Inheritance of Isozyme Variations in Seed Tissues of Abies pinsapo Boiss.

By L. Pascual, F. J. Garcia and F. Perfectti

Departamento de Genética, Facultad de Ciencias,
Universidad de Granada, 18071 Granada, Spain.

(Received 11th May 1993)

Summary

Inheritance of isozyme variations of 15 enzyme systems in four populations of *Abies pinsapo* Boiss., including the putative var. *marocana* has been examined by electrophoresis. Analysis of megagametophytes and embryos from open-pollinated seeds show that the allozymes in these enzyme systems are coded by a total of 33 loci, out of which 22 are monomorphic across all the populations and 11 have at least 2 allelic variants. The confirmation of 1:1 segregation ratios in the seeds of heterozygous trees revealed that, in all but one case, these allozymes exhibited Mendelian inheritance. The differentiation between *Abies pinsapo* and the putative var. *marocana* through the MDH phenotype patterns indicates the genetic divergences between the Spanish and Moroccan populations and the possible existence of varieties in pinsapos.

Key words: Abies pinsapo, var. *marocana*, isozymes, inheritance.

Introduction

Abies pinsapo Boiss. is a fir belonging to the Pinaceae family (Piceaster section) and is endemic only to the western Mediterranean region. Its natural habitat is the moist, cold mountain areas with dry summers in southern Spain and in northern Morocco, where two possible further varieties have been described: *A. pinsapo* var. *marocana* (Trabut) Ceballos and Bolaños and *A. pinsapo* var. *tazaotana* (M. del Villar) Pourtet (Franco, 1950; Liu, 1971; Farjon and Rushforth, 1989).

Research in this species is important due to the following factors: it is an endemic species of limited distribution with reduced, scattered populations which is in an advanced state of regression because of the combined action of many factors, such as climate changes, air pollution, fungal pests and possibly human action; furthermore, the possible existence of two varieties in Morocco provides the opportunity to determine the taxonomy of this species from a genetic point of view; finally, a knowledge of the structure and patterns of the genetic differentiation of this species is important for the preservation of gene-resorcues, improvement and reforestation programs. A prerequisite for such a study is an understanding of the genetic basis for the observed electrophoretic variations.

This paper describes the inheritance of isozymes for 15 enzyme systems in seeds of open-pollinated trees of *Abies pinsapo*. In addition, progenies of heterozygous trees were assayed to determine if the polymorphic loci presented Mendelian inheritance.

Materials and Methods

Open-pollinated cones were collected from individual trees in 4 natural pinsapo populations from Spain and Morocco: the locations and sample sizes of each population are listed in *table 1*. The sample trees were selected with sufficient space between them to avoid the possibility of sampling closely related individuals. The cones were air-dried and the seeds extracted by hand. Wind-pollinated seeds were germinated on moistened filter paper in petri dishes at room temperature until the radicles were 1 mm to 3 mm long. Megagametophytes from 6 to 8 seeds were analysed per tree and for each enzyme.

Megagametophytes and embryos were macerated separately in 50 μl of extraction buffer (Cheliak and Pitel, 1984). The enzyme extracts were absorbed onto paper wicks and subjected to horizontal starch gel electrophoresis using a 12% (w/v) of potato starch from Santiveri S.A., hydrolysed following the procedure of Moretti et al. (1957). Three different buffer systems were used for electrophoresis: I-morpholine/citrate, pH 6.1 (Vallejos, 1983), II-Tris/Histidine, pH 7.0 (Pitel and Cheliak, 1984) and III-LiOH-borate pH 8.1 (Pascual et al., 1988). The gels were run for 15 min, the wicks removed and the electrophoresis continued until the bromophenol blue dye front migrated from the origin towards the anode (12 cm for buffer systems I and II and 8 cm for buffer system III).

Once the electrophoresis was finished, the anodal portion of the gels was cut horizontally in three or four slices and incubated in staining solutions at 37° C 1 to 2 hours in darkness. Stain recipes were modified slightly from recipes supplied by the Institute of Forest Genetics and Plant Physiology, Umeå, Sweden. The lists of the enzymes analysed, electrophoresis buffer systems used, and number of loci observed for each enzyme system are shown in *table 2*. Mobility differences between bands were quantified relative to the buffer front (R_f). Enzyme systems are designated by the enzyme's abbreviation in capital letters (e.g. GOT), and if the enzyme is controlled by more than one locus the faster migrating zone is designated 1 and

Table 1. — Populations of *Abies pinsapo* analysed.

