Retrotransposon-based markers revealed a repartition depending on geographical origin and breeding status of Tunisian pistachio species

Khaled Chatti^{1*}, Sarra Choulak¹, Soumaya Rhouma¹, Karim Guenni², Amel Salhi-Hannachi² and Nouredine Chatti¹

¹ Laboratory of Genetics, Biodiversity and Bioresources Valorisation (LR11ES41), Higher Institute of Biotechnology, University of Monastir, Monastir 5000, Tunisia

² Laboratory of Molecular Genetics, Immunology and Biotechnology, Faculty of Sciences of Tunis, Campus University, 2092 El Manar, Tunis, Tunisia

* Corresponding author: Khaled Chatti e-mail: ckhaled2000@yahoo.fr

Abstract

Retrotransposon movements are considered to be an important factor in evolutionary processes and speciation as well as a source of genetic variation. In order to analyze genetic diversity and population structure in Tunisian pistachio species, nine inter-retrotransposon amplified polymorphism (IRAP) markers were used. As a result, eighty-six amplicons were produced among which 98.15 % were polymorphic. Mean numbers of the effective number of alleles (N_e), Shannon's information index (I) and Nei's genetic diversity (H) were respectively 1.529, 0.478, and 0.310. The average within-population genetic diversity (H_s) was 0.24 and the total diversity (H_t) was 0.3. The Tunisian pistachio populations exhibited high genetic differentiation ($G_{st} = 0.275$) and gene flow ($N_m = 1.888$). The Analysis of Molecular Variance (AMOVA) indicated that variation was very high within populations (83 %). Phylogenetic tree using neighbor-joining (NJ) method and Principal Coordinates Analysis (PCoA) depicted that groupings of Tunisian varieties were made independently of the sex of the trees, but depending on their geographical origin and their breeding status. The model-based Bayesian clustering (STRUCTURE) confirmed these observations. The inter-retrotransposons amplification polymorphism markers were significantly informative at the inter-specific level. Findings reported in our study will be essential toward breeding for new pistachio genotypes with developed chemical and horticultural features.

Keywords: *Pistacia*; Retrotransposon; population structure; gene flow; plant breeding

Introduction

Over time, humans have domesticated plant and animal species to satisfy their needs. This practice has led to a progressive transformation both of them and their landscape (Zeder et al., 2006). The earliest agricultural civilizations were based mainly on cereal crops, which first appeared in the Middle East, and were quickly extended to West Asia, Egypt and Europe (Wadley and Martin, 1993). The cultivation of fruit trees seems to have started three thousand years later, again in the Middle East, and vegetable crops have followed later (Zohary et al., 2012). Since then, Mankind showed a real fascination with fruit trees. Tree species producing organoleptic and nutritional fruits have always been an interesting subject in research and human culture.

Because of its localization on the Mediterranean coast, Tunisia is a shelter for a wealth of plant and animal resources. In particular, crops that are bred in Tunisia adapt to a wide range of environments. There is extensive dryland agriculture where drought and heat are often severe and numerous biotic stresses affect the wide range of crops grown. Among the local plant biodiversity, fruit species occupy a strategic position given their environmental, agricultural and socio-economic roles. These fruit trees, which have an important adaptive capacity, are not very demanding on planting and produce fruits that are highly appreciated by consumers. As a result, these species have recently experienced renewed interest from farmers, breeders, researchers, and consumers.

Pistachio (*Pistacia vera* L. $2n = 30$) is a small fruit species that belongs to the Anacardiaceae family. It is the only

cultivated and commercialized species in the genus *Pistacia* (Zohary, 1996). Indeed, four species belonging to the genus *Pistacia* are existing in Tunisia: *Pistacia atlantica* (Battoum) which is commonly used as a rootstock for vegetative propagation, *Pistacia terebinthus*, *Pistacia lentiscus* and *Pistacia vera* L. The latter is native of arid and semi-arid zones and distributed throughout West Asia, the Middle East zones and the Mediterranean basin such as Greece, Italy, Spain, and Tunisia. The main *P. vera* nut-producing countries are Iran, USA, and Turkey. In Tunisia, the pistachio tree is an ancient crop, particularly in semi-arid and arid zones (Mlika, 1980). It is found in the north (Ariana, Jendouba, Bizerte and Le Kef), the center (Mahdia, Monastir, Kairouan, Kasserine and Sidi-Bouazid), and in the oases of southern Tunisia (Gafsa and El-Guettar). In this area, pistachio cultivation is an important economic activity as evidenced from the national production, which reached more than 3500 t in 2017 making Tunisia rated amongst the top 10 countries in worldwide pistachio production (Faostat, 2017).

