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Full Length Research Paper

Phylogenic analysis in *Acacia senegal* using AFLP molecular markers across the Gum Arabic Belt in Sudan

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Amplified fragment length polymorphism (AFLP) DNA markers were used to characterize the genetic diversity and relationships in gum Arabic tree (*Acacia senegal*). Twenty eight samples of *Acacia senegal* collected from populations distributed throughout the Gum Arabic belt were tested in comparison with samples of *Acacia mellifera* and *Acacia leata*. Nine AFLP selective primer pair combinations generated a total of 433 amplification products with an average of 89.18% detected polymorphisms. Accessions showed the least variation was found within the *A. senegal* accessions in contrast with *mellifera* and *Acacia leata* that presented the highest degree of polymorphism number. According to the cluster analysis two main clusters were obtained in which *A. mellifera* and *A. leata* were placed in a separate group. There were eight subgroups of *A. Senegal.* Three of the eight subgroups of *A. senegal* were clustered according to geographical origin. The variation within population might be correlated with abiotic factors of the environment.

Key words: AFLP marker, gum arabic, *Acacia senegal, Acacia mellifera*, *Acacia leata*, polymorphism.

INTRODUCTION

The genus *Acacia* belongs to family Leguminosea: Mimosoideae, that represents both trees and herbs distributed in the tropical and subtropical regions of the world (Raddad and Luukkanen, 2007). Although there are over 1100 *Acacia* species worldwide, *Acacia senegal* remain the most commercially exploited species of the whole acacia resource (Guinet and Vassal, 1978). The species forms an essential integral component of farming systems in dry and semi-arid regions. The tremendous advantage of the tree lies in 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 by tapping branches and stems of the trees periodically (Verbeken et al., 2003). Many researches have been conducted on the species. 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). Despite the fact that *A. senegal* has a remarkable ecological and socio-economic welfare of areas where it grows naturally,

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most efforts have been exerted towards the gum quantity and quality (Chiveu et al., 2008). For instance, studies on variation in morphological characteristics between populations (Al- Assaf et al., 2005), variation based on gum chemical composition and sources of origin of the species (Chikamai and Odera, 2002), as well as variability in Gum Arabic quality and molecular weight (Motlagh et al., 2006) have been carried out. On the other hand no clear study has so far done in the area of genetic improvement of the species for Gum Arabic yield and quality in Sudan. Efforts to conserve *A. senegal* germplasm resources would be useful to complement gum quantity and quality researches, which would lead to effective utilization of the resources to improve productivity and quality. Our knowledge on the structure of genetic diversity of *A. senegal* in Sudan is still in adequate for conservation methodologies and need to be promoted. In this context, the present study was undertaken in order to understand the genetic diversity of *A. senegal* utilizing the AFLP marker technique (Vos et al., 1995) which will be essential for the elaboration of an efficient strategy for sustainable use and conservation of genetic resources.

MATERIALS AND METHODS

Plant material

Seed samples of 28 *A. senegal* accessions (Table 1) were collected from individuals of natural populations representing eco-geographical distribution within the Gum Arabic belt (Figure 1) Single samples from *Acacia mellifera* (Kitir) and *Acacia 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 the greenhouse for 4 weeks.

DNA extraction

DNA isolation was based on a modified phenol: chloroform method (Doyle and Doyle, 1990). Samples from 4 weeks old seedlings (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 65ºC 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 were precipitated with 0.7 volume of isopropanol and vacuum dried. Finally, the pellets 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.

AFLP analysis

The AFLP analysis was performed as described previously with minor modifications (Vos et al., 1995; Bai et al., 1999). Briefly, isolated genomic DNA (approximately 250 ng) was double digested with *Eco*RI and *Mse*I restriction enzymes. AFLP adapters for both enzymes were subsequently ligated to the digested DNA fragments using T4 DNA ligase. The ligated DNA was pre-amplified using a primer combination based on the sequences of the adapters. Pre-amplification was performed for 20 cycles of 30 sec at 94°C, 1 min at 65°C and 1 min at 72°C.

