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COLLECTION, CHARACTERIZATION AND EXCHANGE OF *PISTACIA* GENUS GERMPLASM

Principal Investigator: Prof. A. Golan

Grantee Institution: Ben-Gurion University of The Negev (BGU, Israel)

Collaborator: Dr. Konstantin P. Popov

Institution: National Institute of Deserts, Flora and Fauna (Turkmenistan)

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Table of Content | Page

Executive summary	3
Research accomplishments	4
Description of project impacts	16
Strengthening of developing countries institutions	16
Future work	16
Managerial issues	16
Budget, Special concerns and Collaboration	17

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 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.

Upon approval of this project seeds of *P. vera* collected in Turkmenistan were germinated and planted at The '*Pistacia* germplasm collection' at The Jacob Blaustein Institute for Desert Research (BIDR). Growth and development of the seedlings is monitored regularly by measurement of seedling stem diameter, canopy diameter and height. Leaf samples were collected and analyzed for polymorphism among accessions and in comparison to other *Pistacia* species in our collection by the Random Amplified Polymorphic DNA technique (RAPD).

In the course of the first year, in December 1999, Mr. Azat Ataev from the Turkmen group joined the Israeli group for training in germplasm collection and characterization. He has made significant progress in mastering the RAPD technique and he is now in the process of molecular characterization of various Turkmen ecotypes. He has been involved in all aspects of physical characterization of the germplasm collection. The expertise acquired by Mr. Ataev will serve the foundation for development of the Turkmen germplasm collection and characterization of its biodiversity.

Seeds were collected in Turkmenistan, at the Badhiz reserve, in the 1999 season. The Turkmen team has identified and marked special character female trees that will be monitored in the course of the project regularly from season to season. Seeds collected are saved as a seed germplasm collection and part of each accession will be germinated for DNA polymorphism analysis. More seeds of selected individual trees will be collected in the 2000 season in the Kopedagh mountains and near the Aphgan border. From each accession a sample will be saved in the seed bank and a sample will be analyzed by RAPD fingerprinting and polymorphism assessment.

Section I

Research accomplishments

The overall research effort in the first year was directed towards development of the RAPD technique for fingerprinting of the *Pistacia* germplasm collection and starting to develop a model for remote senesing monitoring of natural populations of trees for their sex ratio. The results of the RAPD analysis work is presented below in the form of a manuscript in preparation for publication.

Manuscript in preparation

Genetic Relationships Among *Pistacia* Species Evaluated By RAPD

Abstract

Polymorphisms between Mediterranean basin *Pistacia* species and ecotypes within species were assessed by random amplified polymorphic DNA (RAPD) analysis. Twenty-nine *Pistacia* accessions from 7 species, selected from geographically diverse locations in the Mediterranean area, were analyzed. A total of 302 DNA fragments were amplified by 27 primers. There were 264 (87.5 %) polymorphic fragments. Of these, 108 (35.8 %) were polymorphic for the 12 *P. atlantica* accessions, and 90 (29.8 %) were polymorphic for the 7 *P. lentiscus* accessions. Ten *P. atlantica* species-specific and fourteen *P. lentiscus* species-specific RAPD markers were identified. Cluster analyses of the data showed that these *Pistacia* species could be clustered into two groups, one group containing all the *P. lentiscus* accessions and the second group containing all the other accessions. The latter could be divided into two subgroups, one consisting of *P. palaestina*, *P. terebinthus* and *P. chinensis*; the other consisting of *P. atlantica*, *P. khinjuk* and *P. vera*. The latter two species were highly similar as were *P. palaestina* and *P. terebinthus*.

Introduction

Pistacia is a genus of the *Anacardiaceae*. The main monograph study by Zohary (1952) recognizes 11 species in *Pistacia* based on morphology. The systematic taxonomic division of *Pistacia* recognizes four sections (Zohary, 1952) : Eu Lentiscella Zoh., containing *P. mexicana* HBK, and *P. texana* Swingle; Eu Lentiscus Zoh., containing *P. lentiscus* L., *P. saportae* Burnat., and *P. weinmannifolia* Poisson; Eu Butmela Zoh., containing *P. atlantica* desf.; and Eu Terebinthus containing *P. chinensis* Bge., *P. khinjuk* Stocks, *P. palaestina* Bois., *P. terebinthus*, and *P. vera* L. However, the actual level of speciation and relationships within the genus *Pistacia* still remain unclear. Trees of *Pistacia* are characteristic of the Mediterranean basin flora. Five species of the genus grow naturally in the Mediterranean and Middle Eastern areas, *P. lentiscus* , *P. atlantica* , *P. palaestina* , *P. terebinthus* and *P. khinjuk* . *Pistacia vera* originated in central Asia and is cultivated throughout the Mediterranean region. Research efforts have mainly focused on *P. vera* because of its economic importance, while the other species have been overlooked to a large extent. Some of the other species, e.g. *P. atlantica* and *P. khinjuk*, have been used for rootstocks. A better understanding of the genetic relationships among the species would be an advantage for a breeding program in this genus.

