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COLLECTION, CHARACTERIZATION AND EXCHANGE OF *PISTACIA* GENUS GERMPLASM

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Executive summary

The purpose of this project is to develop the *Pistacia* genus from an underutilised crop in Turkmenistan and in the Mediterranean basin into a major crop while conserving the unique germplasm still available before deforestation and desertification processes take place. This program is also aimed at development of a core of young Turkmen scientists who will be trained in the relevant technologies in Israel and will serve as the carriers of the horticultural and conservation objectives of this project.

In the course of the reporting period the two scientists, Mr. Azat Ataev and Dr. Muhamet Nepesov, who trained in the Israeli lab started to apply the technologies and procedures of collection and characterization of *Pistacia*. Mr. Ataev and Dr. Nepesove conducted extensive collection trips to sites of wild *Pistacia vera* stands and collected leaf materials for analyses. DNA was extracted from the samples for RAPD analysis. Aliquots of these samples were transferred to the Israeli group.

A Turkmen trainee, Mr. Atamuradov Nury, was expected to join the Israeli group in the fall of 2001, but he postponed his arrival due to the security situation in the Middle East.

Section I

Research accomplishments

The overall research effort in the third year was directed towards establishment of a biotechnology laboratory and *Pistacia* germplasm collection in Turkmenistan and initial application of the RAPD technique for characterization of the Turkmen *Pistacia* germplasm collection. Morphological and molecular characterization of the developing *Pistacia vera* seedlings from Badghyz (Kepele) and Agashli was carried out and summarized in a form of a manuscript, entitled 'Genetic variability in Turkmen populations of *Pistacia vera* L.', which was accepted for publication in Genetic Resources and Crop Evolution (attached below). Another manuscript entitled 'Comparison of Mediterranean *Pistacia lentiscus* genotypes by random amplified polymorphic DNA, chemical and morphological analyses' (attached below) is in final preparations for submission for publication.

Genetic variability in Turkmen populations of *Pistacia vera* L.

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Abstract

Seedlings of *Pistacia vera* L. developed from seeds of two separate populations in Turkmenistan, Kepele and Agachli, were evaluated for their growth potential and genetic polymorphism. Plant growth rate as well as random amplified polymorphic DNA (RAPD) analysis showed distinct differences between the two populations. In plant height growth rate, 17 Agachli accessions were 1.3 times higher on average than that of 10 accessions of Kepele (significant at p=0.046) and 1.2 times higher for trunk diameter growth rate (p=0.062). Cluster analysis divided most accessions into two main genetic groups according to their geographic origin. The Agachli group was further divided into two subgroups. One Kepele accession (K4), was genetically different from the rest and clustered on a separate outgroup. Two Agachli accessions (A12 and A17) were outside the two main populations clusters. Accessions K9 and K10 from Kepele were exceptional and were clustered in each of the two Agachli's subgroups, indicating a close genetic relationship between the two populations. In addition, high similarity values (0.58-1.00) and small genetic distances reflect plausible gene flow between Kepele and Agachli, which are 100 Km apart. Mantel test revealed significant relationship between the RAPD and the morphological traits matrices, pointing to the genetic basis for the measured differences in the growth rate. Growing the accessions on the same plot, under similar conditions enabled the evaluation of genotypic differences. The combination of morphological traits and molecular markers will further assist in preservation of genetic variability and cultivation of useful genotypes of *P. vera* L.

Introduction

The *Pistacia vera* L. (pistachio) is the only cultivated species among the 11 species of the genus *Pistacia* (Anacardiaceae). It is cultivated in many places around the world, Asia, Middle East, southern Europe, South Africa, Australia and America. Certain characteristics, such as relatively large seeds, rich in fat and proteins, and the ability to withstand fire, make this species suited to extreme environments (Popov, 1994). Based on the analysis of the chloroplast genome it was suggested that *P. vera* L. is the most ancestral species among *Pistacia* sp. (Parfitt & Badenes, 1997).

(Fig. 1)

Based on classical taxonomy (Zohary, 1952) as well as on molecular information (Hormaza et al., 1994), it was concluded that *P. vera* L. originated in central Asia. In Turkmenistan, pistachio grows in the wild in sparse populations, in Badghyz, especially around Kepele, south-eastern Turkmenistan, and around Agachli, along the Afghan border south-east to Badghyz (Fig. 1). These, are among the last remaining places on earth where natural populations of *P. vera* L. are protected in national reserves in their natural habitat (Fig. 2, 3); an area with an average precipitation of ca. 300 mm per year and maximal temperatures of 45-48 ^{0}C (Popov, 1994). In this region, pistachio is cultivated by traditional methods. Seeds, collected from natural stands, are germinated in a nested planting method, where 5-10 seeds are sown together in the same spot. This method ensures the persistence of genetic variability in wild populations of *P. vera* L. However, destruction of natural habitats in central and southwestern Asia, and the process of changing from traditional to modern cultivation, may cause genetic erosion (Hormaza et al., 1998) and loss of important germplasm. Therefore, there is a great importance in preservation of the genetic variability of natural populations of *Pistacia vera* L. This led us to collect seeds from wild populations of pistachio of Turkmenistan in order to establish a live germplasm collection of

these natural trees. Here we report on the genetic variability among accessions from Kepele and Agachli.

Material and Methods

Plant material

Seeds of *P. vera* L. were collected in Turkmenistan from two populations, Kepele and Agachli. The two populations are separated by approximately 100 Km (Fig. 1). A view of the natural stands of *Pistacia vera* L. at Agachli and Kepele is shown in figures 2 and 3, respectively. Seeds were collected from individual trees and in bulk from a mixture of several trees (Table 1). Seeds were germinated and the seedlings were planted in a live germplasm collection plot at the Jacob Blaustein Institute for Desert Research (BIDR), Sede Boker, Israel (Golan-Goldhirsh and Kostiukovsky, 1998). Planting was done in the fall of 1997. Seedlings development was followed by measurements of plant height and trunk diameter, twice a year.

(Figs. 2 & 3)

DNA extraction

Young leaves of 27 accessions of *P. vera* L., representing the two populations, Kepele (10 accessions) and Agachli (17 accessions) (Table 1), were collected for random amplified polymorphic DNA analysis (RAPD). DNA was extracted according to Doyle and Doyle (1987) with the modification of Hormaza et al. (1994).

RAPD analysis

A total of 25 decamer oligonucleotides primers (Operon Technologies, Inc.) were tested for quality of amplification products. Of the 25 primers, 8 were selected for RAPD analysis (Table 2). Total PCR volume was 25 μL, containing 20 ng DNA, 0.1 mM dNTP, 2 mM $MgCl_2$, and 1U of Taq DNA polymerase (Bioline). The amplification reactions were carried out in a BIO-RAD

iCycler under the following conditions: 94 ^{0}C for 3 min, 1 cycle; 94 ^{0}C for 1min, 35 ^{0}C for 1 min, 72 ^{0}C for 2 min, 45 cycles; 72 ^{0}C for 7 min, 1 cycle. Amplified products were analyzed by electrophoresis in a 1.2% agarose gel, after staining with ethidium bromide (0.5 μg/mL), the gel was viewed under UV light. All reactions were repeated twice and only reproducible bands were scored for statistical analysis.

Statistical Analysis

The amplified bands were scored as 1 (present) or 0 (absent). A pairwise similarity matrix was constructed using simple matching coefficient function of the Numerical Taxonomy and Multivariate Analysis System program package for PC (NTSYS-pc, version 2) (Rohlf, 1998). Dendrogram of the 27 accessions was constructed by NTSYS-pc, by applying the unweighted pair group method with arithmetic averages (UPGMA) to the similarity matrix (Nei, 1987).

Differences between accessions in trunk diameter and height were analyzed by ANOVA with repeated measures using SYSTAT software. Based on the correlation coefficients, achieved by regression analysis for trunk diameter and plant height separately, a similarity matrix for trunk diameter and plant height was constructed using NTSYS-pc. A Mantel test, with 1000 permutations (NTSYS-pc software), was used to compare the later to the genetic RAPD matrix.

Results

Plant growth

The average height and trunk growth of *Pistacia vera* L. seedlings, in the live germplasm collection at BIDR, is shown in figure 4. Height growth rate of Agachli seedlings was 0.13 cm/day compared to 0.10 cm/day of Kepele seedlings. There was a significantly (p=0.046) faster height growth rate of seedlings from Agachli than from Kepele. A higher growth rate of trunk diameter was obtained in Agachli accessions (0.025 mm/day), compared to Kepele accessions (0.021 mm/day). This difference was significant at p=0.062.

(Fig. 4)

RAPD analysis

A total of 62 bands were scored, they ranged in size between 300 bp to 2 kb. Each of the primers amplified 6-12 fragments. In total there were 27 polymorphic fragments (Table 2).

