GENETIC DIVERSITY OF *PINUS BRUTIA* IN SYRIA AS REVEALED BY DNA MARKERS

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ABSTRACT

Random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) were used to estimate the genetic diversity between and within 21 populations of *Pinus brutia* collected from five different regions of Syria. After screening 400 Operon Primers, only nine were able to detect polymorphism between the tree samples. The AFLP analysis also confirmed the low genetic variability. Even after digestion of monomorphic RAPD fragments with three restriction enzymes, no increased polymorphism between samples was revealed. The total number of identified polymorphic fragments (loci) between the 311 trees was 111 (74 RAPDs and 37 AFLPs). The highest level of genetic diversity was detected in the region of Latakia and the lowest one was detected in the region of Idleb. The genetic diversity detected within populations was higher than the one detected between populations. A dendrogram based on the results of the polymorphic RAPD and AFLP fragments reflecting the genetic distance between the analyzed *P. brutia* populations was developed. Our results showed that the general level of genetic variability in *P. brutia* populations collected in Syria was low. Relative uniformity of the landscape in the North Western part of Syria might add to the low genetic variability observed.

Key words: *Pinus brutia*, polymorhism, genetic variability, AFLP, RAPD.

INTRODUCTION

Pinus is the largest genus in the family *Pinaceae*, $2n = 24$ chromosomes with about 110–120 species. In the Mediterranean Basin the pine flora includes ten pine species. Although these Mediterranean pine forests cover only 5 % of the total area of the Mediterranean Basin, they comprise around 25 % of the forested area. Of these, *Pinus halepensis* Mill. and *Pinus brutia* Ten. are the most common species (BARBÉRO *et al.* 1998).

The natural distribution of *P. brutia* is confined mainly to the Eastern Mediterranean region covering NE Greece, the Black Sea, Turkey, Crete, Cyprus, Syria, Lebanon and Iraq. It tolerates a broad range of Mediterranean climates (EMBERGER *et al.* 1963), grows from sea level to 1500 m a.s.l. on a wide range of soil types (SELIK 1958; MIROV 1967), and is recognized for its adaptation to drought and alkaline soils (SPENCER 2001).

P. brutia is considered as a highly invasive species, occupying open disturbed sites e.g. through (fires). Due to the extreme Mediterranean climate and/or continuous human interaction, the relatively short-lived *P. brutia* trees are able to form stable pine vegetation associated with broad-leaved species (ZOHARY 1973; BARBÉRO *et al.* 1998). These criteria

have let to an increased interest in this species for commercial plantations during the last decennia, illustrated by breeding and provenance trails carried out in various countries in the Mediterranean region (PANETSOS 1981; ISIK 1986; BARITEAU 1992) and even outside (SOUVANNAVONG *et al.* 1995; SPENCER 1985 and 2001).

It is worrying however, that the *P. brutia* forests in Syria are not included in any of the above mentioned studies. Moreover, publications about these populations are scarce and have a general descriptive tendency (BARBÉRO *et al.* 1976; NAHAL 1983 and 1984). The Syrian forests of *P. brutia* play a vital role in the protection of the environment as they are part of it. Furthermore, it is a valuable seed source for the national reforestation program. Yet, the annual reoccurrence of fires and the continuing conversion of forest areas into agriculture have a negative impact on these natural resources.

Random Amplified Polymorphic DNA (RAPD) markers can be used for fast screening of nuclear genome variation (WILLIAMS *et al.* 1990; WELSH & MCCLELLAND 1990). Despite the frequent use of RAPD markers in crop species for various purposes, e.g. germplasm characterization (ZHANG *et al.* 1996; CHOUMANE *et al.* 1998; FERGUSON *et al.* 1998), the construction of genetic linkage maps (BAI *et al.* 1997; EUJAYL *et al.* 1998a; EUJAYL *et al.* 1999; HOQUE *et al.* 2002), and for the estimation of genetic diversity within and between species (RODRIGUEZ *et al.* 1999; MIGNOUNA *et al.* 1999; VIDAL *et al.* 1999), there are relatively few published reports of RAPD for forest trees (CARLSON *et al.* 1991, GRATTAPAGLIA *et al.* 1992; DALE *et al.* 1992, TULSIERAM *et al.* 1992; NELSON *et al.* 1993; KAYA & NEALE 1993; BINELLI & BUCCI 1994; GRATTAPAGLIA & SEDEROFF 1994; KAYA & NEALE, 1995; LU *et al.* 1995; ADAMS & TURUSPEKOV 1998).

The amplified fragment length polymorphism (AFLP) is another useful marker technology developed by VOS *et al.* (1995). AFLPs are robust and reliable because stringent reaction conditions are used for primer annealing. The AFLP technique combines the reliability of the restriction fragment length polymorphism (RFLP) with the power of the PCR techniques .This technique was widely used for germplasm characterization and genetic mapping in plants over the last five years (MAUGHAN *et al.* 1996; HILL *et al.* 1996; HE &PRAKASH 1997; HONGTRAKUL *et al.* 1997, EUJAYL *et al.* 1998a and b; ANGIOLILLO *et al.* 1999; GRACIA-MAS *et al.* 2000, TEULAT *et al.* 2000) but only few reports exist on its application in forest trees (ÅKERMAN *et al.* 1996, LERCETEAU & SZMIDT 1999, HAYASHI *et al.* 2001, YIN *et al.* 2003).

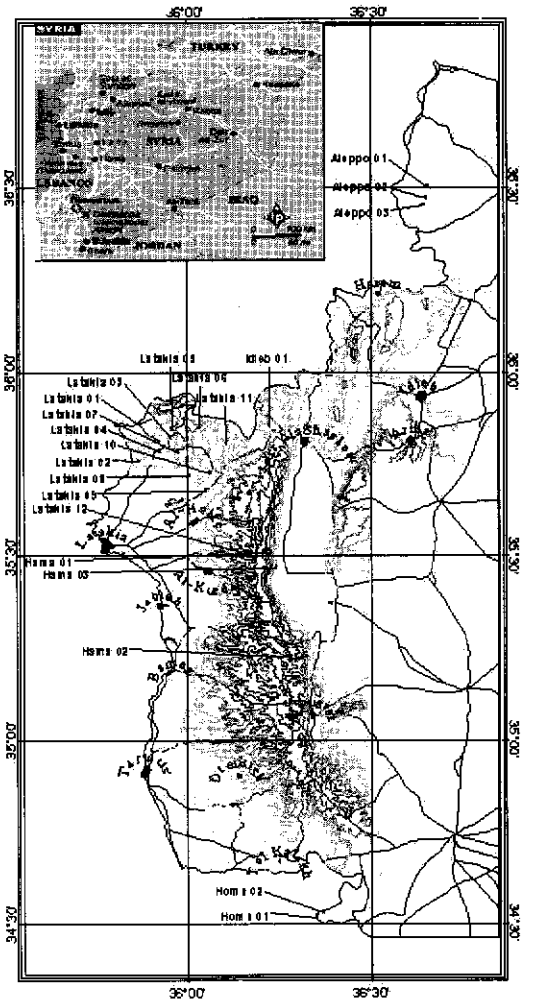

Figure 1. Collection sites of *P. brutia* on geographic map of Syria.

This study is, as far as we know, the first attempt to better understand the genetic processes operating within and between *P. brutia* populations in Syria, which is critical for successful preservation of the ecosystems they live in. For this purpose, RAPD and AFLP markers were used to evaluate the genetic variability within and between Syrian *P. brutia* populations.

MATERIAL & METHODS

Plant Material

Based on an ecogeographical survey, the distribution of *P. brutia* was mapped in Syria (IPGRI, unpublished). Five distinct and geographically separated regions with different climatic conditions were selected. They were Latakia, Aleppo, Hama,

Table 1. Number of populations*, regions and characteristics of the collection sites.

