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ORIGINAL ARTICLE

Genetic Variation among Sumac *(Rhus Coriaria* L.) Samples Collected from Three Locations in Jordan as Revealed by Aflp Markers

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ABSTRACT

Sumac (*Rhus coriaria*) is a medicinal plant grown in upper elevations and distributed between Ajlun, Jerash and Irbid areas of Jordan. Amplified Fragment Length Polymorphism (AFLP) analysis was used to study the genetic relationship among thirty samples of sumac (*Rhus coriaria* L.) trees collected from the Northern regions in Jordan. Three of seven primer pair combinations, produced 42 polymorphic bands. The UPGMA method was employed to construct a dendrogram based on the Jaccard's similarity coefficient. A high similarity index (1.00) was showed between two samples of Jerash, while the lowest similarity (0.12) was registered between the Irbid samples. A dendrogram showed four groups, with the Jerash samples more closely related compared to the rest of the samples. The Ajloun and Irbid samples formed one group. AFLP is considered a powerful tool for assessing the genetic relatedness among either known or unknown samples, species and individuals.

Key words: AFLP, sumac, *Rhus*, Jordan and polymorphism.

Introduction

Rhus coriaria commonly called sumac or sumach, is a perennial plant that belongs to the anacardiaceae family, one of approximately 250 species of the Rhus genus. This species is widely distributed in uplands with elevation (1200m a.s.l) mainly in Ajloun, Irbid and Jerash areas in Jordan. It is characterized by deciduous spirally arranged leaves, pinnately compound, densely panicle or spike flowers with red color and dense clusters of reddish drupes fruits. It is propagated by new sprouts from rhizomes or seeds (Sumac. http:// en.wikipedia.org/ wiki/ sumac).

Rhus coriaria species was planted at the roadsides, ornamental and forest protected areas to conserve the soil from erosion. Moreover, it was used in folk medicine to alleviate stomach problems, diarrhea, bleeding and skin problems[12].

In Turkey and other Arabic countries, the sumac berry was used as a cooking spice (http:// unitproj.library.ucla.edu/bioned/spice/index.cfm); in

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addition it is considered an important ingredient of thyme (Zatter) and sesame mix with olive oil which is desired by many people with breakfast dishes. Özcan and Haciseferogullari[12] reported that sumac is a very popular condiment used as a major souring agent, mixed with freshly cut onions and considered as appetizer. Jordanian people collected the fruits and flowers and processed them to become edible and then marketed to improve their income.

However, the water extract of sumac *Rhus coriaria* has a positive effect on the positive gram bacteria[9,10], also its extract was shown to inhibit the formation of hydroperoxide in natural peanut oil stored at 65ºC for 35 days[2].

Genetic diversity has been studied based on the morphological, physiological and chemical analysis. Goulão *et al.* [6], reported that the traditional methods for characterization and assessment of genetic variability based on morphological, physiological and agronomic traits are often not adequate.

However, recently molecular analysis solved the problem in a few hours compared to months or years. Many molecular markers techniques such as Amplified Fragment Length Polymorphism AFLP, were used to distinguish between individuals, species, accessions and varieties. AFLP markers have been developed by Vos *et al*.,[16] and widely spread due to its reliability and robustness. This technique based on four steps restriction/ ligation, pre-selective, selective and gel electrophoresis.

The use of molecular markers is speeding up plant breeding and clarifying, confirming or even reformulating the systematic taxonomy of several groups of organisms[15]. AFLP markers complement the traditional morphological and phonological descriptors used for the registration of new cultivars. They also contribute to the protection of intellectual property and allow the certification of clonally propagated varieties[4]. The AFLP method[16] has been widely employed in research of plants, fungi and bacteria[1]. The AFLP technique have been used to study the genetic relationships among Mediterranean pistacia species[5], apple cultivars[6], *Miscanthus*[7], *Brassica nigra* accessions[11] and European Rubus[14]. In addition, intraspecific diversity reported in woody plants using AFLP markers is very high such as in olive[13].

The aim of this study was to examine the genetic relationship among sumac samples that were collected from different sites in Jordan based on AFLP markers.

Materials and methods

Plant material

Leaves were collected randomly from 10 sumac trees from Jordan namely, Irbid, Ajloun and Jerash (Table 1).

