

APPLICATION OF RAPD TECHNIQUE FOR THE CONSERVATION OF AN ISOLATED POPULATION OF *Capparis decidua*

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ABSTRACT

Capparis decidua is a rangeland plant species growing in isolated regions in Saudi Arabia. The population in Rawdhat Khuraim was noticed to suffer from lack of new regeneration, in addition to excessive grazing which may reduce the size of the population and reduce the genetic variability. RAPD markers were used to study genetic diversity in this population and a control population. Two criterion were used to study the genetic diversity in population in Rawdhat Khuraim. The first was cluster analysis and genetic distance, and the second was the percentage of polymorphic alleles. Cluster analysis showed that coefficient of similarity within Rawdhat Khuraim population (84-93%) is a lot larger than between Rawdhat Khuraim and the control population (77%). In addition, the percentage of polymorphic alleles in population of Rawdhat Khuraim was 45.8, which is within the range of other endangered plant species. This indicates that the population in Rawdhat Khuraim is isolated, suffering from narrow genetic base and of a particular conservation concern.

Keywords: RAPD markers, isolated population, *Capparis decidua*, ker, tandab, genetic diversity.

INTRODUCTION

Capparis decidua (known as ker or tandab) is a rangeland plant species growing in isolated populations in Saudi Arabia and other parts of the world. In Saudi Arabia, it is found in Riyadh as an isolated population in the northern part of Rawdhat Khuraim, nearly 100 km northeast of Riyadh city. The plant is under heavy browsing from goats and camels. It is feared that excessive grazing may lead to a decrease in population size and therefore, suffers from the genetic consequences of being an isolated small population. This plant has medicinal value. Young parts of the plants are applied to cure boils and swelling and the bark is said to be useful in asthma.

Genetic diversity in natural populations is being lost rapidly due to the process of climate change and deforestation. Both factors have contributed to reducing the size of natural populations, eliminated local populations, or fragmented earlier continuous populations into non-viable fragments. The preservation of genetic diversity both within and among natural populations is a fundamental goal of conservation biology (Keiper and McConchie, 2000).

Successful management and preservation of natural populations depend on accurate assessment of genetic diversity to address questions regarding genetic relationships among individuals as well as levels and structure of genetic variation. In particular, knowledge of population genetic structure provides a historical perspective of evolutionary changes that characterize a species and allow to predict how populations will respond to future events of natural and artificial origin (Wallace, 2002). Genetic diversity within a population is considered to be of great importance for possible adaptation to environmental changes and consequently for long term survival of a species (Hanski and Ovaskainen, 2000). In another word, the loss of genetic variation in a population leads to increasing number of homozygous individuals within a population which is associated with lack of individual fitness (Ellestrand and Elam, 1993). Thus the quantification of genetic variation is currently regarded as a primary goal in conservation efforts and accounts for the current utilization of genetic information in conservation. According to Haines (1994), technologies like: isoenzymes; Restriction Fragment Length

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Polymorphisms (RFLPs); Random Amplified Polymorphic DNAs (RAPD's); DNA cloning and, more recently, micro-satellites must be included in forestry research. In the last decade the use of molecular markers to study genetic diversity, and the systematics and the genetics of populations and species at the DNA and protein levels, has increased greatly.

In recent years, a series of techniques and genetic markers have been developed to analyze and estimate genetic diversity, but no single technique is universally ideal; each available technique has both strengths and weaknesses (Mueller and Wolfenbarger, 1999). Different methodologies using molecular markers are widely used to analyze the pattern of variation within and among natural populations of tree species. Among the various marker systems, RAPDs are one of the most popular DNA-based approaches (Martin and Hernandez Bermejo, 2000; Bekessy *et al.*, 2002). Further, RAPDs are the least technically demanding and offer a fast method for providing information from a large number of loci, particularly in species where no study has previously been undertaken. Moreover, the diversity assessed with RAPDs is comparable with that obtained with other techniques such as isoenzymes (Hamrick and Godt, 1990) or RFLP (Wu *et al.*, 1999). Advantages of RAPDs include suitability for work on anonymous genomes, applicability to work where limited DNA is available, efficiency and low expense (Hadrys *et al.*, 1992). This technique can be used to determine taxonomic identity, assess kinship relationships, detect interspecific gene flow, analyze hybrid speciation, and create specific probes. Also, RAPDs have applications in the identification of hybrid maize variety from the pattern of the corresponding parents (Abdel-Mawgood *et al.*, 2006). Some limitations exist, however, owing to their lack of reproducibility and the identical patterns produced by homozygous and heterozygous individuals.