	Site characteristics[2]								Average tree characteristics[5]		
Site number	Lattitude[1]	Longitude[1]	Altitude[1]	Slope (degree)	Exposure	PMQ[3]	m[4] (gegrees)	Description of collection sites	Region	Height (m)	Dbh[6] (cm)	Height first branch (m)
1	36.501	36.649	501	21	141	55.83	3	Area with extensive agriculture, pasturelands	Aleppo	8.5	23.9	1.8
2	36.472	36.650	479	23	223.5	54.82	2.33	and large patches of pine forests	Aleppo	6.7	24.6	2.6
3	36.458	36.643	516	11	204	55.06	2.47		Aleppo	6.8	25.7	1.8
4	35.507	36.225	618	20	61.5	88.45	1.5	Isolated patch of pine forests bordered by	Hama	7.9	25.7	2.3
6	35.467	36.242	290	16	97.5	72.18	3.73	maquis vegetation, pasture fields and agricultural lands	Hama	7.1	29.1	1.5
5	35.212	36.306	658	22	243	90.54	3.5	Isolated small *Pinus brutia* forest fragment bordered by maquis vegetation and some agricultural lands	Hama	7.2	21.7	1.1
7	34.517	36.344	1291	16	230.4	49.04	0.61	Isolated patch of *Pinus brutia* forest bordered	Homs	7.2	34.8	1.9
8	34.527	36.368	1068	24	132	57.27	1.64	by maquis and extensive pasture lands	Homs	5.9	33.5	1.9
9	35.858	35.867	47	13	171	95.15	6.13	Mosaic of large *Pinus brutia* patches	Lattakia	15.1	32.3	2.8
10	35.754	35.873	130	20	130.5	98.82	5.6	interspersed with agricultural fields,>800 km^2	Lattakia	6.9	25.8	2
11	35.823	35.956	434	24	204.9	96.21	4.16	which extends into Turkey	Lattakia	20	37.9	4
12	35.785	35.925	185	16	193.5	95.59	5.88		Lattakia	14.5	27.2	4.3
13	35.848	35.966	645	23	192.9	91.44	4.06		Lattakia	15.5	30.3	5.1
14	35.846	36.036	517	27	87	85.63	3.57		Lattakia	21.5	33.3	5.7
15	35.779	35.980	547	18	201.9	98.52	4.16		Lattakia	8.5	23.2	2.5
16	35.723	36.010	151	25	238.5	87.55	5.18		Lattakia	14.5	28.4	3.1
17	35.671	36.044	197	16	90	86.66	5.36		Lattakia	18.9	35	4.1
18	35.722	36.066	270	26	144	86.15	5		Lattakia	17.2	30.7	2.2
19	35.801	36.111	381	24	205.5	81.15	4.72		Lattakia	15.1	34	4.8
21	35.848	36.225	309	19	129	70.02	4.06		Idleb	10.1	29.6	2.9
20	35.510	36.142	689	25	222	100.18	3.79	One *Pinus brutia* fragment	Lattakia	10.9	28.9	3.3

* The term populations is being used for the trees sampled from the same geographical site.
[1)] Coordinates are of the weighted middle points of the 15 sampled trees.
[2)] Site characteristics were determined for each location of a sampled tree. The values given in this table are the averages.
[3)] PMQ is the pluviometric quotient.
[4)] m is the mean minimum temperature of the coldest month.

Homs and Idleb (names are derived from the province in which the largest part of the populations occur, Figure1).

In each of these regions, sample sites were selected with distinct environmental conditions (altitude, soil, associate vegetation, different population characteristics), or separated by effective gene flow barriers (distance, mountains), (Table 1). Minimum distance between the sampled trees was 150 meter.

DNA extraction

In total 311 samples of young needles were collected and lyophilized. Genomic DNA was extracted from individual plants. Needles were ground in liquid nitrogen and DNA extraction was performed according to the modified protocol of DOYLE & DOYLE (1990).

RAPD analysis

The protocol of WILLIAMS *et al.* (1990) for RAPD analysis was employed with minor modifications. A total of 400 decamer primers obtained from Operon Technologies, Alameda, Calif. USA (Kits A to X), were used to screen the DNA samples for polymorphism. All amplification reactions were performed using a DNA thermocycler Perkin Elmer 9600. The final reaction volume was 15µl and contained 10 pmol of the 10-mer Operon primers, 25ng of template DNA, 200 µM of each dNTPs, 1.5 mM $MgCl_2$ and 0.8 unit of *Taq* DNA polymerase. After initial denaturation for 4 min. at 94 °C, the reaction was subjected to 35 cycles of 30 sec. at 94 °C, 1 min. at 36 °C and 2 min. at 72 °C , followed by 10 min. at 72 °C. Amplification products were separated by electrophoresis on 1.5 % agarose gels made in 1X TAE buffer (40 mM Tris-Acetate, 1mM EDTA, pH = 8) and were visualized by ethidium bromide staining. Preliminary tests with one sample for each of the five different regions of Syria were conducted to select the RAPD (as well as AFLP) primers able to detect polymorphism.

AFLP analysis

The protocol for the AFLP assay was carried out as described by VOS *et al.* (1995) with minor modifications. 0.5 µg of DNA was digested with the restriction enzymes *Pst*I and *Mse*I. Pre-amplification and selective amplifications were performed as described in the original protocol. Thirteen primer combinations were tested to screen for polymorphism between the DNA samples. The primer combination P100/M301, where *Pst*I-100 has four selective nucleotides (5'GACTGCGTACATGCAG + AACC) and *Mse*I-301 has also four selective nucleotides

(5'GATGAGTCCTGAGTAA + TATA), was selected to analyze the 311 samples. The PCR profile for the pre-amplification program was: 30 sec. at 94 °C, 30 sec. at 60 °C, 1 min. at 72 °C, for 30 cycles. The pro-gram for selective amplification was the following: 30 sec. at 94 °C, 30 sec. at 65 °C, 1 min. at 72 °C, for one cycle. This was followed by 11 cycles over which the annealing temperature was decreased by 0.7 °C per cycle followed by 30 sec. at 94 °C, 30 sec. at 56 °C, 1 min. at 72 °C, for 23 cycles. The amplified fragments were electrophoresed on 6% polyacrylamide gels and stained with silver nitrate (BASSAM *et al.* 1991). The presence and the absence of the bands were visually recorded.

Data analysis

For each marker system (AFLP and RAPD), a matrix of all fragments (bands) scored in the 311 trees was generated using "1" when the band was present and "0" when the band was absent. Fragments of the same molecular weight were scored as identical. Each fragment was presumed to represent a single genetic locus. Genes diversity at each RAPD and AFLP locus and genic variation statistics for all loci were estimated by the software package POPGENE, version 1.32 (YEH *et al.* 1997). Analysis of genetic diversity (*GD*) was calculated following the formula of NEI (1987):

$$GD = n(1 - \Sigma p^2)/(n - 1)$$

where n is the number of samples and p is the frequency of one allele.

Gene diversity was calculated as follows:

$$H = (1 - \Sigma p_{ij}^2) \qquad \text{(WEIR 1990)}$$

where p_{ij} is the frequency of jth allele generated with the primer i.

In the total population, gene diversity (H_t) was divided into the gene diversities within (H_s) and among (D_{st}) populations in the different regions, thus

$$H_t = H_s + D_{st} \qquad \text{(NEI, 1987).}$$

Genetic differentiation (G_{st}) relative to the total population was calculated using the coefficient of gene differentiation (NEI 1973; 1987):

$$G_{st} = D_{st}/H_t$$

G_{st} can take value between 0 (no differentiation between subpopulations) and 1.0 (complete differentiation between subpopulations.

The amount of gene flow between populations, Nm, where N is the population size and m is the fraction of individuals in a population that are migrants, was estimated using the following formula (BOEGER *et al.*, 1993):

$$Nm = 0.5[(1/G_{st}) - 1]$$

If $Nm < 1$, then local populations tend to differentiate; if $Nm > 1$, there will be little differentiation among populations and migration is more important than genetic drift (WRIGHT 1951).

