American Journal of Plant Sciences, **2011, 2, 527-534**
doi:10.4236/ajps.2011.24062 Published Online October 2011 (http://www.SciRP.org/journal/ajps)

DNA Finger Printing of *Prosopis cineraria* and *Prosopis juliflora* Using ISSR and RAPD Techniques

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Received July 17th, 2011; revised August 16th, 2011; accepted September 5th, 2011.

ABSTRACT

Diversity within and among the populations of Prosopis cineraria and Prosopis juliflora collected from different location of Qatar were explored using Inter Simple Sequence Repeat (*ISSR*) *and Random Amplified Polymorphic DNA* (*RAPD*) *markers. A total of one hundred and nine bands were generated from twenty nine ISSR and nineteen bands from seven RAPD primers with an average polymorphism of more than ninety nine percent across all the genotypes. ISSR techniques were capable of distinguishing between P. cineraria and P. juliflora, through twenty one bands. However, of the seven RAPD markers, only three bands were able to distinguish between Prosopis species. The dendrograms for the analysis of genetic similarity show that the individuals from both species can be separated in two highly related groups. Our observations suggest that genetic variations among different accessions of Prosopis are identified using ISSR and RAPD analysis.*

Keywords*: *Ghaf, Ghawiaf, Polymorphism, Dendrogram and Genetic Diversity

1. Introduction

The genus *Prosopis* is one of the commercially important genera of legumes in arid climatic zones with multifarious benefits for mankind. *Prosopis* species are among the most important multipurpose leguminous trees, and are used for revegetation, agroforestry, apiculture, fodder timber, fuel, shade, firewood as well as affecting soil improvement and sand dune stabilization [1,2]. The genus *Prosopis* includes about 44 species that have been described using morphological criteria and these are, in turn, grouped into five sections. Further, these five sections include eight series. However, this classification does not seem to be rigid [3,4]. At the morphological level, leaves and flowers are more or less similar while in case of fruit there is a development from straight to curved and spirally coiled loments. There is also a marked vegetative diversification in the presence or absence of spines, which also provides the foundation for a sectional subdivision of the genus [3]. Similarly, variation of characters such as seed lipids [5], and mineral, crude protein and structural carbohydrate [6], have also been studied for distinguishing species, at least within a section and series.

The accelerated and uncontrolled harvesting of this important natural resource has led to land degradation and desertification, as well as the loss of genetic diversity within and across the *Prosopis* population. Due to multifaceted utility of the species, the pressure for it and its diverse products is mounting. There is a vital need for thorough exploration and exploitation of all the available natural variation in *Prosopis* species so as to manage the available meager resources for sustainable utilization and to establish appropriate reforestation programs for the optimal exploitation of these natural resources [2,7].

Most of the approaches used to determine genetic diversity in this genus have involved isozyme analysis. However, this method has methodological limitations because of restrictions in the number of loci examined and the possible tissue, developmental stage, or environmental specificity of gene expression [8,9]. Yet there were some concerted attempts to record natural genetic variation in various populations of *P. cineraria* using cytogenetical [10] and molecular markers [2]. Other than the above reports of interspecies variations, not much is known about intraspecific genetic variability in *Prosopis* species, especially at the molecular level. Molecular

techniques have been found to be more useful and accurate for determination of both interspecies and intraspecies genetic variation in plants. Sharma *et al*., [2] have suggested that ISSR (Inter Simple Sequence Repeat) markers are the best suited DNA markers for genetic variation analysis in tree species like *P. cineraria*.

ISSR-PCR is a technique, which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multilocus markers. ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology [11]. ISSR is a technique offering the simplicity of RAPDs with an increased level of reliability. It involves PCR amplification of regions between adjacent, inversely oriented microsatellites using a single simple sequence repeat primer. The use of the tandem repeat motifs of di-, tri-, or tetra nucleotides that are abundant in all eukaryotic genomes produces a high number of polymorphic fragments, especially in plants [12]. Since it is simple, fast, cost-effective, highly discriminant and highly reliable, in recent studies, it is widely applied in plant genetic analyses [11, 13-16].