(Table 2)

The similarity matrix among the 27 accessions showed high similarity values (0.58-1.0), indicating close genetic relationship among accessions. The lowest similarity value (0.58) was obtained between the accessions A17 and K4, and the highest value (1.00) was obtained between the accessions A7 to K10 (Table 3). Cluster analysis divided 24 of the 27 accessions into 2 groups according to their geographic population origin (Fig. 5). Accessions that originated from Agachli were further clustered in two subgroups. Interestingly, two accessions, K9 and K10, developed from two different individual trees in Kepele, were grouped together with Agachli accessions, showing higher genetic similarity to Agachli than to Kepele accessions (Table 1, Fig. 5). Two exceptional accessions, A12 and A17 were outside the two main populations clusters, which may point to unique genotypes. Kepele accession K4 was clustered separately from the rest in a disconnected outgroup (Fig. 5). Mantel test was used to compare the RAPD genetic matrix to the morphological traits matrix, and revealed a significant relationship between the two matrices (p=0.4745, out of 1000 random permutations).

(Table 3)

(Fig. 5)

Discussion

Genetic and morphological results showed some differences between Agachli and Kepele *Pistacia vera* L. accessions. Cluster analysis divided 24 of the 27 accessions in two genetic groups, according to their geographic origin. Exceptional were two Kepele accessions that clustered in two of the Agachli subgroups (Fig. 5). This, along with the small genetic distance between and

within the two populations, i.e. high similarity values (0.58-1.00), expressed the close relationship between all accessions. The sparse semisavanna formation of Turkmen *P. vera* L. (Popov, 1994), and dependence on wind pollination for reproduction suggested plausible gene flow via movement of pollen between the two populations.

The average plant height growth rate of Agachli accessions was 1.3 times higher than Kepele accessions and 1.2 times higher for trunk diameter growth rate (Fig. 4). These differences were found significant at 4.6% and 6.2% levels, respectively. The significantly faster growth rate of Agachli seedlings is an important trait that should be considered in further selection for cultivation.

The significant correlation between the genetic and the morphological traits matrices is indicative of the genetic basis for the observed difference between the populations. The fact that all the plants under study, are grown on the same plot under the same environmental conditions excluded effects of environmental conditions on differences in plant growth parameters, and thus the results may reflect ecotypic differences. It is interesting to note that there are reports which showed differences between molecular markers and morphological characteristics e.g., genotypes of *Fragaria virginiana* ssp. *gluaca* (S. Wats.) in northern USA (Harrison et al., 2000), and various varieties of *Hordeum vulgare* L. different in their resistant to barley mild mosaic virus (Bahrman et al., 1999).

The remaining last natural habitats of *P. vera* L. in central Asia were facing extinction due to over-grazing and use for fire wood, leading to loss of biodiversity and as yet untapped germplasm. Therefore, the establishment of the Badghyz natural reserve by the Turkmen government, and scientific research to characterize the biodiversity of *P. vera* L. is the first step in saving this natural resources. An ecogeographical approach, accompanied by a molecular marker approach, create the means to achieve an appropriate allocation of sampling within and among regions, for better handling of germplasm collections (Schoen and Brown, 1993; Harrison et al., 2000).

Continued evaluation of developmental and genetic parameters of *Pistacia* germplasm will be helpful in selection of useful genotypes for crop improvement.

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Table 1. Accessions of *Pistacia vera* L., their origin and abbreviations

Population	Origin*	abbreviation
Kepele	Mix 16	K1
	Mix 17	K2
	Mix 18	K3
	Mix 19	K4
	Mix 20	K5
	Mix 23	K6
	Mix 24	K7
	Mix 25	K8
	Kep 4-15	K9
	Kep 14-43	K10
Agachli	Aga 28-26	A1
	Aga 28-28	A2
	Aga 28-29	A3
	Aga 28-30	A4
	Aga 24-42	A5
	Aga 26-46	A6
	Mix 31	A7
	Mix 32	A8
	Mix 33	A9
	Mix 34	A10
	Mix 35	A11
	Mix 35	A12
	Mix 36	A13
	Mix 37	A14
	Mix 38	A15
	Mix 39	A16
	Mix 41	A17

* Mix- seedlings originated from a collection of bulk of seeds from several trees

Kep, Aga- seedlings originated from seeds of separate individual trees

Table 2. Primers used for RAPD analysis and the number of scored bands

Primer	5'-3' sequence	Total bands	Polymorphic bands
OPA-08	GTGACGTAGG	7	3
OPA-09	GGGTAACGCC	7	3
OPA-12	TCGGCGATAG	7	3
OPC-06	GAACGGACTC	7	3
OPC-19	GTTGCCAGCC	7	4
OPD-05	TGAGCGGACA	9	5
OPD-07	TTGGCACGGG	6	2
OPE-06	AAGACCCCTC	12	4
Total		62	27

Table 3. Matrix of similarity among 27 accessions* of Turkmen *Pistacia vera* L. obtained by using a simple matching coefficient

	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17
K1	1.0																										
K2	0.8	1.0																									
K3	0.8	0.7	1.0																								
K4	0.6	0.6	0.7	1.0																							
K5	0.8	0.8	0.8	0.7	1.0																						
K6	0.8	0.8	0.8	0.7	0.9	1.00																					
K7	0.7	0.8	0.8	0.7	0.8	0.90	1.00																				
K8	0.8	0.9	0.7	0.6	0.8	0.89	0.95	1.00																			
K9	0.8	0.8	0.8	0.7	0.7	0.77	0.77	0.82	1.0																		
K10	0.8	0.7	0.8	0.6	0.8	0.82	0.82	0.84	0.8	1.00																	
A1	0.8	0.8	0.8	0.6	0.8	0.82	0.82	0.87	0.8	0.94	1.00																
A2	0.8	0.7	0.8	0.6	0.7	0.76	0.85	0.84	0.9	0.84	0.84	1.0															
A3	0.8	0.8	0.8	0.7	0.8	0.92	0.89	0.87	0.8	0.90	0.90	0.8	1.00														
A4	0.7	0.7	0.7	0.6	0.7	0.74	0.74	0.76	0.9	0.82	0.79	0.8	0.82	1.0													
A5	0.7	0.7	0.8	0.7	0.8	0.87	0.87	0.85	0.8	0.95	0.89	0.8	0.95	0.8	1.0												
A6	0.7	0.7	0.8	0.6	0.7	0.77	0.84	0.82	0.8	0.85	0.79	0.9	0.85	0.8	0.9	1.0											
A7	0.8	0.7	0.8	0.6	0.8	0.82	0.82	0.84	0.8	1.00	0.94	0.8	0.90	0.8	0.9	0.8	1.0										
A8	0.7	0.8	0.8	0.7	0.7	0.79	0.85	0.87	0.8	0.84	0.87	0.8	0.87	0.8	0.8	0.8	0.8	1.0									
A9	0.7	0.7	0.8	0.6	0.7	0.76	0.79	0.74	0.8	0.84	0.77	0.8	0.84	0.8	0.8	0.8	0.8	0.8	1.0								
A10	0.8	0.8	0.7	0.6	0.8	0.81	0.81	0.85	0.8	0.89	0.92	0.8	0.89	0.8	0.9	0.8	0.8	0.8	0.8	1.0							
A11	0.7	0.8	0.8	0.7	0.8	0.81	0.84	0.89	0.9	0.89	0.89	0.8	0.89	0.8	0.9	0.8	0.8	0.9	0.8	0.9	1.0						
A12	0.6	0.7	0.7	0.6	0.6	0.69	0.76	0.74	0.7	0.74	0.77	0.8	0.74	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	1.0					
A13	0.7	0.7	0.7	0.6	0.7	0.81	0.84	0.82	0.8	0.89	0.89	0.9	0.89	0.8	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.0				
A14	0.7	0.7	0.7	0.6	0.8	0.81	0.81	0.85	0.8	0.89	0.89	0.8	0.89	0.8	0.9	0.8	0.8	0.8	0.8	0.9	0.9	0.7	0.9	1.0			
A15	0.8	0.8	0.8	0.6	0.8	0.82	0.85	0.81	0.8	0.87	0.81	0.8	0.90	0.8	0.9	0.8	0.8	0.8	0.9	0.8	0.8	0.7	0.8	0.8	1.00		
A16	0.7	0.8	0.8	0.6	0.8	0.79	0.85	0.87	0.8	0.90	0.84	0.8	0.87	0.8	0.9	0.9	0.9	0.8	0.8	0.9	0.9	0.7	0.8	0.8	0.94	1.0	
A17	0.7	0.7	0.7	0.5	0.7	0.77	0.74	0.76	0.7	0.73	0.73	0.7	0.76	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.76	0.7	1.0