There is almost no cytogenetic or molecular genetic information available on the various *Pistacia* species. Loukas and Pontikis (1979), Barone et al. (1993) and Dollo (1993) conducted isozyme studies with the aim of distinguishing between varieties of *P. vera*, but insufficient isozyme polymorphisms among closely related cultivars has limited its usefulness for analyses of genetic diversity and relatedness. The problem of insufficient polymorphisms can be mitigated by using DNA-based markers such as restriction fragment length polymorphisms (RFLPs) (Tanksley et al., 1989) or random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland 1990; Williams et al. 1990).

The development of RAPD technology has opened a door for the detection and monitoring of genetic variation in plants in a manner that has not been previously possible. It neither requires previous knowledge of any genomic sequence nor tedious procedures such as for RFLP analysis. The usefulness of RAPDs in genotype identification, population and pedigree analyses, phylogenetic studies, and genetic mapping is well documented (Welsh and MaClelland 1990: Martin et al., 1991; Paran et al., 1991; Halward et al., 1992; Mazzarella et al., 1992; Rerter et al., 1992; Vierling and Nguyen 1992; Wilde et al., 1992). Hormaza et al. (1994a) examined genetic diversity and patterns of relatedness among fifteen *P. vera* cultivars by using RAPD markers. Major phylogenetic papers on *Pistacia* species were reported recently by Parfitt and Badenes (1997 and 1998).

The adaptation of the *Pistacia* trees to harsh desert conditions and their longevity make them ideal candidates for reforestation in arid zones. The availability of an extensive germplasm collection of *Pistacia* from various geographical regions around the Mediterranean (Golan-Goldhirsh and Kostiukovsky, 1998) has made it possible to evaluate their genetic relationships and the evolution of the genus.

In the research reported here, the genetic diversity and phylogenetic relationships among Mediterranean *Pistacia*, *P. chinensis* and *P. vera* were analyzed by RAPD markers.

Materials and Methods

Plant material. Twenty-nine Pistacia accessions (Table 1 and Figure 1), representing seven species in our germplasm collection (Golan-Goldhirsh and Kostiukovsky, 1998) were evaluated in this study. M. annua which is a Euphorbiaceae was used as an outgroup.

DNA isolation. Total DNA was extracted from approximately 5g leaf tissue, according to the method of Hormaza et al. (1994a) based on Doyle and Doyle (1987), with minor modifications of the CTAB buffer (100mM Tris-HCl, 1.4M NaCl, 20mM EDTA, 2% CTAB, 1% PVP, 0.2% β-mercaptoethanol).

PCR amplification and electrophoretic analysis of RAPD products. A preselection of suitable decamer oligonucleotide primers (Operon Technologies, Inc.) was done using 120 primers (Operon kits A, B, C, D, E and F) and 3 Pistacia accessions of P. atlantica, P.vera and P. khinjuk . Of the 120 primers tested, 95 (79%) gave some amplification products, while only 39 (32.5%) revealed polymorphisms and good amplification products. From the latter, 27 primers (Table 2) were selected for further RAPD analysis with all 30 accessions analyzed (Table 1). The protocol reported by Hormaza et al. (1994b) was followed with some modifications. Total PCR volume was 25 μl and contained 10 mM Tris-HCl (pH 8.0), 20 ng of total genomic Pistacia DNA, 100 μM of each dNTP, 50 mM KCl, 2 mM $MgCl_2$, 100 mg/ml gelatin, and 1 unit of Taq DNA polymerase (Advanced Biotechnologies Ltd., U.K.). Two drops of light mineral oil were added

to prevent evaporation. The amplification reactions were carried-out in a MJ-Research Inc. (Watertown, MA) Programmable Thermal Cycler under the following conditions: 94°C for 2 min, 1 cycle; 94°C for 45 seconds, 36°C for 1 min, 72°C for 2 min, 45 cycles; 72°C for 5 min, 1 cycle, followed by storage at 4°C. Amplification products were analyzed by electrophoresis in a 1.5% TBE agarose gel, followed by staining with ethidium bromide 0.5 μg/ml and viewing under UV radiation. All reactions were repeated at least two times and only reproducible bands were scored for statistical analysis.

