GENETIC VARIATION OF ALEPPO PINE (*PINUS HALEPENSIS* MILL.) IN SPAIN

Dolores Agúndez [1], Bernd Degen[2], Georg von Wuehlisch[2] & Ricardo Alia[1]

[1)] Area de Selvicultura y Mejora. CIFOR–INIA. Apdo 8111. E–28080 Madrid. Spain
[2)] Institut für Forstgenetik. Sieker Landstrasse 2. D–22927 Grosshansdorf. Germany.

Received April 1, 1997; accepted August 26, 1997

ABSTRACT

Six populations of *Pinus halepensis* from Spain were analysed by means of starch gel electrophoresis using seven enzyme systems. Four of the 15 analysed loci (22%) were polymorphic at the 95% level. Expected heterozygosity (H_e= 0.063) and effective number of alleles (n_e= 1.07) are low when considering all 15 loci. The populations from Spain have no significant excess of homozygotes (F_{IS}= 0.066). A high variation among populations was detected (G_{ST} = 0.11), in some of the loci analysed, which results in a geographic pattern of variation in populations, from the North-East to the South-East of Spain.

Keywords: *Pinus halepensis*, allozymes, genetic variation, genetic differentiation, inheritance

INTRODUCTION

Aleppo pine (*Pinus halepensis* Mill.) is a widely distributed species all around the Mediterranean Basin, from Jordan in the East to Spain in the West (CRITCHFIELD & LITTLE 1966).

Studies on the genetic variability of Aleppo pine, show lower levels of polymorphism and of genetic differentiation among populations (SCHILLER *et al.* 1986; CONKLE *et al.* 1988; TEISSEIRE *et al.* 1995; KOROL & SCHILLER 1996) than most pine species (HAMRICK *et al.* 1992; PRUS-GŁOWACKI & STEPHAN 1994; SALVADOR *et al.* 1997). Five distinct groups have been defined on the basis of allozyme frequencies (SCHILLER *et al.* 1986): east Mediterranean, west European, east European, Moroccan, and Algerian. A clear pattern of variation in relation to the country of origin was also shown by terpene analysis (SCHILLER & GRUNWALD 1987; BARADAT *et al.* 1995). The most likely origin of Aleppo pine in Spain, France and Morocco is natural, and human influence does not seem to be the main force related to the genetic variability within the French-Spanish-Moroccan area. However Italy has a highly heterogeneous composition in its stands and human intervention over many centuries is the most important fact explaining differentiation among stands (BARADAT *et al.* 1995; SCHILLER & BRUNORI 1992).

The species presents a continuous range in Spain, covering 600,000 ha of scattered natural stands and several marginal populations in the south and centre of the country. Forest fires and farming have been the main forces in fragmentation of the area of the species, which would influence genetic drift and gene flow between populations.

Selection for drought and frost tolerance has led to adaptation of provenances. Marked differences among populations of Aleppo pine in terms of growth and survival have been reported (BARITEAU 1992). Most stands in Spain can be found over a wide range of ecological conditions, in terms of rainfall (from 300 to 900 mm), temperature, frost, and soil conditions (GANDULLO & SÁNCHEZ-PALOMARES 1994). The species is found in Spain mainly on limestone and in mild climates but can live with continental conditions at its westernmost limit of distribution. Additionally, the species has been widely used in afforestation programs in semi-arid conditions.

Eighteen regions of provenance have been defined, based on both ecological variation and geographical distribution (GIL *et al.* 1996) but very little genetic information has been considered.

It is, therefore, necessary to expand our knowledge of the genetic structure of Spanish populations and of the pattern of genetic variation in order to improve the use of forest reproductive material of the species .

The objective of this study was to analyse the level of genetic variation within and among six natural Spanish populations of Aleppo pine by means of isozyme electrophoresis.

MATERIAL AND METHODS

Seed samples were obtained from six natural populations of *Pinus halepensis*, from the natural range of the species in Spain, as shown in Figure 1. A wide range of ecological conditions is covered in these populations. The geographic origin of the sampled populations are presented in Table 1. The bulked seed samples were composed of seeds from 25 trees with a minimum distance of 30 m inbetween.