*For abbreviations, see table 1

Fig. 1. Map of Turkmenistan showing seed collection sites at Kepele and Agachli

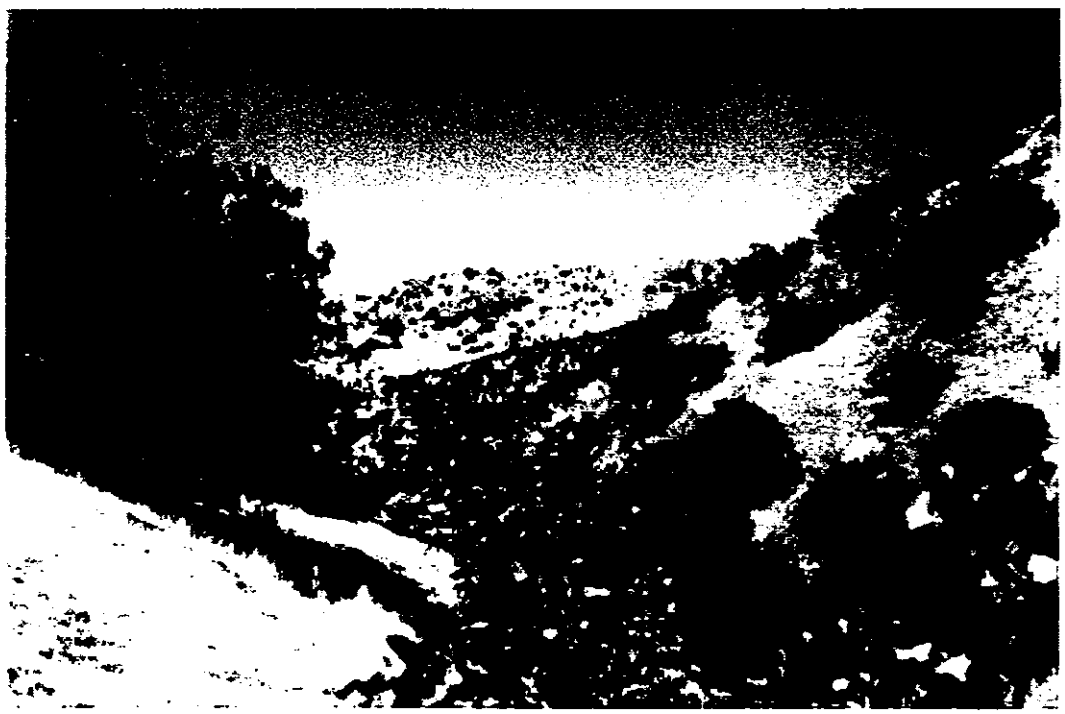


Fig. 2. Natural population of Turkmen *Pistacia vera* L. at Agachli

Fig. 3. Natural population of Turkmen *Pistacia vera* L. at Kepele

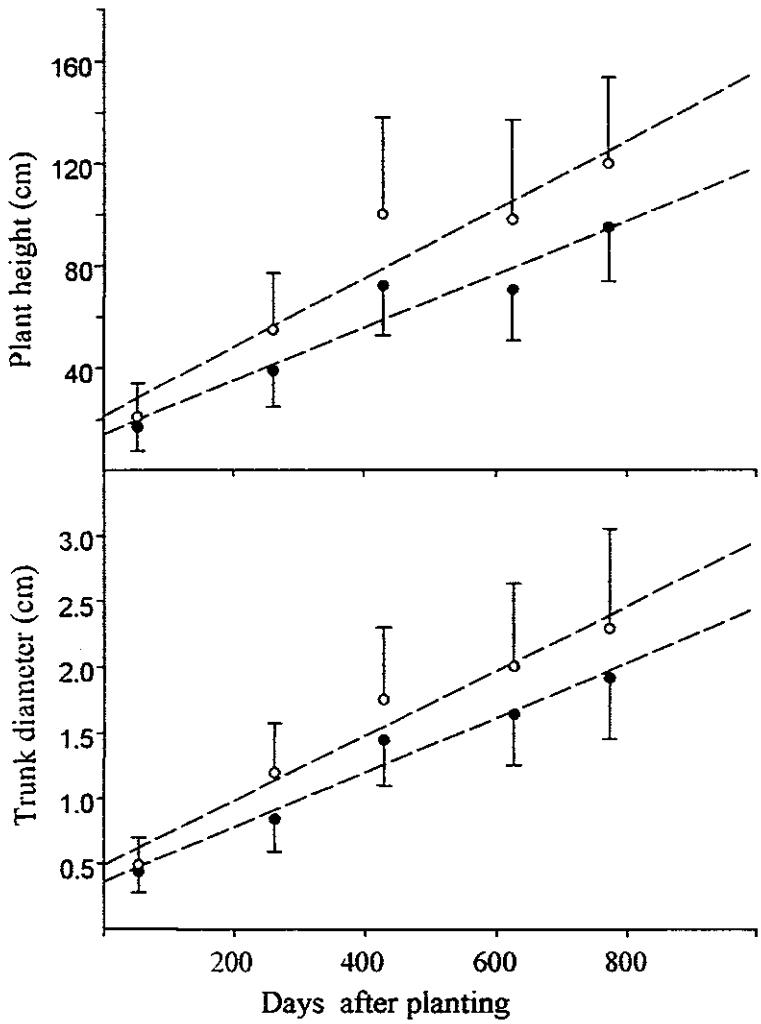
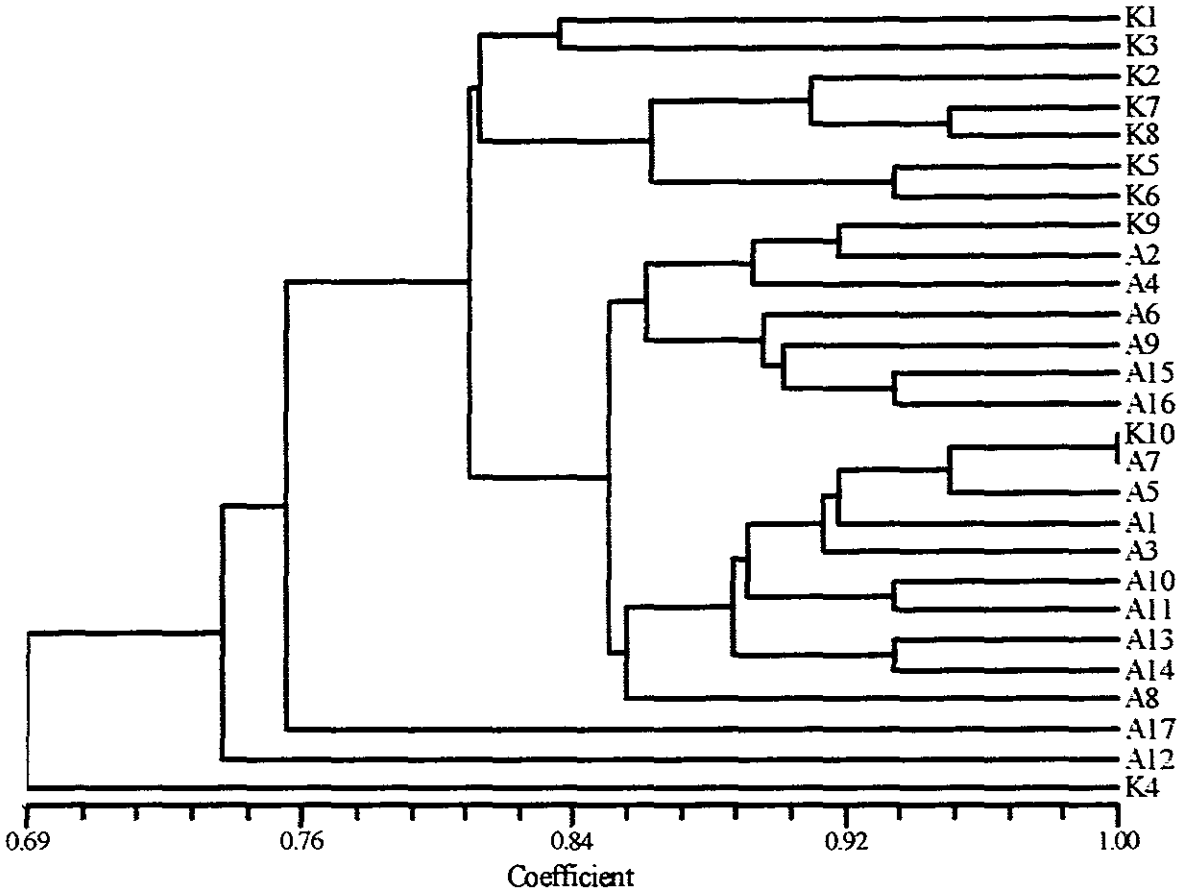


Fig. 4. The average plant height (cm) and trunk diameter (cm) of *Pistacia vera* L. accessions of Agachli (#) and Kepele (!)