A dendrogram of genetic distance between 21 populations was constructed using the Unweighted Pair Group Mean Average Method (UPGMA) (SNEATH & SOKAL 1973) of the software pakage Numerical Taxonomy and Multivariate Analysis System, version 2.01 (NTSYS-pc, ROHLF 1997).

RESULTS

RAPD analysis

Out of 400 Operon primers tested on five samples

Table 2. Number of polymorphic fragments in 21 *P. brutia* populations based on RAPD and AFLP data.

Population and region	# of trees	Number of polymorphic loci (fragments) per										Total number of polym fragments
		RAPD - Operon primers									AFLP	
		B-04	B-20	C-01	C-13	F-15	J-01	P-05	P-14	V-04		
1 Aleppo	14	2	7	6	2	4	6	2	7	7	19	62
2	15	2	6	7	3	4	7	2	6	7	22	66
3	15	2	6	9	2	4	6	4	6	8	11	58
Aleppo	44	2	7	9	3	4	7	4	7	9	25	77
1 Hama	14	3	7	7	2	6	6	2	6	8	15	62
2	14	2	4	8	2	6	7	3	6	7	17	62
3	15	2	3	4	2	3	5	2	5	5	11	42
Hama	43	3	7	9	2	7	7	3	9	9	23	79
1 Homs	15	4	5	8	2	7	7	3	9	7	17	69
2	14	3	7	5	2	2	6	3	7	6	16	57
Homs	29	5	8	9	2	7	8	4	10	8	23	84
1 Latakia	15	3	4	4	2	5	7	4	4	8	20	61
2	15	3	5	5	3	4	6	4	2	4	18	54
3	15	4	5	4	3	5	7	3	7	8	18	64
4	15	4	7	5	2	4	8	3	6	7	20	66
5	15	3	6	5	2	3	9	2	7	8	17	62
6	15	3	7	6	2	5	7	2	8	6	22	68
7	15	5	5	5	2	2	7	4	7	8	14	59
8	15	4	8	6	3	2	7	5	5	6	16	62
9	15	3	5	5	1	4	4	4	5	8	10	49
10	15	5	5	5	2	4	6	4	4	6	21	62
11	15	2	5	6	2	4	6	5	7	6	24	67
12	15	2	5	4	2	4	5	3	5	6	24	60
Latakia	180	7	9	9	4	6	9	6	10	10	32	102
Idleb	15	4	4	5	2	4	6	3	2	5	27	62
The whole collection	311	7	9	9	5	7	10	6	10	11	74	111

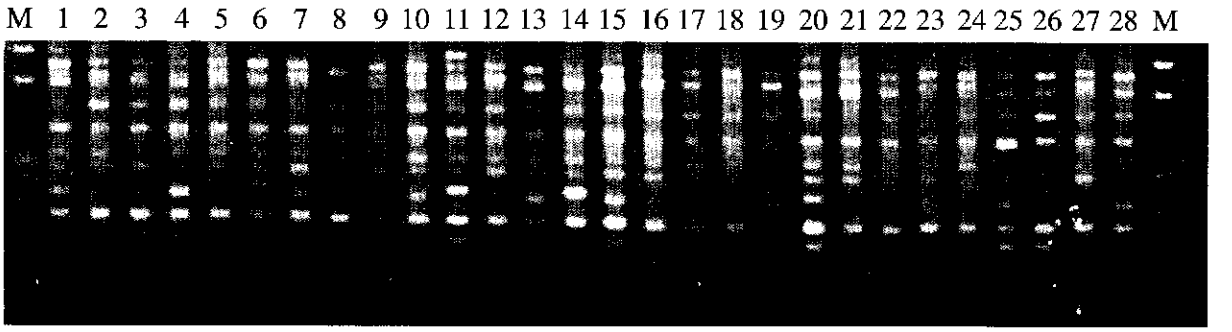

Figure 2. Genomic DNA from populations of *P. brutia* amplified with OPJ-01. Electrophoresis in 1.4% agarose gels. M: molecular marker VI, Mixture of PBR328 cleaved with *BglI* and PBR328 cleaved with *HinfI*, lane 1, lane 2-28 DNA samples of pine.

representing the 5 populations, only nine primers (OPB-04, OPB-20, OPC-01, OPC-13, OPF-15, OPJ-01, OPP-05, OPP-14 and OPV-04) were able to amplify clear and repeatable polymorphic fragments. These primers were then used to analyze the 311 plants representing the 21 populations from the 21 collection sites. The total number of polymorphic fragments was 74. Their molecular weight ranged from 230 to 2500 bp. Fragments with higher molecular weight were mostly monomorphic. The number of polymorphic fragments (polymorphic loci) detected per primer ranged from 5 (with OPC-13) to 11 (with OPV-04, Table 2), which represents an average of 8 polymorphic fragments per primer. The highest number of polymorphic fragments (71) was detected in population 11 of Latakia while the smallest number (44) was detected in population 3 of Hama. An example of amplification with OPJ-01 is shown in Figure 2.

In order to increase the level of polymorphism detected, amplified products of primers producing one or two monomorphic fragments (OPE02, OPF09, OPG16, OPH12, OPN02, OPN03, OPN05, OPS09, OPS13, OPT08, OPD20, OPA18, OPW12, OPW13) were digested with three restriction enzymes (*HinfI*, *EcoRV*, and *TaqI*). Some restriction enzymes cut the amplified fragments (Table 3), but none of these were polymorphic.

AFLP analysis

Distinct and polymorphic fragments (37) of the combination P100/M301 were used for the analysis of the 311 samples. Number of polymorphic fragments varied from 10 (in Population 9 of Latakia) to 24 (in the populations 11 and 12 of Latakia, Table 2). An example of the AFLP amplification is presented in Fig. 3.

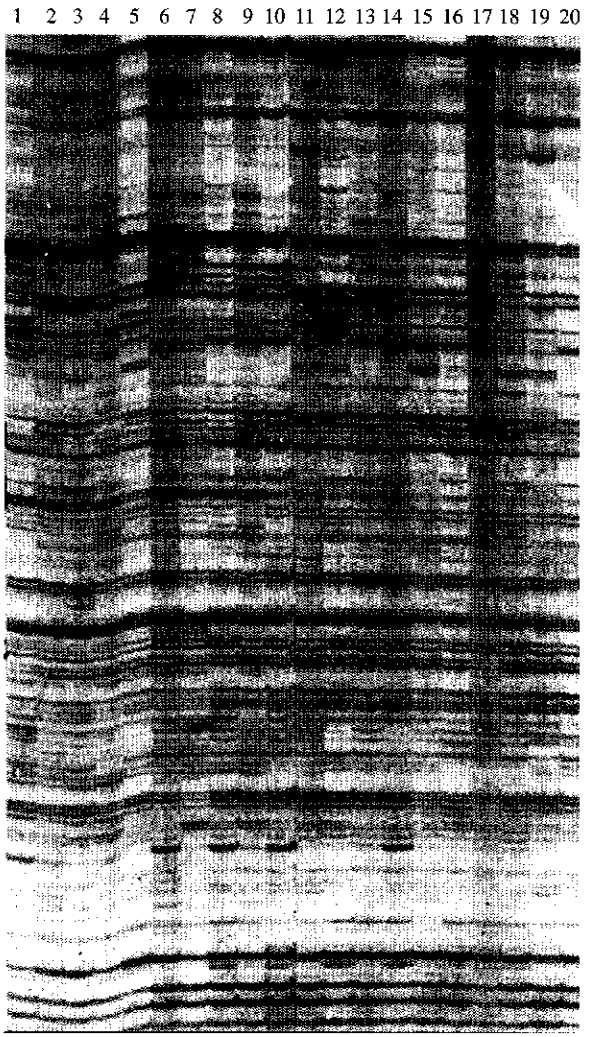

Figure 3. AFLP analysis of populations of *P. brutia* using primer combination P100/M301.