Data analysis.. The amplified bands were scored as 1 (present) and 0 (absent). Only polymorphic amplification bands were used for further analysis. A pairwise similarity matrix and dendrogram were constructed using the Corr and Cluster programs of SYSTAT intelligent software (Wilkinson, 1990), based on Pearson correlation coefficients and Average Linkage methods.

Results

RAPD polymorphism of Mediterranean Pistacia. A total of 302 DNA fragments were amplified by 27 preselected decamer oligonucleotide primers. For each primer used, 6 to 23 polymorphic DNA bands were amplified from a given sample of genomic DNA with an average of 11.2 polymorphic fragments per accession. The fragment size ranged from 300 bp to 2000 bp. Of the 302 DNA fragments, 264 (87.5%) were polymorphic among the 29 Pistacia accessions.

From the 12 P. atlantica accessions analyzed, 135 DNA fragments were amplified, and 108 of them were polymorphic. From seven P. lentiscus accessions analyzed, 125 DNA fragments were amplified and 90 of them were polymorphic. Pistacia atlantica species-specific and P. lentiscus species-specific RAPD markers were also found. An example of amplification products with primer OpC-6 which generated P. atlantica species-specific RAPD marker is shown in Figure 2. Altogether, 10 P. atlantica specific markers and 14 P. lentiscus specific markers were detected by the 27 primers selected.

Genetic similarity among Pistacia accessions analyzed. A similarity matrix among all 30 accessions studied was constructed (Table 3). High similarity values were obtained between the P. vera accessions (26 and 27) and between them and P. khinjuk (28 and 29). The highest similarity value (0.87) between accession 27 (P. vera) and 29 (P. khinjuk) indicate the close relationship between both species. As expected, M. annua, which is the out-group, shared only a small number of markers with Pistacia and had negative Pearson correlation values. The smallest similarity value among all the Pistacia accessions is 0.029, between accession 1 (P. atlantica) and 18 (P. lentiscus).

Within the *P. atlantica* species, the lowest similarity value (0.55) was between accession 9 and 12, the highest similarity value (0.818) was between accession 2 and 5. The latter accessions are from plants growing in Israel; however, accession 2 is from the Galilee (Mediterranean climate) while accession 5 is from the Negev (harsh desert climate) and separated by approx. 300 Km. Accession 9 is from seeds collected in Syria , and accession 12 is from Tunisia.

Among the *P. lentiscus* species accessions, the lowest similarity value (0.529) was detected between accessions 13 and 18, while the highest similarity value (0.794) was between accessions 17 and 19. These values correlate well with their geographical distribution. Accession 13 is from Cyprus, 18 originated in Tunisia and accessions 17 and 19 are both from Tunisia (Figure 1).

Phylogenetic relationships among species as well as within species in the Mediterranean

***Pistacia* genus germplasm collection.** A dendrogram of the 30 accessions was constructed based on the RAPD marker data (Figure 3). The out-group, *M. annua*, represented a separate group as expected. The *Pistacia* accessions could be divided into two major groups, one group containing all the *P. lentiscus* accessions, the second group containing the remaining accessions. The latter could be divided into two subgroups. One consisted of *P. palaestina*, *P. terebinthus* and *P. chinensis* accessions, which could be further divided into 2 groups comprised of (a) *P. chinensis* and (b) *P. palaestina* and *P. terebinthus*. The other consisted of *P. atlantica*, *P. khinjuk* and *P. vera* species, which could be further divided into a group consisting of all the *P. atlantica* accessions and a group consisting of P. *khinjuk* and *P. vera* species.

Within the *P. atlantica* species, accessions 2 and 5, 11 and 6, 4 and 8, as well as 10 and 3 clustered closely (Figure 3). These accessions came from Israel, Spanish germplasm collection, and one accession (8) from Syria (Table 1). Accessions 1 and 9 from Cyprus and Syria, respectively, then clustered more distantly. Finally, accessions 12 from Tunisia and 7 from Syria clustered separately from the others. It appears from the data that the Syrian accessions are genetically more diverse than the others or that they were a source of germplasm for several Mediterranean locations.