K1
K3
K2
K7
K8
K5
K6
K9
A2
A4
A6
A9
A15
A16
K10
A7
A5
A1
A3
A10
A11
A13
A14
A8
A17
A12
K4
0.69
0.76
0.84
0.92
1.00
Coefficient

COMPARISON OF MEDITERRANEAN *PISTACIA LENTISCUS* GENOTYPES BY RANDOM AMPLIFIED POLYMORPHIC DNA, CHEMICAL AND MORPHOLOGICAL ANALYSES

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Abstract- Characterization of the genetic variability of Mediterranean *Pistacia lentiscus* by RAPD, chemical composition of volatiles and morphology is presented. High polymorphism in morphological parameters was found among accessions, with no significant differences in relation to geographical origin, nor to gender. GC-MS analysis of leaves extracted by tert-butyl methyl ether, showed 20 monoterpenes and sesquiterpenes. Cluster analysis divided the accessions into two main chemical groups according to the relative content of the major compounds, with no relation to their geographical origin. In contrast, a dendrogram constructed based on RAPD analysis gave two main clusters according to their geographical origins. Low correlation was found between the genetic and the volatiles content matrices. The high morphological and chemical variability on one hand and the genotypic polymorphism on the other hand, provide ecological advantages that might explain the distribution of *Pistacia lentiscus* over a wide range of habitats. The plants under study are grown together in the same climatic and environmental conditions, thus pointing to the plausible genetic basis of the observed phenotypic differences.

Key Words- Essential oil, genetic diversity, germplasm, morphology, *Pistacia lentiscus*, RAPD

INTRODUCTION

Pistacia lentiscus L. (Anacardiaceae), an evergreen dioecious shrub, is widely distributed along the Mediterranean basin shores (Zohary, 1952). It is a member of a heterogeneous family of eleven species. Morphological characteristics, e.g. leaf partition, leaf rachis, shape and texture of leaflets, presence or absence of terminal leaflet, inflorescence, and fruit are used to differentiate between the species. Zohary (1952), in his monographic study of the genus included *P. lentiscus* in the section *EU Lentiscus*, and disputed the varietal subdivision of this species, that was based mainly on size, shape and number of leaflets.

P. lentiscus (mastic tree) is well known in the Mediterranean countries for its resin, the mastic gum, used since antiquity for incense, as a chewing gum for pleasant breath, for spicing liqueurs and jam, and in the cosmetic industry (Browicz, 1987). Therapeutic effect of mastic gum was demonstrated in healing of duodenal ulcers (Al-Habbal et al., 1984; Al-Said et al., 1986). Antimicrobial activity of the essential oil and resin was also reported (Prokopios et al., 1999). The resin is found in ducts in the bark and leaves. In the Greek island Chios, *P. lentiscus* (referred as *P. lentiscus* var. *chia* by Browicz, 1987), is cultivated and vegetatively propagated by the locals that collect the resin from the bark (Browicz, 1987).

Chemical analysis of the resin and/or essential oils of *P. lentiscus* were reported from different locations around the Mediterranean basin, Spain (Boelens and Jimenz, 1991), Greece (Papageorgiou et al., 1991), Israel (Fleisher and Fleisher, 1992), Corsica (Castola et al., 2000), and Egypt (De Pooter et al., 1991). Comparison of these reports revealed a vast variation in the chemical composition of the leaf essential oils. α-Pinene and myrcene were among the major components in Spanish resin (Boelens and Jimenz, 1991). In

Corsica, leaf oil is composed mainly by tepinene-4-ol and α-pinene (Castola et al., 2000). In Greece, α-pinene constituted 58.9-70% of the essential oil of mastic gum (Papageorgiou et al., 1991). Analysis of the essential oil from Mt. Carmel, Israel revealed mainly high relative content of α-terpineol (Fleisher and Fleisher, 1992). Cultivated *P. lentiscus* accessions grown in Egypt, had car-3-ene as the major component, constituting 65% of the essential oil (De Pooter et al., 1991).

P. lentiscus is a leading component of the low altitude Mediterranean maquis. Its distribution around the Mediterranean basin is extending to North and Eastern Africa and Madeira Island (Zohary, 1996). It was suggested that ecotypic differentiation enabled Israeli *P. lentiscus* to grow in diverse habitats (Shaviv, 1978). At the J. Blaustein Institute for Desert Research (BIDR), located in the Negev desert, Sede Boker, Israel, a live germplasm collection of *Pistacia* spp. was established (Golan-Goldhirsh and Kostiukovsky, 1998). Accessions of *Pistacia lentiscus* collected from different locations around the Mediterranean basin (Israel, Cyprus, Spain and Tunisia) are growing together under similar conditions. The germplasm therefore offers the possibility to assess the genetic basis for the phenotypic variation. Here we report the comparison of chemical and morphological variations among accessions and the use of random amplified polymorphic DNA (RAPD) analysis to evaluate the genetic differences among Mediterranean *P. lentiscus* growing in the germplasm collection.

Methods and Materials

Plant Material. Fruits of *Pistacia lentiscus* were collected during 1995 in 4 locations, Israel at Adoraim, Cyprus at Emba, Limasol area, Spain at Lleida Province and from 3 locations in Tunisia, Aindrham, Takrouna, and Ain Sebaa (Table 1). After germination, seedlings were planted in the *Pistacia* sp. germplasm collection at BIDR (Golan-Goldhirsh and Kostiukovsky, 1998). Plants in the collection are grown in a 3x4 meter distance in row and between rows, respectively. During summer, plants are irrigated by a controlled drip irrigation system, approx. once a week. In winter, run-off is supplemented with irrigation once every 2 to 3 weeks.

All analyses were conducted on male and female trees of 16 accessions of *Pistacia lentiscus* from Spain (6 accessions), Tunisia (6), Cyprus (2) and Israel (2).

RAPD Analysis. A total of 120 primers were pre-tested for the study of the genetic relations among *Pistacia* species, among them 39 revealed polymorphism (data not shown). Of the 39 primers, 9 primers, showed polymorphism among individuals of *P. lentiscus.* Those were selected for further RAPD analysis with all 16 accessions (Table 1). DNA sample from *P. atlantica* was used in the RAPD analysis as an out-group species.

DNA extraction followed the protocol reported by Doyle and Doyle (1987) with the modofocation of Hormaza et al. (1994). For PCR amplification, a 25 µl reaction mixture contained 10 mM Tris-HCl (pH 8.0), 20 ng of genomic DNA, 0.1 mM of each dNTP, 50 mM KCl, 2 mM $MgCl_2$ and 1 U of Taq DNA polymerase (Bioline). The amplification reactions were carried out in a BIO-RAD iCycler under the following conditions: 94 ^{0}C for 4 min, 1 cycle; 94 ^{0}C for 1 min, 35 ^{0}C for 1 min, 72 ^{0}C for 2 min, 45 cycles; 72 ^{0}C for 7 min, 1 cycle. Amplified products were analyzed by electrophoresis in a 1.2% agarose gel, after staining with ethidium bromide (0.5 µg/ml). The gel was viewed under UV

light. All reactions were repeated twice and only reproducible bands were scored for statistical analysis.

Chemical Analysis. The essential oil composition of *Pistacia lentiscus* was determined by GC-MS analysis of methyl *tert*-butyl ether (MTBE) extract of the leaves. Leaves were shaken in MTBE in a ratio of 1:5 w/v, respectively, containing 10 μg per ml isobutylbenzene as an internal standard for 2h and kept in the solvent overnight. The extract was cleaned by passing it through a small column (Pasteur pipette) containing anhydrous Na_2SO_4 and silicic acid (Silicagel 60, 230-400 mesh, Merck), to dry the sample and remove high molecular weight polar substances that interfere with the GC-analyses.

A Hewlett Packard G 1800 B GCD GC-MS system, with an electron ionization detector, was used for the essential oils analysis. Diluted MTBE extracts were injected to the Rtx-5SIL MS column equilibrated with He gas at a flow rate of 1.0 ml/min. Temperature was programmed to start at 70°C for 2 min, then a gradient 4°C/min from 70 to 200°C. The injector temperature was set to 250°C, the detector to 280^0C. The scanning range was recorded from 45 to 450 m/z, with an ionization energy of 70eV. Identification of the main components was done by co-injection of authentic standards and comparison of the EI-MS obtained from authentic standards and complemented with computerized libraries. Quantification of the compounds was done based on total ion chromatographic peak size, as related to the internal standard.