Genetic variability

For each population, the observed number of alleles (na), the gene diversity (h) and the percentage of polymorphic loci for RAPD, AFLP and RAPD +

Table 3. Digestion of monomorphic RAPD fragments and restriction enzymes used.

Operon primers producing monomorphic fragments	Restriction Enzymes		
	Sites for *EcoRV*	Sites for *Hinf*I	Sites for *Taq*I
OPA-18	0	0	1
OPD–20	0	2	1
OPE–02	0	1	1
OPF–09	0	1	2
OPG–16	0	1	1
OPH–12	1	2	0
OPN–03	1	1	0
OPS–09	0	2	3
OPS–13	0	1	0
OPT–08	0	1	0
OPW–12	0	1	0
OPW–13	0	1	0

AFLP were calculated (Table 4). Clear difference between the data based on RAPD and those based on AFLP can be seen. In most populations, the level of polymorphism detected with RAPD marker was higher than the one detected with AFLP. The mean of polymorphic RAPD loci over all regions was 57.6%, where this mean was 48.8% for AFLP loci. We will focus on the results obtained for the combined data (RAPD and AFLP data) because they reflect in a better way, the genetic variability between the samples. The number of polymorphic fragments (loci) varied between populations. It ranged from 42 (in population 3 of Hama) to 69 (in population 1 of Homs) (Table 4). No region specific loci were detected. All loci were presented in all populations but with different frequency. The value of gene diversity varied from 0.15 in the population 3 of Hama to 0.26 in the population 11 of Latakia. The comparison between the 21 populations showed that the observed number of alleles varied from 1.38 in the population 3 of Hama to 1.61 in the population 6 of Latakia.

In order to understand the population structure and to estimate the genetic diversity within and between regions, the different populations were regrouped according to the collection regions (Latakia, Aleppo, Hama, Homs and Idleb) . For each region the values of the observed number of alleles (n_a), the effective number of alleles (n_e), the gene diversity (h), and Shanon's information index (I) were calculated (Table 5). The lowest values for observed and effective number of alleles were in the region of Idleb (1.5586 and 1.3831, respectively) while the highest numbers were in the region of Latakia (1.9189, and 1.4843, respectively). The high values of standard deviation (SD) observed in all parameters showed that the variations within the regions were more important than among the regions.

Total gene diversity (H_t) including the gene diversities within populations (H_s) and among populations (D_{st}), and the amount of gene flow, Nm, between populations, were estimated for each region and over the all regions (Table 6). The higher value of total gene diversity was detected in the region of Latakia (H_t = 0.2887, SD = 0.028) while the lowest value was detected in Idleb (H_t = 0.2183, SD = 0.0447). Concerning the amount of gene flow, the Nm values varied from 1.693 in Latakia to 3.266 in Aleppo, showing the little differentiations among populations in one region and over all regions.

The combined data of the 111 polymorphic fragments derived from RAPD and AFLP analysis was used for the estimation of the Nei's genetic similarity and genetic distance between the 21 populations (Table 7). The highest genetic similarity was detected between populations 3 and 4 from Latakia (0.9694) while the lowest genetic similarity (0.8508) was between population2 from Homs and population12 from Latakia, which means that the biggest genetic distance (0.1616) was between these two populations (Table 7) .

Cluster analysis

A dendrogram based on Nei's genetic distance using UPGMA is presented in Fig. 4. It showed that the 21 populations were clustered together into 2 distinct groups. The first group includes the populations 10, 11, and 12 from latakia with the unique population 21 from Idleb, while the second group

Table 4. The values of observed number of alleles, gene diversity and the percentage of polymorphic loci in the whole samples of Syrian *P.brutia*.

No. of populations	Regions	No. trees/pop	Results with RAPD markers				Results with AFLP markers				Results with RAPD and AFLP markers			
			n_a	h	No. of polym. loci	% of polym. loci	n_a	h	No. of polym. loci	% of polym. loci	n_a	h	No. of polym. loci	% of polym. loci
1	Aleppo	14	1.581	0.25	43	58.11	1.514	0.2	19	51.35	1.56	0.24	62	55.86
2		15	1.595	0.24	44	59.46	1.595	0.3	22	59.46	1.6	0.24	66	59.46
3		15	1.635	0.24	47	63.51	1.297	0.1	11	29.73	1.52	0.2	58	52.25
1	Hama	14	1.635	0.25	47	63.51	1.405	0.2	15	40.54	1.56	0.22	62	55.86
2		14	1.608	0.23	45	60.81	1.46	0.2	17	45.95	1.56	0.22	62	55.86
3		15	1.419	0.17	31	41.89	1.297	0.1	11	29.73	1.38	0.15	42	37.84
1	Homs	15	1.703	0.28	52	70.27	1.46	0.2	17	45.95	1.62	0.26	69	62.16
2		14	1.554	0.23	41	55.41	1.432	0.2	16	43.24	1.51	0.21	57	51.35
1	Lataki	15	1.554	0.22	41	55.41	1.541	0.2	20	54.05	1.55	0.23	61	54.95
2	a	15	1.487	0.18	36	48.65	1.487	0.2	18	48.65	1.49	0.19	54	48.65
3		15	1.622	0.25	46	62.16	1.487	0.2	18	48.65	1.58	0.24	64	57.66
4		15	1.622	0.25	46	62.16	1.541	0.2	20	54.05	1.6	0.25	66	59.46
5		15	1.608	0.24	45	60.81	1.46	0.2	17	45.95	1.56	0.22	62	55.86
6		15	1.622	0.25	46	62.16	1.595	0.2	22	59.46	1.61	0.25	68	61.26
7		15	1.608	0.24	45	60.81	1.378	0.2	14	37.48	1.53	0.22	59	53.15
8		15	1.622	0.24	46	62.16	1.432	0.2	16	43.24	1.56	0.22	62	55.86
9		15	1.527	0.21	39	52.70	1.27	0.1	10	27.03	1.44	0.17	49	44.14
10		15	1.554	0.22	41	55.41	1.568	0.2	21	56.76	1.56	0.22	62	55.86
11		15	1.581	0.25	43	58.11	1.649	0.3	24	64.86	1.6	0.26	67	60.36
12		15	1.487	0.21	36	48.65	1.649	0.2	24	64.86	1.54	0.22	60	54.05
1	Idleb	15	1.473	0.19	35	47.30	1.73	0.3	27	72.97	1.56	0.22	62	55.68
	Mean	14.8	1.58	0.23	42.6	57.60	1.48	0.2	18	48.76	1.55	0.22	61.1	59.82

Table 5. Summary of genic variation statistics for all loci over all the regions.

Regions	No. of trees		RAPD				AFLP				RAPD + AFLP			
			na*	ne*	h*	I*	na*	ne*	h*	I*	na*	ne*	h*	I*
Aleppo	44	Mean	1.7027	1.4482	0.2615	0.3884	1.6757	1.482	0.27	0.3937	1.6937	1.4594	0.2643	0.3902
		St. Dev	0.4602	0.3678	0.1944	0.2757	0.4746	0.403	0.2105	0.2962	0.463	0.3784	0.199	0.2814
Hama	43	Mean	1.7568	1.4247	0.2515	0.3807	1.6216	1.406	0.237	0.351	1.7117	1.4184	0.2467	0.3708
		St. Dev	0.432	0.3651	0.1873	0.2602	0.4917	0.374	0.2022	0.2906	0.455	0.3664	0.1916	0.2697
Homs	29	Mean	1.8243	1.4759	0.2903	0.4391	1.6216	1.41	0.237	0.3506	1.7568	1.4541	0.2726	0.4096
		St. Dev	0.3831	0.3088	0.1595	0.2252	0.4917	0.388	0.2047	0.2922	0.431	0.3369	0.1767	0.2517
Latakia	180	Mean	1.9459	1.4948	0.2937	0.4469	1.8649	1.463	0.2788	0.4239	1.9189	1.4843	0.2887	0.4393
		St. Dev	0.2277	0.3449	0.1666	0.2167	0.3466	0.339	0.1711	0.2335	0.2742	0.3417	0.1675	0.2216
Idleb	15	Mean	1.473	1.3428	0.1895	0.2751	1.7297	1.464	0.2759	0.4094	1.5586	1.3831	0.2183	0.3199
		St. Dev	0.5027	0.4126	0.2184	0.3095	0.4502	0.334	0.1864	0.2691	0.4988	0.3909	0.2115	0.3022
all regions	311	Mean	1.973	1.499	0.2972	0.4535	1.9459	1.486	0.2912	0.4425	1.964	1.4948	0.2952	0.4498
		St. Dev	0.1633	0.3413	0.1615	0.2066	0.2292	0.335	0.1672	0.2229	0.1872	0.3376	0.1627	0.2112

