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Full Length Research Paper

Analysis of genetic diversity in bambara groundnut [*Vigna subterranea* (L.) Verdc] landraces using amplified fragment length polymorphism (AFLP) markers

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Amplified fragment length polymorphism (AFLP) was used to assess genetic diversity among 100 selected bambara groundnut [*Vigna subterranea* (L.) Verdc] landraces from a diverse geographic area of Tanzania. Eleven informative AFLP primer combinations generated a total of 49 scorable polymorphic amplification fragments across the bambara groundnut accessions. Genetic distances between all accessions based on Jaccard's variability index ranged from 0.1 to 0.68, with a total average of 0.3. The results showed that bambara groundnut landraces from Tanzania form a genetically diverse population, and AFLP markers can be effectively employed to assess genetic diversity and to measure genetic relationship among accessions. Cluster analysis revealed that bambara groundnut from Tanzania constitute two major groups in line with their putative geographic origins, one genetically distinct group from the Southern agro-ecological zone and a mixed group with accessions from Central, Lake Victoria and Western agro-ecological zones. The clustering of accessions compared relatively well to clustering based on phenotypic characters. However, correlation of the AFLP marker distances with phenotypic distances showed *r*-values of 0.4 only.

Key words: AFLP markers, genetic diversity, landraces, Tanzania, *Vigna subterranean*.

INTRODUCTION

Bambara groundnut [Vigna subterranea (L.) Verdc] is an indigenous grain legume grown mainly by female subsistence farmers in drier parts of sub-Saharan Africa. The likely centre of origin of bambara groundnut is in West Africa (Hepper, 1963). Its seeds can be consumed fresh when semi-ripe, as pulse when dry and mature or they can be ground into flour (Linnemann and Azam-Ali, 1993). Bambara groundnut is ranked the third most important legume in much of Africa after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) (Sellschop, 1962). The crop is widely adapted to semi arid parts of Tanzania including Central, Western and Southern regions of the country, and to more wet areas around Lake Victoria (Marandu and Ntundu, 1995). In many traditional farming systems, bambara groundnut is intercropped with mainly cereals and root crops (Ntundu, 1997).

Bambara groundnut has several agronomic advantages including high nutritional value, drought tolerance, and ability to produce some yield in soils that are too poor for cultivation of other more favoured species such as common beans and groundnuts (Linnemann and Azam-Ali, 1993; Anchirinah et al., 2001; Azam-Ali et al., 2001). In addition, the crop is relatively free from insect pests and diseases. Bambara groundnut contributes soil nitrogen for other crops by fixing atmospheric nitrogen

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through symbiosis with *Rhizobium* bacteria and is therefore beneficial in crop rotations and intercropping (Mukumbira, 1985; Karikari et al., 1999). Despite the importance of bambara groundnut as a food legume in traditional farming systems in Tanzania, limited breeding efforts have been made to improve this crop. Little information is available about extent of the genetic diversity among bambara groundnut landraces, for long-term conservation and improvement.

Only a few genetic studies on bambara groundnut using molecular techniques have been reported. These are mainly on the population structure and genetic diversity among farmers cultivars using isozymes and RAPD markers (Pasquet et al., 1999; Amadou et al., 2001), respectively. The objectives of the present study were to use AFLP analysis to assess the level of genetic diversity among bambara groundnut accessions from diverse geographic locations in Tanzania, to describe diversity patterns useful for conservation and crop improvement strategies.

MATERIALS AND METHODS

Plant material and DNA extraction

The plant material consisted of one hundred bambara groundnut accessions collected between 1994 and 2000 obtained from the National Plant Genetic Resources Centre in Tanzania (NPGRC). Accessions, previously characterised for morphological variation (Ntundu, 2002), were selected to reflect a wide range of bambara groundnut variation from the Central, Western, Southern and Lake Victoria agricultural ecological zones of the country. Thirty seeds of each accession were sown in pots of 12 cm diameter with peat (Finn peat B2) with pH 5.6-6.4, in a green house with the minimum and maximum temperatures of 22°C and 24°C, respectively. Leaf tissue from 20 three weeks old seedlings of each accession was excised and bulked prior to DNA extraction. Approximately 2 g of bulked young leaf tissue from each accession was frozen in liquid nitrogen, and ground into fine powder with a mortar and pistil. Genomic DNA was extracted following the phenol chloroform method as described by Sharp et al. (1988). DNA concentrations were quantified using a Pharmacia Gene Quant spectrophotometer (Pharmacia Biotech, Columbus, OH) and by visual inspection after electrophoresis in 1% agarose gels stained with ethidium bromide. The concentration of the DNA samples was adjusted to 50 ng/ul and the DNA was stored at –20°C.