Within the *P. lentiscus* species, accessions 17 and 19 clustered most closely (Figure 3). They clustered with accession 18, then with accessions 15 and 16 to form a minor sub-group, with the accessions from Tunisia. This sub-group then clustered with accession 14 from Spain. Accession 13 from Cyprus was last to cluster in this group. These results are in agreement with their geographical distribution (Table 1 and Figure 1).

Discussion

RAPD analysis to study genetic polymorphism in a germplasm collection has an advantage over other methods due to its simplicity and rapidity. The present communication is the first report on the use of RAPD markers to assess polymorphism in Mediterranean *Pistacia* genus germplasm. In these experiments, 264 distinct DNA fragments were produced by 27 primers from *Pistacia*, which implies that 264 loci of the *Pistacia* genome may have been surveyed. The data presented here lay the foundation for future research aimed at using molecular markers to exploit the useful traits of some of the genotypes that we have assessed.

From the total RAPD polymorphic fragments detected in *Pistacia* genus germplasm, approx. 41% were within *P. atlantica*, and 34% within *P. lentiscus*. This indicated that there is a high level of polymorphism in our Mediterranean *Pistacia* genus germplasm collection including *P. atlantica* and *P. lentiscus*. There are two possible reasons for the high polymorphism detected. First, the germplasm in the collection is of diverse origins (Figure 1). Second, the 27 primers were not randomly chosen, they were selected because they showed the most polymorphism out of 120 primers examined using 3 *Pistacia* species accessions.

The dendrogram constructed based on these 27 primers and all the *Pistacia* accessions resulted in 2 major clusters, one of the *P. lentiscus* species and the other remaining species. Of these, *P. vera*/*P. khinjuk* and *P. palaestina* /*P. terebinthus* were very closely clustered, which supported the classification by Zohary (1952) of these species in a separate section, *Eu terebinthus*. All the other species could be separated. *P. atlantica* species was more tightly related to the *P.vera*/*P. khinjuk* group, than to *P. chinensis* and *P. palaestina*/*P. terebinthus* group. The *P. lentiscus* was the most distant species in the Mediterranean *Pistacia* genus. Our results support the suggestion of Parfitt and Badenes (1997), based on chloroplast genome analysis, that the *Pistacia* genus should be divided into two major sections the *terebinthus*

section and the *lentiscus* section, rather than the division into four sections suggested by Zohary (1952) which is based on leaf morphology and geographical distribution.

The RAPD markers of *P. palaestina* and *P. terebinthus* were highly similar. Considering that the two species are similar both morphologically and ecologically (Zohary 1995), our results strongly support the proposal of Engler's (1936) and Yaltirik (1967), that *P. palaestina* should be recognized as a subspecies of *P. terebinthus*.

Also, the *P. vera* and *P. khinjuk* species have a highly similar RAPD fragment pattern. Their close association is consistent with their morphological similarities. *P. khinjuk* and *P. vera* are the only *Pistacia* species with large edible nuts. Both have a similar somewhat unique three-leaflet imparipinnate leaf (Zohary, 1952). These results are in agreement with the chloroplast genome analysis (Parfitt and Badenes, 1997).

It was reported that there are approx. 100 cultivars of *P. vera* worldwide (Maggs, 1973). They are thought to be derived from a few primitive varieties resulting in a high degree of genetic vulnerability. This narrow genetic background cannot meet the needs of breeders working toward improving cultivars for higher yields, better quality, as well as resistance to diseases. For this reason, it is necessary to characterize a larger, more diverse collection of *Pistacia* germplasm with the goal of introducing favorable genes into cultivars from their wild relatives.

At present, there is no published genetic map of *Pistacia* incorporating DNA markers. The first step in developing genetic maps with DNA markers would be to examine the frequency of DNA polymorphism within species and to identify suitable parents showing sufficient DNA polymorphism for efficient mapping. It seems likely that the low level of polymorphism shown within *P. vera* species is not sufficient for the easy construction of a genetic map. Therefore, we suggest employing interspecific crosses for constructing such a map in the future. The data presented in this paper would be useful for determining the best parents for mapping purposes.

