Genetic diversity in bambara groundnut (Vigna subterranea (L.) Verdc) landraces revealed by AFLP markers


Abstract: Bambara groundnut (Vigna subterranea (L.) Verdc), an African indigenous legume, is popular in most parts of Africa. The present study was undertaken to establish genetic relationships among 16 cultivated bambara groundnut landraces using fluorescence-based amplified fragment length polymorphism (AFLP) markers. Seven selective primer combinations generated 504 amplification products, ranging from 50 to 400 bp. Several landrace-specific products were identified that could be effectively used to produce landrace-specific markers for identification purposes. On average, each primer combination generated 72 amplified products that were detectable by an ABI Prism 310 DNA sequencer. The polymorphisms obtained ranged from 68.0 to 98.0%, with an average of 84.0%. The primer pairs M-ACA + P-GCC and M-ACA + P-GGA produced more polymorphic fragments than any other primer pairs and were better at differentiating landraces. The dendrogram generated by the UPGMA (unweighted pair-group method with arithmetic averaging) grouped 16 landraces into 3 clusters, mainly according to their place of collection or geographic origin. DipC1995 and Malawi5 were the most genetically related landraces. AFLP analysis provided sufficient polymorphism to determine the amount of genetic diversity and to establish genetic relationships in bambara groundnut landraces. The results will help in the formulation of marker-assisted breeding in bambara groundnut.

Key words: under-utilized, African legume, molecular markers.

Introduction

Bambara groundnut, an indigenous African legume, plays an important socio-economic role in the semi-arid regions of Africa. It is rich in protein and could help to alleviate nutritional problems in these regions. However, the crop is characterized by variable and unpredictable yields for reasons that have not been identified. In recent years there has been a growing awareness of the potential of bambara groundnut to contribute to increased food production in Africa and the need to improve existing landraces of the crop (Anonymous 1997). Bambara groundnut has not been improved through coordinated breeding programmes and therefore different genotypes of this crop still exist as landraces. By descri-
tion, considerable genetic variation exists within and between landraces (see Zeven (1998) for a review of landraces: definitions and classifications) and hence, landraces are often well adapted to a wide range of environmental conditions. Bambara groundnut landraces have recognizable morphological features like testa colour that can be used to identify them. Commonly, landraces have names based on the colour of the testa and the place where they are grown. Names are also often associated with markets from where the seeds are purchased, which may be unrelated to origin or areas of cultivation. Such an informal method of classification may lead to one landrace having more than a single name as a consequence of seed introductions to or from other places.

Bambara groundnut has a large number of landraces throughout Africa where growers have preserved its genetic diversity without serious attempts by scientists to exploit or improve particular landraces. As awareness of the potential of under-utilized crops like bambara groundnut increases, both in terms of increasing food production and improving crop diversity (IPGRI 1999; Padulosti 2000), there is a need to study and exploit these crops to improve their productivity. Variability of some genotypes has been described in terms of morphological and agronomic traits (e.g., Begemann 1988; Anonymous 1997; Collinson et al. 1997; Squire et al. 1997; Massawe 2000), isozyme markers (Pasquet et al. 1999), and rapid amplified polymorphic DNA markers (RAPDs) (Amadou et al. 2001).

Isozyme diversity in both wild and domesticated forms of bambara groundnut showed high genetic similarities between wild and domesticated forms and protein patterns were found to be similar to those observed in other Vigna species (Pasquet et al. 1999). In the study by Pasquet et al. (1999) that included accessions representing the entire range of morphological variability, the overall isozyme diversity was low. However, Amadou et al. (2001), using RAPD analysis, reported considerable genetic diversity among bambara groundnut accessions.

For an effective improvement program we require efficient techniques for identification, characterization, and development of a genetic linkage map for bambara groundnut. Morphological and agronomic traits, which have traditionally been used for this purpose, usually require growth of the plants to full maturity before identification. Moreover, the morphological characters are often unstable owing to the harsh and variable environmental conditions in which the crop is usually grown. RAPDs have been criticized because their banding patterns are not always reliable or reproducible. AFLPs (amplified fragment length polymorphism) markers (Vos et al. 1995) provide powerful tools for genetic diversity study of bambara groundnut landraces.