Despite the growing value of this fruit species, few studies have dealt with pistachio, especially with regard to the estimation of its genetic diversity and the analysis of population structure and domestication history. Among these few studies, Pazouki et al. (2010) used microsatellite markers for assessing genetic diversity and relationships among *Pistacia* species and cultivars. In 2006, Kafkas et al. detected DNA polymorphism and genetic diversity in a wide pistachio germplasm using different dominant molecular markers. More recently, Motalebi-pour et al. (2016), used SSR markers for genetic linkage map construction in pistachio.

In recent years and with the advent of next-generation sequencing (NGS) technologies, numerous whole genome sequence data from multiple species have become available, which offers a vast possibility for the enhancement of advanced markers in several plant species (Holland et al., 2001). In this context, numerous recent techniques for studying the genomic diversity of higher plants have been described. One of these is the IRAP "Inter-Retrotransposon Amplification Polymorphism" markers. It aims to detect the insertion polymorphism by amplifying a DNA segment comprised between two retrotransposons (Kalendar and Schulman, 2006). These retrotransposons are the most abundant transposable elements in many plant genomes (Kalendar et al., 2014). They have a "copy/paste" transposition mode involving an RNA medium and a reverse transcription process (Kalendar and Schulman, 2006). The transposition mechanism means that the insertions are irreversible and that the retro elements are able to be amplified into a very large number of copies (Mansour, 2008). These retro-elements are considered to be an important factor in evolutionary processes including speciation (Hamdi et al., 2000) as a source of genetic variation (Böhne et al., 2008). Retrotransposons can be applied as molecular markers because their incorporation makes new joints among genomic DNA and their conserved ends. Retrotransposons markers are detected by PCR, which amplifies a sequence of DNA at this joint (Kalendar and Schulman, 2006). This approach has been used to analyze diversity, similarity and genetic relationships in several plants such as rice (*Oryza sativa* L.) (Branco et al., 2007), flax

(*Linum usitatissimum* L.) (Smykal et al., 2011), plum (Senkova et al., 2013), rye (*Secale cereale* L.) (Acherim et al., 2014) sorghum (*Sorghum halepense* L.) (Karimi and Saeidi, 2016) and table grapes (Striato et al., 2019).

Our aim in the present study was to analyze the genetic diversity of pistachio in three Tunisian sites (El-Guettar, Gafsa and Sidi-Bouazid). The main objectives were the estimation of the extent of the molecular diversity, the establishment of phylogenetic relationships between accessions and the genetic structure of the studied populations. Results could be valuable for Tunisian *Pistacia* germplasm collection, conservation, and breeding

Materials and Methods

Plant Materials

Field visits were carried out in the central southern (Gafsa and El-Guettar oasis) traditional areas of pistachio growing in Tunisia. We also included accessions maintained at the Regional Center for Agricultural Research of Sidi-Bouazid (fig S1). Forty-two Tunisian pistachio accessions (20 from El-Guettar, 6 from Sidi-Bouazid and 16 from Gafsa) including 30 female trees, nine male trees (*P. vera*) and three *P. atlantica* were used in the study (Table 1). Plant material comprised fresh leaves of each adult tree. Samples were frozen at -80°C awaiting their use for DNA purification.

DNA Isolation and PCR amplification

DNA isolation is a crucial step for further DNA processing. Total genomic DNA was extracted from frozen leaves of single adult trees by means of a modified MATAB method (Risterucci et al., 2000). The present study consists in detecting the inter-retro transposable region; for this reason, the amplification has been carried out via universal LTR (Long Terminal Repeat) primers described by Smykal et al. (2011) (Table 2). This method requires detecting the insertion polymorphism by amplifying the DNA fragment amongst two retrotransposons (Kalendar and Schulman, 2006) using a single primer complementary to a conserved LTR segment within a family.

Amplification was carried out in 25 µL Reaction Mix containing 1 µL DNA (25ng/µL), 1 µL (20µM) primer, 1 µL (100mM) dNTP mix, 1 unit of Taq polymerase, and sterile ddH₂O. PCR amplification was realized according to the conditions described by Jiang et al. (2014). PCR results were observed by 1.5 % agarose gel containing 0.5 µg/mL of ethidium bromide. Electrophoresis was performed in 1X TBE buffer solution at 5 V/cm. Agarose gel was visualized and photographed under a Compact Digimage System, UVDI series (Major Sciences, USA).

Statistical Analysis

Amplicons generated by the IRAP technique were analyzed and each amplified DNA fragment treated as a di-allelic locus, 1 for presence and 0 for absence. Only polymorphic loci were considered during this analysis. Then, the data set was transformed into a binary matrix used to perform the statistical

Table 1
Label, sex and origin of Tunisian pistachio cultivars studied.

