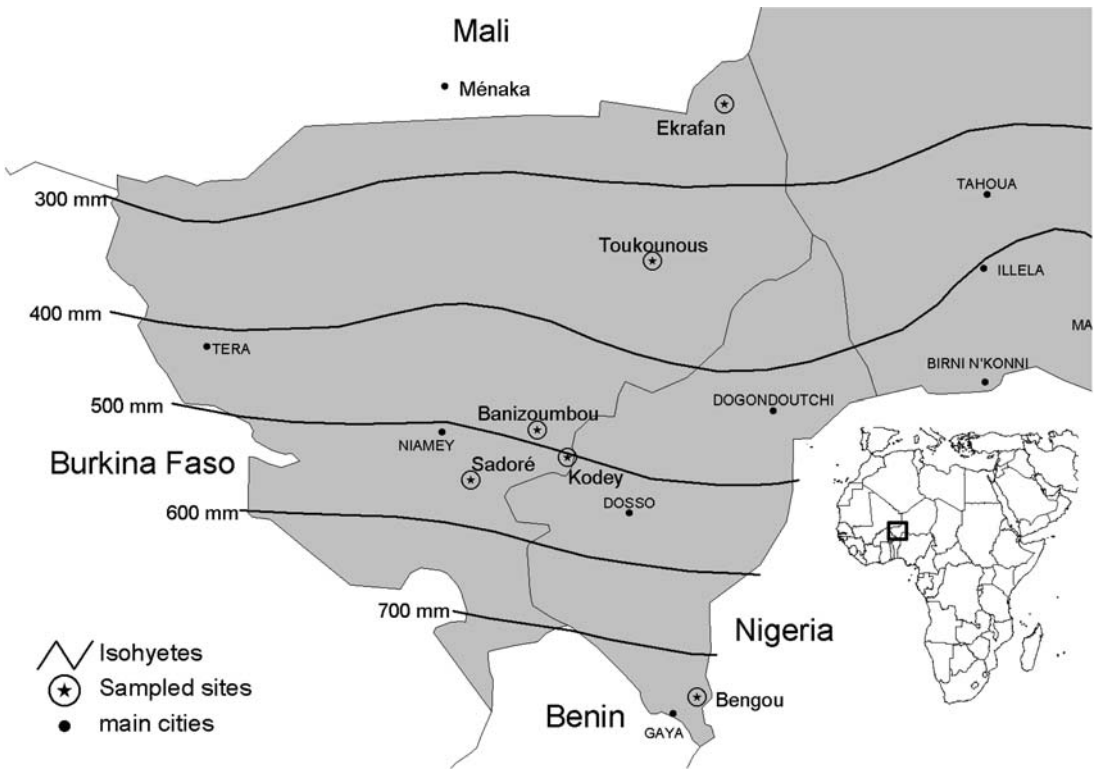


Fig. 1 Situation map of western Niger showing sampling sites and isohyets

between 0.5 and 2.0 kb. Repeatability of each sample was checked and only those bands that appeared in both runs were scored.

Phenotypic data

The following quantitative morphological characters were measured at the time of sampling: Number of vegetative tillers (NTLV), number of flowering tillers NTLF), number of ramifications (NRAM), length of each tiller ($(L1 \rightarrow L_N)$) and number of leaves/tiller ($NF1 \rightarrow NF_N$) for the grasses while for the legumes the number of branches (NTL), number of ramifications (NRAM) and length of each branch ($L1 \rightarrow L_N$) were measured.

Data analysis

Similarity measures based on pairwise comparisons of both AFLP and RAPD bands were calculated by means of Jaccard's coefficient (Jaccard, 1908), Nei and Li's (1979) definition of similarity, and the simple matching (SM) coefficient (Sokal & Michener, 1958), using the software NTSYS-pc version 1.80 (Rohlf, 1993). The matrices of similarity were then analysed using various clustering methods, UPGMA (unweighted pair-group method; Sokal & Michener, 1958), WPGMA (weighted pairgroup method; Sneath & Sokal, 1973), complete linkage (Lance & Williams, 1967) and single linkage (Lance & Williams, 1967). The robustness of the resulting tree topologies were evaluated

by cophenetic correlations using the COPH program of NTSYS-pc. In addition, a principal coordinate analysis (PCO) was carried out in NTSYS-pc using the DCENTER and EIGEN procedures. Mantel analysis (Mantel, 1967) was computed using standardized Mantel's statistics, by the MXCOMP procedure of NTSYS-pc and the significance of the statistic was evaluated by 1000 permutations and expressed as a probability. This test was employed to test whether the similarity matrices obtained for each species based on the AFLP and RAPD data were significantly correlated with corresponding rainfall and grazing data. An analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was used to partition genetic variability using Arlequin software version 2.0 (Schneider et al., 2000), and significance values assigned to variance components based on the random permutation (10,000 times) of individuals assuming no genetic structure.

