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Full Length Research Paper

Genetic variability in Sudanese *Acacia senegal* (L.) assessed by random amplified polymorphic DNA

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Random Amplified polymorphic DNA (RAPD) markers were used to characterize the genetic diversity and relationships among (*Acacia senegal*). A total of 15 primers were tested with the 30 genotypes of *Acacia* spp. (28 *A. senegal*, one *A. mellifera* and one *A. leata*). The results indicated that 7 primers (60%) showed at least 1 consistent polymorphic band. The seven informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships within and among all the Acacia spp under study. The selected primers generated distinctive products in the range of 1.584-5.148 Kbp. Total of 51 amplified fragments were distinguished across the selected primers and the statistical analysis showed 44 polymorphic bands among the 28 genotypes with an average of 7.2 polymorphic bands per primer. The maximum numbers of fragment bands were produced by the primer OPA-09 (11) with 73% polymorphism while the minimum numbers of fragments were produced by the primer OPA-01 (5) with 80% polymorphism Molecular variance (STATISTCA) was used to investigate the genetic diversity among individual. High level of polymorphism among individuals suggested that RAPD technique can be useful for *A. senegal* for the maintenance of germplasm banks and the efficient selection of parents for breeding.

Key words: *Acacia senegal*, Sudan, genotypes, genetic diversity, RAPD.

INTRODUCTION

Acacia senegal (L.) Willd. a leguminous tree species belonging to the Mimosoideae sub family, is an important Gum Arabic producing agroforestry tree species in Sudan. *A. senegal* (L.) Willd is a multipurpose African tree. The species belong to the subgenus, *Aculeiferum* (Arce and Blanks, 2001). The species forms an essential integral component of farming systems in dry and semi-arid regions. The tremendous advantage of the tree lies on its valuable product, limited input requirements, long term productivity, and its multiple purposes attributes specially in the dry regions (Raddad et al., 2006). In Sudan, *A. senegal* is one of the most widespread *Acacia* species and extends over a wide ecological range that is known as the Gum Arabic belt (Awouda, 1990). Within the belt, the tree is adapted to survive under harsh environmental conditions such as low and erratic rainfall, intense solar radiation, and high wind velocity. In this context, the tree has a significant environmental potential in minimizing soil erosion and prevention of desert encroachment (Mark, 1997). Moreover, *A. senegal* is an important socio-economic species in high demand by local communities for its multiple amenities and uses (food, traditional medicine and pharmacy, preservation and improvement of soil fertility, rites and customs, etc.) in addition to the high value Gum Arabic (Glyn et al., 2008). Of many gum producing varieties, *A. senegal* is the most important source of marketable gum (Ballal et al., 2005) which is obtained either from natural exudation or derived by tapping branches and stems of the trees periodically (Verbeken et al., 2003). However, the species remains under pressure as a result of its over-exploitation by human population, shortage of rainfall and inappropriate agricultural practices, leading to the degradation and/or lack of regeneration of *A. senegal* parklands in many zones (Bodil et al., 2005).

Molecular and biochemical studies have been conducted

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Abbreviations: RAPD, Random amplified polymorphic DNA; **PCR,** polymerase chain reaction; **D,** genetic dissimilarity.

on African and Australian *Acacia* species to provide markers useful for plant breeding and conservation programs (Playford et al., 1993; McGranaham et al., 1997; Butcher et al., 1998). In most African species of *Acacia*, isozymes have been used (Boer, 2002). However, data from RAPD analysis have indicated that their diversity is usually similar to or greater than diversity from allozymes in plant species (Esselman et al., 1999, 2000).

RAPD markers are based on amplified arbitrary sequences and sample a wider part of the genome. RAPD has allowed the resolution of complex taxonomic relationships (Cottrell et al., 1997; Casiva et al., 2002).

This study has utilized the advantages of the RAPD molecular markers techniques by using marker's data in estimating the genetic diversity in 28 *Acacia senegal*. Two other species (A. mellifera and one A. leata) were used for comparison. The objective was to understand the current genetic diversity of the species which can be useful for effective management, conservation, and selection for genetic improvement.