Geographic origin	Number	Labels	Sex	Species	Plant nature
El Guetar (Tunisia)	01	GT1	♀	<i>P. vera</i>	Grafted plant
	02	GT2	♀	<i>P. vera</i>	Grafted plant
	03	GT3	♂	<i>P. vera</i>	Grafted plant
	04	GT4	♂	<i>P. vera</i>	Grafted plant
	05	GT5	♀	<i>P. vera</i>	Grafted plant
	06	GT6	♂	<i>P. vera</i>	Grafted plant
	07	GT7	♀	<i>P. vera</i>	Grafted plant
	08	GT8	♀	<i>P. vera</i>	Grafted plant
	09	GT9	♂	<i>P. vera</i>	Grafted plant
	10	GT10	♀	<i>P. vera</i>	Grafted plant
	11	GT11	♀	<i>P. vera</i>	Grafted plant
	12	GT12	♀	<i>P. vera</i>	Grafted plant
	13	GT13	♀	<i>P. vera</i>	Grafted plant
	14	GT14	♀	<i>P. vera</i>	Grafted plant
	15	GT15	♂	<i>P. vera</i>	Grafted plant
	16	GT16	♀	<i>P. vera</i>	Grafted plant
	17	GT17	♀	<i>P. vera</i>	Grafted plant
	18	GT18	♀	<i>P. vera</i>	Grafted plant
	19	GT19	♀	<i>P. vera</i>	Grafted plant
	20	GT20	♀	<i>P. vera</i>	Grafted plant
Gafsa (Tunisia)	21	GF1	♂	<i>P. vera</i>	Grafted plant
	22	GF2	♂	<i>P. vera</i>	Grafted plant
	23	GF3	♀	<i>P. vera</i>	Grafted plant
	24	GF4	♀	<i>P. vera</i>	Grafted plant
	25	GF15	♀	<i>P. vera</i>	Grafted plant
	26	<i>Battoum1</i>		<i>P. atlantica</i>	wild seedling
Sidi Bouzid (Tunisia)	27	SB1	♀	<i>P. vera</i>	Grafted plant
	28	SB2	♀	<i>P. vera</i>	Grafted plant
	29	SB3	♀	<i>P. vera</i>	Grafted plant
	30	SB4	♀	<i>P. vera</i>	Grafted plant
	31	SB5	♂	<i>P. vera</i>	Grafted plant
	32	SB6	♂	<i>P. vera</i>	Grafted plant
	33	SB7	♀	<i>P. vera</i>	Grafted plant
	34	SB8	♀	<i>P. vera</i>	Grafted plant
	35	SB9	♀	<i>P. vera</i>	Grafted plant
	36	SB10	♀	<i>P. vera</i>	Grafted plant
	37	SB11	♀	<i>P. vera</i>	Grafted plant
	38	<i>Matteur1</i>	♀	<i>P. vera</i>	Grafted plant
	39	<i>Matteur2</i>	♀	<i>P. vera</i>	Grafted plant
	40	<i>Irani</i>	♀	<i>P. vera</i>	Grafted plant
	41	<i>Battoum2</i>		<i>P. atlantica</i>	wild seedling
	42	<i>Battoum3</i>		<i>P. atlantica</i>	wild seedling

analyses. Accordingly, to estimate the efficiency of the primers used, some parameters have been calculated such as Polymorphic bands (PB), percentage of polymorphic bands (PPB), Polymorphic information content (PIC) (Ghislain et al., 1999) and the resolving power of the primer (Rp) (Prevost and Wilkinson 1999). In addition, genetic diversity measures (H_t , total gene diversity; H_s , mean genetic diversity within the population; G_{st} , the coefficient of gene differentiation; N_e : effective number of alleles) were tested using POPGENE (Yeh et al. 1999).

Genetic diversity measures for each population (NPB: number of polymorphic band; PPB: percentage of

polymorphic bands; I: Shannon information index of diversity; H : Nei's genetic diversity and N_a : observed number of alleles) were also assessed using POPGENE (Yeh et al. 1999). Furthermore, estimate of gene flow (N_m) among populations was determined according to the formula $N_m = 0.25 \times (1 - G_{st}) / G_{st}$ (McDermott and McDonald, 1993). Additionally, the *Analysis of Molecular Variance* (AMOVA) implemented in the GenAlEx 6.5 program (Peakall and Smouse 2006) was executed, based on 999 permutations, to screen the total IRAP variation within and among populations.

Table 2

Genetic parameters of nine IRAP markers: TB: total number of bands; PB: polymorphic bands; PPB: percentage of polymorphic bands; PIC: Polymorphic information content; Rp: the resolving power of the primer. H_t : total gene diversity; H_s : mean genetic diversity within the population; G_{st} : the coefficient of gene differentiation; Na: observed number of alleles; Ne: effective number of alleles; I: Shannon information index of diversity; H: Nei's genetic diversity.