The morphological data was analysed in GenStat 5 release 3.2 (IACR 1995) using General Linear Regression analysis and analysis of variance (ANOVA). Distance matrices were calculated based on the Euclidean distance measure and analysed using the UPGMA clustering method. A comparison of estimates of genetic distance based on the AFLP data sets and the phenotypic characterisation was carried out using the Mantel Test for correlation between matrices, using the COPH and MXCOMP procedures of NTSYS-pc. The significance of the statistic was evaluated by 1000 permutations and expressed as a probability.

Results

AFLP analysis

Four AFLP primer combinations were selected from a total of 22 on the basis of inter-population variation. Scoring of individuals revealed few fixed differences between populations, with the majority of polymorphisms being intra-population. Among all the scored individuals, a total of 317 clear polymorphisms could be scored between the *B. xantholeuca* accessions, 364 between the *C. biflorus* accessions, 225 between the *A. ovalifolius* accessions and 344 between the *Z. glochidiata* accessions. The AFLP analyses resulted in an average of 79.25 polymorphisms per primer combination for the *B. xantholeuca* accessions, 91 for the *C. biflorus* accessions, 56.25 for the *A. ovalifolius* accessions, and 86 for the *Z. glochidiata* accessions.

Figure 2a–d shows the variation among the populations collected at different points along the rainfall gradient for each species. Due to the difficulties in visualising the large number of screened accessions in one dendrogram, for graphical display purposes, dendrograms were constructed based on the averages of the accessions from each population (from heavily grazed/protected rangeland along a rainfall gradient) using the UPGMA clustering method and Nei and Li's genetic distance.

Among the populations of *C. biflorus* and *A. ovalifolius*, samples collected from sites at 300 mm yr^{-1} and 400 mm yr^{-1} are quite distinct from those collected at other sites along the rainfall gradient. Among the samples of *B. xantholeuca* and *Z. glochidiata* there appears to be a distinction between the populations collected at the lower rainfall sites, $\leq$400 mm yr^{-1}, to those collected at the higher rainfall sites, $\geq$600 mm yr^{-1}. Variation is also seen between the grazed and protected populations at each site along the rainfall gradient for each species, with a few exceptions, e.g. from the *B. xantholeuca* data set, the grazed and protected populations are indistinguishable at the 300 mm yr^{-1} site. The genetic distances between the grazed and protected populations at the lower rainfall sites were in general lower than the genetic distances between these populations at the higher rainfall sites, implying that the level of inter population diversity may be inversely related to the rainfall level, i.e. an increase in aridity is synchronous to a decrease in genetic diversity. Figure 3 shows the PCO plots for each species, based on the averages of accessions within each population. Although these plots reflect the dendrogram clusters as revealed in Fig. 2, in addition they indicate that more variation exists between the populations than was apparent from the 2-dimensional dendrograms e.g. for the *C. biflorus* data set, the grazed population at the 300 mm yr^{-1} site and the protected population at the 400 mm yr^{-1} site are indistinguishable based on Fig. 2B, however 3B clearly differentiates between populations 300G and 400C.

A normalised Mantel statistic (Z) calculated from the similarity between the AFLP and RAPD data with the site data (i.e. rainfall and grazing), where $Z = 1$ represents complete correlation and $Z = 0$ complete dissimilarity is given in Table 2. Overall, the AFLP data shows a lower correlation to the similarity matrices based on grazing and rainfall data in comparison to the