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Table 1. Species and accessions of *Pistacia* in this study

Code #	Species	Origin	Abbrev.*
1	*P. atlantica*	Cyprus	AC1
2	*P. atlantica*	Israel	AI2
3	*P. atlantica*	Israel	AI3
4	*P. atlantica*	Israel	AI4
5	*P. atlantica*	Israel	AI5
6	*P. atlantica*	Israel	AI6
7	*P. atlantica*	Syria	AS7
8	*P. atlantica*	Syria	AS8
9	*P. atlantica*	Syria	AS9
10	*P. atlantica*	Spain**	ASp10
11	*P. atlantica*	Spain**	ASp11
12	*P. atlantica*	Tunisia	AT12
13	*P. lentiscus*	Cyprus	LC13
14	*P. lentiscus*	Spain	LS14
15	*P. lentiscus*	Tunisia	LT15
16	*P. lentiscus*	Tunisia	LT16
17	*P. lentiscus*	Tunisia	LT17
18	*P. lentiscus*	Tunisia	LT18
19	*P. lentiscus*	Tunisia	LT19
20	*P. palaestina*	Israel	PI20
21	*P. palaestina*	Israel	PI21
22	*P. palaestina*	Syria	PS22
23	*P. terebinthus*	Syria	TS23
24	*P. terebinthus*	Greece	TG24
25	*P. chinensis*	California	CC25
26	*P. vera*	Syria	VS26
27	*P. vera*	Syria	VS27
28	*P. khinjuk*	Syria	KS28
29	*P. khinjuk*	Syria	KS29
30	*M. annua*	Israel	MI30

* Abbreviation showing the first letter of the species, first letter of country and code # of accession. Note that only in the case of the outgroup *M. annua* the genus first letter was used instead of the species to avoid confusion with the *atlantica* abbreviation

** Spanish germplasm collection

Table 2. Primers used for the RAPD study

Primer	5'-3' sequence	Number of polymorphic markers detected*
OpA-08	GTGACGTAGG	11
OpA-09	GGGTAACGCC	7
OpA-12	TCGGCGATAG	12
OpB-01	GTTTCGCTCC	11
OpC-02	GTGAGGCGTC	6
OpC-06	GAACGGACTC	7
OpC-11	AAAGCTGCGG	8
OpC-19	GTTGCCAGCC	6
OpD-05	TGAGCGGACA	12
OpD-07	TTGGCACGGG	15
OpD-10	GGTCTACACC	7
OpD-13	GGGGTGACGA	11
OpD-15	CATCCGTGCT	10
OpD-19	CTGGGGACTT	12
OpD-20	ACCCGGTCAC	6
OpE-01	CCCAAGGTCC	6
OpE-03	CCAGATGCAC	9
OpE-06	AAGACCCCTC	15
OpE-16	GGTGACTGTG	18
OpE-17	CTACTGCCGT	6
OpE-18	GGACTGCAGA	11
OpF-02	GAGGATCCCT	9
OpF-03	CCTGATCACC	6
OpF-05	CCGAATTCCC	21
OpF-09	CCAAGCTTGG	13
OpF-10	GGAAGCTTGG	4
OpF-20	GGTCTAGAGG	5
Total		264