Locus	Sequences (5'-3')	TB	PB	PPB (%)	PIC	Rp	Ht	Hs	Gst	Na	Ne	H	I
LTR1	ACCCCTTGAGCTAACTTTTGGGGTAAG	13	13	100	0.422	0.422	0.256	0.218	0.990	2	1.428	0.272	0.428
LTR2	CTTGCTGGAAAGTGTGTGAGAGG	16	16	100	0.379	7.476	0.236	0.185	0.152	2	1.353	0.237	0.387
LTR3	TGTTAATCGCGCTCGGGTGGGAGCA	5	5	100	0.5	7.308	0.387	0.343	0.097	2	1.701	0.388	0.565
LTR4	AGCCTGAAAGTGTGGGTTGTCG	6	5	83.33	0.495	3.571	0.189	0.126	0.302	2	1.411	0.23	0.347
LTR5	CTGGCATTTCATTGTCGTCGATGC	7	7	100	0.483	1.809	0.418	0.305	0.245	2	1.823	0.427	0.605
LTR6	GCATCAGCCTGGACCACTCCTCGTCC	15	15	100	0.385	4	0.224	0.194	0.112	2	1.357	0.237	0.385
LTR7	CACTTCAAATTTTGGCAGCAGCGGATC	6	6	100	0.466	7.142	0.326	0.243	0.196	2	1.539	0.333	0.508
LTR8	TCGAGGTACACCTCGACTCAGG	11	11	100	0.463	11.095	0.365	0.278	0.241	2	1.575	0.332	0.576
LTR9	ATTCTCGTCCGCTGCGCCCTACA	7	7	100	0.487	5.904	0.314	0.268	0.139	2	1.574	0.332	0.498
Mean	-	9.555	9.444	98.15	0.453	4.047	0.302	0.240	0.275	2	1.529	0.310	0.478
total		86	85	-	-	52.774	-	-	-	-	-	-	-

Genetic relationships

Principal Coordinates Analysis (PCoA) was applied to the marker data by means of GenALEX 6.502 program, to construct a bi-dimensional graphic representation of the genetic variability of accessions agreeing to their genetic origin. Based on the similarity index, the Genedist program (Version 3-5726, PHYLIP Software) (Felsenstein, 1995) was used to produce a genetic distance matrix on the basis of the formula of Nei and Li (1979). Thus, the obtained distance matrix was imported into the PHYLIP Software (Felsenstein, 1995) to build the dendrogram based on the *Neighbor-Joining (NJ)* method (Saitou and Nei, 1987). Tree View software (Page, 1996) was used to illustrate the phylogenetic dendrogram.

Population structure

For population structure analysis, *Bayesian model-based clustering* program STRUCTURE version 2.3.3 (Pritchard et al., 2000) was implemented to deduce the most relevant number of clusters (k) to our analysis. The original data matrices were imported into the program and ten independent replicate runs for each k value were performed. Therefore, a possible number of genetic groups (k) in our sample set was investigated by Structure Harvester 0.694 (a web-based program) for collating results produced by the STRUCTURE program, which summarized the results of repeated runs and given the optimal K value using the Evanno method (Evanno et al. 2005). In fact, Structure Harvester 0.694 (Dent 2014: web version) provided a fast way to estimate log-likelihood of k to choose the optimal k value (corresponding to the number of genetic groups that best fit the data).

Results

Polymorphisms of IRAP Markers and genetic diversity analysis

A total of 86 identifiable amplicons were generated using nine prescreened IRAP primer sets. The number of amplicons varied

from 5 (LTR3) to 16 (LTR2) per primer, corresponding to an average of 9.5 amplicons. The percentage of polymorphic bands (PPB) was 100 % for all used primers with the exception of LTR4 whose percentage was 83.33 (Table 2). This result highlights a high degree of genetic diversity within the studied Tunisian pistachios. On the other hand, for every locus, the polymorphic information content was calculated. Values ranged from 0.379 (LTR2) to 0.5 (LTR3) (Table 2). The resolving power (Rp) of the tested primers ranged from 0.422 (LTR1) to 11.095 (LTR8). This result disclosed the efficiency of the primers used to detect diversity at DNA level. Based on the studied IRAP markers, H_s and H_t were calculated for all examined loci. The average within-population genetic diversity (H_s) was 0.24 and the total diversity (H_t) was 0.3. Nei's G_{st} is generally used for describing the average amount of differentiation observed over multiple loci, the multi-locus values of G_{st} were equal to 0.275. On the other hand, the mean effective number of alleles (Ne), Shannon's information index (I) and Nei's genetic diversity (H) were equal to 1.529, 0.478, and 0.310, respectively (Table 2).