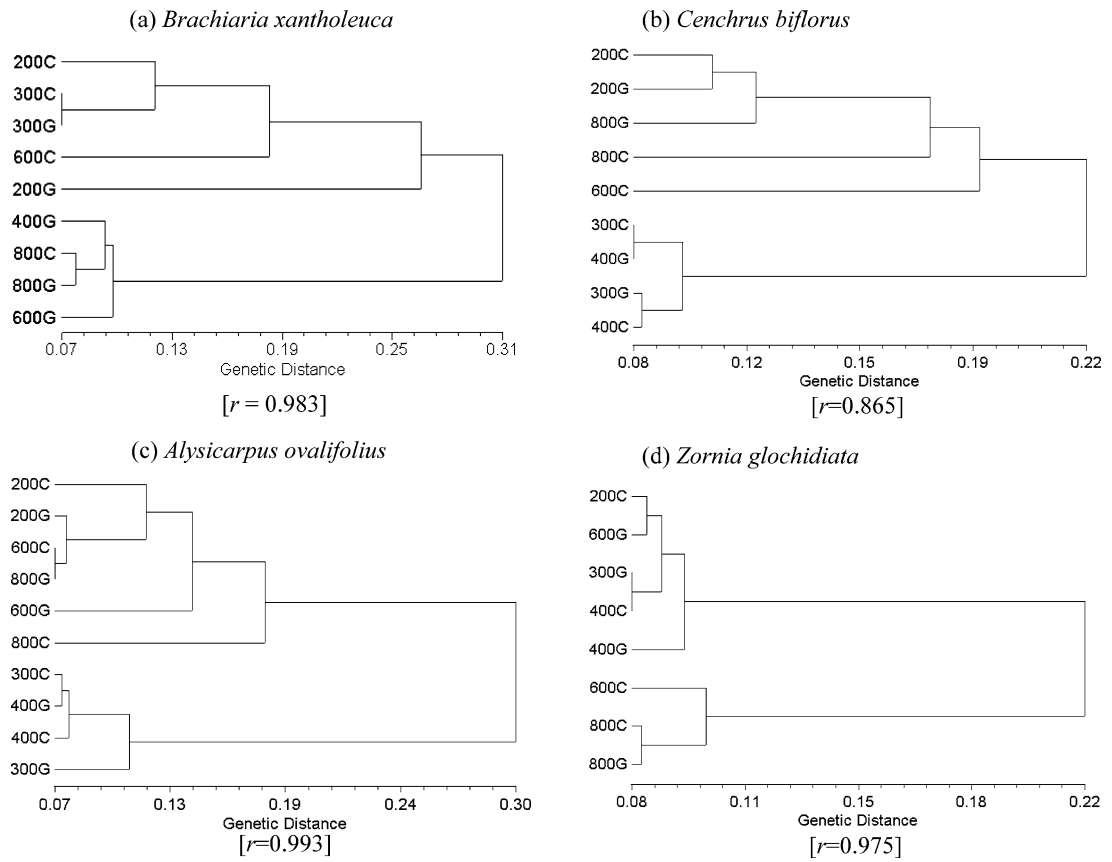


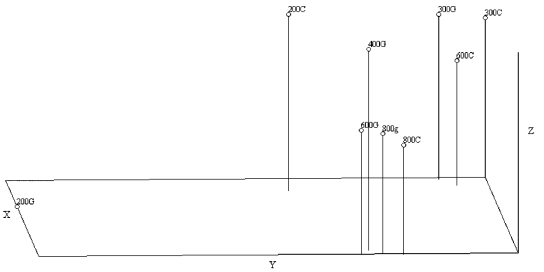
Fig. 2 Four dendrograms constructed based on the averages of the accessions within each population (heavily grazed/protected along a rainfall gradient) for each species using the UPGMA clustering method and Nei and Li's genetic distance, based on the AFLP data. Correlation coefficients (r) is given, [where $r > 0.9$ indicates a very good fit of data to clustering; $r = 0.9$–0.8 indicates a good fit; and $r < 0.8$ indicates a poor fit]

RAPD data. The *A. ovalifolius* AFLP data set shows a higher degree of correlation than the other species to the similarity matrices based on site data, in particular based on the rainfall data ($Z = 0.508$). The AFLP data sets for all the species show a far higher correlation to the similarity matrices based on rainfall data than grazing data, implying that grazing could have had a lower effect on the genetic structure of the populations than rainfall.

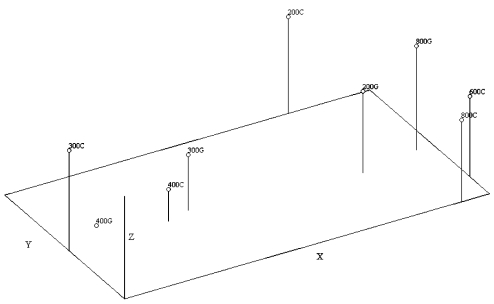
The nested AMOVA (Table 3) was used to partition total genetic variation between individuals, between grazing treatments within a rainfall group, and between rainfall groups. Results show that whereas the majority of variation occurred within grazed/protected populations (51% for *B. xantholeuca*; 81% for *C. biflorus*; 60% for *A. ovalifolius*; 76% for *Z. glochidiata*), the variation between the populations at different points along the rainfall gradient was highly significant ($P < 0.001$ in most cases). The nested AMOVA was also used to partition total genetic variation between individuals, between rainfall treatments within grazed populations, and between grazed/protected groups (data not shown). This revealed an absence of genetic structure based on grazed/protected populations for all four species, through the negative variance components obtained for the between grazed/protected groups partition.