AFLP Analysis

AFLP analysis was performed according to the protocol described by Vos et al. (1995) with minor modifications. 150 ng of bulked template DNA was digested using 5 U *Sse*83871 (Takara Shuzo Company) and 5U *Mse*I (New England Bio-labs). Complementary double stranded adaptors were ligated to the restriction fragments. Pre-amplification was performed in 15 µl reactions with the primers Sse +0 and MseI +1 (one selective nucleotide) following the protocol of Vos et al. (1995). Pre-amplification reactions were diluted 1:25 and used as a template for the selective amplification with Sse +2 primers combined with Mse +2 primers. Selective amplification was performed in 10 µl PCR reactions in 0.6 ml PCR tubes on a Hybaid Touchdown thermocycler (Hybaid Limited, UK).

PCR mixtures for selective amplification contained 1 x reaction buffer (HT Biotechnology Ltd., England), 0.2 mM dNTPs, 12.5 ng Sse-primer, 15 ng MseI-primer, 2.5 µl of the diluted template DNA and 0.15 U Super-Taq polymerase (HT Biotechnology Ltd, England). The selective amplification temperature profile was programmed as: one cycle of 60 s at 94°C, 60 s at 65°C, 1 min at 72°C followed by 9 cycles with 1°C lower annealing temperature in each cycle and 24 cycles of 30 s at 94°C, 30s at 56°C, 60 s at 72°C. The amplification products were stored at –20°C.

For gel analysis, the PCR-reactions were mixed with an equal volume of formamide loading buffer (98% deionised formamide, 10 mM EDTA pH 8.0, 0.05% bromo phenol blue and 0.05% xylene cyanol). The molecular weight marker lambda DNA/Eco471 (MBI Fermentas Ltd) was included on each gel for size determination. Samples were denatured for 5 min at 95 °C, and aliquots 4 µl of the mixture were loaded onto 4.5% denaturing polyacrylamide gels (7M, Urea, 1 x TBE) and the AFLP fragments were separated by electrophoreses for 1 hour at 80W in 1 x TBE buffer. Subsequently the gels were silver stained following the protocol by Bassam et al. (1991) and dried at room temperature for 24 h prior to visually scoring polymorphic bands.

Prior to selective amplification of all accessions, thirty-six selective primer combinations were used to analyse eight selected accessions of diverse geographic origin. Eleven informative selective primer pairs showing high polymorphism were used for the final analysis (Table 1) with all 100 bambara groundnut accessions studied.

Data analysis

Each accession was scored (1) for presence and (0) for absence of each polymorphic band. AFLP bands within accessions were scored as missing if they were poorly resolved on the gel or if the template DNA did not amplify well. Genetic distance was calculated on the basis of Jaccard's coefficient method (Jaccard 1908). The similarity matrix was subjected to cluster analysis by the unweighted pair-group method with arithmetic averages (UPGMA) and a dendogram was created using the TREE procedure (SAS Inc., 1994). Phenotypic distances calculated from 20 quantitative and 7 qualitative morphological traits from a previous phenotypic characterization was correlated with genetic distances based on 49 polymorphic AFLP markers. Morphological measurements used to estimate genetic distances based on phenotypic data were obtained from two growing seasons. Correlation analysis was performed between AFLP marker distances and phenotypic distances calculated from means of quantitative morphological characters for the two combined growing seasons and from each season, separately.