*na = Observed number of alleles
*ne = Effective number of alleles
*h = Nei's (1973) gene diversity
*I = Shannon's Information index

contained the 17 other populations. Three distinct subgroups were identified in the second group. The first subgroup contained two populations from Aleppo (Populations 1 and 2), the second contained three populations from Latakia (populations 7, 8 and 9), and the third subgroup contained 12 populations from 4 different regions (6 from Latakia, 3 from Hama, 2 from Homs and 1 from Aleppo). Within subgroup 3, the populations 1, 2, 3, 4, 5 and 6 from Latakia were closely regrouped together with the population 2 of Hama. The populations Hama 1, Aleppo 3 and Homs 1 formed another cluster while the populations Homs 2 and Hama 3 were relatively isolated in this subgroup.

DISCUSSION

Genetic diversity between and within *P. brutia* populations collected from five different regions of Syria was evaluated using DNA markers. The analysis of these populations with RAPD and AFLP markers showed different level of polymorphism and genetic diversity. The fact that out of 400 Operon primers tested only nine were able to detect polymorphism between the samples, suggests that the level of similarity of the detected loci between the samples was high.

Even the digestion of RAPD fragments with restriction enzymes failed to increase the level of polymorphism detected, confirming the high level of genetic similarity at the sequence level in the Syrian populations of *P. brutia*. Similar results were obtained by KAYA & NEALE (1995) where they found that

Table 6. Analysis of gene diversity in five regions of collections.

Regions	Number of trees		RAPD + ALFP						
			H_t	H_s	D_{st}	G_{st}	Nm	# of polymorph. loci	% of polymorph. loci
Aleppo	44	Mean	0.2646	0.23	0.0351	0.1328	3.266	77	69.37
		St. dev.	0.0398	0.033					
Hama	43	Mean	0.2477	0.197	0.0507	0.2035	1.9568	79	71.17
		St. dev.	0.0368	0.027					
Homs	29	Mean	0.2718	0.233	0.0388	0.1442	2.9681	84	75.68
		St. dev.	0.0312	0.027					
Latakia	180	Mean	0.2887	0.223	0.0657	0.228	1.6932	102	91.89
		St. dev.	0.028	0.02					
Idleb	15	Mean	0.2183	0.218	0.0003	0	α	62	55.86
		St. dev.	0.0447	0.045					
All regions	311	Mean	0.2952	0.275	0.0202	0.0703	6.6121	107	96.40
		St. dev.	0.0265	0.023					

H_t: gene diversity in total population
H_s: gene diversity within populations
D_{st}: gene diversity among populations
Nm^* = estimate of gene flow from G_{st}, e. g., $Nm = 0.5\ (1 - G_{st})/G_{st}$

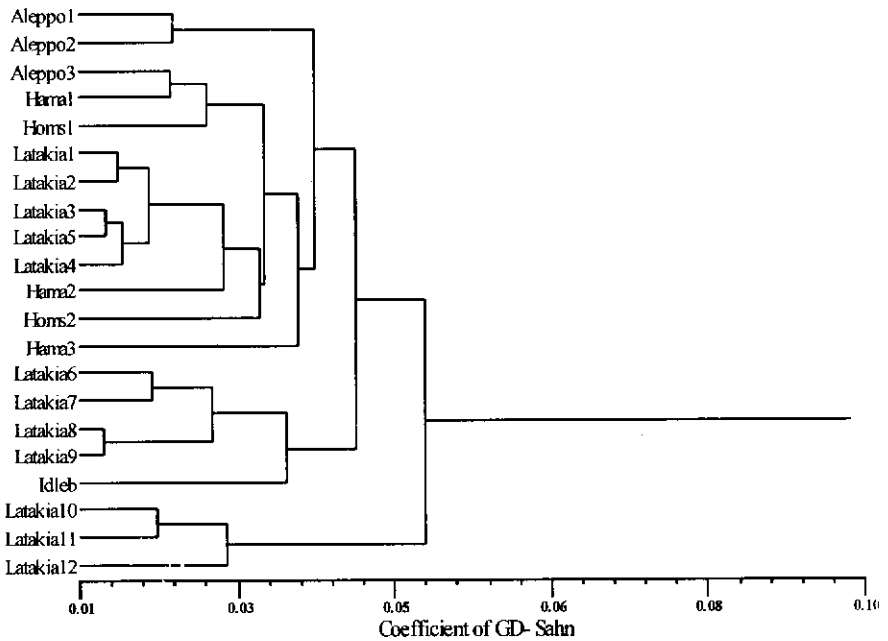

Figure 4. Genetic distance between trees from 21 Syrian populations based on AFLP and RAPD data.

high proportion of RAPD loci detected in Turkish red pine (*P. brutia*) was monomorphic. These results are in disagreement with a study based on isoenzymes carried out on accessions originating from islands of the Northeastern Aegean sea (PANETSOS *et al.* 1998). In that study, the level of genetic diversity detected was higher than the values detected in our populations of Syria. Previous studies on the species

Table 7. Values of genetic similarity (above diagonal) and genetic distance (below diagonal) between the 21 populations of *P. brutia*.