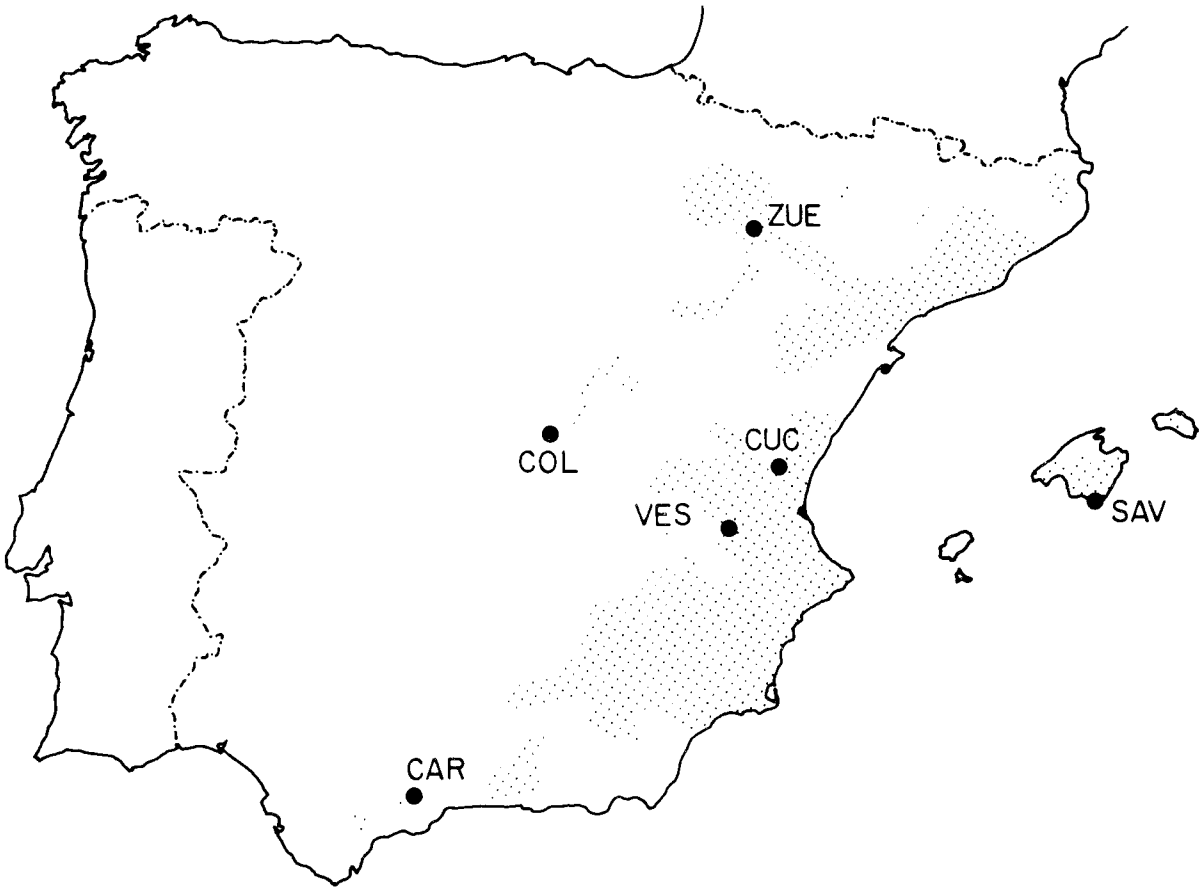

Figure 1. Natural range of *Pinus halepensis* Mill. in Spain and location of six natural populations

Table 1. Location and ecological conditions of the six *Pinus halepensis* populations from Spain

Population	Code	Latitude	Longitude	Altitude (m)	Average annual rainfall (mm)	Average annual temperature (°C)
Zuera	ZUE	41°55'00" N	0°55'40" W	575	410	20.1
Colmenar	COL	40°05'20" N	3°20'10" W	750	480	10.9
Cucalón	CUC	39°47'15" N	0°36'44" W	575	473	20.8
Villa de vés	VES	39°10'44" N	1°14'52" W	850	463	20.6
Carratraca	CAR	36°50'28" N	4°50'40" W	650	567	20.4
S'Avall	SAV	39°17'14" N	3°02'52" E	10	410	16.0

Seeds were germinated until they had a radicle of 2–5 mm in length and then stored at 4 °C to stop embryo growth. Forty to sixty seeds per population were analysed employing ten enzyme systems (AAP, AAT, ACO, ADH, GDH, HK, MDH, 6PGDH, PGM, SKDH) and two different buffer systems were used, as shown in Table 2. The composition of the two buffer systems is the following:

System A: Gel buffer: 0.067 M tris, 0.008 M citric acid, pH 8.7. Electrode buffer: sodium hydroxide 0.06 M, boric acid 0.3 M pH 8.2 (CONKLE *et al.* 1982).

System B: Gel buffer: 0.15 M tris, 0.05 M citric acid, pH 7.5. Electrode buffer: 0.02 M tris, 0.02 M citric acid, pH 7.5 (WENDEL & WEEDEN 1989).

Staining procedure was adapted from CONKLE *et al.* (1982), CHELIAK & PITEL (1984), and WENDEL & WEEDEN (1989). All enzymes of each megagametophyte and the corresponding embryo from each seed were assessed simultaneously. Thus, it was possible to obtain ordered genotypes by comparing them.