RAPD analysis

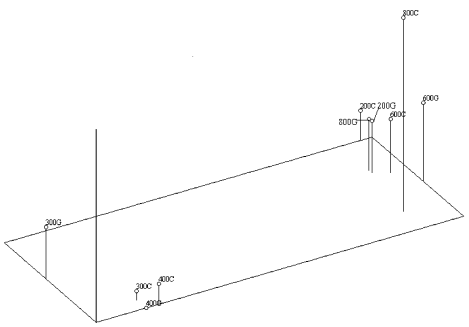
The RAPD technique was carried out on a subset of the total accessions for two of the four selected species; the grass *B. xantholeuca* and the legume *A. ovalifolius*, in order make a comparison with the data produced from the AFLP technique. The 8 RAPD primers used in this study revealed a total of 76 clear polymorphisms scored between the *B. xantholeuca* accessions, with an



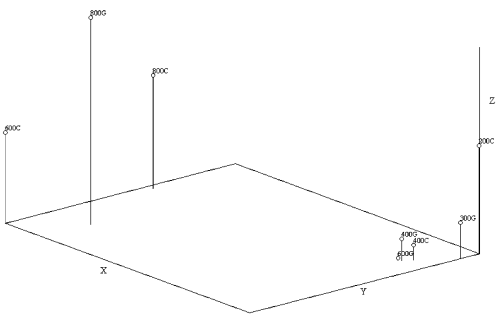
(a) *Brachiaria xantholeuca*
x axis: 34.1%; y axis: 30.13%; z axis: 12.4% (total % variation represented: 76.63%)



(b) *Cenchrus biflorus*
x axis: 28.9%; y axis: 15.1%; z axis :13.7% (total % variation represented: 57.7 %)



(c) *Alysicarpus ovalifolius*
x axis: 31.9%; y axis: 15.7%; z axis: 14.4% (total % variation represented: 62%)



(d) *Zornia glochidiata* x axis: 29.1%; y axis: 20.4%; z axis: 16.5%
(total % variation represented: 66 %)

Fig. 3 Principal co-ordinate analysis plots based on averages of the accessions within each population (heavily grazed/protected along a rainfall gradient) for each species, based on the AFLP data. *X*-axis is principal coordinate 1, *Y*-axis is principal coordinate 2 and *Z*-axis is principal coordinate 3. Labels as in Fig. 2

Table 2 Standardised Mantel's statistic[a] between the similarity matrices obtained for each species based on the AFLP and RAPD data with those obtained by characterising the accessions based on rainfall and grazing

Species	Data type	Grazing and rainfall	Rainfall only	Grazing only
B. xantholeuca	AFLP	0.291	0.269	0.106
C. bifloruss	AFLP	0.240	0.248	0.042
A. ovalifolius	AFLP	0.453	0.508	0.012
Z. glochidiata	AFLP	0.186	0.192	0.053
B. xantholeuca	RAPD	0.581	0.654	0.025
A. ovalifolius	RAPD	0.452	0.459	−0.029[x]

[a]Probabilities (P) evaluated by 1000 permutations were always equal to 0.002, except for[x], where $p = 0.2987$

Table 3 Analysis of molecular variance (AMOVA; Excoffier et al., 1992)

Source of variation	d.f.	SSD	Variance components	% of total variance	*P* value
A					
Between rainfall groups	4	796.095	8.209	36.02	<0.05
Between grazing treatments within rainfall groups	4	150.157	2.925	12.83	<0.001
Within grazing treatments	88	1025.975	11.659	51.15	<0.001
B					
Between rainfall groups	4	366.757	3.476	16.22	<0.05
Between grazing treatments within rainfall groups	4	98.255	0.611	2.85	<0.001
Within grazing treatments	96	1664.646	17.34	80.93	<0.001
C					
Between rainfall groups	4	655.836	5.224	34.03	<0.05
Between grazing treatments within rainfall groups	5	108.858	0.958	6.24	<0.001
Within grazing treatments	127	1164.226	9.167	59.72	<0.001
D					
Between rainfall groups	4	335.072	1.482	8.23	0.314
Between grazing treatments within rainfall groups	3	152.946	2.764	15.33	<0.001
Within grazing treatments	102	1405.21	13.777	76.44	<0.001

Note. I = individuals, P = populations and M = AFLP markers employed) for **A**: *B. xantholeuca* ($I = 97, P = 9, M = 317$); **B**: *C. biflorus* ($I = 105, P = 9, M = 364$ AFLP); **C**: *A. ovalifolius* ($I = 137, P = 10, M = 225$); **D**: *Z. glochidiata* ($I = 110, P = 8, M = 344$). Nested analysis was carried out on all populations. Degrees of freedom (df), sum of squared deviations (SSD) and the significance (*P*) of the variance components are shown

average of 9.5 polymorphisms per primer. Forty-seven clear polymorphisms were scored between the *A. ovalifolius* accessions, with an average of 5.88 polymorphisms per primer. Dendrograms produced using Nei & Li's distance measure and the UPGMA clustering technique for both species, based on both population averages, for a direct comparison with the AFLP data sets, and also based on individuals (the latter being presented due to ease of visualisation of the lower number of accessions employed in the RAPD screening) show clear distinction between the populations sampled at different points along the rainfall gradient (Figs. 4 and 5).