	AL 1	AL 2	AL 3	HA 1	HA 2	HA 3	HO 1	HO 2	LA1	LA2	LA3	LA4	LA5	LA6	LA7	LA8	LA9	LA10	LA11	LA12	Idleb
AL1	****	0.966	0.92	0.904	0.907	0.871	0.88	0.885	0.91	0.898	0.909	0.902	0.919	0.921	0.888	0.875	0.885	0.888	0.919	0.892	0.91
AL2	0.035	****	0.94	0.933	0.921	0.888	0.894	0.909	0.909	0.917	0.907	0.898	0.915	0.919	0.892	0.883	0.881	0.879	0.907	0.883	0.913
AL3	0.083	0.062	****	0.953	0.906	0.911	0.922	0.905	0.911	0.899	0.916	0.904	0.919	0.919	0.905	0.903	0.916	0.881	0.88	0.873	0.89
HA1	0.101	0.07	0.049	****	0.916	0.914	0.937	0.922	0.926	0.926	0.944	0.918	0.936	0.913	0.897	0.89	0.886	0.87	0.879	0.875	0.891
HA2	0.098	0.082	0.099	0.087	****	0.913	0.926	0.917	0.938	0.944	0.958	0.95	0.94	0.953	0.925	0.923	0.902	0.886	0.889	0.891	0.92
HA3	0.138	0.119	0.093	0.09	0.091	****	0.922	0.896	0.908	0.898	0.919	0.906	0.919	0.901	0.905	0.872	0.866	0.89	0.869	0.874	0.865
HO1	0.128	0.112	0.081	0.065	0.077	0.082	****	0.908	0.927	0.915	0.947	0.923	0.929	0.922	0.909	0.886	0.88	0.904	0.896	0.898	0.876
HO2	0.122	0.096	0.1	0.081	0.087	0.11	0.096	****	0.93	0.915	0.915	0.904	0.929	0.91	0.907	0.917	0.881	0.874	0.867	0.851	0.872
LA1	0.094	0.096	0.094	0.077	0.064	0.096	0.076	0.072	****	0.948	0.965	0.955	0.942	0.935	0.922	0.905	0.894	0.901	0.89	0.89	0.896
LA2	0.108	0.087	0.107	0.077	0.058	0.108	0.089	0.089	0.053	****	0.96	0.934	0.944	0.936	0.897	0.896	0.868	0.858	0.866	0.868	0.877
LA3	0.096	0.098	0.087	0.058	0.043	0.085	0.055	0.089	0.036	0.041	****	0.969	0.967	0.962	0.932	0.92	0.905	0.9	0.9	0.905	0.913
LA4	0.103	0.107	0.101	0.086	0.052	0.099	0.081	0.101	0.046	0.068	0.031	****	0.96	0.953	0.919	0.911	0.892	0.898	0.892	0.908	0.9
LA5	0.084	0.089	0.084	0.067	0.063	0.084	0.073	0.074	0.06	0.057	0.034	0.041	****	0.961	0.946	0.936	0.907	0.876	0.883	0.883	0.897
LA6	0.083	0.084	0.085	0.091	0.048	0.104	0.081	0.095	0.068	0.067	0.039	0.049	0.039	****	0.959	0.944	0.922	0.898	0.905	0.912	0.929
LA7	0.119	0.114	0.1	0.109	0.079	0.1	0.096	0.098	0.082	0.109	0.07	0.084	0.055	0.042	****	0.965	0.946	0.913	0.882	0.877	0.912
LA8	0.133	0.124	0.103	0.117	0.08	0.137	0.121	0.087	0.1	0.11	0.083	0.093	0.067	0.058	0.036	****	0.951	0.894	0.892	0.882	0.909
LA9	0.122	0.127	0.088	0.121	0.104	0.144	0.128	0.127	0.112	0.142	0.1	0.115	0.098	0.081	0.056	0.051	****	0.919	0.892	0.893	0.897
LA10	0.119	0.13	0.126	0.14	0.121	0.117	0.101	0.135	0.104	0.153	0.106	0.108	0.132	0.108	0.091	0.112	0.084	****	0.944	0.932	0.913
LA11	0.085	0.098	0.128	0.129	0.118	0.14	0.11	0.143	0.116	0.144	0.106	0.114	0.124	0.1	0.126	0.115	0.115	0.058	****	0.948	0.906
LA12	0.114	0.125	0.136	0.134	0.115	0.135	0.107	0.162	0.117	0.142	0.1	0.097	0.125	0.092	0.131	0.126	0.113	0.07	0.053	****	0.919
Idleb	0.095	0.091	0.116	0.116	0.084	0.145	0.132	0.137	0.11	0.131	0.091	0.105	0.108	0.073	0.092	0.095	0.108	0.092	0.099	0.085	****

of *P. brutia* based on morphological, anatomical, protein, allozymes and resin characteristic have revealed the existence of considerable variation of growth characteristic of this species (ARBEZ 1974; CALAMASSI *et al.* 1988; ISIK 1986; CONKLE *et al.* 1988). But, variation of most of these characteristics appeared to be related mostly to altitude and/ or climatic factors. In our study no relationship was identified between the variations in morphological characters (as crown shape, needles, angle of the stems, height to the first fork, height to the first branch, bends in the stem) and the altitude, the soil and mother rock, or climatic factors (data not presented).

Genetic diversity within and between populations in the same area

The gene diversity within and between populations in each region was estimated separately. The comparison of these values showed that this diversity varied from one population to another within the same region. For example, the difference in gene diversity values was higher between populations from Hama than those from Aleppo. The highest level of gene diversity was detected in the region of Latakia followed by Homs, Aleppo, Hama and the lowest value was in Idleb (Table 5). The detection of more diversity in Latakia populations could be due

to the high number of samples analyzed (180), which are collected from 12 different sites, and to the surface on which these collections were dispersed. On the other side, the variability within the populations was high which could be noticed from the high value of SD for each region (Table 5). This suggests that variation existing within populations and consequently within regions was more important than the values detected between regions.

Gene diversity between populations of different regions

The analysis demonstrated higher level of intra-population diversity compared to inter-populations. Region specific alleles were absent. All alleles detected in this study were present in all populations and all regions but with different frequency, some alleles (OPB-20.4 and OPC-13.4) were very frequent, they were present in 99.8% of the samples while others were rare (OPJ-1.10) and were present in 4% of the total samples (data not presented).

The majority of the gene diversity was detected within the *P. brutia* populations (93.2%, whereas only 6.8% exists between the populations (Table 6). This is in accordance with others studies based on different markers, although the percentages can vary considerably (KAYA & NEALE 1993; KARA *et al.* 1997; PANETSOS *et al.* 1998; KOROL *et al.* 2002).

The small value of genetic differentiation and gene flow in the different regions (0.1328 in Aleppo to 0.228 in Latakia, and 1.693 in Latakia to 3.266 in Aleppo, repectively) showed the little differentiation between populations from the different regions.

At the country level, the values of genetic diversity and genetic distance were calculated for the 21 population sites (Table 7). The highest value of genetic diversity was between populations Latakia 12 and Homs 2 and the smallest value was between populations Latakia 3 and 4.

The dendrogram based on the genetic distance (Fig. 4) showed that the 21 sites (populations) were clustered close to each other and regrouped into two distinguished groups. The most distant group comprises three sites of Latakia (sites 10, 11 and 12) which represent sites localized close to each other and in a relatively separated area of Latakia (Fig. 1) with one site of Idleb. The second group comprises three subgroups, the first one includes the populations 1 and 2 from Aleppo, the second one comprised the three populations from Lata-kia (7,8 and 9) while the other populations of Latakia were in the third subgroup with the populations from Aleppo and Hama and Homs. The populations 1, 2, 3, 4, 5 and 6 from Latakia were more close to each others than the others populations of Latakia. We could notice that in Latakia, the populations which were clustered close to each others in the dendrogram were collected from sites geographically close to each others (eg. Populations 1and 2, then 3, 4, 5, and 6, then 7, 8, and 9). The high level of similarity estimated between the population of Idleb and the populations 10, 11, and 12 of Latakia, and their clustering in the same group in the dendrogram could be due to the geographically close locations (Fig. 1). The situation is completely different for samples collected from Homs. Although the two sites in Homs are much more close to each other than to the other sites of Syria, the two populations analyzed have relatively high values of genetic distance (0.096) and have higher values of similarity with populations from distant sites (Table 7). For the regions of Aleppo, two of the three populations analyzed (1 and 2) were clustered in the same subgroup and were close to each other geographically. The third population, although it is geographically close to the other populations, was clustered with the population of Hama collected from a very distant place. The populations 1, 2 and 3 of Hama were the most dispersed populations. There were all in the same group but in three different subgroups (Fig. 4).

We could conclude from our analysis that although the genetic diversity within populations is higher than that between populations, the general level of genetic variability detected in the Syrian *P. brutia* was not high. Similar results were obtained on Turkish *P. brutia* and it was noted that the genotypes originated from distantly located geographic regions, with unfavorable ecological conditions for growth, had low value in proportion of polymorphic loci (KAYA & NEALE 1993, 1995). The low genetic variability seems to be a character of *P. brutia* where the same information was obtained using the isozymes analysis (KARA *et al.* 1997).

The genetic variability detected within populations of Syria is more important than the one detected between populations. These results confirm the results obtained with isozyme markers on *P. brutia* in the in the Aegean island, Greece populations (PANETSOS *et al.* 1998). Similar results were obtained through the analysis with SSR markers (ELHANS 2001).

The limited level of genetic diversity revealed by RAPD and AFLP markers in the Syrian populations of *P. brutia* could be explained by the fact that these trees were collected from relatively close areas. Seemingly they were subjected to similar conditions

of selection pressures imposed by common environmental influences. The recent development of SSR markers from species of *Pinus* like *P. halapensis* (KEYS *et al.* 2000, SHEPHERD *et al.* 2002) and even from *P. brutia* (ELHANS 2001) could help to reveal higher levels of variations in the Syrian populations. The detection of higher level of genetic diversity and the identification of specific population alleles would provide good information about the genetic structure of the Syrian *P. brutia* populations and could help in selecting the most variable genotypes to conserve and use them as a source for the national forestation program of *P. brutia*.