In order to test the genetic control of loci segregation, χ^2 tests of allozyme frequencies from 49 or 50 endosperms of a single tree were analysed for each polymorphic locus in order to test the 1:1 segregation.

Table 2. Enzyme systems, abbreviations and buffer systems used for electrophoretic analyses

Enzyme system	Abbreviation	E.C. code	Buffer system
Alanine amino peptidase	AAP	E. C. 3.4.11.1	
Aspartate amino transferase	AAT	E. C. 2.6.1.1	
Glutamate dehydrogenase	GDH	E. C. 1.4.1.3	System A
Hexokinase	HK	E. C. 2.7.1.1	
Phosphoglucomutase	PGM	E. C. 2.7.5.1	
Aconitase	ACO	E. C. 4.2.1.3	
Alcohol dehydrogenase	ADH	E. C. 1.1.1.1	
Malate dehydrogenase	MDH	E. C. 1.1.1.37	System B
6-phosphogluconate dehydrogenase	6PGDH	E. C. 1.1.1.44	
Shikimate dehydrogenase	SKDH	E. C. 1.1.1.25	

A product structure test (GILLET 1994) was performed to assess departures from random mating considering the paternal and maternal allele frequency. A chi-square test of homogeneity was performed among maternal and paternal contributions of the allozyme frequencies.

A χ^2 test of allozyme frequencies was used to detect significant heterogeneity in allele frequency among populations (WORKMAN & NISWANDER 1970). The number of polymorphic loci (at the 99% and 95% level), the effective number of alleles (n_e) or diversity (v; GREGORIUS 1978), the total population differentiation δ_T (GREGORIUS 1987), the expected and observed heterozygosity along with the fixation index (F_{IS} = $1-H_o/H_e$; WRIGHT 1965) were computed for each population and all 15 loci as a whole.

For quantification of genetic variation, the diversity v takes into account both the absolute number of different genetic types (alleles or genotypes) and their relative frequency. If all genetic types are equally distributed, then v and the absolute number of alleles are identical. The measure δ_T quantifies the genetic differentiation within a population. If all individuals have the same genotype $\delta_T = 0$ and if all genotypes are different $\delta_T = 1$.

For each polymorphic locus, NEI's (1973) parameters of differentiation within and among populations were estimated. Total genetic diversity (H_T) was partitioned into a within population component (H_S) and an among population component (D_{ST}). Among population variation was compared to total genetic variation to give $G_{ST} = D_{ST}/H_T$. The H_S and D_{ST} values were calculated for each locus and then combined over all polymorphic loci. Therefore, G_{ST} was estimated after the mean H_T and H_S. Gene flow (Nm) was estimated as $Nm = (1-G_{ST})/4G_{ST}$ (NEI 1977).

NEI's (1978) and *D* (PREVOSTI *et al* 1975; GREGORIUS 1984) genetic distances among populations were computed considering each and every locus. Cluster analyses using PREVOSTI distance were performed using the unweighted pair group method algorithm (UPGMA).

The analyses were performed using GSED programme, version 1.1b (GILLET 1994), BIOSYS (SWOFFORD & SELANDER 1981) and the authors own programs.

RESULTS

Genetic structure and genetic diversity of populations

Three of the ten systems analysed (ACO, ADH and SKDH) did not show any reproducible enzyme activity, and they were omitted from subsequent analysis. A total of 15 loci were analysed from seven enzyme systems: AAP (2), AAT (3), GDH (1), HK (1), MDH (4), 6PGDH (2) and PGM (2). Loci and alleles were named after their relative position in the gel, 1 being the more anodal. Enzyme bands phenotypes and genetic interpretation of polymorphic systems are presented in Figure 2.

Segregation analyses in megagametophytes of single trees showed no deviation from the expected 1:1 Mendelian ratio. Data obtained and χ^2 values for polymorphic loci are presented in Table 3.

Table 3. χ^2 - test for allele frequency segregation

Locus	Tree	Allele 1	Allele 2	χ^2 probability
Mdh-4	ZUE-25	23	27	0.32
6-pgdh-2	ZUE-25	26	24	0.08
Hk	CAR-1	22	27	0.51
Pgm-2	COL-23	26	24	0.08

A random mating is assumed, as only the locus *Mdh–4* in VES population and the locus *6Pgdh–2* in COL popula-