Variation is also seen between the grazed and protected populations within each rainfall site from approximately 80% similarity between the grazed and protected populations at the 300 mm yr^{-1} site set to only 20% similarity between the two populations at the 600 mm yr^{-1} site for the *B. xantholeuca* data. This is again indicated in the PCO plots (Fig. 6), the Mantel statistics (Table 2) and also in the AMOVAs (Table 4). The Mantel Statistic (*Z*) indicates that for both species the similarity matrices based on the RAPD data show far higher correlation to the similarity matrices based on rainfall data than grazing data, indicating, as for the AFLP data sets, that grazing appears to have had a lower effect on the genetic structure of the populations than aridity.

Nested AMOVA was used to partition total genetic variation between individuals, between grazing

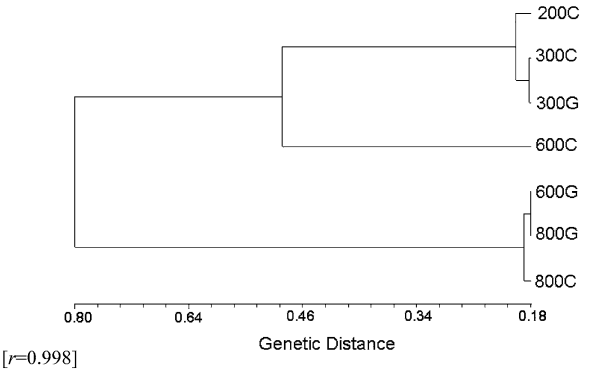


Fig. 4 A dendrogram constructed using the UPGMA clustering method and Nei and Li's genetic distance based RAPD data set for averages of the accessions of *Brachiaria xantholeuca*. Labels as in Fig. 2

[*r*=0.998]

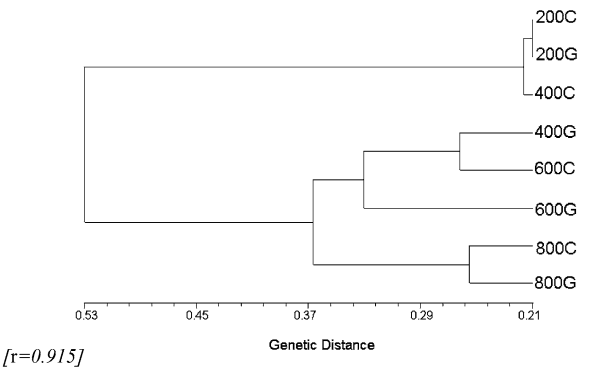
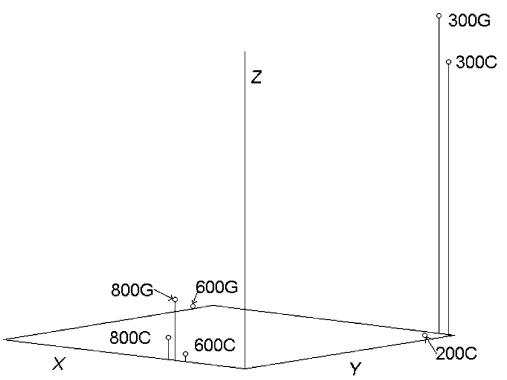


Fig. 5 A dendrogram constructed using the UPGMA clustering method and Nei and Li's genetic distance based RAPD data set for averages of the accessions of *Alysicarpus ovalifolius*. Labels as in Fig. 2

[r=0.915]

(a) *Brachiaria xantholeuca*
x axis: 68.2%; y axis: 18.5%; z axis: 6.9%
(total variation represented: 93.6%)



(b) *Alysicarpus ovalifolius*
x axis: 43.3%; y axis: 19.6%; z axis: 11.8%
(total variation represented: 74.7%)

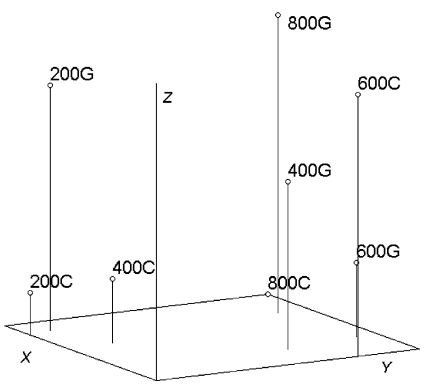


Fig. 6 Principal co-ordinate analysis plots based RAPD data for averages of the accessions within each population (heavily grazed/protected along a rainfall gradient) for each species. X-axis is principal coordinate 1, Y-axis is principal coordinate2 and Z-axis is principal coordinate 3. Labels as in Fig. 2

treatments within a rainfall group, and between rainfall groups. The AMOVAs indicate that the variation between the different rainfall sites was highly significant ($P < 0.01$), accounting for almost 70% of the total variation in *B. xantholeuca* and only 34% in *A. ovalifolius*. The variation that occurred between the grazed and protected populations, although a much smaller proportion of the total (14% for *B. xantholeuca* and *A. ovalifolius*), was also highly significant ($P < 0.001$).

