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Genetic structure of *Bambusa vulgaris* in Ghana and its implications for sampling and conservation

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Abstract: *Bambusa vulgaris* is a non-timber forest product that is gaining popularity as a timber substitute in Ghana. It forms about 95 per cent of bamboo population in Ghana. Increased harvesting and lack of knowledge on proper harvesting and regeneration call for conservation intervention. This study describes the within- and between-population genetic variation using random amplified polymorphic DNA (RAPD). Six deca-mer primers generated 45 different sized fragments (loci) across 76 accessions from four populations in Ghana. The number of bands per primer ranged between five (GEN1-60E) and 10 (GEN1-60A and GEN1-60G) bands with an average of 7.5. The percentage of polymorphic bands ranged from 66.67 to 100 per cent with a mean of 89 per cent. Genetic distances among populations ranged from 10 to 27 per cent. Most of the genetic variation was found to reside within populations (81.5%) with a low index of population differentiation (18.5%), although significant ($P < 0.001$). Cluster analysis produced four major clusters with seven sub-clusters. The evergreen and semi-deciduous accessions grouped separately and may be classified as two different management units (MUs). The results suggest that sampling from moist evergreen in MU1 and dry semi-deciduous zone in MU2 may provide a core collection that could represent the total gene pool of *B. vulgaris* in Ghana.

Key words: *Bambusa vulgaris*, DNA, RAPD, genetic diversity, conservation.

INTRODUCTION

The use of bamboo in Ghana is on the increase particularly for construction, roofing, fencing, cocoa drying platforms, firewood, food, handicrafts, *etc.* Nevertheless, it has now got commercial applications such as flooring and plybamboo. Bamboo is therefore a resource that has potential for the local people as a source of raw material and income generation. When the potential of bamboo is fully exploited, it can reduce the pressure on our dwindling forests as a substitute for timber.

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Bambusa vulgaris Schrad. ex Wendl. is the most predominant bamboo species in Ghana, forming about 95 per cent of bamboo populations in the West Africa (Boko, 2005). The haphazard nature of harvesting *B. vulgaris* in Ghana calls for conservation intervention. The species is not known to set seed, even though flowering may occur sporadically in rare instances. Morphological variation in *B. vulgaris* in Ghana is extensive, probably in relation with the wide range of geographical and ecological niches and climatic regimes. Since morphological characters are under the influence of the environment, DNA marker technique based on Random Amplified Polymorphic DNA (RAPD) was used for the initial evaluation of genetic diversity in *B. vulgaris* population in Ghana. RAPD has a broad taxonomical applicability and does not require prior knowledge of the sequences of genome being analysed (Williams *et al.*, 1990). It has proven valuable in the estimation of genetic diversity in many tropical tree species (Gillies *et al.*, 1999; Lowe *et al.*, 2000; Ofori *et al.*, 2001; 2003), especially species whose nucleotide sequences are unknown. The objective of this study was to assess the genetic diversity within the population of *B. vulgaris* in Ghana and use the information for the planning of sampling and conservation strategies.

MATERIALS AND METHODS

Sampling

Samples were collected from four eco-geographical zones (referred to as populations) where *B. vulgaris* thrives well in Ghana (Table 1). The exact site locations of the sampled clusters were determined using the Geographical Positioning System (GPS 76, Garmin). For each ecological zone visited, leaf samples were collected from 25 culms from different bamboo clusters separated by at least 1 km. The culms sampled were among the largest in the clusters. The leaves were cleaned and dried with silica gel in sealed polythene bags. Accessions were named after the ecological zone, location within the ecological zone and sample number. For example, MS-KIB1 meant accession number 1 located at Kibi in the moist semi-deciduous forest zone.

Genomic DNA isolation

DNA was extracted from the leaf samples using the DNeasy plant mini DNA extraction kit (QIAgen, Germany) following the manufacturer's recommendations. DNA quality

Table 1. Number of samples, ecological zones and their rainfall regimes

Ecological zones	Code for ecological zones	Mean annual rainfall (mm)	Number of samples
Wet evergreen	WE	> 1,750 mm	25
Moist evergreen	ME	1,500 - 1,750 mm	25
Moist semi-deciduous forest zone	MS	1,250 – 1,750 mm	25
Dry semi-deciduous forest / transition zones	DS	1,000 – 1,500 mm	25

and content were determined by electrophoresis of 10 μl of each sample on 1 per cent agarose gel and the intensities of the bands examined under UV light. DNA concentration was determined by comparing the intensities of the DNA bands with those of Hyper-ladder 1 (Bioline, UK Ltd.). DNA stock solutions of 5 ng/μl were then prepared and stored in the deep freezer.