AFLPs have been used in genetic diversity studies, for example in lentils (Lens esculenta) (Sharma et al. 1996), soybean (Glycine max) (Maughan et al. 1996), groundnut (Arachis hypogaea) (He and Prakash 1997), tea (Camellia sinensis) (Paul et al. 1997), sunflower (Helianthus annuus) (Hongtrakul et al. 1997), and cowpea (Vigna unguiculata) (Coulibaly et al. 2002), because of their reliability and effectiveness. The procedure is more efficient than RAPDs for the construction of genetic linkage maps (Lin et al. 1996). AFLPs are not only useful in genetic diversity studies, but also in confirming that the intended crosses or selfing have occurred in breeding programmes (Chen et al. 1999).

AFLPs have potential in bambara groundnut improvement programs like mapping quantitative trait loci (QTLs), e.g., those conditioning characteristics such as drought tolerance, time to flowering, and pod set. AFLPs can also be used to identify different landraces from different geographical locations (Singh et al. 1999 (Azadirachta indica); Angiolillo et al. 1999 (Olea spp.)). AFLPs could also facilitate the identification of diverse parents for use in crossbreeding to establish improved varieties adapted to various environmental conditions.

In the work presented here, AFLP markers were used to study genetic diversity and to establish genetic relationships in contrasting bambara groundnut landraces from different parts of Africa.

Materials and methods

Plant materials

Sixteen bambara groundnut landraces of different geographic origin were used in this study. Their origins and other characteristics are listed in Table 1. Sowing was carried out in plastic pots (21 cm diameter) filled with sand (Silver sand, William Sinclair Horticulture Ltd., Lincoln, U.K.) and plants were maintained at a constant temperature of 30°C and 12 h light (provided by fluorescent tubes and tungsten lamps (400 W)): 12 h dark.

AFLP analysis

Leaf material from 5 to 10 plants of each landrace was harvested from 2-week-old plants for DNA extraction. DNA was extracted from frozen or fresh leaf material using a protocol described by Dellaporta et al. (1983). The analysis of variation within landraces (intra-landrace diversity, used in the present study) determined by RAPD markers was low (Massawe 2000). The DNA extracted from individual plants of each landrace was therefore bulked and used for AFLP analysis. DNA quantity and quality was determined using spectrophotometric measurements (Cecil CE 2041, Scientific and Medical Products, Ltd.) of UV absorption at wavelengths 230, 260, and 280 nm.

AFLP analysis was carried out according to the procedure of Vos et al. (1995) with minor modifications. Approximately 400 ng genomic DNA was digested using 5 U of restriction enzymes Psbl and Msel (New England Biolabs, Inc.) and ligated to 5 pmol Psbl and 50 pmol Msel adaptors (synthesised by Genosys Biotechnologies, Inc., U.K.). The pre-amplification was performed using 5 µL ligated DNA and 30 ng pre-amplification primers (Msel+1 and Psbl+1) (Cruachem Ltd., Glasgow, Scotland, U.K.) using 30 cycles at 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s in a Techne Genius Thermal Cycler (Meadows Instruments, Chicago, Ill.).

The pre-amplified product was diluted at a ratio of 1:20 in TE buffer (10 mM Tris–HCl, 0.1 mM EDTA) and 5 µL of this dilution and 30 ng of selective amplification primers (Msel+3 and Psbl+3) were used for selective amplification. In total, seven selective primer combinations were used. Psbl primers were fluorescently labeled either with HEX or FAM (Cruachem Ltd.). The cycling parameters were as follows: 1
Results

AFLP analysis

AFLPs were used to explore genetic diversity among 16 landraces of bambara groundnut using seven selective primer combinations. A total of 504 fragments were obtained of which 441 (84.0%) were polymorphic (Table 2). On average, each primer combination produced 72 amplified fragments that were detectable by ABI Prism 310 DNA sequencer. However, the maximum number of fragments was found to be 131 and was obtained using primer combination M-ACA + P-GCC, whereas the least number of fragments, 24, was obtained with primer combination M-AGA + P-GCC. The highest level of polymorphism (98.0%) was obtained with primer combination M-ACA + P-GCC, whereas the lowest was 68.0% and was obtained with primer combination M-AAC + P-GGA (Table 2).