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REFERENCES

ADAMS, R. P. & TURUSPEKOV, Y. 1998: Taxonomic reassessment of some Central Asian and Himalayan scale-leaved taxa of *Juniperus (Cupressaceae)* supported by random amplification of polymorphic DNA. *Taxon* **47**: 75–83.

ÅKERMAN, S., TAMMISOLA, J., REGGINA, M., KAUPPINEN, V. & LAPINJOKI, S. 1996: Segregation of AFLP markers in *Betula pendula* (Roth). *For. Genet.* **3** (20): 117–123.

ALI, M. 2001: *Pinus brutia* Ten. **in**: Breugel, P. van and T.O.M. Bazuin. Editors. Development of Research Activities on the Conservation and use of Forest Genetic Resources in Syria. Workshop report, 12–13 May 1998. International Plant Genetic Resources Institute, Rome, Italy: 15–17.

ANGIOLILLO, A., MENCUCCINI, M. & BALDONI L. 1999: Olive genetic diversity assessed using amplified fragment length polymorphisms. *Theor. Appl. Genet.* **98**: 411–421.

ARBEZ, M. 1974: Distribution, ecology and variation of *Pinus brutia* in Turkey. FAO for: Occas. Paper, For. Genet. Resour., Information: 21–33

BAI, Y., MICHAELS, T.E., & PAULS, K.P. 1997: Identification of RAPD markers linked to common bacterial blight resistant genes in *Phaseolus vulgaris* L. *Genome* **40**: 544–551.

BARBÉRO, M., CHALABI, N., NAHAL, I., & QUEZEL, P. 1976: Les formations à conifers méditeranéens en Syrie littorale. *Ecol. Mediter.* **2**:87–98.

BARBÉRO, M., LOISEL, R., QUÉZEL, P., RICHARDSON, D.M.& ROMANE, F. 1998: Pines of the Mediterranean Basin. *in*: Richardson, D.M. Editor. Ecology and Biogeography of *Pinus*. Cambridge University Press, UK: 153–170.

BARITEAU, M. 1992: Variabilité géographique et adaptation aux contraintes du milieu méditerranéen des pins de la section *halepensis*: resultants (provisoires) d'ún essai en plantations comparatives en France. *Ann. Sci. For.* **49**: 261–276.

BASSAM, B.J., CAETANO-ANOLLES, G., AND GRESSHOFF, P.M. 1991: Fast and sensitive silver staining of DNA in polyacrilamide gels. *Analy. Biochem.* **196**: 80–83.

BINELLI, G., & BUCCI, G. 1994: A genetic linkage map of *Picea abies* Karst., based on RAPD markers, as a tool in population genetics. *Theor. Appl. Genet.* **88**: 288 –288.

BOEGER, J.M., CHEN, R.S. & MCDONALD, B.A. 1993: Gene flow between geographic populations of *Mycosphaerella graminicola* (Anamorph *Septoria tritici*) detected with restriction fragment length polymorphism markers. *Phytopathology* **83**: 1148–1154.

CALAMASSI, R., PUGLISI, S. R. & VENDRAMIN, G.G. 1988: Genetic variation in morphological and anatomical needle characteristics in *Pinus brutia* Ten. *Silvae Genet.* **37**(5 –6): 199–206.

CARLSON, J. E., TULSIEARAM L. K, GLAUBITA J. C., LUK, V.W.K., KAUFFELDT, C & RUTLEDGE R. 1991: Segregation of random amplified DNA markers in F_1 progeny of conifer. *Theor. Appl. Genet.* **83**:194 –200.

CHOUMANE, W., ACHTAR, S., VALKOUN, J., AND WEIGAND, F. 1998: Genetic variation in core and base collections of barley from WANA as revealed by RAPD's. pp. 159–164 *in*: A.A Jaradat (Ed.), Triticeae III. Science Publishers, Inc., Enfield, NH, USA

CONKLE, M. T., SCHILLER, G. & GRUNWALD, C. 1988: Electrophoretic analysis of diversity and phylogeny of *Pinus brutia* and closely related taxa. *Syst. Bot.* **13**(3):411–424.

DALE, G., GATES, C., VOGEL,H., DAGGARD, G., & TEASDALE, B. 1992: Mapping of the interspecific hybrid between *Pinus caribaea* and *Pinus elliottii* using RAPD markers. **in**: 6th International conifer Biotechnology Working Group. April 23-28, Reaserch Triangle Park, N.C.

DOYLE, J.J. & DOYLE J.L. 1990: Isolation of plant DNA from fresh tissue. *Focus* **12**:13–15.

EMBERGER, L., GAUSSEN, H., KASSA, A. & DE PHILLIPS, A. 1963: Bioclimatic map of the Mediterranean zone. Ecological study of the Mediterranean zone. Arid Zone Research. UNESCO-FAO, Paris. 58 pp. and two maps.

ELHAN, E. O. 2001: The pattern of genetic variation of *Pinus brutia* populations from southern Turkey determined by nuclear SSR markers. Middle East Technical University , Department of Biology, Ankara , Turkey. M Sc Thesis, September 2001, 163 pp.

EUJAYL, I., BAUM, M., POWELL, W., ERSKINE, W., & PEHU, E. 1998a: A genetic linkage map of lentil (*Lens* sp.) based on RAPD and AFLP markers using recombinant inbred lines. *Theor. Appl. Genet.* **97**: 83–89.

EUJAYL, I., ERSKINE, W., BAYAA, B., BAUM, M. & PEHU, E. 1998b: *Fusarium* vascular wilt in lentil: Inheritance

and identification of DNA markers for resistance. *Plant Breed.* **117**: 497–499.

EUJAYL, I., ERSKINE, W., BAYAA, B., BAUM, M. & PEHU, E. 1999: Inheritance and linkage analysis of frost injury in lentil. *Crop Sci.* **39**(3): 639–642.

FERGUSON, M.E., NEWBURY, H.J., MAXTED, N., FORD-LLOYD, B.V. & ROBERTSON L.D. 1998: Population genetic structure in *Lens* taxa revealed by isozyme and RAPD analysis. *Genet. Res. Crop Evol.* **45** (6): 549–559.

GRACIA-MAS, J., OLIVER, M., GOMEZ-PANIAGUA, & VINCENTE M.C.DE. 2000: Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. *Theor. Appl. Genet.* **101**: 860–864.

GRATTAPAGLIA, D., CHAPPARRO, J., WILCOX, P. , MCCORD, S., WERNER, D., AMERSON, H., MCKEAND, S. E., BRIDGWATER F., WHETTEN R., O'MALLEY D., & SEDEROFF R. 1992: Mapping in woody plants with RAPD makers: Application to breeding in forestry and horticulture. Joint Plant Breeding Symposia Series on Applications of RAPD technology to Plant Breeding, Novembre 1, Minneapolis, Minnesota. Pp.40–50.

GRATTAPAGLIA, D. & SEDEROFF R. 1994: Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus uraphylla* using a pseudo-testcross: mapping strategy and RAPD markers. *Genetics* **137**: 1121 –1187.

HAYASHI, K., KONO, T., TERADA K. & KAWASAKI. 2001: AFLP markers linked to gene for resistance to pine needle gall midge in Japanese black pine (*Pinus thunbergii*). Poster. Plant & Animal Genome IX Conference, San Diego, CA, January 13–17.

HE, G.H. & PRAKASH C.S. 1997: Identfication of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). *Euphytica* **97**: 143–149.

HILL, M., WITSENBOER, H., ZABEAU, M., VOS, P., KESSELI, R. & MICHELMORE, R. 1996: PCR-based fingerprinting using AFLPs as a tool for studying genetic relationships in *Lactuca* spp. *Theor. Appl. Genet.* **93**: 1202–1210.

HONGTRAKUL, V., HUESTIS, G.M. & KNAPP, S. 1997: Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. *Theor. Appl. Genet.* **95**: 400–407.