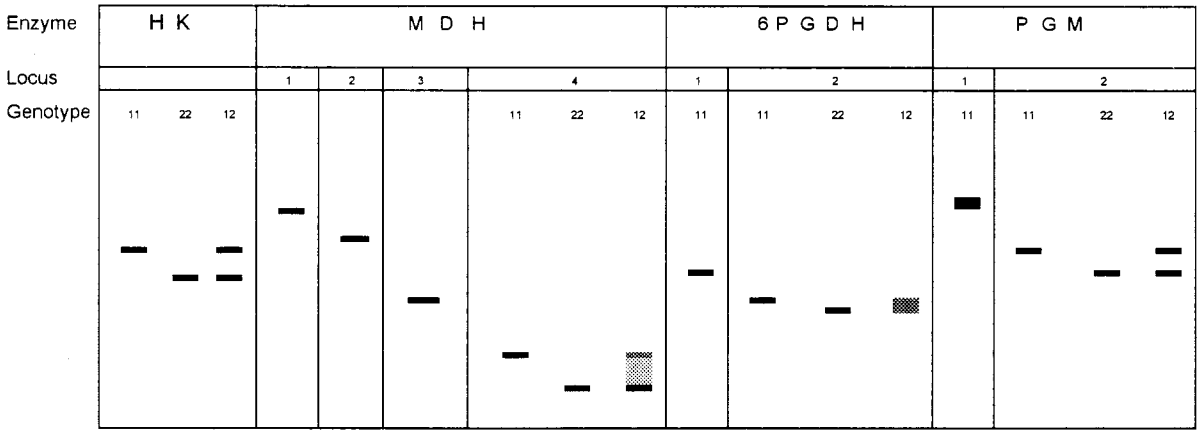

Figure 2. Genetic interpretation of enzyme bands phenotypes for 4 polymorphic loci in embryo tissue of *Pinus halepensis* Mill.

Table 4. Allele frequencies of the polymophic loci by populations

Locus	Allele	ZUE	COL	CUC	VES	CAR	SAV	Mean
Mdh-4	1	0.9479	0.9500	0.8875	0.8583	0.8205	0.9800	0.9074
	2	0.0521	0.0500	0.1125	0.1417	0.1795	0.0200	0.0926
	N^1	96	80	80	120	78	100	554
6-pgdh-2	1	0.8854	0.8000	0.9750	0.9000	0.8382	0.8333	0.8720
	2	0.1146	0.2000	0.0250	0.1000	0.1618	0.1667	0.1280
	N^1	96	80	80	80	68	78	482
Hk	1	0.0938	06154	0.4500	0.3250	0.7321	0.3382	0.4257
	2	0.9063	0.3846	0.5500	0.6750	0.2679	0.6618	0.5743
	N^1	96	52	80	80	56	68	432
Pgm-2	1	0.7396	0.9500	0.8250	0.9054	1.0000	0.9625	0.7399
	2	0.2604	0.0500	0.1750	0.0946	0.0000	0.0375	0.2601
	N^1	96	80	80	74	70	74	474

[1] Sample size (number of alleles)

tion showed deviation from the product structure. Paternal and maternal contributions to allele frequencies do not differ significantly, as shown by a χ^2 test for each locus and population. Thus, paternal and maternal contributions were pooled for subsequent analysis. Allele frequencies of the four polymorphic loci are shown in Table 4.

There is significant heterogeneity among populations for allozyme frequencies of the four loci, as shown in Table 5. *Hk* and *Pgm–2* are loci with the highest heterogeneity among populations. *Mdh–4, 6Pgdh–2* and *Pgm–2* showed allele 1 as the most frequent in all three loci. Hk is the most polymorphic locus in every population, with allele 1 as predominant in two populations (COL and CAR).

Four of the 15 loci analysed (22%) were polymorphic at the 95% level: *Mdh–4, 6Pgdh–2, Hk* and *Pgm–2*, as shown in Table 6. In these four loci the number of alleles per locus averaged to 1.8 alleles, and the 15 loci as a whole gave a value of 1.2 alleles per locus.

Descriptive genetic parameters for the six populations are shown in Table 6. A population from Mallorca island (SAV), has the lowest level of polymorphism (13%). The species displays a low level of genetic diversity, in terms of effective number of alleles (1.07 for the six populations). Total population differentiation is quite similar among populations and identical to expected heterozygosity due to the sample size. Observed and expected heterozygosities have low mean values (0.059 and 0.063 respectively). This figure is similar to that reported by SCHILLER et *al.* (1986) for the eastern group of provenances and higher than in France (TEISSEIRE et *al* 1995). Wright's fixation index shows that there is a slight excess of homozygosity (0.066) in the overall populations. The values vary from an excess of heterozygosity in the ZUE population (–0.023) to a slight excess of homozygosity in COL and CUC populations (0.203 and 0.152 respectively).

