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Assessment of Genetic Variations in *Cordia africana* (Lam) in Sudan Using Random Amplified Polymorphic DNA Marker

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Abstract

Cordia (*Cordia africana*) is one of the important forest trees in Sudan. The study was carried out to assess the genetic diversity among four accessions of *C. africana* growing naturally in different geographical areas in Sudan using DNA molecular markers. The DNA was extracted from 250 mg of fresh leaf materials and subjected to PCR using 10 RAPD primers. The primers generated 45 polymorphic bands out of 64 total bands. Application of INTYS $_{PC21-}$ program in the cluster analysis, showed two diverse groups among the examined accessions. High similarity was observed between Ad-Damazin and Kas accessions (77%). Cordia species from Ad-Damazin, Kas and Zalingei populations formed a cluster group, whereas the species from Diling was diverged from them. The tested accessions of *C. africana* were found to have considerable genetic diversity. These results indicate that RAPD could be efficiently used for studying genetic diversity of wild plant species.

Keywords :*Cordia africana*, RAPD marker, Genetic variation, DNA.

Introduction

Cordia africana is a flowering tree of the family Boraginaceae (Nadja *et al*., 2002). The tree is native to tropical Africa. In Sudan, the tree is known as "gumbail" and widespread in Ad-Damazin, Darfur and Kordofan (Drummond, 1981). *C. africana* is fast-growing tree valued for food, firewood and in manufacturing of high quality furniture (Bekele-Tesemma *et al*., 1993). Conservation of plant genetic diversity has recently generated a lot of interest in the tropics as a result of many years of mismanagement; adverse environment as well as socio-economic changes (Young and Merriam, 1992). Genetic variation within and among populations can be investigated by employing biochemical markers (proteins and/or isozymes/allozymes), DNA molecular markers or direct DNA sequencing (Weising *et al.*, 2005). Until recently, research on the tropical trees was largely confined to allozyme studies of genetic structure of adults in continuous forests. The use of molecular markers in the investigation of genetic variation is getting a wide acceptance and broad application in fields such as phylogeny, taxonomy ecology, and genetics and breeding (Abayneh, 2007). RAPD data can detect genetic diversity between related species (Harvey and Botha, 1996) and within species (Van Oppen *et al*., 1996). It has also been used in

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the study of inter- and intra-specific variability among populations from the different or the same geographic regions (Pazoutova *et al*., 2000; Walker *et al*., 2001).

Maintenance of genetic diversity is considered crucial for long- term survival and evolutionary response of populations to changes in the environment (Hueneke, 1991). Recent development of molecular markers has complemented and drastically reduced the time taken in generating information required in making decisions for conservation and management. In the present study, the RAPD technique (Williams *et al*., 1990) was used to characterize genetic variability within populations of *C. africana* from different regions in Sudan.

Materials and Methods

Plant material and DNA extraction

Seed samples of 4 accessions of *C. africana* were collected from naturally growing individuals representing eco-geographical distribution namely, Zalingei, Kas, Dilling and Ad-Damazin (Fig.1). Seeds of each accession were grown in 30 cm diameter earthen pots in a greenhouse. Samples were collected from the young leaves of seedlings (200 mg), placed into coffee grinders, covered with dry ice, pulverized to form fine powder and then immediately transferred to the DNA extraction solution. Total genomic DNA was extracted using a modified cetyl trimethyl ammonium bromide (CTAB) procedure (Gillies *et al*., 1997). The amount and quality of the extracted DNA were checked on 1.5% agarose gel and stored at -20 $^{\circ}$C to be used later.

RAPD analysis

Genetic diversity within the populations of *C. africana* was assessed by RAPD analysis using 10 primers. For polymerase chain reaction (PCR), mixtures were prepared in 25µl volumes containing 2.5µl 10X *Taq* buffer, 1.5µl $MgCl_2$, 2.5µl dNTPs, 2µl random primer, 0.5µl *Taq* DNA polymerase and 1µl of the extracted DNA (10ng). The mixture was made up to 25µl by addition of sterilized distilled water.

RAPD/PCR (Bogani *et al*., 1994) reactions were initiated using an Applied Biometra Thermal Cycler programmed to repeat the thermal profile. Amplification conditions were based on three steps. Step one, was an initial denaturation 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 48°C for 1min, and ended by extension at 72°C for 2 min. Step three, was a final extension cycle that performed at 72°C for 5 min. The PCR machine was adjusted to hold the product at 4°C.The amplification products were mixed with gel loading dye. The amplified DNA fragments and the standard marker (λ Hind III digested DNA) were separated on 1.5% agarose gel. After staining with ethidium bromide gels were visualized under ultra violet light and then photographed.