HOQUE, M.E., MISHRA, S.K. & SARKER A. 2002: Inheritance and linkage relationship between morphological and RAPD markers in lentil (*Lens culinaris* Medik). *Indian J. Genet.* **62**(1):5–10

ISIK, K. 1986: Altitudinal variation in *Pinus brutia* Ten.: Seed and seedling characteristics. *Silvae Genet.* **35**(2–3): 58–67.

KARA, N., KOROL, L., ISIK, K. & SCHILLER, G. 1997: Genetic diversity in *Pinus brutia* Ten.: Altitudinal variation. *Silvae Genet.* **46**(2-3): 155–161.

KAYA, Z. & NEALE, D.B. 1993: Randomly identified DNA (RAPD) polymorphisms in *Pinus nigra* var pallasiana and *P. brutia*. *Turk. J. Agric. For.* **17**: 295–306.

KAYA, Z. & NEALE, D.B. 1995: Utility of random amplified polymorphic DNA (RAPD) markers for linkage mapping in Turkish red pine (*pinus brutia* Ten.). *Silvae Genet.* **44** (2–3): 110–116.

KEYS, R.N., AUTINO, A., EDWARDS, K.J., FADY, B., PICHOT, C. & VENDRAMIN, G.G. 2000: Characterization of nuclear microsatellites in *Pinus halepensis* Mill. and their inheritance in *P. halepensis* and *Pinus brutia* Ten. *Mol. Ecol.* **9**: 2155–2234.

KOROL, L., SHKLAR, G. & SCHILLER, G. 2002: Genetic variation within and among *Pinus brutia* Ten. seed stands in Turkey in their isoenzymes. *For. Genet.* **9**: 233–242.

LERCETEAU, E. & SZMIDT, A.E. 1999: Properties of AFLP markers in inheritance and genetic diversity studies of *Pinus sylvestris* L. *Heredity* **82**: 252–260.

LU, M. Z., SZMIDT, A. E. & WANG, X. R. 1995: Inheritance of RAPD fragments in haploid tissues of *Pinus sylvestris* (L.). *Heredity* **74**: 582–589.

MAUGHAN, P.J., SAGHAI-MAROOF, M.A., BUSS, G.R, HUESTIS, G.M. 1996: Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance and nearisogenic line analysis. *Theor. Appl. Genet.* **93**: 392–401.

MIGNOUNA, H.D., NG, N.Q., IKEA, J. & THOTTAPILLY, G. 1999:.Genetic diversity in cowpea as revealed by random amplified polymorphic DNA. *J. Genet. & Breed.* **53**: 151–159.

MIROV, N.T. 1967: The Genus *Pinus*. The Roland Press Company, New York, N. P.252–254.

NAHAL, I. 1983: Le Pin Brutia (*Pinus brutia* Ten. subsp. *brutia)*. Première partie. *Forêt Méditerr.* **5** (2): 165–172.

NAHAL, I. 1984: Le Pin Brutia (*Pinus brutia* Ten. subsp. *brutia*). Deuxième partie. *Forêt Méditerr.* **6** (1): 5–18.

NEI , M. 1973: Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA* **70**: 3321–3323.

NEI, M. 1987: Molecular Eolutionary Genetics. Columbia University Press, New York, NY.

NELSON, C.D., NANCE, W.L. & DOUDRICK, R.L. 1993: A partial genetic linkage map of slash pine (*Pinus elliotti* Engelm. var. *elliottii*) based on randomly amplified polymorphic DNAs (RAPD). *Theor. Appl. Genet.* **87**: 145–151.

PANETSOS C.P., 1981: Monograph of *Pinus halepensis* (Mill.) and *P. brutia* (Ten). *Ann. Forest.* **9**(2): 39 = 77.

PANETSOS, K. P., ARAVANOPOULOS, F.A. & SCALTSOYIANNES, S. 1998: Genetic variation of *Pinus brutia* from islands of the Northeastern Aegean sea. *Silvae Genet.* **47** (2–3): 115–120.

RODRIGUEZ, J..M., BERKE, T., ENGLE, L., & NIENHUIS J. 1999: Variation among and within *Capsicum* species revealed by RAPD markers. *Theor. Appl. Genet.* **99**: 147–156.

ROHLF, F.J. 1997: NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistical Inc., New York.

SELIK, M. 1958: Botanical investigation on *Pinus brutia* especially in comparison with *P. halepensis*. Ist Univ.

Orm. Fab. Derg. 8a: 161–198.

SHEPHERD, M., CROSS, M., MAGUIRE, T.L., DIETERS, M.J., WILLIAMS, C.G. & HENRY R.J. 2002: Transpecific microsatellites for hard pines. *Theor. Appl. Genet.* **104**: 819–827.

SNEATH, P.H.A & SOKAL . R. 1973: Numerical Taxonomy. Freeman. San Francisco. 573 pp.

SOUVANNAVONG, O., MALAGNOUX, M. & PALMBERG-LERCHE, C. 1995: International cooperation in the conservation of Mediterranean forest genetic resources. *Diversity* **11** (1–2): 19–20.

SPENCER, D.J. 1985: Dry country pines: Provenance evaluation of the *Pinus halepensis* = *P.brutia* complex in the semi-arid region of south-east Australia. *Austral. For. Res.* **15**: 263–279.

SPENCER, D.J. 2001: Conifers in the dry countries. A report for the RIRDC/L7W Australia/FWPRDC Joint Venture Agroforestry Program. RIRDC Publication No. 01/46. RIRDC Project No. CSF-57A.

TEULAT, B., ALDAM, C., TREHIN, R., LEBRUN, P., BARKER, J.H.A., ARNOLD, G.M., KARP, A., BAUDOUIN, P. & ROGNON, F. 2000: An analysis of genetic diversity in coconut (*Cocos nucifera*) populations from across the geographic range using sequence-tagged microsatellites (SSRs) and AFLPs. *Theor. Appl. Genet.* **100**: 764–771.

TULSIERAM, L.K., GLAUBITZ, J.C., KISS, G. & CARLSON J.E. 1992: Single tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. *Biol/Technol.* **10**: 686–690.

VIDAL, J.R. COARER, M. & DEFONTAINE A. 1999: Genetic relationships among grapevine varieties grown in different French and Spanish regions based on RAPD markers. *Euphytica* **109**: 161–172.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M., VAN DE LEE, T. , HORNES, M., FRITJERS, A., POT, J., PELEMAN, J., KUIPER, M. & ZABEAU, M. 1995: AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res.* **23**: 4407–4414.

WEIR, B.S. 1990: Genetic Data Analysis. Sinauer Associates, Sunderland, Mass.

WELSH, J & MCCLELLAND, M. 1990: Finger-printing genomes using PCR with arbitrary primers. *Nucl. Acids Res.* **18**:7213–7218.

WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFALSKI, J.A. & TINGEY, S.V. 1990: DNA polymorphism amplified by arbitrary primers as useful as genetic markers. *Nucl. Acids Res.* **18**: 6531–6535.

WRIGHT, S. 1951: The genetical structure of populations. *Ann. Eugenics* **15**: 323–354.

YEH, F.C., BOYLE, T.J.B. 1997: Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belg. J. Bot.* **129**: 157.

YIN, T M., WANG, X-R., ANDERSSON, B., LERCETEAU-KOHLER, E. 2003: Nearly complete genetic maps of *Pinus sylvestris* L. (Scots pine) constructed by AFLP marker analysis in a full sib family. *Theor. Appl. Genet.* **106** (6): 1075–1083.

ZHANG, X.Y., WANG, R.R.C. & DONG, Y.S. 1996: RAPD polymorphisms in *Aegilops geniculata* Roth (Ae. *ovata auct.*). Genetic resource crop evolution. Dordrecht, The Netherlands : Kluwer Academic Publishers, c1992-. Oct 1996. **43** (5): 429–433.

ZOHARY, M. 1973: Geobotanical Foundations of the Middle East. Gustav Fischer Verlag, Stuttgart.