The main aim of this study was to use RAPD markers to identify the genetic diversity present within an isolated population of *Capparis decidua* and to use this information for better management and conservation of this population.

MATERIALS AND METHODS

Plant Materials:

Samples for DNA extraction were collected from twelve plants from Rawdhat Khuraim. Two additional plants from another population in Madina (960 Km. west of Riyadh) were used as a control. Plant materials were juvenile parts of the plant or leaves, if present. Plant materials were washed several times with distilled water before subjecting to lyophilization. Lyophilized materials were ground to fine powder.

DNA Extraction:

DNA was extracted from dried plant material. Twenty milligrams of lyophilized powder was used for genomic DNA extraction using the DNeasy Plant Mini Kit (Qiagen Inc., Mississauga, ON, Canada) according to the manufacturer's directions. Extracted DNA was quantified by spectrophotometer followed by dilution to 25 ng μL^{-1} for RAPD analysis.

RAPD Technique:

Twelve different RAPD primers were used in this study. A list of these primers is presented in table 1. Amplification of PCR was carried out in a 25 μl reaction mixture with 25 ng of genomic DNA using RAPD-PCR beads (Amersham, USA) according to the manufacturer's direction. Optimal amplification conditions for RAPDs were one cycle of 5 min at 94°C (initial denaturation), followed by 45 cycles of 30 seconds at 94°C (denaturation), 1 min at 36°C (annealing) and 2 min at 72°C (extension). A final step of 10 min at 72°C ensured full extension of all amplified products. The RAPD bands were separated in 2.0 % agarose gel, stained in ethidium bromide and visualised by UV transillumination.

Data Analysis:

To examine the genetic relationship within the population, a dendrogram was constructed using a UPGMA analysis as implemented by NTSYS-pc, Version 2.02c (Rohlf, 1997). The PCR data generated from the fourteen individual plants were scored into 0 and 1. For each genotype, the presence of a band (1) or its absence (0) was scored.

Table 1. List of RAPD primers used in this study.

Primer	Nucleotide sequences
RAPD 1	5' CACACCGTGT '3
RAPD 2	5' GTCCTCGTGT '3
RAPD 3	5' ACGGTTCCAC '3
RAPD 4	5' GTCTTGGGCA '3
RAPD 5	5' GTCACCTGCT '3
RAPD 6	5' GGC GCGTTAG '3
RAPD 7	5' GACGAGCAGG '3
RAPD 8	5' GGCTGCCAGT '3
RAPD 9	5' TGGAGTCCCC '3
RAPD 10	5' CCCGTCTACC '3
RAPD 11	5' ACCGTCCGT '3
RAPD 12	5' TGACCAGGCA '3

RESULTS AND DISCUSSION

Understanding of the genetic structure of a species is essential for defining strategies and actions for its management in a sustainable use, the establishment and management of plantations, and genetic improvement and conservation *in* and *ex situ*. The present situation of the *C. decidua* population in Rawdhat Khuraim attracted our attention since no new individuals appeared in the last 15 years of working there. In addition, this population suffers from excessive grazing which may reduce the population size and consequently decreasing genetic variability in this population. To our knowledge, the first study that used molecular techniques to characterize the genetic diversity among a population of *Cappris decidua* was by Abdel-Mawgood *et al.*, 2005.

The 12 random primers generated a total of 39 RAPD polymorphic and 46 monomorphic loci ranging in size from 1670 to 280 bp. This set of loci is expected to give a good sampling of the total

genome and a good assessment of the genetic diversity. The number of bands per primer varied from 3 to 15. Figure 1 is a representative agarose gel for PCR products of the RAPD primer number 2. A dendrogram showing genetic interrelationship among individuals of Rawdhat Khuraim and between Rawdhat Khuraim and the control population is presented in Figure 2. The dendrogram shows that the 14 individuals can be divided into two groups, the Madina group (M1 and M2) and the Rawdhat Khuraim group (K1-K12). Genetic similarity between individuals of Rawdhat Khuraim was very high and the coefficient of similarity ranged from 84 to 93%. This indicated that individuals in Rawdhat Khuraim population are quite similar. Moreover, coefficient of similarity between Rawdhat Khuraim and the control population was 77% which is a lot lower than among individuals of Rawdhat Khuraim. Genetic similarity between Rawdhat Khuraim and the control population was used as an indication of how much genetic diversity is present in the population under study. This indicates that the population in Rawdhat

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Khuraim has very low genetic variation between its individuals. In another study, Fu *et al.*, (2003) used RAPD technique for the analysis of genetic diversity in *Changium smyrnioides*, an endangered plant, and the similarity coefficient of different populations of the same plant ranged from 0.22 to 0.89 with an average of 0.31.