The assessment of the genetic variation within different geographic areas of pistachio showed an average observed number of alleles (Na), Shannon's information index (I) and Nei's genetic diversity (H) equal to 1.732, 0.35, and 0.229, respectively (Table 3). The population El-Guetar displayed higher values of I (0.421), H (0.274) and genetic polymorphism (PPB: 90.7 %) than the other two populations. In contrast, the population of Gafsa exhibited lower genetic polymorphism (46.51), lower Shannon information index of diversity (I = 0.235) and lower Nei's genetic diversity (H = 1.465). The average IRAP polymorphism in Tunisian pistachio population was important (73.26%). N_m estimation was performed on the 86 obtained IRAP loci. The mean value of gene flow (N_m) for all loci was estimated by the formula $0.25 \times (1 - G_{st}) / G_{st}$. It is considered high since $N_m > 1$ ($N_m = 1.888$).

Moreover, AMOVA was performed to test the significance of the partitioning of genetic variance between and within populations. The result indicated that variation was very high

Table 3.

Genetic Variation Statistics within different geographic region of Tunisian Pistachio revealed by IRAP markers

NPB: number of polymorphic band; PPB: percentage of polymorphic bands; I: Shannon information index of diversity; H: Nei's genetic diversity; Na: observed number of alleles; Nm gene flow.

Population	N	NPB	PPB (%)	I	H	Na	Nm
El-Guetar	20	78	90.70	0.4213	0.2745	1.9070	-
Gafsa	06	40	46.51	0.2358	0.1556	1.4651	-
Sidi-Bouزيد	16	71	82.56	0.3947	0.2570	1.8256	-
Mean		63	73.26	0.3506	0.2290	1.7325	1.8887

Table 4

Analysis of molecular variance (AMOVA) of the pistachio accessions revealed by IRAP markers

Source	df	SS	MS	Est. Var.	%	P-value*
Among Pops	2	99.209	49.604	2.829	17%	p<0.001
Within Pops	39	526.529	13.501	13.501	83%	p<0.001
Total	41	625.738		16.330	100%	p<0.001

df: Degree of freedom; P-value: probability of null hypothesis

*Significance tests after 1000 permutations

within the populations (83 %) but low (17 %) among them (Table 4).

Genetic relationships

To increase the cluster analysis information, a multivariate approach was used. Fig. 1 illustrates the distribution of the ecotypes according to the first two components (axes1 and 2). Outcomes showed that almost 37.29 % of the total variability was described by the first three PCoA. Varieties were clustered in three different groups in PCoA plot: El-Guetar (pop1), Gafsa (pop2), and Sidi-Bouزيد (pop3) (Fig. 1). This result is confirmed by the dispersion of cultivars exposed by the *NJ* tree (Fig. 2). In fact, the dendrogram classified the 42 studied accessions into three major clusters. The first one (I) contained all 'El-Guetar' genotypes (1-20). The second clade (II) represented all Gafsa accessions (21-25), and the third clade (III) grouped Sidi-Bouزيد (27-37), 'Irani'(26), and 'Matteur' varieties (38, 39) (Fig. 2). Otherwise, phylogenetic tree with neighbor-joining (*NJ*) method depicted that groupings of Tunisian varieties were made independently of the sex of the trees, but depending on their geographical origin and their breeding status. Indeed, El-Guetar and Gafsa varieties were clearly separated from those from Sidi-Bouزيد. Moreover, the three ecotypes of *Pistacia atlantica* were placed in a separate subgroup in the third clade (Fig. 2).

Population structure

Population structure of the 42 Tunisian pistachio accessions was surveyed under the Hardy-Weinberg Equilibrium assumption, using STRUCTURE V2.3.3 software. Via a model-based Bayesian clustering, the log likelihood between following *k* values (ΔK) exhibited a higher level of clustering at *k* = 2 for the considered pistachio accessions

(Fig. 3b). The Structure at *k* = 2 enabled differentiating two major clusters (Fig. 3a). Cluster 1: included accessions from Sidi-Bouزيد and wild pistachio accessions (Battoum). Cluster 2 was made of accessions from El-Guetar and Gafsa. As it turned out from the clustering analysis, a clear differentiation based on the geographic origin arise. At *k* = 3 and *k* = 4, there was not a clear discrimination between cultivars, although we observed heterogeneity in groups.

Discussion

IRAPs have been widely used to study genetic variability, genetic relationships, and genetic material management (Tehen et al., 2004). Retrotransposons are very abundant and scattered in the majority of plant genomes. The dispersal and ubiquity of these elements in plant genomes provide an extraordinary basis for improving molecular marker systems (Kalendar et al., 1999; Waugh et al. 1997). Geological, topological and climatic variability are presented as an important factor in plant biodiversity. Thanks to this variability, the Mediterranean region is considered one of the cradles of biodiversity in the world (Myers et al. 2000). Among the Mediterranean regions, Tunisia is a center of diversification of several agricultural species where there are several cultivated varieties that often bear the name of a locality or that of a farmer. This diversity is of interest for species such as apricot, almond, pomegranate, date palm, pistachio.