Table 5. χ^2-test for significant allele frequency heterogeneity among 6 *Pinus halepensis* populations from Spain

Locus	Allele	χ^2	DF	Significance
Mdh-4	1	2.10		
	2	20.56		
	Total	22.66	5	***
6-pgdh-2	1	2.22		
	2	15.15		
	Total	17.37	5	**
Hk	1	60.71		
	2	45.00		
	Total	105.71	5	***
Pgm-2	1	5.32		
	2	46.39		
	Total	51.71	5	***

However, these values do not differ significantly from the equilibrium.

Table 7 shows Nei's genetic parameters of variation within and among populations. Total genetic variation for the species, considering all the 15 analysed loci, is low (0.071). However, 11% of this variation is due to among population variation (G_{ST}), with a large difference depending on the locus in question. This value varies from 18% for *Hk* to 3% for *6Pgdh–2*.

Genetic distance among populations

Nei's and Prevosti distances, combining all the loci, are shown in Table 8. Nei's genetic distances among populations varies from 0.002 to 0.036, with a mean of 0. 010

Table 7. Nei's genetic parameters of variation within and among 6 populations of *Pinus halepensis* from Spain

Locus	H_T	H_S	D_{ST}	G_{ST}	Nm
Mdh-4	0.168	0.1620.2	0.006	0.037	6.357
6-pgdh-2	0.223	17	0.006	0.029	8.384
Hk	0.489	0.403	0.086	0.176	1.169
Pgm-2	0.186	0.169	0.016	0.086	2.651
Average[1]	0.266	0.238	0.029	0.109	2.044
	(0.071)	*(0.063)*	*(0.007)*	*(0.099)*	*(2.275)*

[1] Average: 4 polymorphic loci included; in brackets and italics, values when all 15 loci are included

and Prevosti distances are higher, varying from 0.017 to 0.072, with a mean of 0.036.

The cophenetic correlation between the ultrametric distance obtained by the cluster algorithm and the Prevosti genetic distance is 0.70. We can therefore use the dendrogramme obtained by the UPGMA method (Figure 3), to display the relationship among populations. A population from northern Spain (ZUE) shows the longest genetic distance from the others. The main group of populations is split in two groups: the first one includes the populations from the island of Mallorca (SAV) and the eastern populations (VES, CUC); the second one includes a population from southern Spain (CAR) and an isolated population from central Spain (COL).

DISCUSSION

TEISSEIRE *et al.* (1995) made a complete revision of different enzyme systems in Aleppo pine. The genetic interpretation presented in this study are in accordance with those presented by TEISSEIRE *et al.* (1995). We have

Table 6. Descriptive genetic parameters of 15 isozyme loci in 6 *Pinus halepensis* populations from Spain

Population	99 % criterion		95 % criterion		Effective number of alleles	Heterozygosity		Fixation index
	# of alleles per locus	% of polymorphic loci	# of alleles per locus	% of polymorphic loci		expected	observed	
ZUE	1.3	27	1.3	27	1.06	0.057	0.058	–0.023
COL	1.3	27	1.3	27	1.07	0.066	0.052	0.203
CUC	1.3	27	1.2	20	1.07	0.069	0.058	0.152
VES	1.3	27	1.3	27	1.07	0.069	0.064	0.062
CAR	1.2	20	1.2	20	1.07	0.064	0.066	–0.021
SAV	1.3	27	1.1	13	1.06	0.055	0.056	–0.009
Average	1.3	26	1.2	22	1.07	0.063	0.059	0.066

Table 8. Nei's (1978) genetic distances among populations for 15 loci (below diagonal) and Prevosti genetic distances (above diagonal)

Population	ZUE	COL	CUC	VES	CAR	SAV
ZUE	–	0.055	0.039	0.033	0.072	0.037
COL	0.023	–	0.035	0.035	0.022	0.024
CUC	0.010	0.006	–	0.021	0.044	0.032
VES	0.006	0.008	0.002	–	0.040	0.017
CAR	0.036	0.002	0.010	0.013	–	0.040
SAV	0.008	0.006	0.004	0.002	0.013	–