DNA isolation

Total cellular DNA was extracted using a modified CTAB isolation protocol. Approximately (30 mg) fresh leaves was ground in liquid nitrogen and mixed with 750 µl of fresh and preheated 2x CTAB solution with 0.8g PVPP in 2ml tubes then placed at 65ºC for 30 min. The mixture was mixed with 750 µl of chlorophorm/ isoamyl alcohol (24:1) then vortexed a few seconds, then centrifuged at 14000g for 20 min. The upper phase (supernatant) was placed in 2ml tubes with 600ml isopropanol and the tubes were then shaked until the thread of DNA appeared before they were centrifuged for 20 min at 14000g. The solution was poured off and the tubes left to dry before adding 600 µl of cold 70% ethanol and then placed overnight at –20ºC. The ethanol was then poured off and the tubes dried. 100µl of TE was added and placed at 65ºC for 30min. Four micolitter of RNAse (10mg/ml) were added per tube and left for 45min at 37ºC. DNA quantitation was performed using a S2100 UV/VIS DIODE-Array-Spectrophotometer, Version 1.7.

AFLP procedure

The AFLP procedure was performed as previously described by Vose *et al*.[16]. Sumac DNA (500ng) was double digested with *EcoRI* and *Tru91* (an isochizomer of *MseI*) and double stranded adaptors were ligated to the ends of DNA fragments, generating template DNA for subsequent PCR amplification (pre-amplification followed by selective). Restriction and ligation reactions were carried out simultaneously in a single reaction tube[16].

To carry out the reaction, an enzyme master mix for 35 reactions was prepared containing 2µl *EcoRI* buffer, 2µl *Tru91* buffer, 0.25µl *EcoRI* enzyme (12u/µl), 0.25µl *Tru91* enzyme (12u/µl), 0.625µl 1mg/ml BSA, 9.875µl ddH_2O and 5µl genomic DNA (500ng), the mix was centrifuged for a few seconds then stored at 37ºC for 3 hr. The restriction-ligation reactions consisted of 0.5µl 10x T4 buffer, 0.2µl T4 DNA ligase (3u/µl), 0.5µl *Tru91* adaptor forward, 0.5µl *Tru91* adaptor reverse, 0.5µl *EcoRI* adaptor forward, 0.5µl *EcoRI* adaptor reverse and 2.3µl ddH_2O. Then distributed 5µl for the restriction tubes, then stored at 37ºC for 3hr. The restriction-ligation reactions were diluted 1:4, then used in pre-amplification step. The *Tru91* complementary primer had a 3'-C and the *EcoRI* complementary primer had a 3'-A.

The pre-amplification (preselective) mix was prepared by adding 2µl of 5-fold diluted DNA from the restriction-ligation reaction, 2.5µl 10xbuffer (PCR), 2.5µl dNTPs (5mM), 1µl AFLP preselective primer (*EcoRI*+ A-3'), 1µl AFLP preselective primer (*Tru91*+ C-3'), 0.2µl *Taq* polymerase (5u/µl) and 15.8µl ddH_2O. The pre-amplification (preselective)

Table 1: Oligonucleotides adaptors and primer combinations used for AFLP analysis.

Name	Sequence
EcoRI adaptor	5'- CTCGTAGACTGCGTACC-3'
	3'- AATTGGTACGCAGTC-5'
Tru91 adaptor	5'-GACGATAGTCCTGAG-3'
	3'-TACTCAGGACTCAT-5'
Primers used in pre-amplification	
EcoRI+1-A	5'-GACTGCGTACCAATTCA-3'
Tru91+1-C	5'-GATGAGTCCTGAGTAAC-3'
Primers used in selective amplification	
EcoRI+3-ACA	*5'-GACTGCGTACCAATTC+ACA-3'*
EcoRI+3-AAG	*5'-GACTGCGTACCAATTC+AAG-3'*
EcoRI+3-AAC	*5'-GACTGCGTACCAATTC+ATA-3'*
EcoRI+3-ATA	*5'-GACTGCGTACCAATTC+ATA-3'*
EcoRI+3-AAT	*5'-GACTGCGTACCAATTC+AAT-3'*
MseI (an isochizomer of Tru91)	
MseI+3-CAT	5'-GATGAGTCCTGAGTAAC+CAT-3'
MseI+3-CAG	5'-GATGAGTCCTGAGTAAC+CAG-3'
MseI+3-CAA	5'-GATGAGTCCTGAGTAAC+CAA-3'
Primer pair combinations (*EcoRI/ MseI).*	
1-ACA/CAT	
2-AAG/CAG	
3-AAG/CAT	
4-AAC/CAT	
5-AAC/CAG	
6-ATA/CAA	
7-AAT/CAT	

amplification was carried out in a thermal cycler programmed at 72ºC for 2min followed by 20 cycles of 94ºC for 30 sec, 56ºC for 40 sec and 72ºC for 50 sec and finally incubated at 4ºC. The pre-amplification DNA was diluted 5-fold with ddH_2O and selective amplifications were carried out by using different *EcoRI* and *Tru91* primer combinations (Alpha DNA).