During the last decade, molecular markers such as Random Amplified Polymorphic DNA (RAPD) have been widely used to detect polymorphism [17], analyze phylogenetic relations [18,19], identify cultivars [20,21], discriminate between wild and cultivated species [21,22], and detect agronomic traits [23,24]. The RAPD markers in particular, have been successfully used to determine intraspecies genetic diversity in tropical and semitropical forest plants. These include *Cedrus* [25] and *Pinus* [26, 27]. In contrast, fewer reports are available on the distribution of forest trees in arid and semiarid regions. In the *Prosopis* genus, recent RAPD studies have shown differences in allele frequencies among species of section *Algarobia* and it has identified species from South America, Africa and Asia [9,28,29]. These markers have been used to differentiate accession levels of the *Prosopis* genus [30]. Randomly amplified polymorphic DNA (RAPD) markers, in particular, have been successfully employed for determination of intra-species genetic diversity in several plants. These include date palm [31], papaya [32], poplars [33] and amaranths [34]. The RAPD technique was also used for detecting inter- and intraspecies variation amongst *Prosopis* accessions and species [35]. The present paper describes the RAPD and ISSR profile variation between *P. cineraria* and *P. juliflora* as well as variation within species of *Prosopis* and determination of the relative heterogeneity of the species.

2. Materials and Methods

Twelve samples, including six *P. cineraria* (Arabic name: Ghaf) and six *P. juliflora* (Arabic name: Ghawiaf) were chosen randomly from different locations in Qatar. The frozen young leaf tissues of *Prosopis* were first cleaned carefully with distilled water. Then, one gram of leaf sample was cut into small pieces and ground into fine powder using liquid nitrogen. The DNeasy Plant Maxi kit protocol (QIAGEN) was used to extract DNA by following the manual instructions of the kit (DNeasy Plant Handbook). The obtained DNA was quantified and quailfied using a Nanodrop Spectrophotometer. For further estimation of the DNA quantity 2 µl was loaded on 0.85% agarose gel at 100 Volts for thirty min. The gels mixture were stained with ethidium bromide and visualized under UV light.

Thirty ISSR and fifteen RAPD primers were custom synthesized from Integrated DNA Technologies (IDT), Inc. USA. These primers were screened using polymerase chain reaction (PCR) in a total reaction mixture of 20 µl containing 2 µl (20 - 30 ng) of total genomic DNA, 10µl of AmpliTaq Gold 360 Mastermix (Applied Biosystems), 1 µl (5 pmol/µl) of primers each and 7 µl of nuclease free water. Amplification was carried out in a Veriti 96 Well Fast Thermal cycler (made by Applied Biosystems) under the following conditions: initial denaturation 95˚C for 10 min, 35 cycles (denaturation 95˚C for 30 s, annealing temperature depending on primer for 30 s, extension 72˚C for one min), final extension 72˚C for 10 min.

The amplified DNA fragments, 5 µl and 2 µl of loading dye (making a total volume of 7 µl), were loaded on to the gel using the 1.5% agarose at 30 Volts for 180 min. in 1X TAE buffer (30 mM), the gels were stained in ethidium bromide and visualized on a UV transilluminator and documented using gel documentation system AlphImager EC by Alpha View software V.3.0.0.0.

ISSR and RAPD bands were precisely measured by the gel documentation system software and scored for each genotype. Each reproducible polymorphic DNA band at a particular position on the gel was treated as a separate character and scored as present (1) or absent (0) to generate a binary data matrix. The data was then computed with the PowerMarker software V 3.0 [36] to detect the major allele frequency, number of alleles, gene diversity and polymorphism information content (PIC) value (which is commonly used in genetics as a measure of polymorphism) for a marker locus used in linkage. The phylogenetic relationship among the genotypes was drawn by Past software V 1.91 [37] on the basis of Hamming similarity index with 100 bootstraps.