The pistachio tree is one of the ancient plant species cultivated. It is a tree of great interest because of its productivity, the nutritional quality of its fruits and its ability to adapt to arid

Fig 1

Principal coordinates analysis (PCoA) for the first and second coordinates estimated for IRAP markers (Axis 1 and 2 explain 30 % of the total variability). Pop1=El-Guetar, Pop2=Gafsa and Pop3=Sidi-Bouزيد.

Fig 2

NJ dendrogram of Tunisian pistachio accessions constructed with Nei's genetic distances based on IRAP alleles. Clade I (El-Guetar), clade II (Gafsa) and clade III (Sidi-Bouزيد).

A

B

Fig.3

Estimated population structure based in IRAP markers using STRUCTURE software. a: Population structure, each color indicates a Group with the same genetic background. Bold lines represent the limit of the two genetic groups. b: Mean log likelihood $\ln P(X|K)$ as a function of the number of genetic clusters (K), in our case $K = 2$. Geographic origin: (1) El-Guetar, (2) Gafsa & (3) Sidi-Bouزيد.

and semi-arid regions. Like the Maghreb countries, Tunisian pistachio trees are constantly threatened by various abiotic and biotic stresses that have far-reaching environmental and socioeconomic impacts.

The main reason for exploring the genetics of *P. vera* L. in this study was to document conclusively the strong intra-specific variation in Tunisia, there was not enough data on this subject. In addition, our previous study (Chatti et al. 2017), in which we used morphological parameters, did not give clear results regarding the genetic organization of the Tunisian pistachio tree. The present study revealed a remarkably high level of genetic diversity in *P. vera* species based on IRAP markers. A total of 86 bands and 85 polymorphic bands with 98.15 % of polymorphism were detected in this species. This result shows clearly the abundance of IRAP amplification bands, which indicates the large-scale dispersion of retrotransposons in the genome. Moreover, Nei's gene diversity index (H) values within populations varied from 0.23 to 0.427 with an average of 0.31. Our study clearly reveals that there is wide genetic variability among Tunisian *P. vera* germplasm. In fact, all tested primers are efficient to detect DNA polymorphisms in pistachio germplasm. This hypothesis is strongly supported in view of the high resolving power ($R_p=52.774$) and PIC (0.453) rates. Clustering and structure analysis based on IRAP markers indicated that Sidi-Bouزيد population is most divergent from the other populations. The reason for this divergence can be its geographical background; the plant material was collected from the experimental orchard situated in the experimental station of the Agriculture Research Center (CRRRA, Sidi-Bouزيد). The growing region is characterized by a steppe Mediterranean type of weather with an annual rainfall of 200 mm and a reference evapo-transpiration of more than 1300 mm. The soil is heavy and calcareous and has a clay-sandy texture (Abidi, 2016). While the oasis of El-Guétar has a very old irrigation system flowing from the bottom of the Orbat Mountains. The pistachio tree, adapted to the environment of the region, is the symbol of the city. Wild originally, it has been growing for thousands of years in Jebel Orbat. Thanks to Chott El-Guétar that has an area of 3730 ha, El-Guétar pistachios are closely associated with specific geological, geomorphological, and pedological features (Hachicha and Ben Aissa, 2014). The Chott acts as an evaporative system of various salts. These natural conditions give El-Guétar pistachios a special taste that distinguishes them from other regions. On the other hand, the mean value of gene flow (N_m) for all loci was very high since $N_m > 1$ ($N_m=1.888$). Gene flow is a process occurring both in time and in space, across pollen and seeds, which is strongly related to the horticultural system (Hamrick et al., 1993). In pistachio, as angiosperm species, plastids are exclusively inherited maternally. Thus, plastid population genetic structure should be determined by gene flow from seeds (Ellstrand, 2014). In Tunisia, seeds are rarely exchanged among regions of pistachio cultivation. The vegetative propagation is widely used (Maggs, 1973). Therefore, between these locations, female varieties are exchanged in order to provide a warranty for production in unfavorable growing areas.

Conclusion

In conclusion, it is crucial to figure out the genetic polymorphism and variation among and within accessions to adopt the efficient plan for conservation and sampling management. In this study, IRAP marker, in theory comparable to the ISSR and RAPD marker, was an efficient system to assess genetic variation and to detect polymorphism among *P. vera* accessions. The study of IRAP profiles of 42 accessions showed a wide genetic diversity in the Tunisian Pistachio germplasm and a clear pattern of genetic variability could be elucidated between populations. This novel information is very essential for opening extensive horizons for growing new cultivars with developed chemical and horticultural features. In addition, our findings will be an important resource for the study of sex determinism, seed propagation and controlled crosses.