Primers selected for the selection amplification were from available AFLP selective primers that were purchased and stored at –20ºC at NCARE lab. The *EcoRI* primers contained three selective nucleotides with sequence (Table 1) while the *Tru91* primers had the selective nucleotides starting with G (Table 1).

For the selective amplification, the reactions were set up as follows: 2µl of 5-fold diluted pre-selective amplification reaction product, 1.0µl *Tru91* primers, 1.0µl *EcoRI* primers, 2µl of 10 x buffer, 2µl dNTPs (0.25mM), 0.2µl*Taq* polymerase (5u/µl), 0.25µl of $MgCl_2$ (15mM) and 11.55ddH_2O.

Selective amplification was carried out in a thermal cycler programmed at 94ºC for 2min, followed by 13 cycles of 94ºC for 20 sec, 68ºC for 30 sec and 72ºC for 1min, and 23 cycles of 94ºC for 30 sec, 59ºC for 30 sec and 72ºC for 1min and a final incubation at 4ºC. The selective amplification reaction product (6µl) was mixed with 4ul of loading buffer (98% deionized foramide, 10mM EDTA (pH: 8), 0.05% bromofenol, 0.05% xylene cyanol), from which 6µl was finally loaded onto a 6% polyacrylamide gel run on a vertical gel sequencing apparatus (Cleaver, Scientific, Ltd.).

Nine primer combinations (*EcoRI- / Tru91-*) were used in analysis (Table 1), thirty samples from sumac trees were subjected to the selective amplification with these primer combinations, and treated under the same conditions. To determine the size of the AFLP fragments, we used an AFLP DNA marker (50bp step ladder) (Promega), ranging in length from 50 to 800bp.

Data analysis

AFLP polymorphic bands were scored as present (1) or absent (0) and estimates of similarity among all tested samples were calculated according to Ne and Li, (1979). The matrix of similarity was analyzed by the Unweighted Pair-Group Method (UPGMA) and the dendrogram was obtained by using SPSS program, version 10.

Results and Discussion

Out of nine primer pair combinations that were tested only three pair combinations ATA/CAA, AAG/CAG and AAT/CAT showed amplified fragments (Table 2). A high number of amplified fragments 25 and 17 were detected by the combinations ATA/CAA and AAG/CAG, respectively (Table 3). The primer pair combination AAT/CAT showed the lowest number of bands. The similarity ranged from 0.12 % to 1.00 % (Table 2). A high similarity index (1.00) was showed among the samples that were collected from Jerash governorate. These samples indicate that these sumac trees probably came from the same source of seeds and nursery of Jerash forest, which propagated them and then distributed to the farmers in the same region, but the lowest similarity (0.12 %) registered between samples of Irbid and Irbid-Ajloun (Table 2).

However, the AFLP analysis show that their is a genetic variation among the sumac trees studied. This judgment based on the dendrogram (Fig 1) was generated among samples. Four main clusters were generated from this dendrogram.