Another criterion used to study the level of genetic diversity in Rawdhat Khuraim was the percentage of polymorphic loci. As indicated above, the 12 random primers generated a total of 73 RAPD polymorphic and 60 monomorphic loci. This means that the percentage of the polymorphic loci in the population under study is 54.87. It is useful to compare the level of genetic variation in *C. decidua* with other plants with a similar life history, geographic range, and breeding system using RAPD technique. For example the percentage of the polymorphic loci within the individuals in this study was 45.8 (39 RAPD polymorphic and 46 monomorphic loci). The percentage of polymorphic loci in other endangered plant species, e.g. *C. smyrnioides* was 69% (Fu *et al.*, 2003), *Lactoris fernandeziana* was 24.5% (Brauner *et al.*, 1992), *Paeonia suffruticosa* was 22.5% and *Paeonia rockii* was 27.6% (Pei *et al.*, 1995), and *Dacydium pierrei* was 33% (Su *et al.*, 1999). This indicates that *C. decidua* population in Rawdhat Khuraim may fall within the range of endangered plant species. Generally speaking, although *C. decidua* is an

endangered species, this population still has enough genetic diversity that should be able to fit environmental variation. However, the excessive grazing, and human activities that damage its habitat, may make the populations of these species decrease in size and their habitats be island-like in distribution.

Since the population of *C. decidua* in Rawdhat Khuraim is isolated, it has therefore no way of increasing its genetic diversity through immigration. In addition, field surveying showed that the population size was around 200 individuals only (data not shown). Ellstrand and Elam (1993), indicated that, in isolated populations, genetic drift may reduce genetic variation, increase levels of inbreeding and consequently, reduce the potential of a species to adapt to environmental changes. This indicates that the population in Rawdhat Khuraim may be of particular conservation concern as it is unlikely to recover from any stochastic extinction events that may occur.

The application of molecular biological techniques for studying genetic diversity between and among natural populations is an emerging application in conservation biology providing an appropriate focus for conservation management or monitoring (Newton *et al.*, 2002). Our results showed that the population at Rawdhat Khuraim may merit individual conservation attention. This population appears to be particularly distinctive and should perhaps be accorded highest priority for conservation.

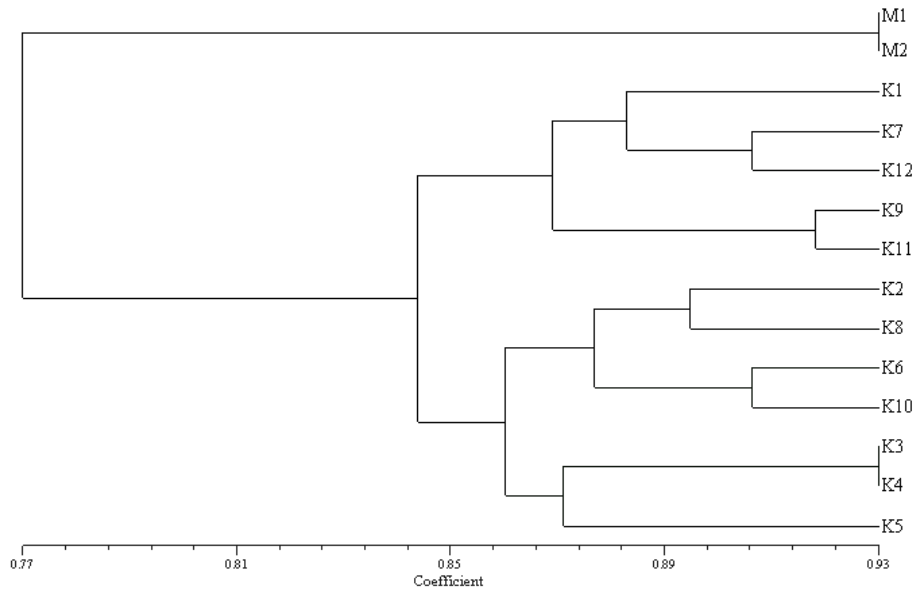


Figure 1. Cluster analysis showing the interrelationships between control population (M1 and M2) and Rawdhat Khuraim population (K1 to K12) of *Capparis decidua*.