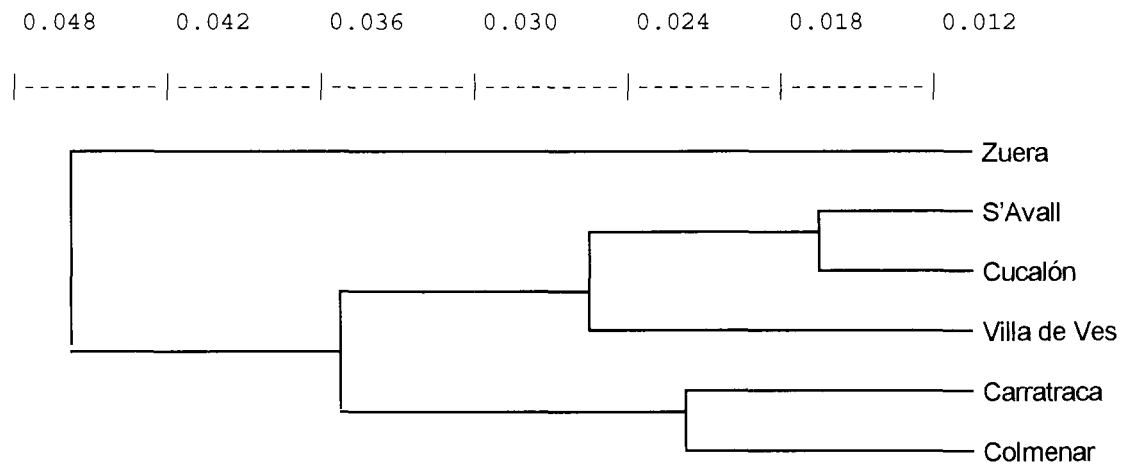

Figure 3. UPGMA cluster analysis of Prevosti genetic distances between 6 populations of *Pinus halepensis* (mono- and polymorphic loci included).

followed LOUKAS *et al.* (1983) for the interpretation of *Hk*, not studied by the former authors. The locus *Hk* displays the largest variability in this study. Absence of alleles reported probably as the result of introgression with *P. brutia* (SCHILLER *et al.* 1986) stresses the natural origin of Aleppo pine in Spain and the fact that no material was introduced from the eastern Mediterranean (BARADAT *et al.* 1995; SCHILLER & MENDEL 1995).

Our results are not fully comparable with those reported in other studies due to the different sample size and enzyme systems analysed. SCHILLER *et al.* (1986) studied only two provenances from a limited area in south-eastern Spain. They found lower frequencies in *Mdh–4* and *6Pgdh–2* allele 1 than those shown in our study. The two populations were reported as polymorphic for *Mdh–3* and *Aap–1* (5 and 1% level respectively). The percentage of polymorphic loci is related to the sample size. In this study the sample size is greater than 80 per population which result in a probability larger than 95% of detecting alleles with a minimum frequency of 7% (GREGORIUS 1980); however it is not enough to detect alleles with frequency of 1%. Therefore, it will be necessary to include some more provenances and larger sample sizes in a subsequent study in order to have a clear pattern of variation for the whole area.

Pgm–2 and *Hk* are polymorphic loci that have not been analysed in Spanish populations before; some differences have been found among populations within the natural range. There is no deficiency of allele 1 in *Pgm–2* as was found in French populations (TEISSEIRE *et al.* 1995), and there is no evidence of gametic selection against this allele. Moreover, the most frequent allele found in Spain is the most anodal band (allele 1), which is not the case in French populations. Allele frequencies of *Hk* and *Pgm–2* have similar values to those shown by LOUKAS *et al.* (1983) in Greek populations. The difference is that Greek populations showed two more rare alleles that have not been found in the Spanish populations.

The proportion of polymorphic loci is slightly larger in our study (22%) than in the whole range of the species (15%, SCHILLER *et al.* 1986), and clearly larger than in French populations (14%, TEISSEIRE *et al.* 1995). Genetic diversity of the analysed populations, measured by the level of heterozygosity, is similar to that obtained for the whole range of the species. It is considered low in comparison to that of other conifers (HAMRICK *et al.* 1992).

The analysed Spanish populations do not show significant deviation from Hardy-Weinberg equilibrium. The equilibrium among maternal and paternal contribution suggest an efficient sampling of the mother trees to define the genetic structure of the populations. The lower level of heterozygosity was found in two populations from central Spain (COL) and from the island of Mallorca (SAV). However, the slight difference in the values does not permit us to conclude any loss of diversity, measured both in terms of v and heterozigosity, due to isolation in both populations.

Significant differences have been found in allozyme frequencies mainly in the loci *Hk* and *Pgm–2*. In spite of showing low genetic diversity, genetic differentiation among populations is higher than that obtained in other species. HAMRICK *et al.* (1992) gave an average coefficient of genetic differentiation for gymnospermes of 7.3%, while we have estimated in this study 11% for Aleppo pine. In other pine species in Spain, considerably lower values have been reported: 4% in 7 *Pinus sylvestris* populations (PRUS-GŁOWACKI & STEPHAN 1994) and 5.5% in 12 *Pinus pinaster* populations (SALVADOR *et al.* 1997). Gene flow measured by the number of migrants shows that there is a slight isolation effect in Aleppo pine (2.27) in comparison to *P. pinaster* (4.66).

Genetic differentiation, as the result of local adaptation, does not necessarily mean a wider ecological range in comparison with the other two species. GANDULLO & SÁNCHEZ-PALOMARES (1994) have found that the habitat of Aleppo pine is less variable in terms of water supply and soil drainage. Nevertheless, Aleppo pine can grow in a wider range of drought intensity and duration of dry periods than other pine species.