The PCR products of the pre-amplification reaction were diluted 50 times to be used as templates for the selective amplification. The final selection amplification was performed by combining nine *EcoR*1-*Mse*1 primer pairs, each containing three selective nucleotides (Table 2). The following touchdown thermal profile was used in all selective amplifications: 2 min at 94°C; 13 touchdown cycles at 94°C for 30 sec, 65°C for 30 sec (decreasing the temperature by 0.7°C per cycle) and 72°C for 60 sec; 23 cycles at 94°C for 30 sec, 56°C for 30 sec and 72°C for 60 sec. The PCR products were separated by electrophoreses on a 6% (w/v) denaturing polyacrylamide gel under standard sequencing conditions. The patterns of the separated AFLP fingerprint were visualized after silver staining of the gels (Heukeshoven and Dernick, 1988). The experiments were repeated twice for each genotype to confirm the repeatability.

Data analysis

For each individual DNA fingerprints, only intense unambiguous AFLP-bands were manually scored for their presence (1) or absence (0). Data were compiled for each accession in a data matrix and were analyzed using the mathematical model for studying genetic variation (Nei and Li, 1979). Coefficient similarity trees were produced by clustering the similarity data with the un-weighted pair group method using statistical software package STATISTCA- SPSS (Stat Soft Inc.). The similarity coefficient (Rohlf, 1993) was used to construct a dendrogram by the un-weighted pair group method with arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

AFLP patterns

Various numbers of bands were produced depending on the primer combinations. Reproducibility of the AFLP products was very high. The non-repeatable bands were mainly faint bands that showed up in some PCRs, but not in others. These results are consistent with previous re--ports (Zhang et al., 1999; Rouf Main et al., 2002) regarding the reproducibility of AFLP markers and further confirm that the AFLP technique generates highly reproducible DNA profiles for *A. senegal.*

Genetic diversity and relatedness

A total of 45 combination primers were tested with the 28 *A. senegal* in addition to *A. mellifra* and *A. leata* accessions. Only 9 (20% of the total) primer combinations, that generated optimally clear AFLP profiles, were selected to analyze the diversity of the samples. Each of the 9 primer combinations was able to generate an informative AFLP fingerprints across the all DNA samples (Figure 2). The

Table 1. Sources of acacia seeds, locations in Sudan and soil types of the site.

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

Acacia mellifera*; * Acacia leata.*

nine selective primer combinations amplified 433 complete bands, with an averaging of 48.1 bands per primer combination (Table 2). Of the 433 bands scored, 386 (89.18%) were polymorphic by virtue of their absence in at least 1 of the 30 accessions. Primer combination E/ACC-M/CAA produced the maximum total number of bands (71) and the highest number of polymorphic bands (64), whereas E/ACC-M/CAA produced the least total number (27) and polymorphic (16) bands (Table 2). The large number of markers generated with this technique indicating the presence of large AFLP variation (Figure 2).

The genetic similarity coefficients based on the AFLP data ranged between 0.06 and 0.60. The highest similarity coefficients (0.61) for pair wise comparisons among the 30 accessions was between accessions 30, that is, *A. mellifera* and 25 from *A. senegal* of the central part of Sudan. The least similarity coefficients value (0.06) was for the pair wise comparisons of 16 and 22 (both of from different locations of West of Sudan). The genetic rela-

Figure 1. The Gum Arabic belt in Sudan.