RESULTS

AFLP Analysis

Eleven informative AFLP primer combinations generated a total of 346 reproducible amplification fragments across all bambara groundnut accessions, among which 49 bands were polymorphic (Table 1). The number of amplified AFLP bands per primer pair varied from 14 to 45 with an average of 31.5 bands. The average number of polymorphic bands detected was 4.5 per primer combination, and the fragment sizes, determined by comparing the amplicons with the standard DNA ladder, ranged from about 80 to 650 base pairs (bp). Two primer

Table 1. AFLP primer pairs and their number of amplified and polymorphic bands for diversity study of bambara groundnut [*Vigna subterranea* (L.) Verdc] landraces.

AFLP primer pair	Amplified bands	No. of polymorphic bands
M12/S11 M-AC/S-AA	45	2
M12/S17 M-AC/S-CG	44	9
M13/S11 M-AG/S-GA	32	4
M13/S17 M-AG/S-CG	14	2
M14/S23 M-AT/S-TA	37	2
M14/S11 M-AT/S-GA	28	5
M14/S12 M-AT/S-GA	30	2
M14/S15 M-AT/S-GC	27	2
M14/S16 M-AT/S-CC	20	6
M14/ S19 M-AT/S-GG	32	7
M14/S22 M-AT/S-GT	37	8
Total	346	49
Mean	31.5	4.5

combinations (M12/S17 M-AC/S-CG and M14/S22 M-AT/S-GT) generated 9 and 8 polymorphic bands, respectively, a relatively higher numbers of polymorphisms compared to the other primers used in this study.

Cluster analysis

Genetic distances between all pairs of the 100 bambara groundnut accessions based on AFLP markers varied from 0.10 to 0.68, with a total average genetic distance of 0.30. Three pairs of accessions TZA1482 and TZA1486; TZA1478 and TZA1479; TZA1495 and TZA1487 all collected from the Southern agro-ecological zone produced identical patterns. Accession TZA 800 from the Lake Victoria zone was quite different from the remaining accessions studied. It had a genetic distance of 0.68 to the average of all other accessions studied. The UPGMA dendogram based on Jaccard's coefficient of genetic distance suggested the existence of two major clusters (II and III) mainly along their lines of geographic origin (Figure 1). Four accessions TZA509, TZA512, TZA598 and TZA1491 collected from the Central and Southern agro-ecological zones formed a separate small group (I) from the two major clusters. The second cluster (II) included 48 accessions, dominated by 38 accessions collected from the isolated regions (Lindi and Mtwara) of the Southern agro-ecological zones in Tanzania.

Grouping of accessions from the Southern zone suggests the influence of geographic isolation on genetic polymorphism. Another Nine accessions from Central (TZA 1848, TZA1992, TZA2010), Lake Victoria (TZA2114, TZA2538) and Western (TZA1932, TZA1961, TZA1976, TZA1977, and TZA1978) agro-ecological zones were also included in cluster II (Figure 1). Cluster III grouped together 48 bambara groundnut accessions from Central, Lake Victoria, Southern and Western agro-ecological zones. These bambara groundnut accessions in cluster III did not form a distinct group with respect to geographical origin.

Correlations between AFLP markers and phenotypic distances

Correlations between AFLP markers and phenotypic distances calculated from a combination analysis of 7 qualitative and 20 quantitative morphological traits ranged from weak (r = 0.35) to modest (r = 0.48) for different correlation combinations considered in this study. The correlation between AFLP marker distances and phenotypic distances for combined qualitative and quantitative characters for two seasons showed a correlation coefficient of r = 0.41 and 0.40 when the quantitative characters were considered alone. When the first season was considered separately, correlation between AFLP marker distances and phenotypic distances for combined quantitative and qualitative characters and quantitative characters considered separately showed values of correlation coefficients of r = 0.38 and r = 0.35, respectively. While the second season resulted in slightly higher values of r = 0.46 and r = 0.47 for both quantitative and qualitative traits, respectively and quantitative characters considered separately. Bi plotting of phenotypic distances against AFLP marker distances (figure not shown) did not indicate any clear pattern.

DISCUSSION

In the present study, extent and organization of genetic diversity within 100 selected accessions of bambara groundnut from Tanzania was assessed with 49 polymorphic AFLP bands. Grouping of the bambara groundnut accessions according to geographic origin indicates considerable genetic divergence probably due to different growing environments. The results of this study are in accordance with previous studies by Massawe et al. (2000) based on AFLP molecular marker analysis revealing extensive genetic diversity between 12 African bambara groundnut landraces from diverse origin. Amadou et al (2001) also reported considerable genetic diversity among 25 African bambara groundnut accessions from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, using Random Amplified Polymorphic DNA (RAPD) markers, and demonstrated two main groups of accessions mainly along the lines of their geographic origin.