Pinus halepensis in Spain has a more continuous range than the other two pine species. However, forest fires are quite frequent in Aleppo pine stands and could have produced bottleneck effects in the past. More than 300,000 ha have been affected by forest fires during the period 1985–1995 (DGCONA 1996, data not published). Several adaptations to forest fires have been recently described for the Aleppo pine (GIL 1996, pers. com.), such as early flowering and cone serotinity (ROLDAN *et al.* 1992) that would result in a rapid recolonization of the burned area by a small number of genotypes. Therefore, reproduction after forest fires and selection by resistance to extreme drought conditions could be suggested as factors explaining the larger genetic differentiation (G_{ST}) among certain populations.

The large genetic variation in Scots pine and Maritime pine is explained by a refugia of the species during the glaciations in the Iberian peninsula (PRUS-GŁOWACKI & STEPHAN 1994; BARADAT & MARPEAU 1988). The evolution of *Pinus halepensis* forests in Spain since 15,000 B.P. have been recently described (GIL *et al.* 1996). One centre of expansion after glaciation is fully recorded by paleontological data in the south of Spain (Málaga) in the pleniglacial era. In the coldest period the species could only be found just close to the Mediterranean shore, from Valencia to Cataluña. During the tardiglacial era (15,000 –10,000 B.P.) *Pinus halepensis* was found in inner locations of Alicante and Cataluña. Paleontological studies show an increment of records during the Atlantic period (7,500 –4,500 B.P.), but it is not possible to conclude whether Aleppo pine had reached the centre of Spain and the Ebro basin, as today. Therefore, the existence of a refugia during the glaciations suggest the loss of genetic variability before glaciations.

The results inferred by the interpretation of Nei's and Prevosti genetic distances and the UPGMA dendrogramme show the relationship among populations. However, the small number of populations studied do not allow the definition of a general pattern of variation in the species.

The structure of the genetic variation is in accordance with the clinal pattern shown in morphological traits (AGUNDEZ *et al.* 1996) and in resin monoterpenes (SCHILLER & GRUNWALD 1987). The Aleppo pine stands in the southern range of distribution in Spain are smaller than in the rest of the range. Human influence by seed transfer in afforestation is not an explanation of the genetic differences, as natural regeneration is produced in these areas.

Further investigations including some more populations are necessary to establish the pattern of variation of the species in Spain and to establish the role of genetic drift, gene flow, selection and post glacial migration.

It would be necessary to have some more information in order to interpret the significance of the low level of genetic variation detected, in comparison with the high viability (physiological adaptability) in marginal environmental conditions.

ACKNOWLEDGEMENTS

We would like to thank Dr. Hans Muhs for giving us the opportunity to carry out this study at the Institut for Forest Genetics (Grosshansdorf, Germany). A special mention is given to Alexandra Tusch for providing technical assistance. We also thank Dr. Gabriel Schiller and Dr. Luis Gil for an early review of the paper, and two anonymous referees for their valuable suggestions.

The study was founded by a grant of the INIA to the first author and by the projects CC93–195 INIA–ICONA and FAIR CT95–0097. It was conducted in the frame of the Spanish-German Cooperation Programme.

REFERENCES

AGUNDEZ, D., ALIA, R. & GIL, L. 1996: Relationship between phenotypic variation and phytoclimate in *Pinus halepensis*

provenances from Spain. IUFRO International Conference, "Diversity and Adaptation in Forest Ecosystems in A Changing World". Vancouver, Canada.

BARADAT, Ph. & MARPEAU, A. 1988: Le Pin maritime *Pinus pinaster* Ait. Biologie et génétique des terpènes pour la conaissance et l'amélioration de l'espèce.Thèse. Université de Bordeaux.

BARADAT, Ph., MICHELOZZI, M., TOGNETTI, R. & KHOUJA, M.L. 1995: Geographical variation in the terpene composition of *Pinus halepensis* Mill. *In:* Populations genetics and genetic conservation of forest trees. (eds.: Ph. Baradat, W.T. Adams & G. Müller-Starck). SPB Academic Publishing. pp: 141–158.

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

CHELIAK, W. M. & PITEL, J. A. 1984: Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report Pl–X–42. Petawawa National Forestry Institute. Canadian Forestry Service. 49 pp.

CONKLE, M. T., HODGKISS, P. D., NUNNALLY, L. B. & HUNTER, S. C.1982: Starch gel electrophoresis of conifer seeds: a laboratory manual. General Technical Report PSW–64. Pacific Southwest Forest and Range Experiment Station. USDA Forest Service. 18 pp.