Morphological Measurements. Morphological traits, quantitative and qualitative, were measured and evaluated. For each accession, 5 measurements were done of leaf length, number of leaflets per leaf, leaf length, leaflet length and width. Qualitative traits evaluated were: leaflet shape, color and texture. Color and texture were assessed

according to an evaluation scale, 1 (light green) to 5 (dark green) for color, and from 1 (flexible) to 5 (leathery) for leaf texture.

Data Analysis. RAPD data as well as the relative content of essential oil compounds were subjected to similarity matrix and cluster analyses, using the Numerical Taxonomy and Multivariate Analysis System program package for PC (NTSYS-pc, version 2). The relative content of essential oils constituents were normalized by transformed values (arcsin), and the similarity index was constructed using similarity for interval data with Euclidean distances. For RAPD analysis, amplified bands on a gel were scored as 1 (present) or 0 (absent), and a similarity matrix was constructed using simple matching coefficient function. Both dendrograms were constructed by applying the unweighted pair group method with arithmetic averages (UPGMA) to the similarity matrix (Nei, 1987).

Mantel test, with 1000 permutations was conducted by NTSYS-pc to compare the chemical and genetic similarity matrices.

RESULTS

RAPD Analysis. A total of 79 bands were scored, ranging between 300 bp to 2 kb, an average of ca. 9 bands for each primer (Table 2). Seventy out of the 79 scored bands were polymorphic (Table 2).

Similarity values, showing genetic relations among the accessions are presented in table 3. High similarity values were obtained among the Spanish accessions (0.75-0.89), Tunisian (0.81-0.90), Israeli (0.94) and Cyprian accessions (0.86). High similarity values were found between Tunisian and Spanish accessions, (0.67-0.86), indicating close relations between *P. lentiscus* from these two countries. The Israeli accessions showed

higher similarity values to Tunisian accessions (0.69-0.80) than to the Spanish accessions (0.58-0.73). The Cyprian accessions showed lower similarity values to all other accessions (0.56-0.73). The similarity values between all 16 accessions and *P. atlantica*, as an out-group, was relatively low (0.35-0.45) (Table 3).

A dendrogram, divided the 16 accessions into two distinct genetic groups (Fig. 1). One group included the Tunisian, Spanish and Israeli accessions and the second genetic group the Cyprian. The Tunisian accessions were clustered together, in the same phylogenic subgroup of the Spanish accessions (Fig. 1). The Israeli accessions were genetically closer to the later two than to accessions from Cyprus. *P. atlantica* was in a separate outgroup, distinctly different from the rest (Fig. 1).

Out of 1000 permutations, Mantel test, conducted to analyze the relation between the genetic and chemical content matrices indicated low correlation (R=-0.25) with no significant relation between the matrices (P=0.02).

Chemical Composition of the Volatile Fraction of Pistacia lentiscus. GC-MS analysis of MTBE extract from leaves of *Pistacia lentiscus* showed 20 main constituents, including 14 different monoterpene hydrocarbons and 6 sesquiterpene hydrocarbons (Table 4). Among them, α-pinene, sabinene, limonene, caryophyllene and germacrene D were the major compounds (Table 4). The relative content of the five main compounds indicates close chemical resemblance among geographically distinct Mediterranean *P. lentiscus* (Table 4). Nevertheless, minor compounds were specific to certain accessions. α-Muurolene was found in a relatively low concentration (0.4-0.8%) just in 4 of the Spanish accessions (Sp-m2, Sp-f2, Sp-f3, Sp-f4). cis-β-Ocimene was present in three accessions, an Israeli female (0.15% of total volatiles) and in two Tunisian accessions

(Tn-m2, Tn-f1) (relative content of *ca.* 1.7%). In addition, high relative content of α-thujene (11-12.3%) was found in two Spanish accessions, Sp-f3 and Sp-m1 (Table 4).

Cluster analysis divided the 16 accessions into 2 main chemical groups according to the relative content of one of the five main compounds (Fig. 2). The first, was characterized by high relative content of the monoterpene limonene (28.7-45.5%), and was further divided into two chemical subgroups: (I) limonene/α-pinene, included the Israeli and Cyprian male accessions, exhibiting high relative content of α-pinene (13.8-24.1%); (II) limonene/caryophyllene subgroup, included two Spanish (Sp-m2, Sp-f1) and Israeli female accessions, exhibiting high content of the sesquiterpene caryophyllene (13.2-22.4%). The second main chemical group was divided into 3 subgroups: (I) The Cyprian female and two Tunisian (Tn-f1, Tn-m2) accessions were clustered together in α-pinene chemical group (35.1-49.0%). (II) Characterized by relatively high concentration of sabinene (24.1-45.8%) four Tunisian (Tm1, Tn-f2, Tn-f3, Tn-f4) and two Spanish (Sp-m1, Sp-f3) accessions were clustered together. (III) Germacrene D chemical group (41.1-45.9%), clustering two Spanish female accessions (Sp-f4, Sp-f2) (Fig. 2).

Four of the six Spanish accessions were clustered in separate subgroups within each of the limonene dominant and the sabinene dominant groups (Fig. 2). None of the 6 Tunisian accessions was clustered in the limonene group, nor the Israeli or the Cyprian accessions in the sabinene chemical subgroup II (Fig. 2). In addition, α-muurolene was found in a low relative concentration (0.4-0.8%) just in 4 of the Spanish accessions; cis-β-ocimene was present in three accessions, one Israeli (0.15% of total volatiles) and two Tunisian (Tn-m2, Tn-m1) accessions (relative content of ca. 1.7%) (Table 4). High relative content of α-thujene (11-12.3%) was present in two Spanish accessions, Sp-f3

and Sp-m1 (Table 4).

Morphological Characteristics. The number of leaflets pairs ranged from 2-3 in Cyprian accessions, 3-4 in the Israeli, and 4-6 in both Spanish and Tunisian accessions (Table 5). While in 5 accessions (Cy-m, Sp-f1, Tn-m2, Tn-f3, Sp-f3, and Is-m) the number of leaflet pairs was equal in all the counted leaves, in others, variability was found (Table 5). A terminal leaflet was observed in a few leaves of some of the accessions. In some accessions, e.g. Sp-m1, Tn-f2 and others, uneven number of leaflets was also found.

Leaf length ranged from 3.0 cm (Cy-f, Cy-m, Is-f) to 5.8 cm (Tn-m1), and leaflet length and width between 1.94 and 0.62 cm (Sp-m1) to 3.3 and 1.24 cm (Sp-m2), respectively (Table 5). Accession Tn-m1 had the thin trunk diameter (2.3 cm), while Spf-3 was wider (5.6 cm). Plant height ranged from 60 (Cy-f) to ca. 160 cm (Sp-m2), and canopy area from 0.5 (Sp-f4) to 2.5 m^2 (Cy-f). Correlation analysis didn't indicate a significant relation between plant height to canopy area (R= 0.13). However, relatively high correlation coefficient was found, as was expected, between the number of leaflets and leaf length (R=0.67), between leaflet length and leaf length (R=061), and between leaflet width and leaflet length (R=0.76) (Table 6).

The Spanish plant Sp-m2 was distinctly different from all other accessions. It was significantly higher than all other plants (156cm), and had exceptionally long leaf (7.8cm), leaflet length (3.3cm) and width (1.24cm) (Table 5). 'Wing-like' rachis of ca. 1-2 mm was measured in all accessions.

Interestingly, the average length of the compound leaves of male accessions of Spain and Tunisia was longer than that of female accessions (5.5cm vs. 4.5cm, respectively) (Table 5). However, ANOVA analysis conducted to compare between all accessions, when sorted by gender, didn't show significant differences in any of the quantitative

morphological traits, nor when sorted on the basis of geographic origin, or chemical composition.

Leaflet shape ranged from acuminate (e.g. Is-m, Sp-f2) to blunt apex (e.g. Sp-f1, Tn-f4), and from almost lanceolate (e.g. Sp-m1, Tn-f3) to ovate shape (e.g. Tn-f4, Cy-f) (Table 5). Leaves of accessions from Cyprus and Israel were characterized as having the most leathery leaflets, while the others had more flexible texture (Table 5).

DISCUSSION

DNA molecular markers (RAPD), morphological characteristics and leaf essential oil content were used to evaluate the genetic and phenotypic diversity of 16 representatives of Mediterranean *Pistacia lentiscus*.

A phylogenetic tree that was constructed based on the results of RAPD analysis, divided the 16 accessions according to their geographic origin (Fig. 1). Interestingly, the Israeli accessions were genetically closer to Tunisian and Spanish accessions than to the Cyprian accessions. The latter were clustered in a separate group, genetically distinct from the rest (Fig. 1), indicating the genetic isolation of *P. lentiscus* growing in Cyprus and the development of distinctive genotypes.