3. Results and Discussion

Among thirty ISSR and fifteen RAPD primers tested for their ability to generate bands patterns in *Prosopis* geno-

types, twenty nine ISSR and seven RAPD primers successfully produced clear bands in most of the studied genotypes. So far, one ISSR primer namely BT26 (GGAT)4, and eight RAPD primers (A12, D10, OPBO4, OPA12, OPA19, OPC-06, OPC-10 and OPC-15) did not amplify clear bands in our genetic materials, even when different PCR conditions were used.

The twenty nine ISSR primers used in this study provided a total of 109 bands, 108 of which were polymorphic (99% polymorphism). The percentage of polymorphism produced by each primer differed from one primer to another. The maximum value of polymorphism was 100%, produced by all primers except BT25, which produced the minimum value of polymorphism, 50%. There was an average polymorphism of 99.1% across all the genotypes.

A total number of 109 amplified DNA bands were generated across the studied genotypes with average of 3.76 bands per primer. The ISSR profiles of the amplified products of each primer are shown in (**Table 1**). A maximum number of eight bands were amplified with primer BT10 and a minimum of one band with primer BT3. The number of monomorphic bands was primer dependent and only one of these bands was generated by primer BT25. The Hamming genetic similarity coefficient, recognized the two studied *Prosopis* species (**Figure 1**).

Table 1. Code and sequence of the thirty DNA ISSR primers used for identifying the *Prosopis* species and types of the amplified DNA bands.

ISSR		No. of bands			
Primers	**Sequence**	**Total**	**Polymorphic**	**Monomorphic**	**% Polymorphic**
BT1	(AC) 8T	6	6	0	100
BT2	(ACC) 6	3	3	0	100
BT3	(ACTG) 4	1	1	0	100
BT4	(AG) 10C	3	3	0	100
BT5	(AG) 10T	7	7	0	100
BT6	(AG) 8T	5	5	0	100
BT7	(AGG) 6	6	6	0	100
BT8	(ATG) 6	4	4	0	100
BT9	(CA) 6AC	7	7	0	100
BT10	(CA) 6GG	8	8	0	100
BT11	(CA) 6GT	3	3	0	100
BT12	(CA) 8A	4	4	0	100
BT13	(CAC) 3GC	2	2	0	100
BT14	(CT) 10A	4	4	0	100
BT15	(CT) 10G	4	4	0	100
BT16	(CT) 10T	4	4	0	100
BT17	(CT) 8AC	2	2	0	100
BT18	(CT) 8GC	3	3	0	100
BT19	(CT) 8TG	2	2	0	100
BT20	(CTC) 3GC	3	3	0	100
BT21	(GA) 6CC	3	3	0	100
BT22	(GA) 6GG	4	4	0	100
BT23	(GA) 8C	3	3	0	100
BT24	(GACA) 4	3	3	0	100
BT25	(GAG) 3GC	2	1	1	50
BT27	(GT) 6CC	2	2	0	100
BT28	(GT) 6GG	5	5	0	100
BT29	(GTG) 3GC	2	2	0	100
BT30	(TC) 10C	4	4	0	100
Total		**109**	**108**	**1**	**99**
Average		**3.76**			