tionship among the AFLP patterns of different acacia accessions as presented in the clustering dendrogaram in Figure 3. According to the cluster analysis we found six main groups originated in the phylogenic tree. Group one contained accessions 1, 2, 3, 4, 5, 6 and 7 which were originally clay soil of Eastern Sudan. Group two contained the accessions 10, 11, 12, 14,15,16,17, 18, 19 and 20. The accessions 10, 11 and 12 were from clay soils of East of Sudan while the rest either from sandy clay or sandy soils of the Western areas. Group three contained three accessions (21, 22 and 23) were also from West of Sudan. The accessions 26, 27, 28 and 29 are from the same subgroup. Accessions of this group were collected from clay soils of the Blue Nile area in the central region of the Sudan. Although the accessions 13 are from 24 different locations (Table 2) they stay in same subgroup/ cluster. The last group, contained two 25 and 30. Both of them were from different genotypes, *Acacia leata* and *A. mellifera* which are known as kitir and Shubahi respectively. From the dendrorgram (Figure 3) we can observe that the accessions 8 and the accessions 9, (although they were from clay area of the East of Sudan), were placed in separate subgroups/clusters.

This study provides us with good knowledge about

Table 2. Primer combinations for selective amplification, and total polymorphic bands scored in *A. senegal, A. mellifera* and *A. leata* AFLP profiling.

Primer pair	Total number of Scored bands	Number of polymorphic bands	Polymorphism (%)
*E/AGG-M/CAG	32	28	87.5
E/AAG-M/CAC	60	55	91.6
E/ACA-M/CTT	54	51	94.4
E/ACA-M/CTC	47	43	91.4
E/ACT-M/CAT	56	53	94.6
E/ACC-M/CAA	71	64	90.1
E/AGC-M/CAC	39	34	91.8
E/AGC-M/CTG	47	42	89.3
E/AGG-M/CTC	27	16	59.2
Average	48.1	42.9	89.18

*E = Primer of *Eco*RI; M = Primer *Mse*I.