Table 4 Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992)

Source of variation	d.f.	SSD	Variance components	% of total variance	*P* values
A					
Between rainfall groups	3	615.670	13.874	69.25	<0.01
Between grazing treatments within rainfall groups	3	69.109	2.791	13.93	<0.001
Within grazing treatments	48	161.767	3.370	16.82	<0.001
B					
Between rainfall groups	3	166.902	3.192	34.43	<0.01
Between grazing treatments within rainfall groups	4	52.912	1.294	13.96	<0.001
Within grazing treatments	45	215.393	4.786	51.62	<0.001

Note. I = individuals, P = populations and M = RAPD markers employed for **A**: *B. xantholeuca* ($I = 55$, $P = 7$, $M = 76$; **B**: *A. ovalifolius* ($I = 53$, $P = 8$, $M = 47$). Nested analysis was carried out on all populations. Degrees of freedom (df), sum of squared deviations (SSD) and the significance (P) of the variance components are shown

Morphological data analysis

Tables 5–6 show the results of the analysis of variance carried out for each population of all four species whereby grazing and rainfall are the fixed effects. In the majority of cases, there is a significant difference between the selected morphological characters from the grazed and protected populations along a rainfall gradient. For all the morphological characters measured, with the exception of mean number of ramifications per plant for the *Z. glochidiata* data set, a significant difference was found between the populations from different grazing sites, or populations along the rainfall gradient or from an interaction of the two. Overall, in the four species, the mean length of tillers/branches per plant decreased with an increase in rainfall level and grazing pressure while the mean number of ramifications per plant increased with grazing pressure. For the grasses, the mean number of vegetative tillers and the mean number of leaves per tiller increased per plant with grazing pressure. For the legumes, the mean number of branching per plant also increased with grazing pressure.

Comparison of morphological and AFLP data sets

A comparison of estimates of genetic distance based on the AFLP data sets and the phenotypic characterisation, using the Mantel Test, did not reveal statistically significant correlations between the two data sets for all four species. The probability of correlation between the molecular and phenotypic data sets, based on 1000 permutations, for each species are as follows; for the two *B. xantholeuca* data sets, $P = 0.1518$, for the two *C. biflorus* data sets, $P = 0.4226$, for the two *A. ovalifolius* data sets, $P = 0.2907$, and lastly for the two *Z. glochidiata* data sets, $P = 0.1508$.

Discussion

In this study, both AFLP and RAPD proved to be high resolution techniques for detecting genetic variation within the four selected wild forage species. However, notable differences between the AFLP and RAPD data sets were obtained. Overall, a significant genotypic distinction was revealed between populations under increased aridity stress at the lower rainfall isohyets and those at the higher rainfall isohyets, although the majority of variation was found to occur within populations. For the *Brachiaria* populations, 36% of the total variation was accounted for by between rainfall sites based on the AFLP data (Table 3) and 69% based on the RAPD data set (Table 4). However for the *Alysicarpus* populations the total variation explained by between rainfall groups was identical (34%) based on the AFLP and RAPD data sets. A significant difference was revealed between the grazed and protected populations within rainfall sites, whereby the proportion of the total variation accounted for by the within rainfall groups treatment was low both for the AFLP data sets (13% for *B. xantholeuca*; 2.85% for *C. biflorus*; 6.2% for *A. ovalifolius*; 15.3% for *Z. glochidiata*) and the RAPD data sets (14% for both *B. xantholeuca* and *A. ovalifolius)*. The majority of the total variation found within the *B. xantholeuca* RAPD data set was accounted for between rainfall groups, and not between individuals, as might have been expected from the results from the

Table 5 Analysis of variance for the morphological measures taken from the grass species, *B. xantholeuca* and *C. biflorus*