*The total number of polymorphic fragments including the outgroup was 302

Table 3. Matrix of Similarity among 30 accessions

	AC1	AI2	AI3	AI4	AI5	AI6	AI7	AI8	AI9	AI10	AI11	AI12	IC13	IS14	IT15	IT16	IT17	IT18	IT19	PI20	PI21	PS22	TS23	TG24	CC25	VS26	VS27	KS28	KS29	MI30
AC1	1																													
AI2	0.751	1																												
AI3	0.666	0.739	1																											
AI4	0.734	0.751	0.685	1																										
AI5	0.731	0.818	0.771	0.762	1																									
AI6	0.707	0.793	0.764	0.788	0.752	1																								
AI7	0.561	0.657	0.587	0.588	0.657	0.613	1																							
AI8	0.75	0.786	0.732	0.784	0.763	0.774	0.645	1																						
AI9	0.651	0.739	0.634	0.719	0.682	0.726	0.644	0.7	1																					
AI10	0.691	0.726	0.746	0.742	0.774	0.714	0.606	0.74	0.676	1																				
AI11	0.631	0.736	0.72	0.751	0.749	0.81	0.58	0.75	0.704	0.726	1																			
AI12	0.619	0.619	0.726	0.622	0.617	0.646	0.574	0.703	0.551	0.626	0.561	1																		
IC13	0.089	0.181	0.141	0.175	0.184	0.179	0.174	0.163	0.135	0.131	0.217	0.146	1																	
IS14	0.087	0.159	0.121	0.152	0.18	0.104	0.136	0.143	0.078	0.092	0.141	0.108	0.591	1																
IT15	0.092	0.164	0.107	0.175	0.15	0.163	0.14	0.13	0.101	0.079	0.2	0.075	0.636	0.698	1															
IT16	0.079	0.188	0.131	0.163	0.173	0.15	0.165	0.135	0.124	0.102	0.224	0.117	0.662	0.687	0.713	1														
IT17	0.092	0.144	0.093	0.168	0.162	0.14	0.112	0.113	0.099	0.097	0.195	0.081	0.58	0.672	0.697	0.723	1													
IT18	0.029	0.1	0.061	0.147	0.086	0.099	0.034	0.12	0.038	0.033	0.136	0.017	0.529	0.646	0.727	0.68	0.716	1												
IT19	0.089	0.162	0.122	0.175	0.148	0.161	0.094	0.145	0.08	0.112	0.217	0.089	0.563	0.666	0.729	0.737	0.791	0.751	1											
PI20	0.272	0.326	0.331	0.316	0.31	0.304	0.259	0.295	0.226	0.26	0.29	0.299	0.187	0.221	0.208	0.232	0.206	0.125	0.225	1										
PI21	0.179	0.267	0.217	0.24	0.216	0.28	0.258	0.236	0.186	0.184	0.267	0.222	0.112	0.128	0.115	0.175	0.149	0.105	0.167	0.693	1									
PS22	0.267	0.338	0.308	0.292	0.321	0.298	0.297	0.272	0.221	0.255	0.32	0.241	0.112	0.146	0.151	0.193	0.166	0.105	0.201	0.693	0.787	1								
TS23	0.253	0.328	0.348	0.301	0.312	0.325	0.331	0.331	0.28	0.24	0.346	0.296	0.112	0.123	0.147	0.19	0.115	0.081	0.161	0.736	0.706	0.743	1							
TG24	0.266	0.376	0.342	0.349	0.323	0.354	0.325	0.362	0.255	0.271	0.376	0.31	0.175	0.193	0.179	0.222	0.164	0.169	0.175	0.633	0.716	0.716	0.717	1						
CC25	0.23	0.266	0.305	0.311	0.268	0.299	0.349	0.325	0.237	0.271	0.339	0.273	0.122	0.103	0.182	0.187	0.164	0.135	0.179	0.368	0.399	0.417	0.367	0.418	1					
VS26	0.345	0.382	0.386	0.372	0.383	0.431	0.414	0.405	0.352	0.296	0.382	0.393	0.127	0.07	0.091	0.08	0.063	0.066	0.07	0.262	0.241	0.259	0.296	0.31	0.254	1				
VS27	0.363	0.4	0.386	0.407	0.401	0.467	0.434	0.405	0.37	0.314	0.418	0.393	0.127	0.052	0.131	0.08	0.099	0.081	0.089	0.225	0.241	0.259	0.296	0.29	0.235	0.829	1			
KS28	0.404	0.442	0.465	0.488	0.444	0.512	0.473	0.485	0.431	0.353	0.412	0.472	0.138	0.12	0.144	0.168	0.151	0.131	0.158	0.375	0.41	0.391	0.432	0.406	0.348	0.707	0.707	1		
KS29	0.32	0.356	0.362	0.363	0.374	0.421	0.371	0.362	0.345	0.272	0.392	0.368	0.115	0.07	0.13	0.098	0.095	0.083	0.069	0.221	0.272	0.272	0.312	0.306	0.251	0.777	0.87	0.678	1	
MI30	-0.16	-0.18	-0.14	-0.18	-0.13	-0.08	-0.1	-0.18	-0.15	-0.14	-0.14	-0.09	-0.15	-0.09	-0.16	-0.16	-0.13	-0.09	-0.11	-0.16	-0.14	-0.16	-0.14	-0.18	-0.17	-0.19	-0.15	-0.09	-0.15	1

Figure 1: Map of the Mediterranean basin and Middle East showing the origin of the accessions used in this study. The numbers on the map correspond to the code # in Table 1.