RAPD analysis

Ten deca-mer random primers (GEN1 60A - GEN1 60J; Bioline, UK Ltd.) were evaluated with eight DNA samples randomly chosen from all the four populations for their ability to amplify bamboo DNA. Criteria for selection included reproducibility, clarity of RAPD profile, number of bands and proportion of polymorphic bands. Six primers were finally selected and used for analysis. The six selected primers were used to amplify DNA from the 100 accessions. Each 25 μl reaction volume consisted of 25 ng template DNA, 2.5 mM $MgCl_2$, 1× buffer, 200 μM dNTPs, 0.5 μM primer and 1U of Taq polymerase (Bioline, UK Ltd.). Amplification was performed in a 96-well block thermocycler (PCR Express, Hybaid, UK Ltd.) under the following conditions: 3 min at 94°C for 1 cycle, followed by 36 cycles of 1 min at 94°C, 1 min at 48°C and 1 min at 72°C, and a final extension of 5 min at 72°C. The PCR products were held at 4°C until they were analysed. PCR products were subjected to 1.4 per cent agarose gel electrophoresis in 1× TAE buffer at 120V (BIO-RAD Power PAC 300) for 2.5 h at room temperature. The PCR reactions were performed twice to check for reproducibility. DNA bands were detected by ethidium bromide staining, visualised by fluorescence under UV light. The gel images were photographed using a digital image documentation system (Flowgen). DNA bands were scored using ChemiImager 5.5 software and the banding patterns were recorded on Excel spreadsheet.

Data analysis

PCR products that were reproducible in two successive amplifications were selected. Bands were defined by their molecular weights using DNA standard size marker (Hyperladder 1, Bioline, UK Ltd.). The RAPD products were scored in a binary matrix as present (1) or absent (0). Pairwise RAPD distances between the accessions were estimated from the relativized Euclidean distances (RED) computed as:RED = E/m, where m is the total number of polymorphic bands, and E is Euclidean distance (Excoffier *et al.*, 1992) defined it as: $E = \Sigma[(n_x - n_y)^2]^{1/2}$ where n_x and n_y are bands unique to trees *x* and *y* respectively using PCORDWIN programme (version 3.17). The resultant genetic distance matrix was exported to Dar-Win 3.5 and used for construction of dendrogram using un-weighted paired group method with arithmetic averages (UPGMA).

The non-parametric analysis of molecular variance (WINAMOVA 1.5; Excoffier *et al.*, 1992), which uses the analyses of variance framework, was used to partition the total variation into within- and between-populations. This was based on a simulation of 1000

random permutations of individuals across populations. A pairwise distance (D) between populations was also computed from the following equation: $D = {}^{xy}\Sigma E_{ij}(xy)/n_x n_y$ where $E_{ij}(xy)$ is the distance between tree *i* from population *x* and tree *j* from population *y* and n_x and n_y are the number of trees from population *x* and population *y* respectively. Furthermore, principal component analysis was performed to obtain an alternative visualization and additional information on genetic relationships among the accessions using PCORDWIN 3.17.

RESULTS

The RAPD profile

Out of the 100 accessions, 76 produced clear and reproducible bands which were used for the analysis. The six random deca-mer primers chosen for the analysis generated a total of 45 different sized fragments (loci) that ranged in size from 1,200 bp to 400 bp. The number of bands per primer ranged between five (GEN1-60E) and 10 (GEN1-60A and GEN1-60G) and the grand average over the four populations was 7.5. The percentage of polymorphic bands ranged from 66.67 to 100 per cent with a mean of 89 per cent (Table 2). No amplification products were observed in control reactions that did not have any template DNA.

Genetic relationships among individual accessions

The genetic dissimilarity distance among individual accessions ranged from 0.0 to 0.87. Most often, distances between accessions in close proximity were low. The UPGMA dendrogram analysis gave four main clusters with seven sub-clusters (Fig. 1). Each major cluster consisted of genotypes from different populations. However, on the average cluster 1 was made up of mostly accessions from the evergreen zones (82%) while 18 per cent were from moist semi-deciduous and dry semi-deciduous populations. Similarly, clusters 2, 3 and 4 were mostly accessions from the semi-deciduous zones forming 81 per cent, while 19 per cent were from the evergreen

Table 2. Attributes of random oligonucleotide primers used for generating RAPD profile from 76 individuals of *B. vulgaris*

Primer	Nucleotide sequences	Number of bands produced	Percentage of polymorphic bands
GEN1-60A	5'CGCAGTACTC 3'	10	100
GEN1-60C	5'CTACACAGGC 3'	8	75
GEN1-60D	5'GTCCTTAGCG 3'	6	100
GEN1-60E	5'GTCCTCAACG 3'	5	100
GEN1-60F	5'CTACTACCGC 3'	6	66.67
GEN1-60G	5'GAGTCAATCG 3'	10	90
	Total	45	89