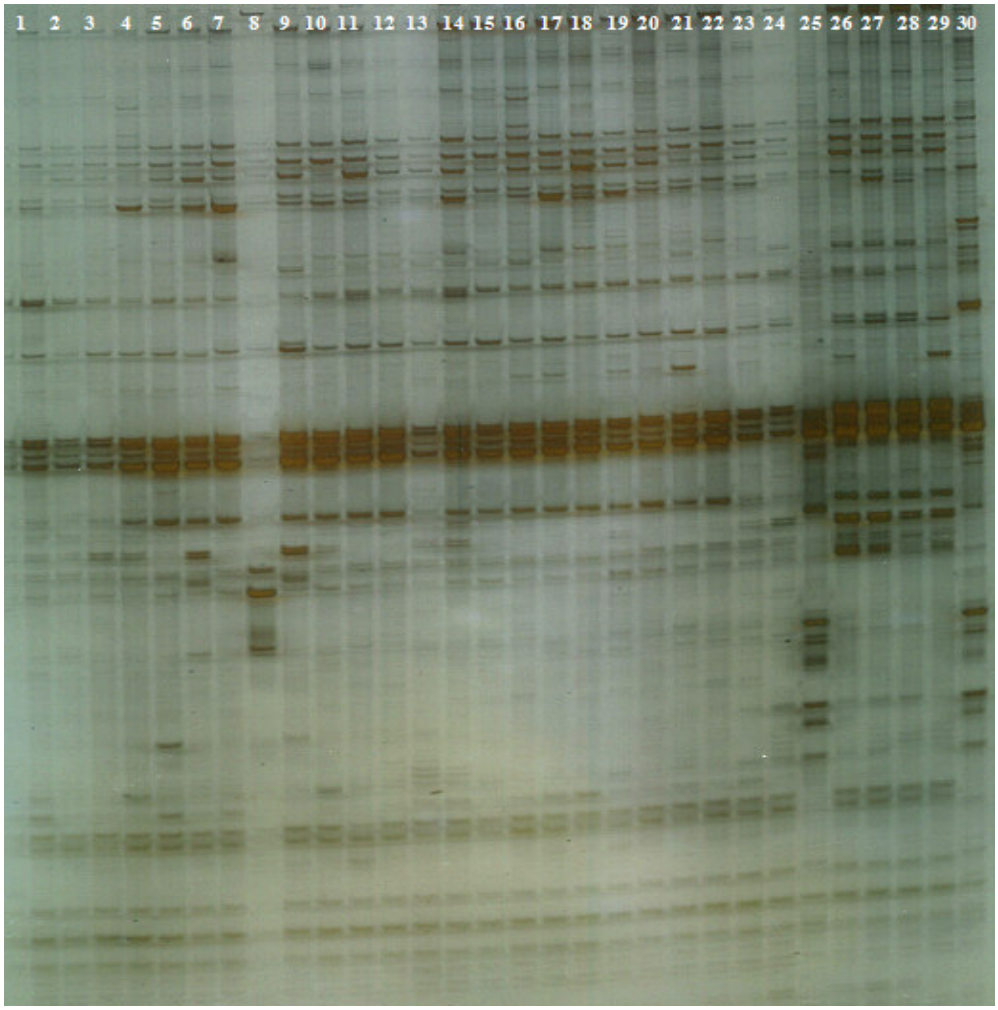

Figure 2. Normalized AFLP band patterns generated from 30 accessions of acacia using the primer combination E/ACC-M/CAA.

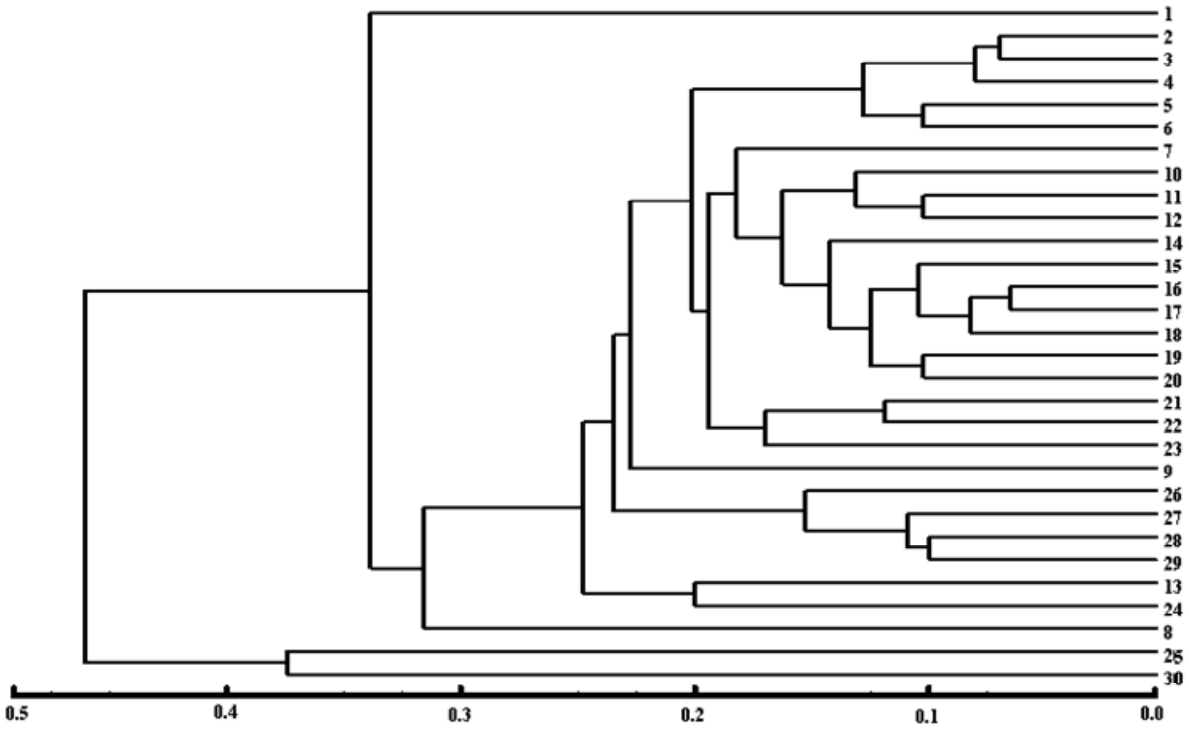

Figure 3. Combined cluster analysis derived from AFLP data by NTSYS and UPGMA to estimate the genetic distance analysis of 30 acacia accessions using 9 AFLP primer combinations.