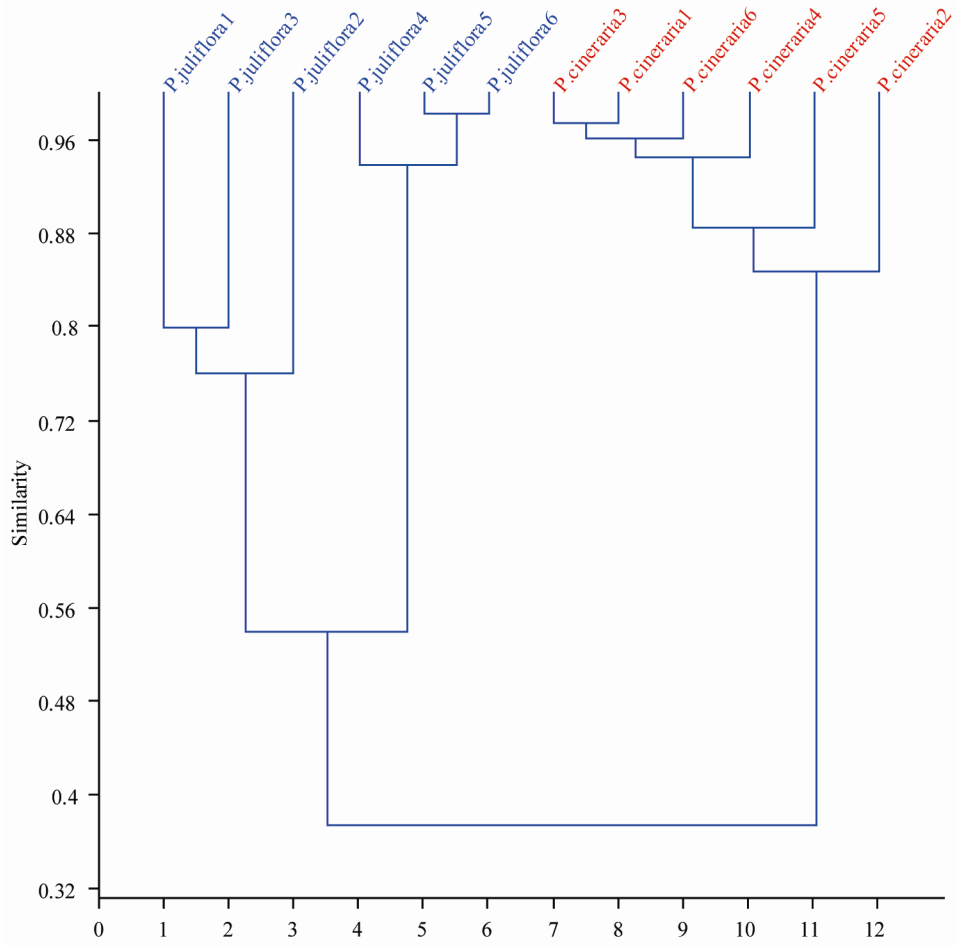

Figure 1. Dendrogram of Qatari *Prosopis* species based on ISSR technique according to hamming similarity coefficient.

The highest similarity value was 0.02 which was recorded between *P. juliflora* 5 and *P. juliflora* 6. The next highest similarity value was 0.03 between *P. cineraria* 1 and *P. cineraria* 3 and between *P. cineraria* 3 and *P. cineraria* 6. The lowest similarity value was 0.82 between *P. cineraria* 1 and *P. juliflora* 5 and 6, *P. cineraria* 3 and *P. juliflora* 5 and 6 and between *P. cineraria* 6 and *P. juliflora* 5 and 6. The next lowest similarity value was 0.81 between *P. cineraria* 4 and *P. juliflora* 5 and 6.

Similarity coefficient matrices were used to generate a dendrogram of *Prosopis* genotypes based on Hamming analysis (**Figure 1**). The analysis divided the twelve genotypes into two distinct clusters. The first cluster included *P. cineraria*, while the second cluster contained *P. juliflora* genotypes.

The identification and characterization of species become possible through fingerprinting of each species since DNA is a source of informative polymorphism [38]. Based on twenty nine ISSR markers twenty one bands are capable of to distinguishing between *P. cineraria* and *P. juliflora*. Those bands were 2, 1, 1, 6, 2, 2, 1, 1, 1, 1, 2 and 1 represented in BT1, BT4, BT5, BT9, BT10, BT11, BT12, BT14, BT15, BT18, BT23 and BT30 respectively, whereas of the seven RAPD markers, only three bands are distinguishable between *P. cineraria* and *P. juliflora*. However those bands were only one band each in DO5, OPC02 and D20. The distinguishable bands in all species appeared in *P. cineraria* but not in *P. juliflora*.