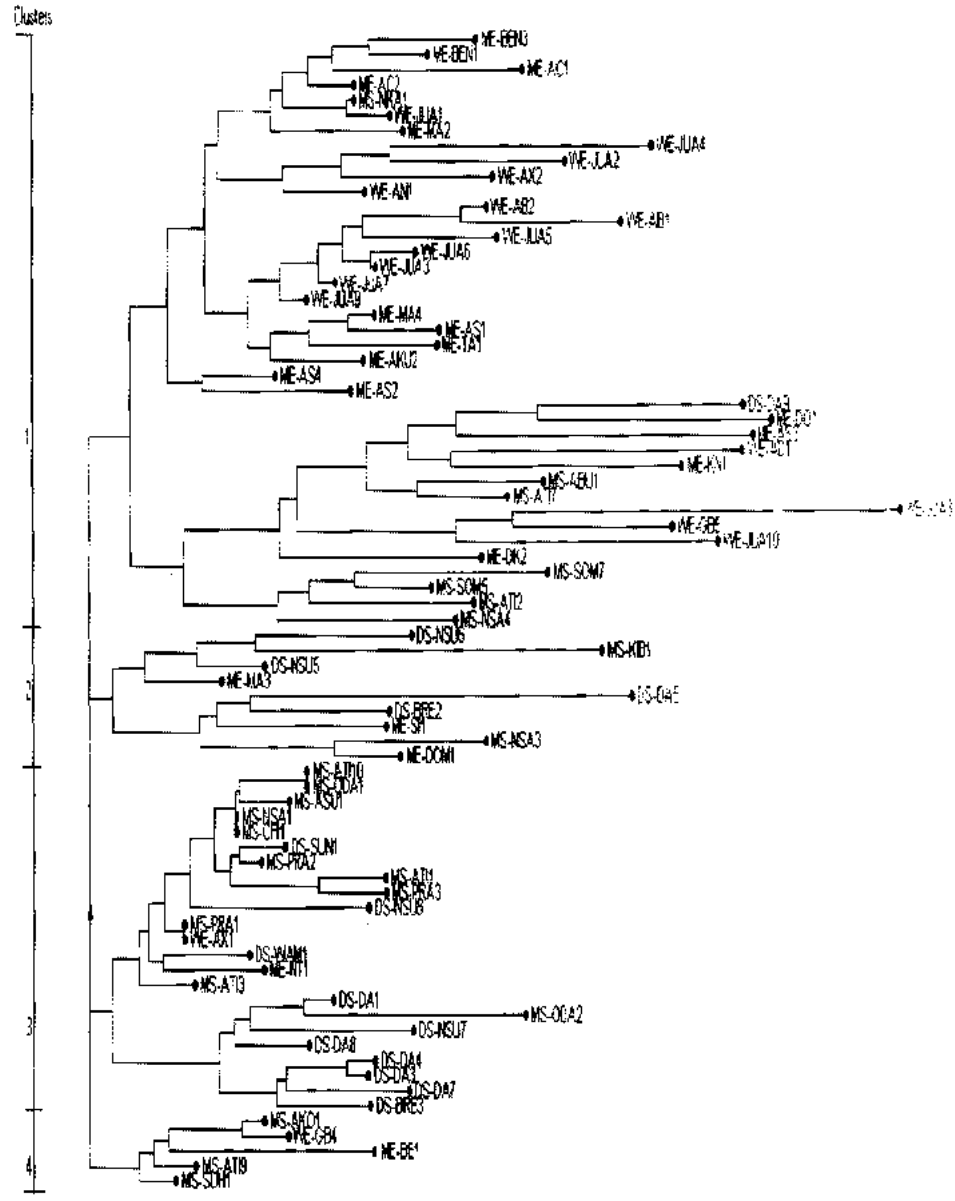

Figure 1. Dendrogram showing genetic relationships among 76 accessions of *B. vulgaris*.

populations. Principal component analysis defined by axes 1 and 2 (Fig. 2) gives a clear view of the dispersion of accessions and the putative separation of the evergreen and semi-deciduous accessions along axis 1. Within these major groups, accessions in close proximity and/or from adjoining localities grouped together, notably DS-DA1, DS-DA3 and DS-DA4S.

Figure 2. Principal component analysis of 76 accessions of *B. vulgaris* with 45 RAPD loci.