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

CRITCHFIELD, W. & LITTLE, E. 1966: Geographic distribution of the pines of the world. Miscellaneus Publication 991. Forest Service. USA. Washington D.C.

GANDULLO, J. M. & SANCHEZ-PALOMARES, O. 1994: Estaciones ecológicas de los pinares españoles. Colección Técnica. ICONA. Madrid. 188 pp.

GIL, L., DIAZ-FERNANDEZ, P., JIMENEZ, M. P., ROLDAN, M., ALIA, R., AGUNDEZ, D., DE MIGUEL, J., MARTIN, S. & DE TUERO ,M. 1996: Regiones de procedencia de *Pinus halepensis* Mill. DGCONA. Madrid. 112 pp + 22 maps.

GILLET, E.M. 1994: GSED. Genetic Structures from Electrophoretic Data. Version 1.0. User's manual. Abteilung für Forstgenetik und Forstpflanzenzüchtung der Universität Göttingen: 1– 49.

GREGORIUS, H. R. 1978: The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. *Mathematical Bioscience* **41**:253–271.

GREGORIUS, H. R. 1980: The probability of losing an allele when diploid genotypes are sampled. *Biometrics* **36**:643 –652.

GREGORIUS, H. R. 1984: A unique genetic distance. *Biometrical Journal* **26**:13–18.

GREGORIUS, H. R. 1987: The relationship between the concepts of genetic diversity and differentiation. *Theoretical and Applied Genetics.* **74**:397–401.

HAMRICK, J. L., GODT, M. J. & SHERMAN-BROYLES, S. L. 1992: Factors influencing levels of genetic diversity in woody plant species. *In:* Population Genetics of Forest Trees (eds.: W. T. Adams, S. H. Strauss, D. L. Copes & A. R. Griffin). pp: 95–124.

KOROL, L., & SCHILLER, G. 1996: Relations between native Israeli and Jordanian Aleppo pine (*Pinus halepensis* Mill.) based on allozyme analysis: a note. *Forest Genetics* **3** (4):197–202

LOUKAS, M., VERGINI, Y. & KRIMBAS, C. B. 1983: Isozyme variation and heterozygosity in *Pinus halepensis* L. *Biochemical Genetics* **21**(5/6):497–509.

NEI, M. 1973: Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. USA.* **70** (12):3321–3323.

NEI, M. 1977: F-statistics and analysis of gene diversity in subdivided populations. *Ann. Human. Genet* **41**:225–233.

NEI, M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**:283–290.

PREVOSTI, A., OCAÑA, J. & ALONSO, G. 1975: Distances between populations of *Drosophila subobscura*, based on chromosome arrangement frequencies. *Theor. Appl. Gene. Band* . **45** (S). 231 – 241.

PRUS-GLOWACKI, W. & STEPHAN, B.R. 1994: Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. *Silvae Genetica* **43**(1):7–14.

ROLDAN, M., CATALAN, G., GIL, L. & PARDOS, J. A. 1992: Flowering and fructification in a clonal seed orchard of *Pinus halepensis* Mill. AFOCEL-IUFRO Workshop: Production de varietés génétiques améliorées pour les essences forestiéres, Bordeaux.

SALVADOR, M. L., SEISDEDOS, M. T., ALIA, R. & GIL, L. 1997: Estudio de la variabilidad genética en doce poblaciones naturales de *Pinus pinaster* con marcadores isoenzimáticos. *Cuadernos de la SECF* **5**:119–124.

SCHILLER, G. & GRUNWALD, C. 1987: Resin monoterpenes in range-wide provenance trials of *Pinus halepensis* Mill. in Israel. *Silvae Genetica* **36**(3/4):109–114.

SCHILLER, G. & BRUNORI, A. 1992: Aleppo pine (*Pinus halepensis* Mill.) in Umbria (Italy) and its relation to native Israeli populations. *Israel Journal of Botany* **41**:123–127

SCHILLER, G., CONKLE, M. T. & GRUNWALD, C. 1986: Local differentiation among Mediterranean populations of Aleppo pine in their isoenzymes. *Silvae Genetica* **35** (1):11–19.

SWOFFORD, D. L. & SELANDER, B. R. 1981: BIOSYS–1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72**:281–283.

TEISSEIRE, H., FADY, B. & PICHOT, Ch. 1995: Allozyme variation in five French populations of Aleppo pine (*Pinus halepensis* Miller). *Forest Genetics* **2** (4):225–236.

WENDEL, J. F. & WEEDEN, N. F. 1989: Isozymes in Plant Biology. Ed. Dioscorides Press. Oregon. 40 pp.

WORKMAN, P. L. & NISWANDER, J. D. 1970: Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am. J. Human Genet.* **22**:24–49.

WRIGHT, S. 1965: The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**:395-420.