Acknowledgements

This work was supported by grants from the Tunisian Ministry of Higher Education and Scientific Research. The paper is dedicated to the late Professor **Imen Said**.

References

- Abidi W (2016) Pomological and physical attributes of pistachio (*Pistacia vera* L.) varieties grown in west-central Tunisia. Journal of new sciences, Agriculture and Biotechnology 28(4), 1582-1588
- Achrem M, Kalinka A, Rogalska SM (2014) Assessment of genetic relationships among *Secale* taxa by using ISSR and IRAP markers and the chromosomal distribution of the AAC microsatellite sequence. Turkish Journal of Botany 38(2), 213-225. <https://doi.org/10.3906/bot-1207-1226>
- Böhne A, Brunet F, Galiana-Arnoux D, Schultheis C, Volff JN (2008) Transposable elements as drivers of genomic and biological diversity in vertebrates. Chromosome research. 16(1), 203-215 <https://doi.org/10.1007/s10577-007-1202-6>
- Branco CJS, Vieira EA, Malone G, et al. (2007) IRAP and REMAP assessments of genetic similarity in Rice (*Oryza sativa*). Journal of Applied Genetics 48(2), 107-113. <https://doi.org/10.1007/BF03194667>
- Chatti K, Choulak S, Guenni K, Salhi-Hannachi A (2017) Genetic diversity analysis using morphological parameters in Tunisian Pistachio (*Pistacia vera* L.). Journal of Research in Biology Sciences, 02: 29-34
- Dent EA (2014) STRUCTURE HARVESTER version 0.694. <http://taylor0.biology.ucla.edu/structureHarvester/>.
- Ellstrand NC (2014) Is gene flow the most important evolutionary force in plants? Am. J. Bot. 101(5), 737-753. <https://doi.org/10.3732/ajb.1400024>
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611-2620. <https://doi.org/10.1111/j.1365-294x.2005.02553.x>
- Faostat (2017) <http://www.fao.org/faostat/en/data/QC>
- Felsenstein J (1995) PHYLIP (Phylogeny Interference Package) version 3.5 c. Department of Genetics, University of Washington, Seattle, Washington
- Ghislain M, Zhang DP, Fajardo D, Huamán Z, Hijmans RJ (1999) Marker-assisted sampling of the cultivated Andean potato *Solanum phureja* collection using RAPD markers. Genetic Resources and Crop Evolution 46, 547-555. <https://doi.org/10.1023/A:1008724007888>