Within and between population variability

The partitioning of genetic variation was examined by AMOVA. The results of the AMOVA (Table 3) indicated that 18.48 per cent of the total variation was attributable to the differences among populations while 81.52 per cent was due to the variation among individuals within populations. Thus, the overall genetic diversity among or between populations was low compared to diversity within populations. A low

Table 3. Analysis of molecular variance among four populations of *B. vulgaris*

Variance component	d.f.	SS	MSS	Variance (%)	*P* value
Among populations	3	63.511	21.170	18.48	< 0.0010
Within populations	72	289.002	4.014	81.52	< 0.0010
PHIst = 0.185					

Table 4. Estimation of within-population diversity of four populations of *B. vulgaris*

Populations	Within population diversity (%)
Moist evergreen	28.9
Wet evergreen	25.7
Moist semi-deciduous	26.9
Dry semi-deciduous	18.5

coefficient of population differentiation (PHIst) of 0.185 was obtained but was significant ($P < 0.001$). Examination of the within population diversity gave a range of 18.5 per cent (dry semi-deciduous) to 28.9 per cent (moist evergreen; Table 4).

Pairwise comparisons of genetic distances among bamboo populations are shown in (Table 5). The pairwise genetic distance index among populations ranged from 0.10 (between WE and ME) to 0.27 (between WE and DS) with an average distance among populations being 0.185. These results thus indicated that accessions from the two evergreen populations (wet evergreen and moist evergreen zones) have close genetic identity (D = 0.10). Similarly, those from the semi-deciduous zones (moist semi-deciduous and dry semi-deciduous) also had close genetic identity (D = 0.13). On the other hand, populations from the evergreen zones were quite dissimilar to those from the deciduous zones.

DISCUSSION

The dendrogram analysis shows admixture of genotypes in each cluster. However, there is an evidence of genetic differentiation between the evergreen and the semi-deciduous populations. This is confirmed by the significant ($P < 0.001$) index of population differentiation of 18.5 per cent and the genetic distances among populations (Table 5), most of which could be related to the differentiation of DS from the other populations. The level of genetic diversity within and among populations of bamboo

Table 5. Estimation of genetic distances among four populations of *B. vulgaris*

	ME	WE	MS
WE	0.10		
MS	0.16	0.22	
DS	0.23	0.27	0.13

in Ghana (PHIst = 18.5%) is typical of perennial outcrossing species that maintains most variation within populations (Hamrick, 1989; Lachenaud *et al.*, 2004). However, since *B. vulgaris* is not known to reproduce through seed, the diversity should be attributed to induced mutations and ecological distribution of diverse forms within populations. Nonetheless, a clear pattern of genetic differentiation is emerging between the evergreen and semi-deciduous populations as depicted in Figures 1 and 2. Judging from the genetic distances among populations, the bamboo populations in Ghana may be considered as two management units (MUs), defined by Moritz (1994) as populations with significant divergence in allele frequencies regardless of the phylogenetic distinctiveness of alleles. These are the evergreen populations (MU1) and semi-deciduous populations (MU2). Dry semi-deciduous is even evolving fast dissociating itself from the other three populations and may in the near future become a separate MU. This indicates that loss of dry semi-deciduous populations could result in the loss of a significant amount of genetic variation in the bamboo populations in Ghana.

Implications for sampling and conservation

Generally, planning for sampling and conservation of genetic resources of bamboo should not be exclusively based on genetic diversity. The degree of adaptation as well as growth and culm quality obtained from replicated field tests must also be taken into consideration. However, for the purposes of biodiversity conservation, suggestions are made based on the genetic diversity observed. Sampling from ME and DS may be considered for biodiversity conservation. ME has the largest within population diversity in MU1 (evergreen zone). Comparing the genetic distances between ME and the two populations within MU2 (semi-deciduous zone), the distance between DS (0.23) was larger than that between MS (0.16). Sampling widely within these populations may represent the total gene pool of *B. vulgaris* in Ghana. Caution should however be taken to avoid sampling from adjoining clusters since most of the clusters in close proximity have very close genetic identities, which may be the result of the mode of gene flow through vegetative propagation. During sampling, it was noted that the number and sizes of bamboo clusters in DS were considerably lower than the other three populations suggesting that bamboo populations in DS might be undergoing through some bottlenecks. This is supported by the low genetic diversity within DS representing 6.5 per cent lower than the mean diversity. Attention should therefore be paid to this population since reduction in size and loss of genetic variation seriously threaten their ability to persist.

CONCLUSION

RAPD technique is powerful enough for effective detection of polymorphisms and population genetic patterns in *B. vulgaris*. The results may be used as a basis for developing diversity-rich core collections for conservation and plantations in Ghana.

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