Species	Source	Character	Sum of squares	df	Mean squares
	Rainfall level	NTLV	17.500	4	4.375*
		NTLF	299.936	4	74.984**
		NRAM	751.140	4	187.785**
		LX	45.753	4	11.438
		NFX	92.661	4	23.165**
	Grazing	NTLV	7.165	1	7.165*
		NTLF	9.471	1	9.471
		NRAM	.247	1	.247
		LX	13.764	1	13.764
		NFX	10.164	1	10.164**
	Rainfall*	NTLV	12.044	3	4.015*
	Grazing	NTLF	462.566	3	154.189*
		NRAM	1010.387	3	336.796**
		LX	288.301	3	96100**
		NFX	97.565	3	32.522**
	Error	NTLV	129.817	99	1.311
		NTLF	701.142	99	7.082
		NRAM	4133.538	99	41.753
		LX	737.952	99	7.454
		NFX	129.832	99	1.311
C biflorus	Rainfall level	NTLV	161.921	4	40.480**
		NTLF	34.262	4	8.566**
		NRAM	211.190	4	52.798**
		LX	75.432	4	18.858
		NFX	22.196	4	5.549
	Grazing	NTLV	31.431	1	31.431**
		NTLF	2.220	1	2.220
		NRAM	.723	1	.723
		LX	191.695	1	191.695**
		NFX	14.213	1	14.213*
	Rainfall*	NTLV	70.062	3	23.354**
	Grazing	NTLF	4.588	3	1.529
		NRAM	399.328	3	133.109**
		LX	15.089	3	5.030
		NFX	27.505	3	9.168*
	Error	NTLV	465.000	110	4.227
		NTLF	269.313	110	2.448
		NRAM	1311.34	110	11.921
		LX	1400.966	110	12.736
		NFX	291.695	110	2.652

Note. Character codes as in Table 3. Sum of Squares, df (degrees of freedom), Mean Squares and F values are shown, where $^{*}P = 0.05$ and $^{**}P = 0.01$

AFLP data analyses and taking into account the outbreeding nature of the species. The amount of between individual variation was also much lower for the *A. ovalifolius* RAPD data set, compared with the AFLP data set.

Among all species, the level of genotypic differentiation between the grazed and protected populations at different rainfall sites was quite variable. Of interest was the observation that less genetic variation was revealed between the grazed and protected populations when the aridity stresses were increased. For instance, for the *C. biflorus* and *A. ovalifolius* AFLP data sets, the grazed and protected populations grouped together at approximately 90% similarity each at the 200 mm yr^{-1} rainfall site and this again decreased to 82% similarity each at the 800 mm yr^{-1} rainfall site. However differences due to the two molecular approaches were apparent, e.g. for the *B. xantholeuca* populations, in the

Table 6 Analysis of variance for the morphological measures taken from the legume species, *A. ovalifolius* and *Z. glochidiata*

Species	Source	Character	Sum of squares	df	Mean square
A. ovalifolius	Rainfall level	NTLV	24.386	4	6.097**
		NRAM	1172.854	4	293.213**
		LX	430.762	4	107.690**
	Grazing	NTLV	20.313	1	20.313**
		NRAM	20.942	1	20.942
		LX	381.893	1	381.893
	Rainfall*	NTLV	39.074	3	9.768**
	Grazing	NRAM	282.422	3	70.605
		LX	74.428	3	18.607
	Error	NTLV	217.188	138	1.574
		NRAM	6593.125	138	47.776
		LX	2907.138	138	21.066
Z. glochidiata	Rainfall level	NTLV	142.521	4	35.630**
		NRAM	170.706	4	42.676
		LX	222.155	4	55.539**
	Grazing	NTLV	77.223	1	77.223**
		NRAM	21.437	1	21.437
		LX	95.368	1	95.368**
	Rainfall*	NTLV	11.113	2	5.557
	Grazing	NRAM	129.395	2	64.697
		LX	204.460	2	102.230**
	Error	NTLV	515.583	111	4.645
		NRAM	2902.313	111	26.147
		LX	1275.841	111	11.494

Note. Character codes as in Table 3. Sum of Squares, df (degrees of freedom) and Mean Squares are shown, where $^{**}P = 0.01$

AFLP data set, the grazed and protected population at the 600 mm yr^{-1} site grouped together at a level of approximately 70% similarity, whereas in the RAPD data set, this was represented by 20% similarity.

The observed differences between the AFLP and RAPD data sets reflect differences between the two marker systems; the AFLP technique has a higher multiplex ratio than RAPDs, e.g. a ten-fold increase in the number of loci that can be assayed at one time in maize (Pejic et al., 1998) and lentil (Sharma et al., 1996) copared to RAPDs. The comparatively smaller RAPD data sets in this study for *B. xantholeuca* (76 markers, compared to 317 generated by the AFLP technique; a four-fold increase) and *A. ovalifolius* (47 markers, compared to 225 generated by the AFLP technique; a five-fold increase), in addition to the higher resolution of the polyacrylamide gels used for AFLPs as opposed to the agarose gels used for RAPDs, may account for the reduced intra-population discrimination of the RAPD data set.

Overall, the AFLP molecular analyses revealed more intra-population variation for the outbreeding grass species than for the inbreeding legume species, indicating that geneflow may have contributed to the homogenisation of the genetic structure of the populations. This common phenomenon has been demonstrated in other outbreeding species (e.g. *Manilkara zapota*, Heaton et al., 1999), whereby variation within populations, i.e. between individuals, is frequently greater than between populations.