- Hachicha M, Ben Aissa I (2014) Managing salinity in Tunisian oases. *Life Sciences Journal* 8(9), 775-782. <https://doi.org/10.17265/1934-7391/2014.09.007>
- Hamdi HK, Nishio H, Tavis J, Zielinski R, Dugaiczky A (2000) Alu-mediated phylogenetic novelties in gene regulation and development. *Journal of Molecular Biology* 299(4), 931-939. <https://doi.org/10.1006/jmbi.2000.3795>
- Hamrick JL, Murawski DA, Nason JD (1993) The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetation* 107(1), 281-297. <https://doi.org/10.1007/BF00052230>
- Holland JB (2001) Epistasis and plant breeding. *Plant Breeding. Reviews*. 21, 2792. <https://doi.org/10.1002/9780470650196.ch2>
- Jiang LF, Qi X, Zhang XQ, Huang LK, Ma X, Xie WG (2014) Analysis of diversity and relationships among orchardgrass (*Dactylis glomerata* L.) accessions using start codon-targeted markers. *Genetic Molecular. Research* 13, (2): 4406-4418. <https://doi.org/10.4238/2014.june.11.4>
- Kafkas S, Hakan O, Bekir E, Izzet A, Halit Seyfettin A, Sonay K (2006) Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm: Comparison of AFLP, ISSR, and RAPD markers." *Journal of the American Society for Horticultural Science* 131.4, 522-529. <https://doi.org/10.21273/JASHS.131.4.522>
- Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A (1999) IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. *Theoretical and Applied Genetics* 98: 704-711. <https://doi.org/10.1007/s001220051124>
- Kalendar R and AH Schulman (2006) IRAP and REMAP for retrotransposon-based genotyping and fingerprinting. *Nature Protocols*. 1(5), 2478-2484. <https://doi:10.1038/nprot.2006.377>
- Kalendar R, Schulman AH (2014) Transposon-based tagging: IRAP, REMAP, and iPBS. *Methods Molecular Biology* (1115) 233-55. https://doi:10.1007/978-1-62703-767-9_12
- Karimi A, Saeidi H (2016) Genetic diversity of *Sorghum halepense* (L.) Pers. in Iran as revealed by IRAP markers. *Plant. Genetic. Resources*. 14(2), 132-141. <https://doi.org/10.1017/S1479262115000167>
- Maggs DH (1973) Genetic resources in pistachio. *Plant. Genet. Resources. Newsletter*. 29: 7-15
- Mansour A (2008) Utilization of genomic retrotransposons as cladistics markers. *Journal of Cell and Molecular Biology*: 7, 17-28
- McDermott JM and BA McDonald (1993) Gene flow in plant pathosystems. *Annual Review of Phytopathology*: 31(1), 353-373
- Mlika M (1980) Contribution à l'étude du pistachier en Tunisie : Choix de variétés mâles et femelles à floraison synchrone- Anatomie des fleurs. Mémoire de fin d'études du cycle de spécialisation INAT, Tunisie
- Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*. 403(6772), 853-858. <https://doi.org/10.1038/35002501>
- Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*. 76(10): 5269-5273. <https://doi.org/10.1073/pnas.76.10.5269>
- Page RDM (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* 12, 357-358. <https://doi.org/10.1093/bioinformatics/12.4.357>
- Pazouki L, Mardi M, Shanjani PS, et al. (2010) Genetic diversity and relationships among *Pistacia* species and cultivars. *Conservation Genetics* 11, 311-318. <https://doi.org/10.1007/s10592-009-9812-5>
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6, 288-295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Prevost A, Wilkinson M (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics* 98, 107-112. <https://doi.org/10.1007/s001220051046>
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*. 155, 945-959
- Risterucci AM, Grivet L, N'goran J, Pieretti I, Flament MH, Lanaud C (2000) A high-density linkage map of *Theobroma cacao* L. *Theoretical and Applied Genetics* 101(5), 948-955. <https://doi.org/10.1007/s001220051566>
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology Evolution* 4(4), 406-425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Senkova S, Ziarovska J, Bezo M (2013) Utilization of IRAP technique for plums genotypes differentiation. *Biosci. Res.* 10(1), 01-07
- Smykal P, Bacova-Kertesova N, Kalendar R, Corander J, Schulman AH, Pavelek M (2011) Genetic diversity of cultivated flax (*Linum usitatissimum* L.) germplasm assessed by retrotransposon-based markers. *Theoretical and Applied Genetics* 122(7), 1385-1397. <https://doi.org/10.1007/s00122-011-1539-2>
- Striato D, Kuhn B, Nagata W, Marinelli G, Oliveira-Collet S, Mangolin C, Machado M (2019) Development and use of retrotransposons-based markers (IRAP/REMAP) to assess genetic divergence among table grape cultivars. *Plant Genetic Resources: Characterization and Utilization*. 17(3), 272-279. <https://doi:10.1017/S1479262119000029>
- Techen N, Crockett SL, Khan IA, Scheffler BE (2004) Authentication of medicinal plants using molecular biology techniques to complement conventional methods. *Current Medicinal Chemistry* 11(11), 1391-1401. <https://doi.org/10.2174/0929867043365206>
- Wadley G, Martin A (1993) The origins of agriculture: a biological perspective and a new hypothesis. *Australian Biologist* 6, 96-105
- Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas BBT, Powell W (1997) Genetic distribution of BARE-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). *Molecular and General Genetics* 253, 687-694. <https://doi.org/10.1007/s004380050372>
- Yeh F, Yang R, Boyle T (1999) POPGENE Version 1.31, Microsoft Windows-Based Freeware for Population Genetic Analysis. Quick User Guide; University of Alberta. Edmonton, AB, Canada
- Zeder MA, Emshwiller E, Smith BD, Bradley DG (2006) Documenting domestication: the intersection of genetics and archaeology. *Trends in Genetics* 22, 139-55. <https://doi.org/10.1016/j.tig.2006.01.007>
- Zohary D (1996) The genus *Pistacia* L. In *Taxonomy, distribution, conservation and uses of Pistacia genetic resources*, Padulosi S, Caruso T, Barone E (eds) (IPGRI, Palermo), pp. 1-11
- Zohary D, Hopf M, Weiss E (2012) Domestication of Plants in the Old World. The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. Oxford University Press, UK. 264p. <https://doi.org/10.1093/acprof:osobl/9780199549061.001.0001>
- Ziya Motalebi-pour E, Kafkas S, Khodaei-aminjan M, et al. (2016) Genome survey of pistachio (*Pistacia vera* L.) by next generation sequencing: Development of novel SSR markers and genetic diversity in *Pistacia* species. *BMC Genomics* 17, 998. <https://doi.org/10.1186/s12864-016-3359-x>