MATERIALS AND METHODS

Plant material

28 of *Acacia senegal* genotypes collected from individuals of natural populations representing eco-geographical distribution within the Gum Arabic belt (Table 1). Single samples from *A. mellifera* (Kitir) and *A. leata* (Shubahi) that also produces good quality of Gum Arabic were used for comparisons. All samples were collected from mature, healthy, and productive acacia trees and the selection parameters were based on the productivity, type of soil and location. Seeds of each accession were grown in 30 cm diameter earthen pots in a greenhouse for 4 weeks.

DNA extraction

DNA isolation was based on a modified phenol: chloroform method (Doyle and Doyle, 1990). Leaf samples (2.5 g) were placed into coffee grinders, covered with dry ice, and pulverized to form fine powders. Powdered plant materials were immediately transferred into 13 ml Falcon tubes containing 4 ml of pre-warmed lyses solution, (4 M NaCl, 100 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 1% β-mercaptoethanol (v/v) and 1.0 % SDS (w/v)), and the tubes were then incubated at 65°C for 30 min. The DNA was extracted sequentially with an equal volume of phenol/chloroform (1:1), and chloroform/ isoamyl alcohol (24:1). Total nucleic acids was precipitated with 0.7 volume of isopropanol and vacuum dried. Finally, the pellet were re-dissolved in water and treated with RNase A at 37°C for 1.5 h to remove RNA, and the RNase was inactivated at 60°C for 1 h. Final DNA concentrations were determined by measuring with a UV spectrophotometer and the integrity of each DNA sample was examined with an agarose gel.

RAPD analysis and primer selection

Fifteen (OPA- obtained from operon technologies) primers were used for polymerase chain reaction (PCR) amplification.Six primers that produced strongly amplified polymorphic bands with these test templates were selected for RAPD-PCR analysis (Table 2). The PCR reaction mixtures were prepared in 25 µl volumes containing 2.5 µl of 10X Taq buffer, 1.5 µl $MgCl_2$ (50 mM), 2.5 µl dNTPs (2 mM/µl), 2 µl random primer (10 pmol/µl), 0.5 µl Taq DNA polymerase (5 U/µl) and 1 µl of the extracted DNA (10 ng). The mixture was made up to 25 µl by addition of sterilized distilled water. RAPD/PCR reactions were optimized initiated using an applied Biometra thermal cycler programmed to repeat the thermal profile.

Setting of the PCR program was based on three steps. Step one, was an initially initial denaturation step at 94°C for 5 min. Step two, was run for 40 cycles, each starting with denaturation at 94°C for 1 min, followed by annealing at 36°C for 1 min and ended by extension at 72°C for 1 min. Step three, was a final extension cycle performed at 72°C for 7 min. The PCR machine was adjusted to hold the product at 4°C. The PCR product was mixed with 1 - 3 µl of loading dye (0.25% bromophenol blue, 0.25% Xylene Cyanol and 40% Sucrose, w/v) and spun briefly in a micro centrifuge before loading. The PCR products and 1 kp DNA ladder were electrophoresed using 2% agarose gel at 100 volts followed by staining with ethidium bromide, then fragments were separated and were visualized with an ultraviolet (UV) transilluminator.

Data analysis

Polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The genetic dissimilarity (D) matrix among genotypes was estimated according to Nei and Lei (1979). Coefficient of similarity trees were produced by clustering the similarity data with the unweighted pair group method using statistical software package STATISTCA-statistical package for the social sciences (SPSS) (Stat soft Inc.).