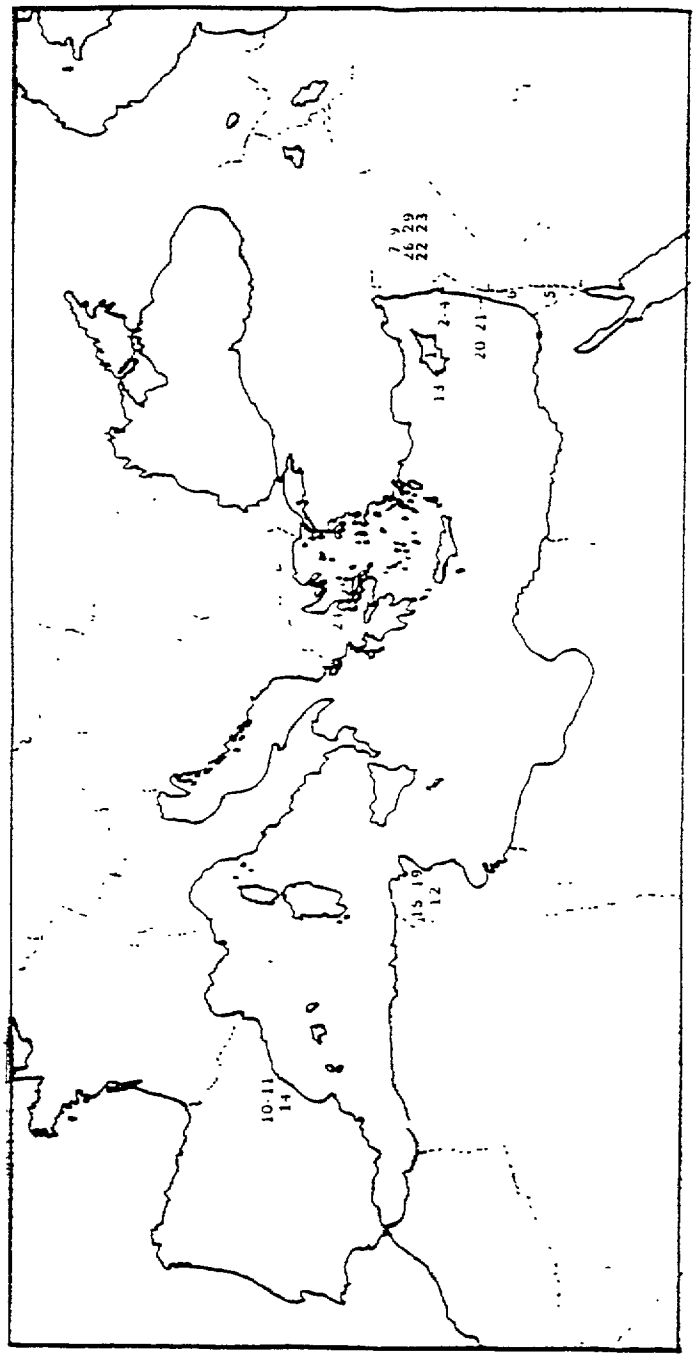


Figure 2: Amplification products obtained with primer OpC-6. Lanes 1-31, from left to right, refer to AmpliSize™ DNA size standard and the accessions listed in Table 1 in the same order.