Statistical analysis

For each primer, the number of polymorphic and monomorphic bands was determined. Bands clearly visible in at least one genotype were assigned score (1) for present, (0) for absence and entered into a data matrix. Fragment size was estimated by interpolation from the migration distance of marker fragments.

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Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The genetic dissimilarity matrix among genotypes was estimated. Coefficient of similarity trees were produced by clustering the similarity data with the un-weighted pair group method using statistical software program INTYS $_{PC21}$.

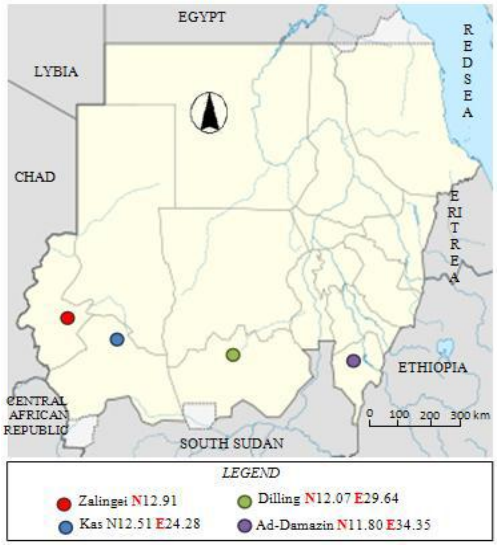

Fig. 1. Locations of the investigated *C. africana* in Sudan

Results and Discussion

The informative primers (10) were selected and used to evaluate the degree of polymorphism and genetic relationships between four accessions of *C. africana*. A total of 64 amplified fragments were distinguished across the selected primers and the statistical analysis. Table 1 shows 45 polymorphic bands among the four accessions, with an average of 4.5 polymorphic bands per primer. The maximum number of fragment bands (10) was produced by the primer OPA-10 with 90% polymorphism while the minimum numbers of fragments were produced by the primer OPA-03 and OPA-17 (4) with 25% and 75% polymorphism respectively. The amplification was repeated to confirm the reproducibility where only reproducible bands were considered for scoring and analysis. The pattern of the produced RAPD fragments is shown in Fig. 2.

RAPD analysis

Cluster analysis with INTYS pc21 using genetic distances was performed to generate a dendrogram (Fig.3) illustrating the overall genetic relationships within the four accessions. The dendrogram based on RAPD data divided into two groups. Group 1 includes accession collected from Diling and group 2 includes other accessions. In the present study, there was main group not related to soil type or rainfall regime as clustered. The group contained Ad-Damazin, Kas as

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sisters and Zalingei and showed genetic closeness. Diling came out of group. The high similarity 77% was between Ad-Damazin and Kas, and the low similarity 51% was between Ad - Damazin, Kas, Zalingei in one hand and Diling in other hand. Although the Eco-geographical factors between *Cordia* species, Ad - Damazin, Kas and Zalingei populations formed a cluster that excluded Diling. This may be due to effective gene flow among the three populations and limited gene flow with Diling or through foraging. This is expected since earlier morphological data showed that the Diling population varied from others in growth, germination and leaf margin (Hamza, 1990).

This is the first study to demonstrate splitting in *C. africana* accessions collected from different regions of Sudan. It may provide useful keys to select the species for further screening for bioactivities based on the RAPD profiles. However, more research in field experiments at plant stage is needed. It offers practical information for the future conservation of *Cordia* species and highlights some factors that may have influenced the partitioning of genetic diversity in this species across Sudan.

Table 1. Sequences of the primers used for RAPD and their polymorphic bands

Primer code numbers	*Primer sequence (5'-3')	Total number of band	Number of polymorphic band	Polymorphic band (%)
OPA-00	ATCAGCGCACCA	5	1	20
OPA-01	AGCAGCGCCTAC	6	3	50
OPA-02	GCCAGCTGTACG	8	7	87.5
OPA-03	TGCCTCGCACCA	4	1	25
OPA-04	GCCCCGTTAGCA	7	5	71.4
OPA-06	ACTGGCCGAGGG	7	7	100
OPA-10	GCCTGCCTCACG	10	9	90
OPA-12	CTCCTGCTGTTG	7	6	85.7
OPA-17	GGTTGGGAATG	4	3	75
OPA-19	AAGGCGCGAACG	6	3	50
Total		64	45	654.6
Average		6.4	4.5	65.5

*Primers from Operon Technology, Alameda, CA, USA.