RESULTS AND DISCUSSION

A total of 15 primers were tested with the 30 genotypes of Acacia spp. (28 *A. senegal*, one *A. mellifera* and one *A. leata*). The results indicated that 7 primers (60%) showed at least 1 consistent polymorphic band. The seven informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships within and between all the *Acacia* spp under study. The selected primers generated distinctive products in the range of 1.584 - 5.148 Kbp. A total of 51 amplified fragments were distinguished across the selected primers and the statistical analysis showed 44 polymorphic bands among the 28 genotypes with an average of 6.3 polymorphic bands per primer. The maximum numbers of fragment bands were produced by the primer OPA-09 (11) with 73% polymorphism while the minimum numbers of fragments were produced by the primer OPA-01 (5) with 60% polymorphism (Table 2). Pattern of RAPD fragments produced by the 10-mer primer OPA -01, OPA-03, OPA-09 OPA-13 OPA- 14 OPA- 17, and OPA-20 is as shown in Figure 2.

The dendrogram showed two main clades (Figure 2). The first clade contained two groups, the first group contained (Algadamblia 2, Simmer 3, as sisters and (Algadamlia, Elrrahad) showed gentic closeness, the second group contained (macrmgrees 2, Algabsha, Algadamblia 4, simmer 2, Simmer 1 and Nabg as sisters, (Alphil 1, alphil 2 as sisters). Algadamblia 3, Nawa, Bara

Table 1: Sources of Acacia (Hashab) seeds, locations in Sudan and soil types of the site.

Sample No.	Common name	Site of collection	Location	Type of soil
1	Hashab	Algadamblia 1	Eastern Sudan	Clay
2	Hashab	Algadamblia 2	Eastern Sudan	Clay
3	Hashab	Algadamblia 3	Eastern Sudan	Clay
4	Hashab	Algadamblia 4	Eastern Sudan	Clay
5	Hashab	Simmer 3	Eastern Sudan	Clay
6	Hashab	Simmer 1	Eastern Sudan	Clay
7	Hashab	Simmer 2	Eastern Sudan	Clay
8	Hashab	Alphil 1	Eastern Sudan	Clay
9	Hashab	Alphil 2	Eastern Sudan	Clay
10	Hashab	Macrm grees 1	Eastern Sudan	Clay
11	Hashab	Macrm grees 2	Eastern Sudan	Clay
12	Hashab	Simmer belt 3	Eastern Sudan	Clay
13	Hashab	Algabsha	Western Sudan	Sand
14	Hashab	Nawa	Western Sudan	Sandy clay
15	Hashab	Bara Aldankong	Western Sudan	Sandy clay
16	Hashab	Aldmokia 1	Western Sudan	Sand
17	hashab	Alsmeeh	Western Sudan	Sandy clay
18	Hashab	Hamra Elgoaz	Western Sudan	Sand
19	Hashab	Nabg	Western Sudan	Sandy clay
20	Hashab	Elrrahad	Western Sudan	Sand
21	Hashab	Aldamokia 2	Western Sudan	Sand
22	Hashab	Algabsha	Western Sudan	Sand
23	Hashab	Abualgur	Western Sudan	Sandy clay
24	Hashab	Wadalneil	Blue Nile	Clay
25	Kitir	*Kitir	Blue Nile	Clay
26	Hashab	Bout	Blue Nile	Clay
27	Hashab	Altakamul	Blue Nile	Clay
28	Hashab	Abugumi	Blue Nile	Clay
29	Hashab	Kur Dunia	Blue Nile	Clay
30	Shubahi	**Shubahi	Blue Nile	Clay

**Acacia mellifera* ** *Acacia leata*

aldankong, and macrm grees showed genetic closeness. The second clade contained (Simmer belt 3, Aldamokia 1), (Algabsha, Bout), (Abugumi, Kur donia) as sisters, Abualgur, Hamraalgoz and Altakamul came in different group. Kitir and shubahi came out of group. This study provided us with good knowledge about genetic variability of *Acacia* which may allow more efficient and effective use of resources in plant improve-ment programs. In this study we utilized RAPD markers for better assessment of relationship of the accessions of *Acacia* collected from different locations. The genetic dissimilarity values obtained with RAPD have been introduced for measuring genetic relationships in many plant species for easiness of the method, which only requires PCR technology. The low reproducibility of RAPD (Karp et al., 1997), introduces problem in their use for cultivar identification compared with other marker applications. From the results of the RAPD profiling, it was observed that *A. senegal* genotypes produced good number of amplified bands but few showed less number of amplified bands on

Table 2. Polymorphism detected by the use of 15 random primers on 30 *Acacia* individuals.