Differently from the genetic clustering, morphological as well as content of essential oil showed high phenotypic variability among accessions of *Pistacia lentiscus*, and did not indicate differences among the geographic locations (Tables 4-5). Based on the relative content of the 20 constituents of the leaf essential oil, cluster analysis divided the different accessions according to what appears to be chemotypic groups rather then to their geographic origin or gender (Fig. 2). Thus, indicating rather close resemblance in the chemical content of *P. lentiscus* around the Mediterranean. Accordingly, the 16

Mediterranean accessions were clustered in two main chemical groups (Fig. 2). The first was characterized by high relative content of limonene (29-45%). The second, was further divided to 3 chemical subgroups characterized by high relative content of germacrene D (41-46%) and sabinene (24-46%), or α-pinene (35-49%) (Fig. 2, c.f. Table 4).

Mantel test, used to compare the volatiles content to the genetic matrices, indicated a low, not significant relation between the matrices, suggesting that there was no chemotypic differentiation (cf. Fig. 2). However, it is possible that the dominant nature of RAPD markers and the limitation of the techniques to detect polymorphism in cases of heterozygosity (Parker et al., 1998) may account for the low correlation between the matrices. Alternatively, genetic RAPD analysis was correlated with the volatile oil constituents of accessions, like in the cases of *Ocimum gratissimum* (Vieira et al., 2001), and *Cymbopogon* (Sangwan et al., 2001). Therefore, it is possible that using more accessions, and/or additional differential primers will result in better correlation between the two matrices.

The results point to the plausible effect of environmental conditions on phenotypic plasticity. The relatively high content of sabinene and germacrene D was unique in this study compared to previous reports on the content of essential oil of *P. lentiscus* of Spain (Boelens and Jimenz, 1991), Corsica (Castola et l., 2000), Egypt (De Pooter et al., 1991) and Israel (Fleisher and Fleisher, 1992). The effect of stress conditions on the content of essential oil was reported in several studies: Llusia and Penuelas (1998) reported that under drought conditions the total terpenes concentration of *P. lentiscus* was increased by 21.3% compared to control, grown under irrigated conditions. Drought and nutrient deficiency (phosphorus and/or nitrogen) enhanced allocation of fixed carbon into the

biosynthesis of essential oils in *Rosmarinus officinalis* and *Lavandula latifolia* (Ross and Sombrero, 1991). UV-B radiation enhanced the level of most of the major volatiles in *Ocimum basilicum* (Johnson et al., 1999). Therefore, it is possible that under the extreme desert conditions at our germplasm collection in the Negev desert, the concentration of specific compounds (e.g. sabinene, germacrene D) increased. However, to verify this assumption, the effect of environmental conditions on the content of essential oil of *P. lentiscus* should be further studied under controlled conditions.

More definite indication to the effect of the environmental desert conditions on *P. lentiscus* was supplemented by morphological characteristics. Asymmetry is an indicator of environmental stress in both plants and animals (Graham et al., 1993). Therefore, the uneven number of paired lateral leaflets and appearance of terminal leaflet, suggested that *P. lentiscus* is not well adapted to growth under desert conditions. Morphological variability was expressed in most of the analyzed parameters, e.g. leaflets characteristics, plant height, trunk diameter, and canopy area (Table 5). When comparison was done after sorting the accessions by geographic origin, or gender, statistical analysis did not show significant differences in any of the parameters, reflecting the high variability within accessions from each of the geographic regions. Similarly, no morphological differences were observed between genders of *P. lentiscus* in Portugal (Barradas and Correia, 1999).

The large morphological and chemical variability found among accessions of *P. lentiscus* on one hand, and the genotypic differentiation on the other hand, probably contribute to the wide distribution of *P. lentiscus* along a range of habitats around the Mediterranean basin. *P. lentiscus* was found as one of the most drought tolerant plant among other evergreen species of the Mediterranean maquis (Gratani, 1995). Different eco-physiological characteristics as drought resistance, fast regeneration after fires and

resistance to herbivores attribute to the potential of *P. lentiscus* for forestry in our region. Consequently, strengthening the importance in preservation of the genetic variability. However, the data reported in this work is based on the accessions available in the live germplasm collection at BIDR. It is not yet enough to extrapolate from these results to the genetic variability in nature. Further sampling is therefore needed for determination of the genetic variability and the establishment of conservation management practices.

The fact that the plants grow together, under similar conditions reduced the effect of varied environmental conditions on the measured differences. Thus, *Pistacia* spp. live germplasm collection at BIDR enabled the study of the genetic basis of the observed and measured phenotypic polymorphisms. Nevertheless, while genetic analysis separated the accessions according to their geographic origin, no significant phenotypic differences, i.e. content of essential oil and morphological parameters, were observed. Thus pointing to the significance of DNA molecular marker techniques when ecotypic or chemotypic differentiation is discussed.

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TABLE 1 *PISTACIA LENTISCUS* GROWING IN THE GERMPLASM COLLECTION FIELD IN SEDE BOKER AND THEIR ORIGIN

No.	Country	Site of origin	gender	Abbreviation
1	Israel	Adoraim	male	Is-m
2	Israel	Adoraim	female	Is-f
3	Cyprus	Limassol area	male	Cy-m
4	Cyprus	Limassol area	female	Cy-f
5	Tunisia	Aindrham	male	Tn-m1
6	Tunisia	Takrouna	male	Tn-m2
7	Tunisia	Takrouna	female	Tn-f1
8	Tunisia	Ain Sebaa	female	Tn-f2
9	Tunisia	Takrouna	female	Tn-f3
10	Tunisia	Ain Sebaa	female	Tn-f4
11	Spain	Lleida Province	male	Sp-m1
12	Spain	Lleida Province	male	Sp-m2
13	Spain	Lleida Province	female	Sp-f1
14	Spain	Lleida Province	female	Sp-f2
15	Spain	Lleida Province	female	Sp-f3
16	Spain	Lleida Province	female	Sp-f4

TABLE 2. PRIMERS USED FOR RAPD ANALYSIS AND THE NUMBER OF SCORED BANDS

Primer	5'-3' sequence	Total bands	Polymorphic bands
OPA -08	GTGACGTAGG	12	10
OPB-01	GTTTCGCTCC	3	2
OPC-02	GTGAGGCGTC	16	14
OPD-03	GTCGCCGTCA	12	12
OPD-05	TGAGCGGACA	10	9
OPD-07	TTGGCACGGG	8	8
OPD-13	GGGGTGACGA	8	8
OPD-20	ACCCGGTCAC	4	3
OPE-06	AAGACCCCTC	4	4
Total		77	70

TABLE 3. MATRIX OF SIMILARITY VALUES OF *PISTACIA LENTISCUS* ACCESSIONS

Accession*	Is-m	Is-f	Cy-m	Cy-f	Tn-m1	Tn-m2	Tn-f1	Tn-f2	Tn-f3	Tn-f4	Sp-m1	Sp-m2	Sp-f1	Sp-f2	Sp-f3	Sp-f4	P. atlantica
Is-m	1.00																
Is-f	0.94	1.00															
Cy-m	0.68	0.73	1.00														
Cy-f	0.66	0.69	0.86	1.00													
Tn-m1	0.74	0.78	0.65	0.66	1.00												
Tn-m2	0.69	0.75	0.60	0.56	0.85	1.00											
Tn-f1	0.71	0.76	0.64	0.63	0.84	0.84	1.00										
Tn-f2	0.74	0.79	0.64	0.65	0.81	0.84	0.83	1.00									
Tn-f3	0.75	0.80	0.68	0.64	0.83	0.90	0.86	0.86	1.00								
Tn-f4	0.73	0.78	0.63	0.64	0.83	0.88	0.81	0.91	0.88	1.00							
Sp-m1	0.58	0.63	0.58	0.59	0.70	0.68	0.74	0.74	0.70	0.78	1.00						
Sp-m2	0.66	0.71	0.66	0.65	0.76	0.74	0.83	0.83	0.81	0.86	0.86	1.00					
Sp-f1	0.60	0.66	0.59	0.59	0.69	0.69	0.75	0.75	0.71	0.79	0.84	0.83	1.00				
Sp-f2	0.59	0.65	0.58	0.56	0.73	0.73	0.71	0.74	0.70	0.80	0.88	0.86	0.76	1.00			
Sp-f3	0.69	0.73	0.63	0.61	0.78	0.75	0.84	0.81	0.80	0.85	0.83	0.89	0.84	0.83	1.00		
Sp-f4	0.65	0.70	0.65	0.64	0.73	0.68	0.76	0.74	0.73	0.75	0.83	0.84	0.75	0.80	0.83	1.00	
P. atlantica	0.41	0.44	0.38	0.38	0.50	0.44	0.45	0.44	0.48	0.44	0.39	0.41	0.35	0.39	0.38	0.40	1.00