In contrast, based on isozyme analysis, Pasquet et al. (1999) reported that both wild and domesticated bambara groundnuts were characterized by low genetic diversity,

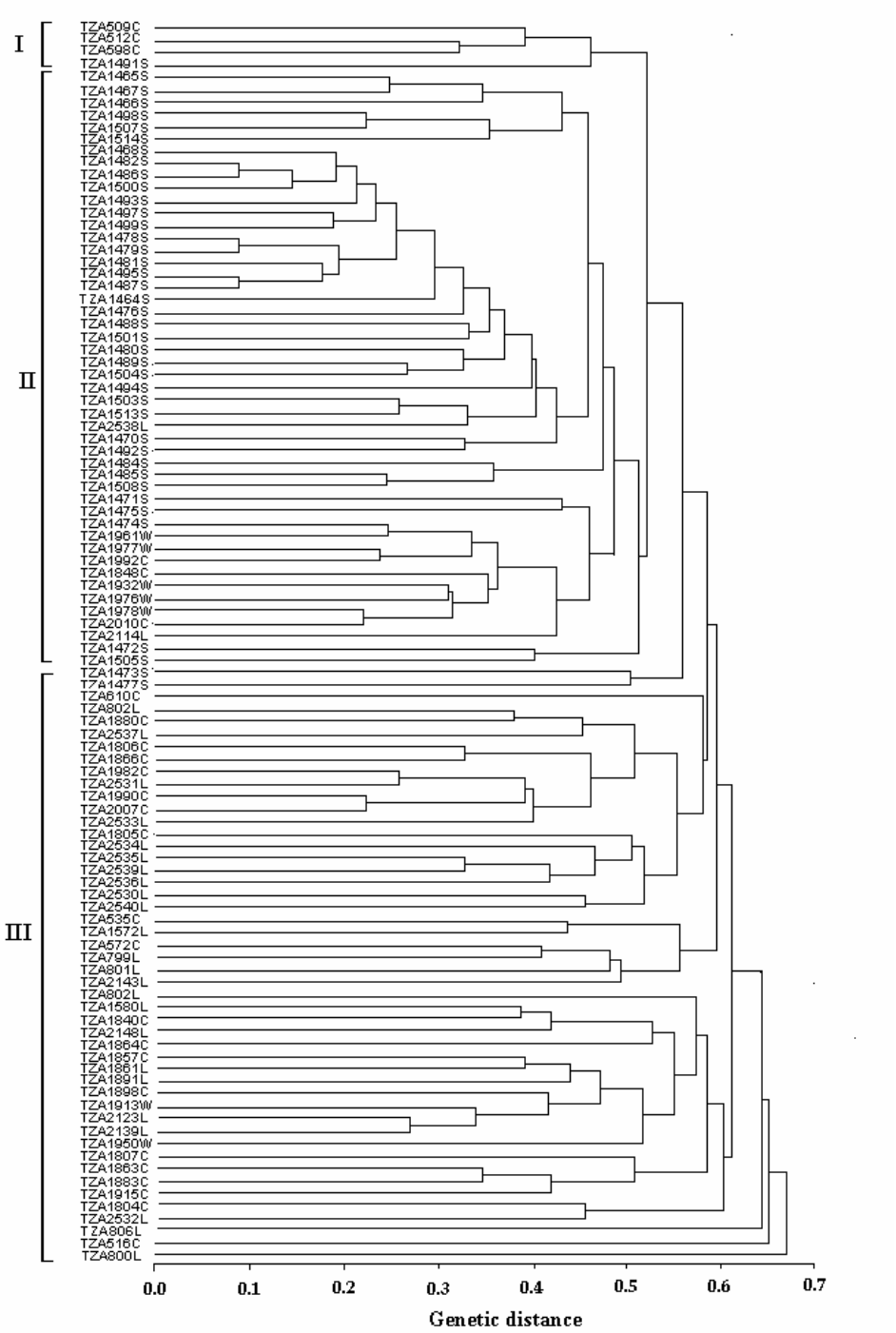

Figure 1. Dendogram of genetic distances among 100 bambara groundnut accessions based on 49 AFLP markers. The prefix TZA before numbers is a designation for Tanzania accessions code, and the suffix letters C, S, L and W following accession numbers indicate collection zones namely Central, Southern, Lake Victoria and Western zones, respectively.