Overall, the phenotypic analyses (Tables 5 and 6) indicated significant differences both between the sites along the rainfall gradient and between the grazed and protected populations within each rainfall site. Hence the phenotypic differentiation reflected the appropriateness of the characters selected to investigate adaptive traits to aridity and grazing. It is well documented that adaptive traits for grazing and drought are often closely associated (Breman & Cissé, 1977; Coughenour, 1985; Milchunas et al., 1995). Indeed, if the benefit from the photoperiodism response that controls the duration of the growth cycle (Penning de Vries & Djitèye, 1982) is more clearly related to aridity, and the adherent bristles of the spikelets or the ability to grow after defoliation (Tuomi et al., 1994; Hiernaux & Turner, 1996) are more explicitly related to grazing, some of the traits such as the ability to adjust tiller numbers in grasses, and low branching in legumes, to available resources

depending on plant density, soil fertility and moisture regime, could be seen as adaptive traits to both aridity and grazing stress (Hiernaux et al., 1994; Volesky, 1994).

In our study, Mantel tests indicated a lack of correlation between both AFLP and RAPD loci with adaptive phenotypic traits for grazing and aridity. This may imply that gene migration overrides selection, and would therefore provide no evidence to support the environmental theory of genetic diversity (Nevo, 1988), which would have predicted that natural selection would maintain useful genetic variance for adaptation to the grazing and aridity stresses. Instead, the results could contribute to the evidence in support of the neutral theory of molecular evolution (Kimura, 1983), which assumes that functionally neutral changes of the genome escape the editing effects of Darwinian selection but instead are fixed in populations by random genetic drift (Owuor et al., 1997). In fact many of the polymorphisms revealed by the RAPD and AFLP techniques are likely to be neutral markers that are not linked to differences in morphology or adaptation to habitat. Such indications, showing no significant correlation between genetic and morphological markers, have also been shown in other recent studies, e.g. Heaton et al. (1999); Venable et al. (1998). However, a number of studies have found a direct correlation between adaptation to ecological stresses and DNA differentiation, e.g. aridity (Li et al., 2001) and altitudinal gradients (Semagn et al., 2000). In order to explore further the possibility of adaptive DNA differentiation for aridity and grazing stresses, additional primer sets for the AFLP and RAPD techniques could be used, in order to try and cover a greater proportion of the genome. Alternatively, it may be considered that the morphological differences associated with the grazing and aridity stresses are the result of phenotypic plasticity, which has been found to occur in several species usually in response to changes in climate (Heaton et al., 1999). Reciprocal transplants of individuals from grazed sites under drought stresses with those from protected sites at higher rainfall isohyets would ultimately be necessary to provide indications to the presence or absence of a genetic correlate with the observed morphological responses.

The results from the present study are unable to address the issue of identifying grazing and aridity adapted genotypes that could be used to reclaim degraded arid land and provide high quality fodder. The results do, however, highlight a number of important issues for Sahelian forage genetic resources and rangeland management. In particular, the results from the molecular analyses reveal that, geographically, genetic variation is not randomly distributed. This supports results presented in previous studies on wild forage species. Kußerow (1997) reported the existence of genetic variability between different habitats in the Sahelian region in five wild forage species. Huff et al. (1993) reported large genetic differences between natural populations of the outcrossing buffalograss, *Buchloë dactyloides*, between regions and between populations within an adaptive region. The present study also indicates that grazing stresses are not a major contributing factor to the loss of biodiversity for the four wild forage species studied, however with increasing aridity stresses, the impact of grazing on the genetic diversity of populations may increase. Consequently, the different habitats of the Sahelian region can be related to a genetic diversity distribution for each forage species, as a first step for future conservation activities. As different habitats can contribute different levels of diversity, ideally the region as a whole should be protected. Such a conclusion is reinforced by Kußerow's (1997) findings that over a seventeen–year period, a 90.7% decrease of areas around Niamey, which were once occupied by forages in a secondary vegetation type, are now converted to millet fields. Conservation strategies need to take this information into account in order to establish a sustainable system with a regional approach and an increase in on – farm activities, in order to slow down any loss in genetic diversity caused by human pressure on the environment, principally through livestock grazing, combined with desertification processes. With respect to *ex-situ* conservation strategies, areas of high diversity need to be targeted and the results of the present study highlight the usefulness of molecular techniques for assessing the geographic distribution of genetic variability of wild forage species. Traditional approaches to the characterisation of rangeland diversity have relied upon the ability to resolve differences in morphological characters at the species level and not the genotype level. A molecular component is valuable as it avoids many of the complications of environmental effects acting upon characters by looking directly at variation controlled at the genetic level, in addition to being able to reveal a far larger number of characters in much less time. Molecular, in combination with phenotypic, approaches can contribute to better rangeland

management, through knowledge of breeding systems and gene flow between populations, and potentially introducing drought and grazing survival traits into populations.

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