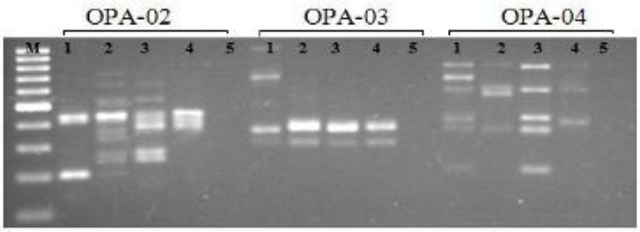

Fig.2. RAPD profiles of *Cordia* species from different sites of Sudan

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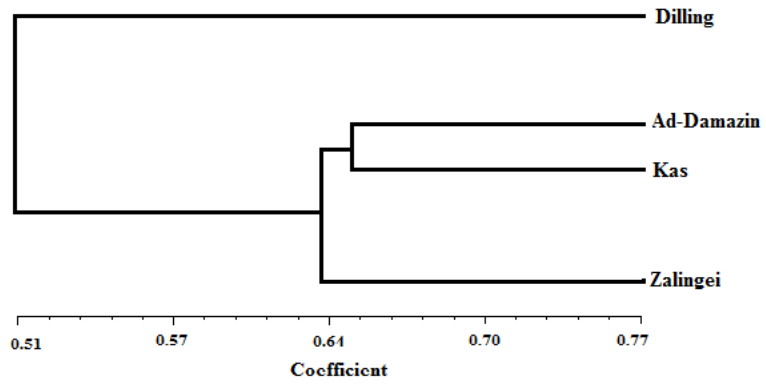

Fig.3. Cluster analysis derived from RAPD data, to estimate the genetic dissimilarity for 4 populations of *Cordia* from different regions of Sudan using 10 RAPD primers.

In Ethiopia Abayneh, (2007) carried out the genetic variation of 8 farmland populations *C. africana* in comparison with 4 continuous forests broadleaved tree species of *C. africana* Lam using AFLP technique. The findings reveal that the scattered trees on farmlands harbor substantial genetic diversity comparable to the continuous populations, and can be used as sources of genetic materials for tree planting, tree improvement and conservation activities in areas where the natural forest has been denuded. Also Abayneh *et al*., (2011) assessed genetic variation in a total of 22 populations of the tree species *C. africana* Lam., the analysis of the AFLP data revealed high diversity in all investigated populations. The observed patterns and levels of genetic variation within and among the populations indicated that efficient gene flow via pollen and seed is likely to be the main factor contributing to the maintenance of genetic diversity in natural and disturbed conditions.

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تقدير التباين الوراثى لأشجار القمبيل (*Cordia africana* Lam.) فى السودان بإستخدام واسمات التضخيم العشوائى لسلسلة تفاعل الحمض النووى

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مستخلص البحث

يعتبر القمبيل (*Cordia africana*) من أشجار الغابات المهمة بالسودان ونظراً لزيادة الإهتمام بحفظ وصيانة المصادر الوراثية الطبيعية فقد أُجريت هذه الدراسة للكشف عن التباينات الوراثية بإستخدام الحمض النووى (DNA) لأربعة أنماط محلية، جمعت من مواقع جغرافية مختلفة بالسودان. تم إستخلاص الحمض النووى من عينات بزنة 250 ملجم من الاوراق الخضراء للنباتات المدروسة ومن ثم أُخضعت لفحص التباين الوراثى بإستخدام تقنية التفاعل التضاعفي العشوائي لسلسلة الحمض النووى (RAPD) بإستعمال عشر من البادئات الجزيئية (primers). أظهرت النتائج وجود تباين فى 42 حزمة من أصل 64. كما أظهرت نتائج التحليل العنقودي بواسطة برامج INTYS PC21- لتحديد درجة القرابة. تفرقت الأصول المدروسة الى مجموعتين. حيث إجتمعت الاصول الواردة من الدمازين وكاس وزالنجى فى مجموعة عنقودية بينما إبتعدت عنها الأصول الواردة من الدلنج. وتبين أيضا أن هنالك نسبة عالية من التشابه الوراثى (77 %) بين الأصول الواردة من مناطق الدمازين وكاس. أثبتت الدراسة وجود درجة من التباين الوراثى بين أنماط القمبيل (cordia) الموجودة بالسودان. كما أشارت النتائج إلى مقدرة تقنية التضخيم العشوائى لسلسلة تفاعل الحمض النووي (RAPD) فى إظهار التباين الوراثي الجزيئي فى القمبيل.

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