Consequently, techniques of molecular genetic markers have an important potential for the detection of genetic differences among species [39].

At the same time, it is necessary to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation and collection of germplasm, and to increase the utilization of plant genetic resources.

A detailed account of molecular phylogeny and the diversification history of thirty species of *Prosopis* were previously done by Catalano *et al.* [40]. It suggested that *Prosopis* is not a natural group, but one that adopted an ancient occupation of arid environments. This study indicated that old world species of *Prosopis* are not closely

related with the new world (American) species. The present investigation of *P. cineraria* revealed that there is a greater genetic variation of 88.2% within the populations, whereas the variance among populations was only 11.8% only. This is in support of observations by Sharma *et al.* (2010) [2], who reported high natural genetic variation at the DNA level in thirty accessions of *P. cineraria* collected from different districts of Rajasthan.

During recent years, different studies have supported the hypothesis that dry-adapted taxa in different regions of the world diverged concomitantly with the expansion of arid environments. That is the view of Phylica [41], Ruchoideae [42], Tiquillia [43] and Agave [44] in North America, and Rheum [45] in East Asia.

Genetic differentiation depends on the scale of environmental heterogeneity and the balance of selection and higher gene flow especially amongst trees [46]. This fact supports our study that *Prosopis* is not a natural group, but a species that in ancient times, adopted and occupied arid environments in which its populations were adaptable, and where they had the highest level of fitness The study of sequence variation also provides means to detect loci responsible for local adaptation [47]. When populations become locally adapted to contrasting environments, alleles entering a new population may be eliminated by selection, so that the gene flow is partly restricted. The stronger the selection, the more rapidly immigrant alleles of lower fitness will be eliminated from the population thereby reducing effective migration rates and increasing the time to coalescence [48].

The seven RAPD primers used in this study provided a total of nineteen bands; all of them were polymorphic (100% polymorphism). The total number of nineteen amplified DNA bands was generated across the studied genotypes with an average of 2.7 bands per primer. The RAPD profiles of the amplified products of each primer are shown in **Table 2**. A maximum number of five bands were amplified with primer OPC11 and a minimum of two bands were amplified with primers A13, D20, A10 and D07.

Using the Hamming genetic similarity coefficient, recognized the *Prosopis* species, the highest similarity value was 0.0, which is identically recorded between (*P. cineraria* 1 and *P. cineraria* 4) and (*P. juliflora* 4, 5 and 6 were identical as will), the next highest similarity value was 0.05 between (*P. cineraria* 1 and *P. cineraria* 3) which was the same result with ISSR markers and (*P. cineraria* 3 and *P. cineraria* 4) while the lowest similarity value was 0.95 between (*P. cineraria* 1 and *P. juliflora* 4, 5 and 6), (*P. cineraria* 4 and *P. juliflora* 4, 5 *and 6*), the next lowest similarity value was 0.89 between (*P. cineraria* 3 and *P. juliflora* 4, 5 and 6).

Similarity coefficient matrices were used to generate a dendrogram of *Prosopis* genotypes based on Hamming analysis (**Figure 2**), the analysis divided the twelve genotypes into two distinct clusters, the first cluster includes *P. cineraria*, while the second cluster contains *P. juliflora* genotypes.

The molecular markers obtained by the ISSR technique revealed a remarkable molecular discrimination among *Prosopis* species more than RAPD technique. The phylogenetic analysis on the basis of ISSR (**Figure 1**) discriminate among *P jouliflora* 4, 5 and 6, while RAPD markers used (**Figure 2**) cannot revealed almost the same pattern. Also ISSR technique (**Figure 1**) discriminate among *P cineraria* 1 and 4, while RAPD markers (**Figure 2**) did not. However, Ajibade *et al.* [49] and Galvan *et al*. [15] concluded that ISSR would be a better tool than RAPD for phylogenetic studies. Nagaoka and Ogihara [50] have also reported that the ISSR primers produced several times more information than RAPD markers in wheat.