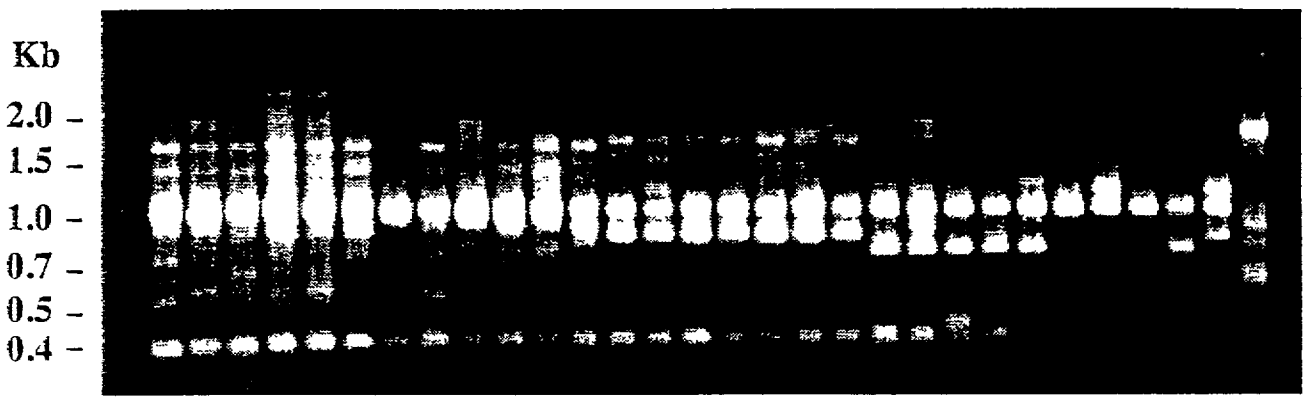
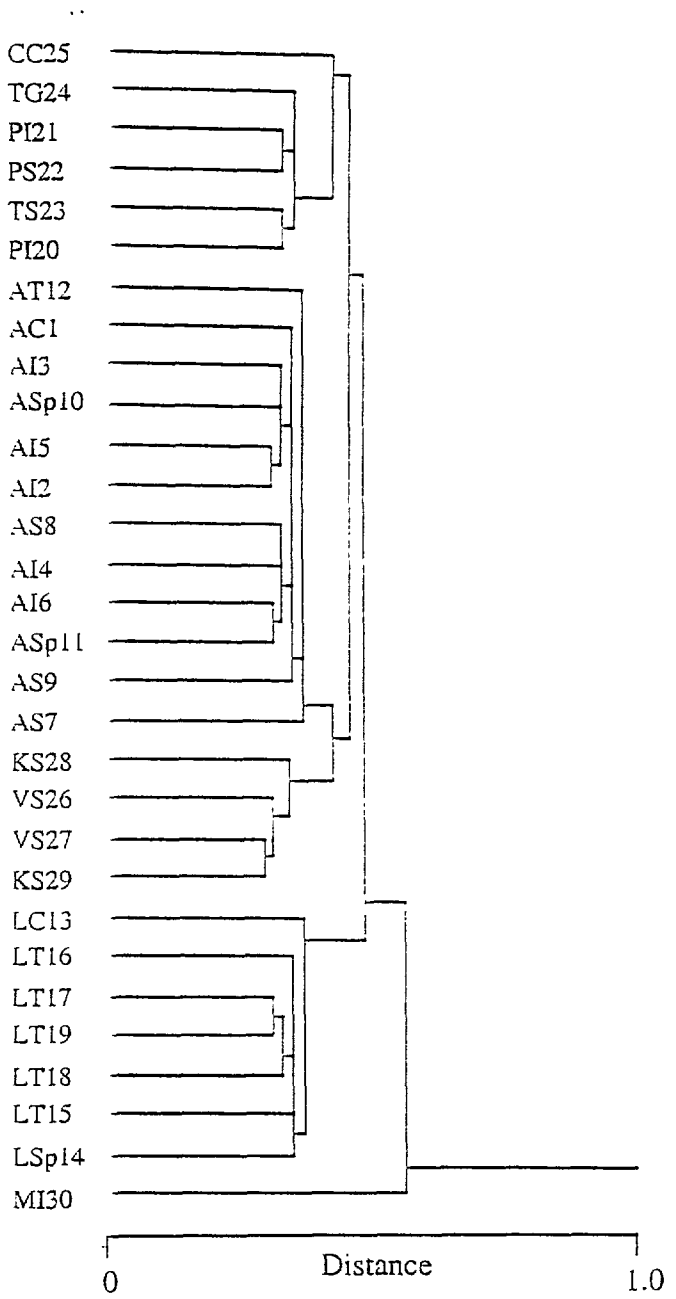


Figure 3: Dendrogram of Mediterranean *Pistacia* based on RAPD marker analysis.



C) Scientific impact of collaboration: Seed samples were collected in Turkmenistan to start establishment of a seed germplasm collection and identification of unique wild accessions. Samples of seeds were transferred to the Israeli group. Training of a Turkmen scientist, Mr. Azat Ataev, started in the Israeli laboratory.

D) Description of project impacts: The project impact can be evaluated at 3 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 the Badhiz reserve. 3. Training of a Turkmen scientist 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 establishment the *Pistacia* conservation laboratory in Turkmenistan. The instruments are: PCR, Power supply and electrophoretic equipments. All these pieces of equipment were purchased from American suppliers and are being tested in the Israeli laboratory before transfer to the Turkmen laboratory.

F) Future work:

Major effort will be made to intensify collection of seeds from as diverse as possible locations in Badhiz, 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. Work will continue to develop a working model for assessment of tree's gender by remote sensing based on reflection. Fingerprinting of Turkmen accessions by RAPD will continue.

Section II

A) Managerial Issues:

Fund transfer from Israel to the Turkmen group had a slow start, because of a name change of the Turkmen institute. It was changed from Institute for Desert Research, The Academy of Science of Turkmenistan to National Institute of Deserts, Flora and Fauna. It is hoped that now funds can be transferred regularly.

B) Budget:

No budget changes were made.

C) Special Concerns:

No changes

D) Collaboration, Travel, Training and Publications:

Mr. Azat Ataev from Turkmenistan is scheduled to stay in training in Israel until December 2000. Prof. Golan will visit the Turkmen group in Aug/Sep 2000 for coordination of the project, seed collection mission and selection of an additional trainee.