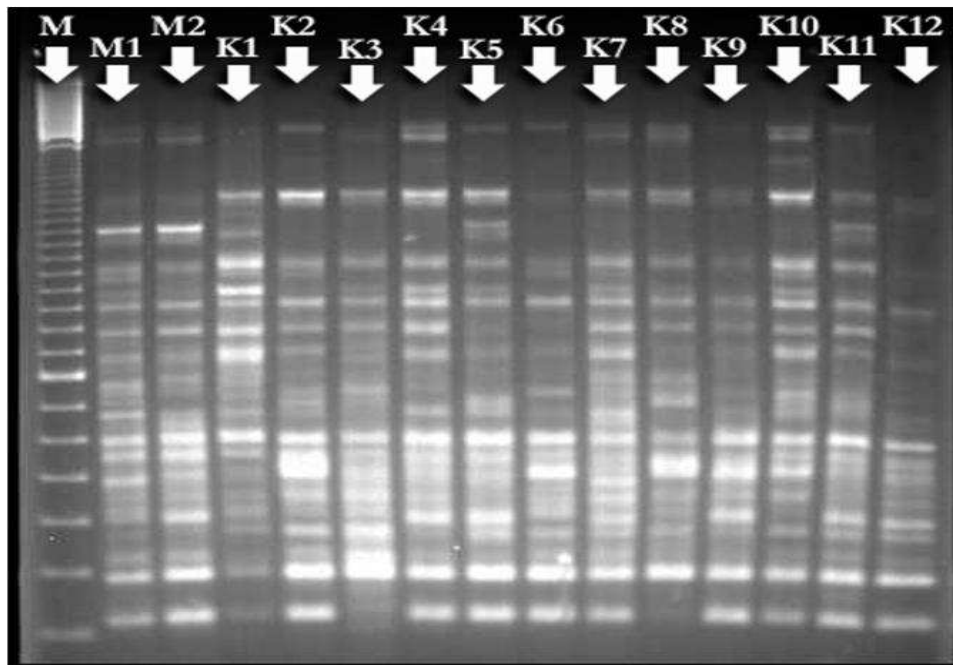


Figure 2. Amplified DNA fragments from 12 individuals from Rawdhat Khuraim (k1 to K12), and two individuals from the control population in Madina (M1 and M2). M is Molecular weight standards (100 bp ladder) are shown in the far left lane.

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الملخص العربي

دراسة التباين الوراثي في عشيرة معزولة من نبات التنضب

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يعد التنضب *Capparis decidua* أحد نباتات المراعي التي تنمو في مناطق معزولة في المملكة العربية السعودية. وقد لوحظ أن عشيرة التنضب في روضة خريم قرب مدينة الرياض تفتقر إلى التجديد في أفرادها، إضافة إلى تعرضها للرعي الشديد مما يهدد بتقلص حجم العشيرة وانخفاض التنوع الوراثي فيها. استخدمت طريقة RAPD لدراسة التباين الوراثي في عشيرة التنضب في روضة خريم ومقارنتها بعينة من عشيرة أخرى للاستفادة من النتائج في إدارة هذه العشيرة والمحافظة عليها. وقد اتبع في هذه الدراسة أسلوبان: الأول التحليل العنقودي وقياس القرابة الوراثية، والثاني قياس نسبة الأليلات متعددة المظهر polymorphic alleles. أظهرت نتائج التحليل العنقودي أن معامل التماثل بين أفراد عشيرة روضة خريم (٨٤-٩٣%) كان أعلى من مثيله بين عشيرة روضة خريم ككل وبين عشيرة المقارنة (٧٧%). وإضافة إلى ذلك، بلغت نسبة الأليلات متعددة المظهر ٤٥,٨ وهذه النسبة تعد في حدود النسب المعروفة للأنواع النباتية الأخرى المهتدة بالانقراض. يستنتج من هذه الدراسة أن عشيرة التنضب في روضة خريم تعاني من نقص في تنوعها الوراثي وبحاجة إلى المحافظة عليها.