genetic variability of acacia which may allow more efficient and effective use of resources in plant improvement programs. In this study we utilized AFLP markers for assessment of relationship of the accessions of acacia collected from different locations. The dendrogram obtained was analogous with the results of AFLP because they both placed genotypes (25) shubahi and (30) Kitir, in the same major group. This reflects the fact that they are more closely related to each other, belong to different species and not related to *A. senegal* (Figure 3). Generally, samples collected in the same climatic zones belong to the same cluster group. These results also indicate that the genetic structuring of the sampled individuals is correlated with their geographic origin. However, additional mapping studies are needed to identify specific genes or genome regions that might have a direct influence on the observed variation.

The high level of genetic variation in *A. senegal* observed in this study is consistent with its wide geographic range as is also the case with other acacias. Many studies in population genetics of *Acacia* species revealed that the variation among the species was related to their geographical distribution (Butcher et al., 1998). Wickenesswari and Norwati (1993) found that the population structure of the tropical *A. auriculiformis* reflected its distribution in three geographical regions. Similar results were also obtained by McDonald et al. (2003) when they studied *A. tumida.* High level of differentiation in *A. aulacocarpa* was related to its complex geographical distribution (McGranahan et al., 1997; McDonald and Maslin, 2000). Pohlman et al. (2005) suggested that adaptation to harsh environmental condition might lead to the genetic diversity. In addition gene flow among populations via pollen dispersal might have contributed to genetic diversity (Dangasuk and Gudu, 2000).

Since, intra-specific diversity has become a fundamental parameter for the management of species with the aim of maintaining their evolutionary potential (Rajagopal et al., 2000) exploration of the genetic diversity of the economically important trees is required for efficient genetic resource management. The AFLP combination of two different sources of variation leads to highly reproducible, efficient and reliable results that make this procedure an extremely useful tool for molecular variability studies in plant breeding and conservation programs.

REFERENCES

Al-Assaf S, Phillips GO, Aoki H, Williams PA (2005). Studies on *Acacia* gum exudates. Part I: The molecular weight of *Acacia senegal* gum exudates. Food Hydrocol. 19: 647-660.

Awouda E (1990). Indicators for present and future supply of Gum Arabic. In: Phillips G, Wedlock Williams P. Gums and stabilizers for the food industry, Published by IRL press at Oxford University Press,