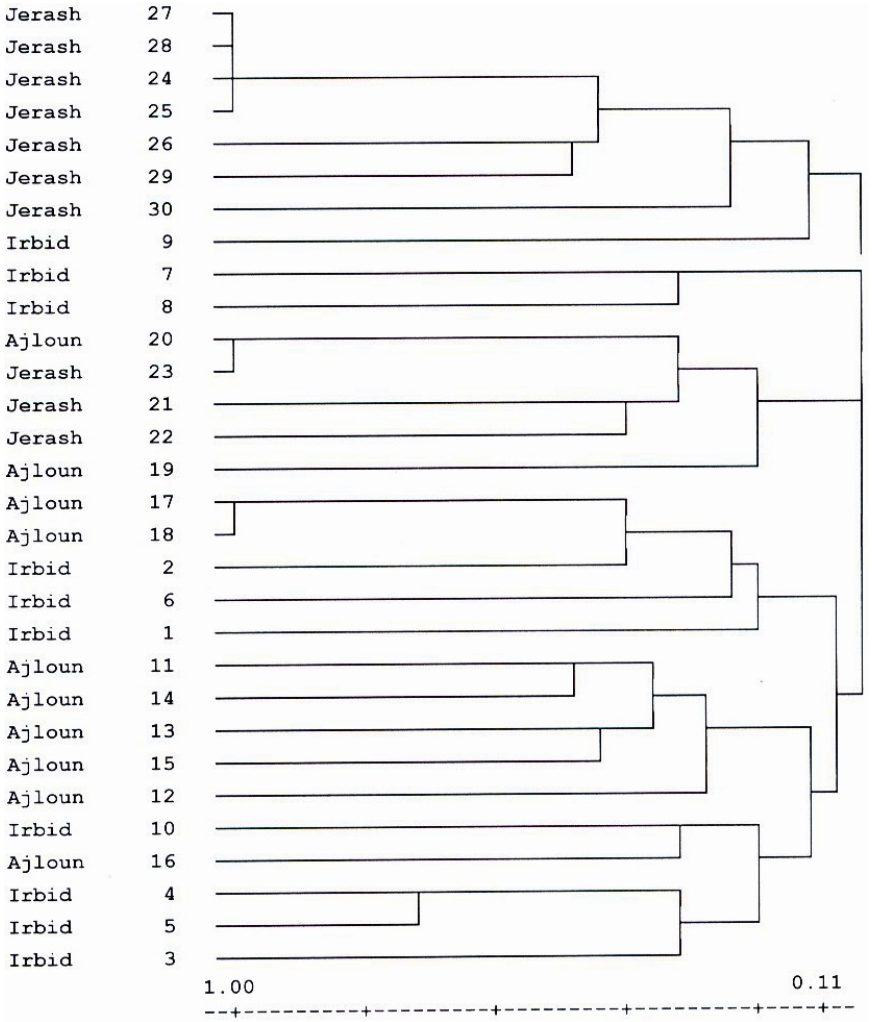

Fig. 1: A dendrogram of sumac samples generated by the UPGMA method using the Jaccard's similarity matrix based on AFLP markers obtained with three primer pair combinations.

The first group includes three subgroups, the first one formed 4 samples were more closely and the second subgroup formed two samples from Jerash the third showed one individual of Irbid. The second group formed two individuals from Irbid. The third group has three subgroups the first has one sample from Ajloun and one from Jerash, the second group included two samples from jerash but the final sub group has one Ajloun individual. The fourth group included three sub clusters the first has four sub- sub cluster, the second sub cluster has three sub-sub clusters but the rest sub cluster has two sub-sub cluster.

High genetic diversity was found among the three populations collected from three locations (Figure 1 and Table 2). For Jerash region, with the exception of the pair of samples 25, 24, 28 and 27, all other entries are genetically distinct but they formed two separated groups, the first included 7 individuals, the second included 3 individuals. For Irbid region, two individuals formed one group, one individual included with the first group, and seven individuals were grouped with Ajloun samples. Ajloun region relatively showed varied individuals through its formed two samples with Jerash and eight samples with Irbid in distinct groups. High similarity was showed between 17and 18 samples (Figure 1 and Table 2).

Irbid population has shown the highest intra population diversity since 7 sub-clusters can be visualized; five of them included one individual each. High similarity was showed between one sample from Ajloun (20) and Jerash (23). The accessions 76 and 18 are the closest. High genetic diversity within a population was found. The similarity matrix allowed to confirm the high relatedness between the samples in the pairs (24, 25, 26, 27 and 28) of Jerash and (17 and 18) of Ajloun. The variability that was found may be related to the eco-geographical condition which plays a great role for creating variation through climatic changes over the past years.