indicating that wild bambara groundnut is the progenitor of the domesticated form. However, isozymes are generally limited by the low levels of polymorphism detectable and may fail to discriminate cultivars differing only slightly in genetic make up.

In the present study, accessions from the southern agro-ecological zones formed a clear group (Cluster II) together with a few accessions from Central, Western and Lake Victoria agro-ecological zones. It indicates that this group is genetically distinct from the rest of the material studied. This may be attributed to the fact that the Southern agro-ecological zone is a rather isolated area geographically, with poor infrastructure as compared to the other zones included in this study. As a result,

movement of plant germplasm between the southern and the other regions may have been limited, which may have created isolation from the rest of the bambara groundnut seed material in the country.

Clustering of the nine accessions from other agro-ecological zones in cluster II on the other hand, may suggest that origin of these accessions is not from the location at which they were collected. The independent grouping of accessions TZA509, TZA512, TZA598, TZA1491 and TZA800 from the Central, Southern and Lake zone, respectively (Figure 1) indicates that these accessions deviate from the two major groups of accessions. Morphological evaluation of these accessions provides no explanation (Ntundu, 2002) as to why these accessions are genetically distinct. Such divergent accessions may have good breeding value, which may be utilised for direct selection and as parents of crosses with accessions from different clusters. The mixture of accessions in cluster III mainly from Central, Lake Victoria and Western agro-ecological zones indicates that bambara groundnut accessions in this group constitute a more heterogenic group, with variable genetic background. The explanation, which may be proposed in this regard, is the high frequency of bambara groundnut seed exchange by farmers over wide geographic–ethnic regions. This could be in part due to a relatively good transportation in the area through the central railway system, which links the Central, Western and Lake Victoria agro-ecological zones. Small farmers in Eastern Africa generally seem to exchange seeds frequently. A farmers field survey (Ntundu, 2002) indicated that at least 44% of farmers in Tanzania obtain their bambara groundnut seeds from others farmers within (39%) and outside (5%) of their regions, annually. In their survey on seed market assessment in the Dodoma, Iringa and Morogoro regions in Tanzania, Ashimogo and Rukulantile (2000) reported that 35.4% of farmers obtain maize (*Zea mays* L.) seeds from their fellow farmers, while 60.1% use only their own seeds. Similar practise has been reported to be common among growers of cucurbits (*Cucurbita moschata* Duch) in Zambia where at least 40% of the farmers obtain their seeds from other farmers (Gwanama and Nichterlein, 1995) within their neighbouring growing regions.

In applied breeding, phenotypic or genetic distances have been expected to provide predictors for heterosis. Our results for bambara groundnut landraces generally indicate rather modest correlations between genetic distances based on marker data and genetic distances based on morphological measurements. Non-significant correlations between phenotypic distances and molecular genetic distances in plants seem to be a widespread phenomenon (Bar-hen et al., 1995; Roldan-Ruiz et al., 2001). In other studies moderate correlations between the two types of distance measurements have been reported (Burstin and Charcosset, 1997), supporting our findings in this study.

Our study reported herein shows that bambara groundnut landraces from Tanzania form a genetically diverse population, and AFLP markers can be effectively employed to assess genetic diversity and to measure the extent of genetic relationship among accessions. Knowledge of the degree of genetic relationships between bambara groundnut accessions will be of importance for crop improvement and may help to establish a core collection as part of the germplasm collection management to sample a maximum of genetic variation in a minimum of accessions. This study reveals that bambara groundnut accessions from Tanzania constitute two major groups in line with their putative geographic origin including a genetically distinct group from Southern agro-ecological zone and a mixed group with accessions originating from the Central, Lake Victoria and Western agro-ecological zones.

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