Name of primer	Sequence of primer (5'- 3')	Total number of bands	Number of polymorphic bands	% of polymorphic bands
OPA-01	CAGGCCCTTC	5	3	60
OPA-03	AGTCAGCCAC	6	5	83
OPA-09	GGGTAACGCC	11	8	73
OPA-13	CAGCACCCAC	7	7	100
OPA-14	TCTGTGCTGG	6	6	100
OPA-17	GACCGCTTGT	10	10	100
OPA-20	GTTGCGATCC	6	5	83.3
Total		51	44	599.3
Average		7.2	6.3	85.6

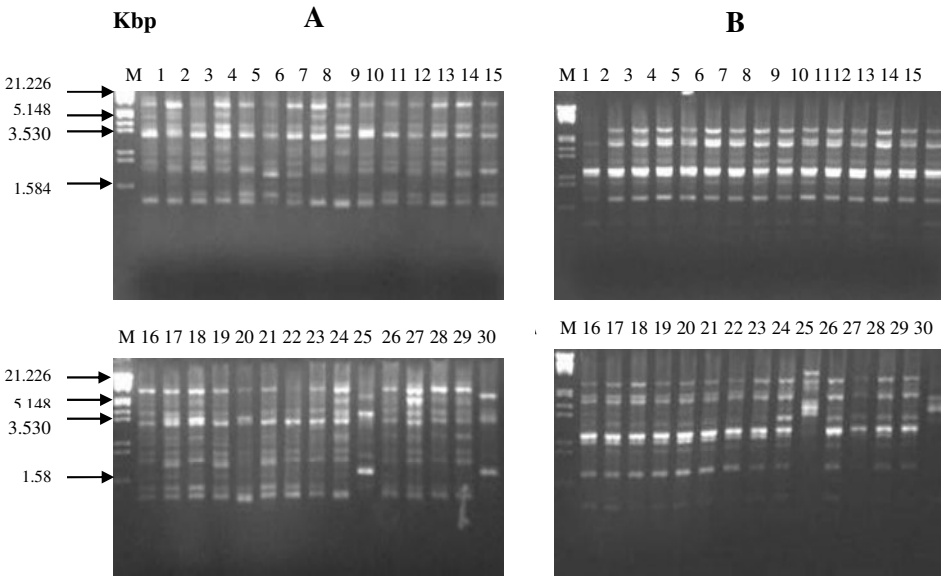

Figure 1. A- RAPD fingerprints of *Acacia* genotypes (1-30) generated with the primer OPA-17, M- DNA marker (λ Hind III digested DNA). B- RAPD fingerprints of *Acacia* genotypes (1 - 30) generated with the primer OPA-09, M- DNA marker (λ Hind III digested DNA).

some primer. Similarly, unique patterns were observed differentiating all 30 genotypes from each other by using seven 7-mer RAPD primers. It can be that concluded that RAPD markers could be used for differentiating *Acacia senegal* genotypes and it might help in generating molecular data base for germplasm conservation.

Effective plant improvement programs depend on the availability of genetic diversity. It is well known that landraces are of variation in plants and are still the major source for crop improvement programs in developing countries. To assess the genetic diversity present in Gum Arabic belt, seeds were collected from different locations in Sudan. The collected accessions showed variation in morphological and characteristic of the produced gum. However; morphological variation does not always reflect real genetic variation and that could be attributed to the control of interaction of the genotype with environment by unknown genetic traits (Smith and Smith, 1992).

In our study the genetic analyses showed low levels of diversity across the geographic range, although samples were collected from geographically distant locations from Eastern and Western Sudan. There was limited differentiation between provenances within these regions, and sometimes high diversity within provenances. Limited genetic differentiation between provenances seems to be the result of genetic stability due to long overlapping generations, and a very low mutation rate. Limited marker polymorphism and limited differentiation between provenances within broad regions are common features in *A. senegal* (Fenyo and Vandevelde, 1990).