pp. 45-54.
Bai H, Tefera H, Ayele M, Nguyen H (1999). AFLP analysis of tef and its relatives. Crop Sci. 39: 819-24.
Ballal M, Elsiddig A, Elfadl M, Luukkanen O (2005). Gum Arabic yield in differently managed *Acacia senegal* stands in western Sudan. Agroforestry Forum. 63: 237-245.
Bodil E, Lennart O, Eltighani E, Andrew W (2005). A traditional agro forestry system under threat: an analysis of the Gum Arabic market and cultivation in the Sudan Agroforestry Systems, 64: 211-18.
Butcher PA, Moran GF, Perkins HD (1998). RFLP diversity in the nuclear genome of *Acacia mangium*. Heredit. 81: 205-213.
Chikamai BN, Odera JA (2002). Commercial plants, gums and resins in Kenya, Nairobi, Kenya, pp. 47-52.
Chiveu CJ, Dangasuk OG, Omunyin ME, Wachira FN (2008). Genetic diversity in Kenyan populations of *Acacia senegal* (L.) willd revealed by combined RAPD and ISSR markers. Afr. J. Biotechnol. 7: 2333-2340.
Dangasuk OG, Gudu S (2000). Allozyme variation in 16 natural populations of *Faidherbia albida* (Del.) A. Chev. Hered. 133: 133-145.
Doyle J, Doyle J (1990). A rapid total DNA preparation procedure for fresh plant tissue. Focus, 12: 13-15.
Glyn O, Takashi O, Kazunari U (2008). The regulatory and scientific approach to defining Gum Arabic (*Acacia senegal* and *Acacia seyal*) as a dietary. Food Hydrocolloids, 22: 24-35.
Guinet P, Vassal J (1978). Hypotheses on the differentiation of the major groups in the genus *Acacia* (Leguminosae). Kew Bull. 32: 509-27.
Heukeshoven J, Dernick R (1988). Improved silver staining procedure for fast staining of sodium dodecyl sulfate gels. Electrophoresis, 9: 28-32.
Mark S (1997). Economic policy and desertification in arid and semi-arid developing countries. Pak. Dev. Rev. 36: 191-201.
McDonald MW, Maslin BR (2000). Taxonomic revision of the salwoods: *Acacia aulacocarpa* Cunn. Ex. Benth; and its allies (Leguminosae: Mimosoideae: section juliflorae). Aust. Syst. Bot. 13: 21-78.
McDonald MW, Buther PA, Bell JC, Nguyen CV (2003). Clinal variation and genetic divergence in *Acacia tumida* (Mimosaceae). Aust. Syst. Bot. 16: 57-67.
McGranahan M, Bell JC, Moran GF, Slee M (1997). High genetic divergence between geographic regions in the highly out crossing species *Acacia aulacocarpa* (Cunn. Ex Benth). Forest. Genet. 4: 1-13.
Motlagh S, Ravines P, Karamalla K, Qifeng M (2006). The analysis of *Acacia* gums using electrophoresis. Food Hydrocolloids. 20:848-854.
Nei M, Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc. Natl. Acad. Sci. USA, 76: 5269- 5273.
Pohlman CL, Nicotra AB, Murray BR (2005). Geographi range size, ecophysiology and phenotypic plasticity in Australian *Acacia* species. J. Biogeogr. 32: 341-351.
Raddad Y, Luukkanen O (2007). The influence of different *Acacia senegal* agro-forestry systems on soil water and crop yields in clay soils of the Blue Nile region. Sudan Agric. Water Manage. 87: 61-72.
Raddad Y, Luukkanen O, Salih A, Kaarakka V, Elfadl M (2006). Productivity and nutrient cycling in young *Acacia senegal* farming systems on vertisol in the Blue Nile region, Sudan. Agroforestry syst. 68: 193-207.
Rajagopal J, Bashyam L, Bhatia S, Khurana D, Srivastava P, Lakshmikumaran M (2000). Evaluation of genetic diversity in the Himalayan poplar using RAPD markers. Silvae Genetica, 49: 60-66.
Rohlf F (1993). NTSYS-pc. Numerical taxonomy and multivariate analysis system. version 1.18. New York: Exeter: Setauket.
Rouf Mian M, Hopkins A, Zwonizer J (2002). Determination of genetic diversity in tall fescue with AFLP markers. Crop Sci. 42: 944-50.
Verbeken D, Dierckx S, Dewettinck K (2003). Exudate gums: occurrence, production, and applications. Appl. Microbiol. Biotechnol. 63: 10-21.
Vos P, Hogers R, Bleeker M, Reijans V, Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP a new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407-14.
Wickenesswari R, Norwati M (1993). Genetic diversity of natural populations of *Acacia auriculiformis.* Aust. J. Bot. 4: 65-77.
Zhang H, Ozias-Akins P, Kochert G, Kresovich S, Dean R, Hanna W (1999). Differentiation of bermudagrass (*Cynodon* spp.) genotypes by AFLP analyses. Theor. Appl. Genet. 98: 895-902.