Table 2: Similarity matrix based on the AFLP markers among thirty trees of sumac collected from three locations in Jordan during 2007.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.40																													
3	0.24	0.38																												
4	0.18	0.40	0.50																											
5	0.17	0.38	0.38	0.75																										
6	0.36	0.43	0.33	0.27	0.43																									
7	0.20	0.27	0.27	0.29	0.27	0.31																								
8	0.29	0.19	0.19	0.29	0.27	0.21	0.46																							
9	0.20	0.12	0.19	0.13	0.19	0.21	0.14	0.14																						
10	0.20	0.27	0.36	0.39	0.27	0.31	0.23	0.23	0.23																					
11	0.18	0.24	0.31	0.18	0.24	0.16	0.20	0.13	0.20	0.29																				
12	0.18	0.24	0.24	0.18	0.24	0.27	0.29	0.20	0.20	0.20	0.43																			
13	0.27	0.33	0.25	0.19	0.25	0.50	0.21	0.13	0.21	0.42	0.46	0.36																		
14	0.12	0.25	0.25	0.19	0.25	0.29	0.21	0.13	0.21	0.21	0.58	0.46	0.39																	
15	0.13	0.19	0.19	0.29	0.36	0.31	0.23	0.23	0.23	0.46	0.50	0.39	0.55	0.50																
16	0.20	0.46	0.27	0.39	0.36	0.31	0.23	0.23	0.14	0.46	0.39	0.29	0.31	0.31	0.46															
17	0.31	0.50	0.20	0.13	0.13	0.33	0.15	0.15	0.15	0.25	0.21	0.21	0.33	0.23	0.15	0.36														
18	0.31	0.50	0.20	0.13	0.13	0.33	0.15	0.15	0.15	0.25	0.21	0.21	0.33	0.23	0.15	0.36	1.00													
19	0.14	0.13	0.21	0.14	0.13	0.15	0.17	0.17	0.27	0.17	0.33	0.23	0.15	0.50	0.27	0.27	0.30	0.30												
20	0.13	0.20	0.29	0.21	0.20	0.14	0.25	0.25	0.18	0.15	0.21	0.21	0.14	0.23	0.15	0.25	0.17	0.17	0.30											
21	0.13	0.29	0.39	0.21	0.20	0.14	0.15	0.15	0.25	0.15	0.42	0.31	0.14	0.33	0.25	0.50	0.27	0.27	0.44	040										
22	0.13	0.19	0.36	0.29	0.27	0.21	0.14	0.14	0.14	0.14	0.39	0.13	0.13	0.31	0.23	0.33	0.15	0.15	0.40	050	0.50									
23	0.13	0.20	0.29	0.21	0.20	0.14	0.25	0.25	0.18	0.15	0.21	0.21	0.14	0.23	0.15	0.25	0.17	0.17	0.30	1.00	0.40	0.50								
24	0.23	0.21	0.13	0.14	0.13	0.25	0.17	0.17	0.27	0.40	0.23	0.14	0.36	0.15	0.27	0.27	0.30	0.30	0.20	0.18	0.18	0.17	0.18							
25	0.23	0.21	0.13	0.14	0.13	0.25	0.17	0.17	0.27	0.40	0.23	0.14	0.36	0.15	0.27	0.27	0.30	0.30	0.20	0.18	0.18	0.17	0.18	1.00						
26	0.15	0.14	0.14	0.15	0.14	0.17	0.17	0.18	0.30	0.30	0.15	0.25	0.27	0.17	0.30	0.18	0.20	0.20	0.22	0.33	0.20	0.18	0.33	0.57	0.57					
27	0.23	0.21	0.13	0.14	0.13	0.25	0.17	0.17	0.27	0.40	0.23	0.14	0.36	0.15	0.27	0.27	0.30	0.30	0.20	0.18	0.18	0.17	0.18	1.00	1.00	0.57				
28	0.23	0.21	0.13	0.14	0.13	0.25	0.17	0.17	0.27	0.40	0.23	0.14	0.36	0.15	0.27	0.27	0.30	0.30	0.20	0.18	0.18	0.17	0.18	1.00	1.00	0.57	1.00			
29	0.14	0.13	0.21	0.23	0.21	0.15	0.17	0.17	0.40	0.27	0.14	0.14	0.25	0.15	0.27	0.17	0.18	0.18	0.20	0.18	0.18	0.27	0.18	0.50	0.50	0.57	0.50	0.50		
30	0.15	0.14	0.23	0.15	0.14	0.17	0.18	0.18	0.18	0.30	0.15	0.15	0.17	0.17	0.18	0.18	0.20	0.20	0.22	0.20	0.20	0.18	0.20	0.38	0.38	0.43	0.38	0.38	0.38	

Further study should be conducted to survey all of sumac species grown in Jordan and extend the research to include the studying of the chemicals (for example, Malic acid, citric acid and tartaric acid) and the physical properties. More efficient results will be obtained if all findings correlated with eco-geographical information and if it is possible to compare them with other species grown within neighbor countries of Jordan such as Syria and Turkey etc. Knowledge of the genetic structure of a landrace is fundamental in elaborating strategies which involve the local farmers, allowing us to improve and safeguard the genetic integrity of landrace genetic resources[8]. AFLP markers enable a quick and reliable assessment of intraspecific genetic variability[3].

In conclusion, AFLP is a useful tool that can facilitate the collection and evaluation accessions or genetic resources and shortening the time needed for assess the genetic variation among and within individuals and species in the fields.

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