The amplified products ranged from 50 to 400 bp and molecular markers specific for each landrace are presented in Table 3. Some amplified fragments were monomorphic across all landraces while some fragments were landrace specific. For example, a fragment of 335 bp was present only in LunT1995 and not in any other landraces when the M-ACA + P-GCC primer combination was used.

Cluster analysis

The dendrogram constructed using UPGMA (Fig. 1) summarizes the interrelationships observed among different landraces. The resultant dendrogram grouped the 16 landraces into three clusters, A, B, and C. Cluster B formed two subclusters, I and II, and both were further subdivided into two groups. DipC1995 and Malawi5 showed the highest coefficient of similarity (closest pair) and were clustered together. DodC1997 and DodC1994 (both cream in testa colour), both of which originated from Tanzania, were grouped together under the subcluster also including two other landraces from Tanzania (DodR1995 and DodR1997) and one landrace from Malawi (Malawi1). Malawi3, NTPS,
ZimbR1993, Malawi4, and Malawi2 (from southern Africa) formed a separate subcluster. Ankpa-4 from Nigeria and Tiganicuru from Mali were grouped together under the cluster that also included Yola from Nigeria. LunT1995 from Sierra Leone formed its own cluster.

Discussion

The objective of the present work was to investigate the amount of genetic diversity and to establish genetic relationships among contrasting bambara groundnut landraces from different growing regions in Africa using fluorescence-based AFLP markers. Landraces used in this study represented the best possible range of morphological variability and geographical origin available in the cultivated form of bambara groundnut.

The study demonstrates that there is considerable diversity in landraces of bambara groundnut and there were no two landraces that produced identical electropherograms. These results suggest that it may be possible to produce a unique fingerprint for each landrace. Genetic relationships observed among bambara groundnut landraces from different regions in Africa using AFLP were related to their place of collection and, to a great extent, to their testa colour. Amadou et al. (2001) reported similar findings using RAPDs in which considerable genetic diversity was found among 25 bambara groundnut accessions. Landrace-specific fragments are being used to produce landrace-specific markers for identification purposes. These could also be employed to identify molecular markers linked or associated with specific characteristics of interest like photoperiod sensitivity for fruit filling (Limemann 1991) and in the development of a molecular genetic map.

Although all of the primer pairs used in the present study produced polymorphic fragments, not all produced landrace-specific fragments. For example, the M-ACC + P-GGA primer combination did not produce a landrace-specific fragment. This has implications for the choice of primer pairs for use in determining unique molecular marker fragments in bambara groundnut landraces. The M-AAC + P-GGA, M-ACA + P-GCC, and M-ACA + P-GGA primer pairs are recommended for the detection of landrace-specific fragments in bambara groundnut. For those landraces that did not produce fragments specific to them, more primer pairs could be

<table>
<thead>
<tr>
<th>Primer combination</th>
<th>Total no. of fragments</th>
<th>% polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-AGA + P-GCC</td>
<td>24</td>
<td>88</td>
</tr>
<tr>
<td>M-AGA + P-GGA</td>
<td>32</td>
<td>81</td>
</tr>
<tr>
<td>M-AAC + P-GCC</td>
<td>92</td>
<td>82</td>
</tr>
<tr>
<td>M-AAC + P-GGA</td>
<td>72</td>
<td>68</td>
</tr>
<tr>
<td>M-ACA + P-GCC</td>
<td>131</td>
<td>98</td>
</tr>
<tr>
<td>M-ACA + P-GGA</td>
<td>125</td>
<td>96</td>
</tr>
<tr>
<td>M-ACC + P-GGA</td>
<td>28</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>504</td>
<td>NA</td>
</tr>
<tr>
<td>Average per primer pair</td>
<td>72</td>
<td>84</td>
</tr>
</tbody>
</table>

Note: NA, not applicable.
explored that would produce unique fragments for these landraces. There was no association between the number of unique molecular marker fragments and geographic origin of these landraces. For example, DodR1997 from Tanzania and LunT1995 from Sierra Leone had the highest number of unique fragments, but came from two distinct geographic origins not associated with centres of origin or domestication of this species.