Molecular markers can be used to study the genetic diversity and genetic relationships among *A. senegal* accessions at the DNA level. The RAPD method also made it possible to sort out the mislabeling of different

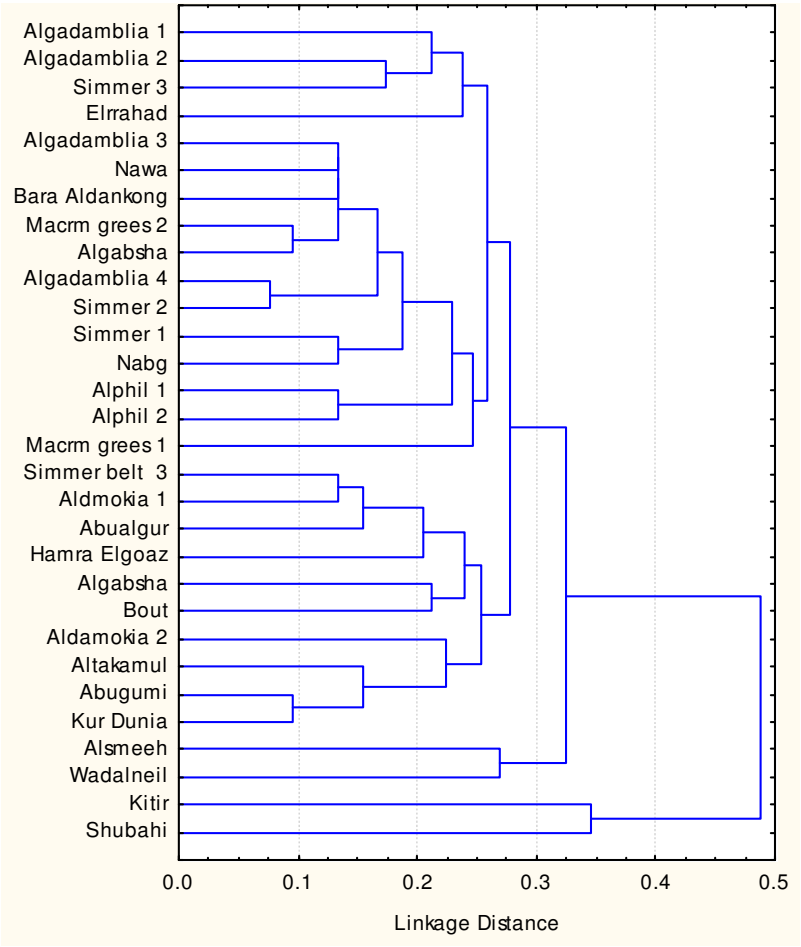

Figure 2. Combined cluster analysis derived from RAPD data by STATISTCA-SPSS to estimate the genetic distance analysis of 30 acacia accessions using 7 RAPD primers.

ecotypes based on the obtained banding pattern. The technique has been used in the same manner previously, to discriminate fig genotypes (Elisario et al., 1998; Galderisi et al., 1999; De Masi et al., 2003). The RAPD procedure can easily be used for a large number of samples and ecotypes and/or universal primers would generate fingerprints to identify the genetic background of the plants, a knowledge which is necessary for rational management of this important fruit crop. Moreover, the genetic diversity analyses in *A. senegal* shows, in comparison with related *A. melifra* and *A. leata*, how important this technique is in the establishment of a national germplasm collection and also how to molecularly identify. The high level of genetic variation in *A. senegal* obser-ved in this study is consistent with its wide geographic range which is also the case with other acacias (Habeballa and Gaali). Genetic studies of other domesticated *Acacia* species throughout the world have identified many species with moderate to high levels of population differentiation that was geographically structured (Butcher et al., 1998).

In conclusion, the results of this study indicated that RAPD analysis can be successfully applied to the study of genetic diversity, and relationships within and among accessions also is a useful tools for molecular variability studies in plant breeding and conservation programs.

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