*For abbreviations see table 1

TABLE 4. ESSENTIAL OIL CONTENT OF *PISTACIA LENTISCUS* LEAVES

		Relative content (%) in different accessions*															
		Is-m	Is-f	Cy-m	Cy-f	Tn-m1	Tn-m2	Tn-f1	Tn-f2	Tn-f3	Tn-f4	Sp-m1	Sp-m2	Sp-f1	Sp-f2	Sp-f3	Sp-f4
α-thujene	923	0.00	0.00	0.32	0.00	0.55	0.00	0.00	0.48	0.00	0.00	11.06	0.00	0.00	0.00	12.26	0.00
α-pinene	933	13.77	16.19	24.06	49.03	18.73	42.74	35.08	19.02	21.98	25.95	22.75	5.07	6.51	22.25	19.38	13.20
camphene	950	0.52	0.97	0.00	1.19	0.00	0.90	0.00	0.37	0.00	0.71	2.80	0.00	0.00	0.89	2.68	1.32
sabinene	972	0.74	0.74	1.21	12.19	32.77	12.62	19.86	24.13	42.34	45.83	24.27	10.96	10.17	9.61	28.56	21.66
β-pinene	979	1.85	3.95	7.43	8.54	14.13	4.57	3.04	6.27	5.05	2.03	4.44	0.00	0.86	7.30	3.50	2.14
myrcene	988	1.89	1.09	1.59	1.00	0.82	0.65	1.06	0.79	0.79	1.23	1.09	1.33	1.54	0.35	1.65	0.41
α-phellandrene	1007	1.11	0.35	0.00	0.84	0.65	1.28	2.83	4.75	2.30	1.35	4.03	0.00	1.06	0.00	0.27	0.00
limonene	1029	45.54	29.49	40.56	6.11	5.39	6.69	15.36	9.33	8.05	7.35	3.34	28.73	29.69	1.58	3.66	0.75
β-phellandrene	1031	3.33	4.24	1.18	6.42	4.90	5.56	9.90	8.56	8.21	6.45	3.05	0.87	1.25	1.47	3.06	1.02
β-ocimene Z	1034	0.00	0.15	0.00	0.00	0.00	1.70	1.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
β-ocimene E	1044	0.00	0.57	0.00	0.00	0.62	5.21	5.29	1.94	0.00	1.76	0.00	0.00	1.43	0.71	0.00	0.00
γ-terpinene	1057	0.00	0.00	0.00	0.18	1.03	0.00	0.23	0.55	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00
2-undecanone	1293	1.11	0.69	0.33	0.00	0.78	0.50	0.00	0.00	0.00	0.80	0.00	0.00	0.55	0.60	0.20	0.79
β-elemene	1388	1.11	0.00	2.94	0.00	0.00	0.00	0.86	0.00	0.63	0.00	0.00	0.81	0.81	0.00	0.65	0.91
caryophyllene	1418	5.44	13.16	0.00	3.71	3.73	7.13	1.06	2.78	0.80	3.10	10.73	22.38	20.71	6.05	9.04	6.78
α-humulene	1454	1.26	2.41	1.17	0.66	1.39	0.00	0.00	0.48	0.40	0.00	1.75	3.21	2.93	3.00	1.77	2.73
γ-muurolene	1476	0.00	0.00	0.37	0.00	1.33	0.00	0.00	0.97	0.46	0.00	0.00	1.14	1.03	2.16	1.69	0.98
germacrene D	1480	22.33	26.01	18.84	10.04	11.79	10.45	3.66	18.75	8.25	3.44	10.18	23.68	20.35	41.11	9.67	45.87
α- muurolene	1498	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.00	0.78	0.60	0.37
δ-cadinene	1519	0.00	0.00	0.00	0.00	1.40	0.00	0.00	0.82	0.74	0.00	0.31	1.30	1.12	2.14	1.35	1.07
Total		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Total μg/g		974	545	1181	1084	1305	706	829	671	1347	683	793	904	762	749	1040	1514

*For abbreviations see table 1

TABLE 5. MORPHOLOGICAL CHARACTERISTICS OF MEDITERRANEAN ACCESSIONS OF *PISTACIA LENTISCUS* GROWING AT BIDR

Accession*	Leaf length (cm)	Leaflets			Trunk diameter (cm)	Plant height (cm)	Canopy area (m^2)	Qualitative traits		
		No.	Length (cm)	Width (cm)				texture	color	shape
Is-m	4.00 (0.00)	4.00 (0.00)	2.02 (0.15)	0.82 (0.04)	3.70	79.00	1.75	3	4	1
Is-f	3.40 (0.55)	3.40 (0.55)	2.02 (0.04)	0.86 (0.09)	2.80	93.00	1.90	4	4	1
Cy-m	3.02 (0.04)	2.00 (0.00)	2.56 (0.35)	1.04 (0.05)	3.40	125.00	1.00	5	2	1
Cy-f	3.00 (0.71)	3.20 (0.84)	2.32 (0.70)	1.00 (0.00)	3.60	61.00	2.51	4	3	2
Tn-m1	5.80 (0.45)	5.80 (0.45)	2.22 (0.23)	0.80 (0.07)	2.30	80.00	0.95	2	1	1
Tn-m2	5.00 (0.00)	5.00 (0.00)	2.14 (0.13)	0.80 (0.00)	4.80	96.00	1.30	3	3	1
Tn-f1	5.60 (0.55)	5.60 (0.55)	1.98 (0.27)	0.62 (0.11)	3.60	75.00	1.70	3	2	1
Tn-f2	4.60 (0.55)	4.60 (0.55)	2.26 (0.11)	0.82 (0.08)	2.90	68.00	1.00	2	4	2
Tn-f3	4.00 (0.00)	4.00 (0.00)	2.72 (0.19)	0.76 (0.09)	3.10	77.00	1.40	2	3	1
Tn-f4	4.40 (0.55)	4.40 (0.55)	2.74 (0.18)	1.08 (0.08)	3.80	83.00	1.30	2	3	2
Sp-m1	4.20 (0.37)	5.60 (0.55)	1.94 (0.31)	0.62 (0.13)	3.50	93.00	1.40	2	4	2
Sp-m2	5.60 (0.55)	5.60 (0.55)	3.30 (0.21)	1.24 (0.09)	3.30	156.00	1.95	2	2	1
Sp-f1	4.00 (0.00)	4.00 (0.00)	2.24 (0.05)	0.86 (0.05)	3.30	135.00	2.30	2	3	2
Sp-f2	4.20 (0.45)	4.20 (0.45)	2.26 (0.30)	0.84 (0.11)	3.40	76.00	1.10	3	5	1
Sp-f3	4.00 (0.00)	4.00 (0.00)	2.76 (0.11)	0.92 (0.04)	5.60	82.00	1.85	2	2	2
Sp-f4	5.32 (0.63)	4.80 (0.45)	2.54 (0.25)	0.78 (0.08)	4.50	95.00	0.50	2	3	1

*For abbreviations see table 1

Number in brackets indicate standard deviation of 5 measurements of each accession

TABLE 6. THE CORRELATION COEFFICIENTS AMONG THE DIFFERENT QUANTITATIVE MORPHOLOGICAL TRAITS

	Leaf length	Leaflet no.	Leaflet length	Leaflet. width	Trunk diameter	Height	Canopy area
Leaf length	1.00						
Leaflets no.	0.67	1.00					
Leaflet length	0.61	-0.05	1.00				
Leaflet width	0.32	-0.34	0.76	1.00			
Trunk diameter	0.07	-0.05	0.19	0.03	1.00		
Height	0.28	-0.01	0.46	0.50	-0.04	1.00	
Canopy area	-0.24	-0.19	-0.01	0.24	0.01	0.13	1.00

FIG. 1. Dendrogram of genetic RAPD analysis relations among *Pistacia lentiscus* accessions, obtained by applying unweighted pair group method with arithmetic averages (UPGMA)

For abbreviations, see Table 1

FIG. 2. Dendrogram of essential oil content relations among *Pistacia lentiscus* accessions, obtained by applying unweighted pair group method with arithmetic averages (UPGMA)