The high levels of genetic polymorphism observed in this study indicate that most of these landraces are highly diverse from one another. The major contributory factor to high levels of genetic diversity in many species is the nature of their breeding systems (Perera et al. 1998; Singh et al. 1999), with out-crossing species having higher diversity than self-fertilized species. Although bambara groundnut is largely self-fertilising, Doku and Karikari (1971) reported that cross-pollination occurs and is aided by ants. These ants can be observed even when the crop is grown in the glasshouses at the University of Nottingham. However, it should be noted that isozyme diversity in both wild and domesticated bambara groundnut accessions from different regions in Africa indicated low total genetic diversity between accessions, but there was high intra-population diversity (Pasquet et al. 1999). Further studies are therefore needed to compare results from different genetic marker systems.

Cluster analysis of the 16 landraces of bambara groundnut provide an insight into the origin and relationships between these landraces. The colour of the testa proved to be an important indicator of the relationships among landraces. Those landraces with the same colour of the testa, for example red (DodR1995, DodR1997), were grouped together. This has significant implications in genetic relationships in bambara groundnut landraces because most landraces have names based on the colour of the testa. LunT1995, which has been reported to have marked morphological and physiological differences from other landraces (Massawe et al. 1999; Collinson et al. 1999), formed its own cluster and had some landrace-specific markers that were absent in other landraces.

DipC1995 and Malawi5 were the most genetically related landraces (Cluster A); however, this cluster was the most genetically diverse from the rest of the clusters. DipC1995 and Malawi5, are originally from Botswana and Malawi, respectively, and the present study suggests that these two landraces may have either originated from the same location and are closely related or that seed material might have been moved from one country to another. Amadou et al. (2001) reported similar findings in which bambara groundnut accessions from Zimbabwe were grouped together with those from Zambia. In the present study, Jaccard’s coefficient of similarity values ranged from 0.59 to 0.88 and, compared with 0.69 to 0.97, were lower than those reported by Amadou et al. (2001) using RAPD analysis. This would suggest that AFLPs are capable of detecting more polymorphisms than RAPDs. The reliability and reproducibility of AFLPs means that the molecular markers identified could be used to develop specific primers for the different landraces for easy identification. The use of semi-automated fluorescence-based detection of AFLPs improved fragment scoring and data handling significantly (see Zhang et al. 1999).

Minor crops, such as bambara groundnut, could benefit from the application of technologies in molecular breeding. These techniques should be used to accelerate the acquisition of knowledge for marginal crops cultivated by resource-
poor farmers. Results reported here offer the basis of an effective improvement program on bambara groundnut. Considering these findings, future research should be aimed at the development of a molecular genetic linkage map of bambara groundnut using AFLPs. Landrace-specific markers offer a powerful tool for identification purposes and in breeding programs. Selection of parents for mapping studies could also greatly benefit from the present study. For example, to maximize genetic diversity, artificial hybridization should be done between landraces that are genetically distinct (with low genetic similarity values), such as between DipC1995 and Tiganicuru.

Acknowledgements

The first author is grateful for the financial support from the University of Nottingham, Overseas Research Scheme (U.K.), and the Open University of Tanzania.

References


© 2002 NRC Canada
This article has been cited by:


5. Vishnu Bhat, Deepmala Sehgal, Soom Nath Raina. Applicability of DNA Markers for Genome Diagnostics of Grain Legumes 497-557. [CrossRef]