Table 2. Code and sequence of the seven DNA RAPD primers used for identifying the Prosopis species and types of the amplified DNA bands.

RPAD		No. of bands			
Primers	**Sequence**	**Total**	**Polymorphic**	**Monomorphic**	**% Polymorphic**
DO5*	TGAGCGGACA	3	3	0	100%
OPC11	AAAGCTGCGG	5	5	0	100%
OPC02*	GTGAGGCGTC	3	3	0	100%
A13	CAGCACCCAC	2	2	0	100%
D20*	ACCCGGTCAC	2	2	0	100%
A10	GTGATCGCAG	2	2	0	100%
DO7	TTGGCACGGG	2	2	0	100%
Total		19	19	0	
Average		2.714			100%

*Band distinguish between species.

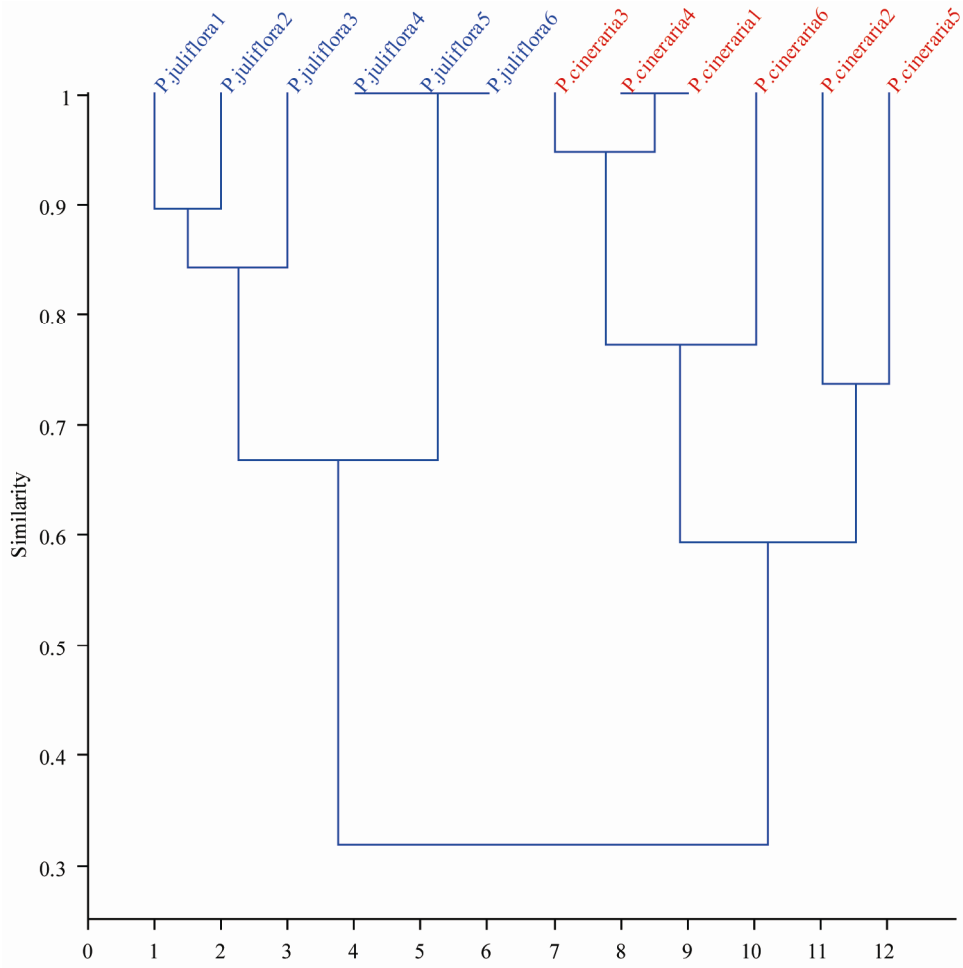

Figure 2. Dendrogram of Qatari *Prosopis* species based on RAPD technique according to hamming similarity coefficient.

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