For abbreviations, see Table 1

Figure 1

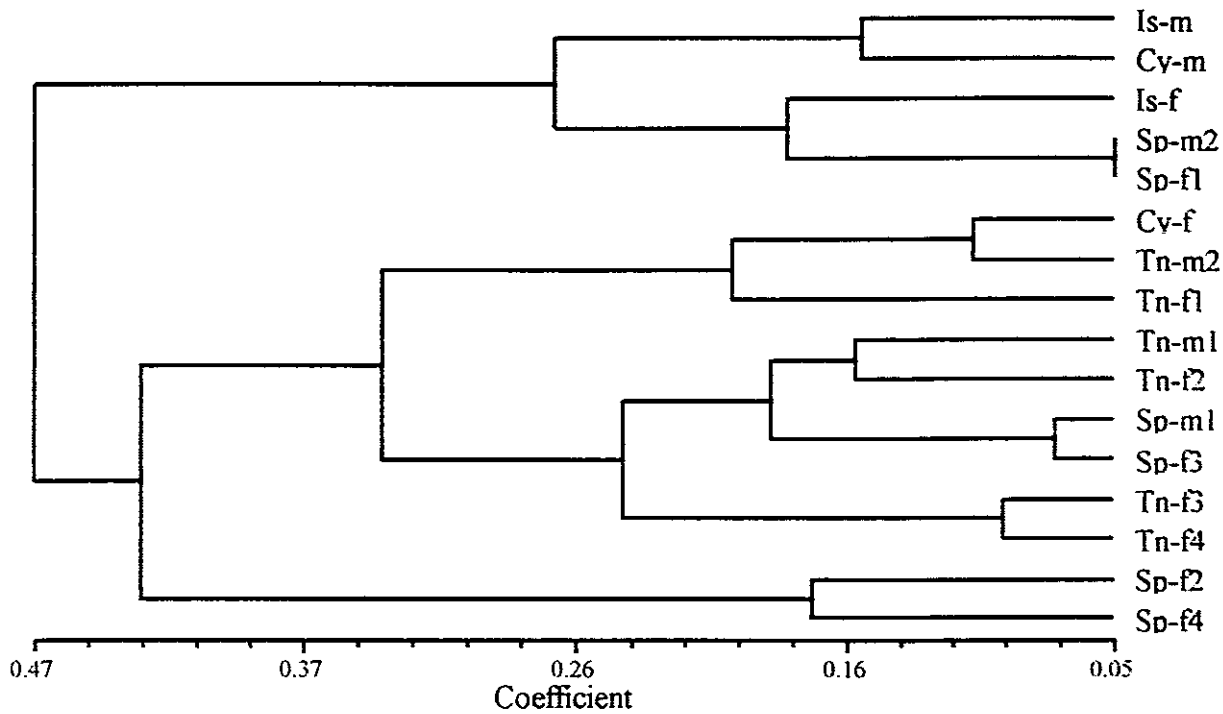
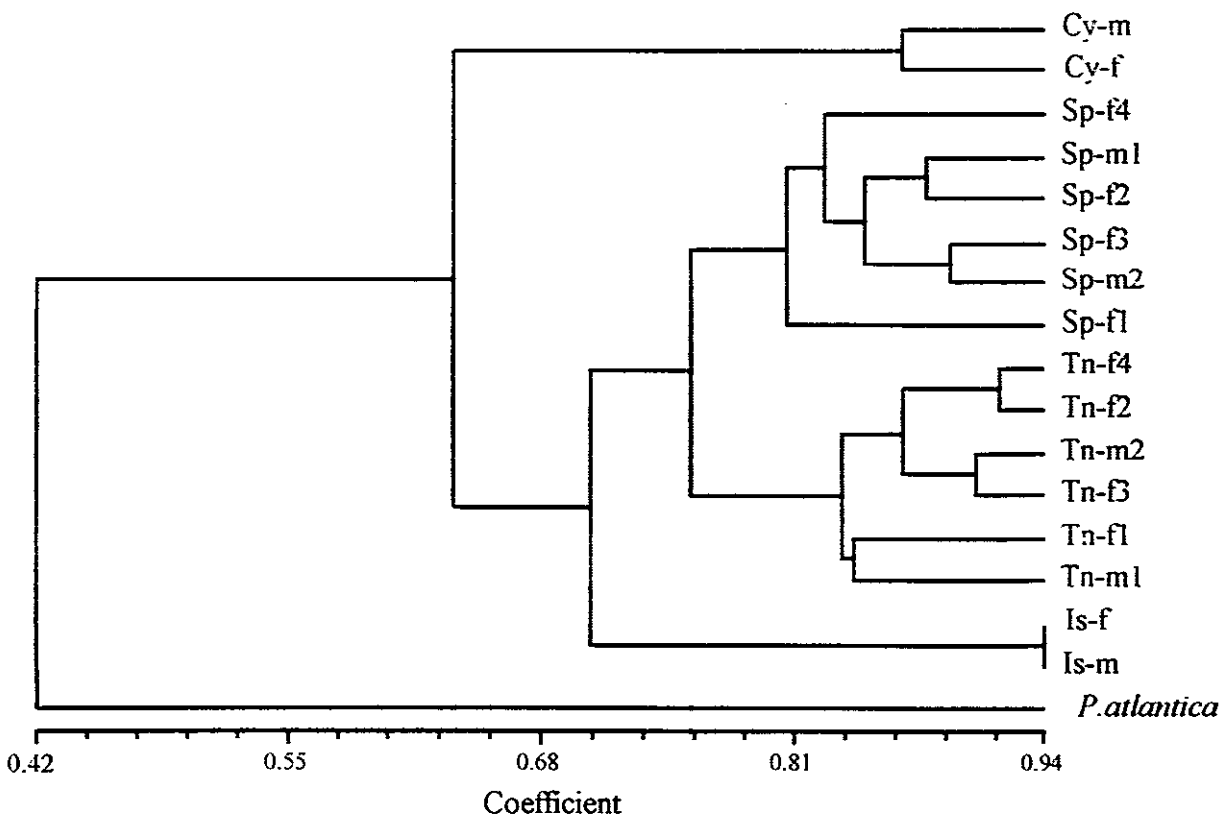


Figure 2



C) Scientific impact of collaboration: Initiation of characterization of Turkmen *Pistacia vera* wild germplasm constituted a major step towards evaluation of this germplasm for future use. Training of Turkmen scientists in the Israeli laboratory will enable establishment of a similar laboratory in Turkmenistan for studies on their plant genetic resources. True collaborative work on samples collection and analysis, preparation of manuscript for publication and preparation of scientific reports will have impact on the ability of the Turkmen group to continue this research and attract more funding

D) Description of project impacts: The project impact can be evaluated at 4 levels. 1. Establishment of the RAPD technique for evaluation of the *Pistacia* germplasm for fingerprinting of unique accessions and evaluation of biodiversity. 2. Beginning of establishment of a Turkmen germplasm collection and characterization of the unique wild accessions available in Turkmenistan. 4. Purchase of equipments and chemicals and their transfer to Turkmenistan, for establishing a laboratory for RAPD analysis at the National Institute of Deserts, Flora and Fauna 3. Training of Turkmen scientists in techniques and methods for development and monitoring of biodiversity and molecular fingerprinting.

E) Strengthening of developing countries institutions: We have purchased essential equipment for the molecular markers analysis by RAPD for the laboratory in Turkmenistan. The instruments are: PCR, Power supply and electrophoretic equipments, automatic pipettes, supplies and chemicals. All the major equipments were purchased from American suppliers and tested in Israel and already transferred to the Turkmen laboratory.

F) Future work:

Major effort will be made to intensify collection of seeds from as diverse as possible locations in Badghyz, Kopedagh Mountains and near the Aphgan border in Turkmenistan. During collection trips we shall mark unique individual trees for yield and tree structure and shape. Morphological and molecular characterization of Turkmen accessions will continue. Seedlings of all accessions selected will be prepared for transfer to the selected site at Ak Tokai for establishment of the Turkmen live germplasm collection. Experiments to test the effect of salinity on *Pistacia vera* seedlings from Turkmenistan in comparison to other *Pistacia* species will be conducted.

Section II

A) Managerial Issues:

Fund transfer, which was problematic in the first year was solved since the introduction of the NGO, Ecoforest channel to the money transfer procedure. Contacts between the groups is maintained via electronic mail and telephone. We expect to have a new trainee from Turkmenistan join the Israeli group in the fall of 2002.

B) Budget:

No budget changes were made.

C) Special Concerns:

No changes

D) Collaboration, Travel, Training and Publications:

Samples of last season collection trips of leaves from various sites in Turkmenistan were prepared for analysis by Mr. Azat Ataev in Turkmenistan and aliquots sent to the Israeli group. A new trainee from Turkmenistan is expected to join the Israeli group in the fall of 2002. The Turkmen PI, Dr. Muhamet Durikov is expected to visit the Israeli group toward the final stages of the project in 2002/03 for summary and planning future scientific collaboration. So far 2 manuscripts were prepared for publication (see manuscripts attached). One was accepted for publication and the second is in the final stages of preparation for submission for publication.