LOCATION	NO. TREES	HEIGHT (M)
SIERRA DE LAS NIEVES (RONDA-MÁLAGA-SPAIN)	104	1000 - 1800
SIERRA DE GRAZALEMA (CADIZ-SPAIN)	40	1000 - 1650
SIERRA BERMEJA (ESTEPONA-MÁLAGA-SPAIN)	15	1300 - 1400
JBEL TISSOUKA (CHAOUEN-MOROCCO) (PUTATIVE VAR. *MAROCANA*)	27	1500 - 2038

the slower zones 2, 3, etc. Isozyme loci are written with an initial upper case letter and each locus numbered in the same way (e.g. Got3). For the alleles the number 1 is assigned to the most mobile band at any locus and those lacking stain activity are designated as null and numbered 0. A number with an asterisk is assigned to the isozyme activity that is only expressed in embryos.

To confirm Mendelian inheritance of the isozyme phenotypes for each polymorphic locus, the segregation data obtained from heterozygous trees was pooled. The goodness of fit to a 1:1 segregation hypothesis was measured by means of a χ^2 test, and heterogeneity test among the trees (*Table 3*) was computed. These procedures are standard in this type of study (Adams and Joly, 1980; El-Kassaby et al., 1982, 1987; Millar, 1985; Harry, 1986).

Results and Discussion

Monomorphic enzymes

ACO

One zone of activity was observed on gel stained for ACO (*Fig. 1*). This monomeric enzyme has been reported to be coded by a single, polymorphic locus in several conifers (Moran et al., 1980; El-Kassaby, 1982; Wheeler and Guries, 1982; Ledig and Conkle, 1983; Ernst et al., 1987). There is no variation in this zone in the populations analysed thus far, and both megagametophyte and embryo tissues show one single band (*Fig. 1*), presumably coded by one monomorphic locus.

GDH

Gels stained for GDH were monomorphic for a single band (*Fig. 1*). The same GDH band as that found in megagametophytes also occurs in embryos, suggesting that same monomorphic gene encodes GDH in both seed tissues. A similar monomorphic phenotype has been described for GDH in *Pinus albicaulis* (Furnier et al., 1986), *Pinus attenuata* (Strauss and Conkle, 1986), *Pinus monticola* (Steinhoff et al., 1983), *Abies balsamea* (Jacobs et al., 1984) and in most European populations of *Abies alba* (Bergmann et al., 1990). In many other conifer species, a single variable locus GDH segregating for several alleles has been reported for this multimeric enzyme (Mitton et al., 1979; Adams and Joly, 1980; Yeh and El-Kassaby, 1980; Guries and Ledig, 1982).

ME

ME gels were also invariant, but in this case a single band was found in each of the two zones, ME-1 and ME-2, in both seed tissues (*Fig. 1*). In the ME-2 zone it is possible that there may exist another slightly slower allelic product with a minute migration difference, which is often difficult to distinguish under our electrophoretic conditions. In other species of *Abies*, only one monomorphic zone has been reported (Jacobs et al., 1984).

Table 2. — Abbreviations for enzymes used in text; enzyme commission reference number; buffer systems used for electrophoresis; and number of loci observed.

ENZYMES	ABBREV.	EC N°	BUFFER SYSTEMS	N° OBS. LOCI
ACID PHOSPHATASE	ACPH	3.1.3.2	II	3
ACONITASE	ACO	4.2.1.3	II	1
ALCOHOL DEHYDROGENASE	ADH	1.1.1.1	II	3
DIAPHORASE	DIA	1.6.4.3	II	4
GLUCOSE 6 PHOSPHATE DEHYDROGENASE	G6PD	1.1.1.49	I and II	1
GLUTAMATE DEHYDROGENASE	GDH	1.4.1.3	III	1
GLUTAMATE OXALOACETATE TRANSAMINASE	GOT	2.6.1.1	III	3
ISOCITRATE DEHYDROGENASE	IDH	1.1.1.42	I and II	2
LEUCINE AMINOPEPTIDASE	LAP	3.4.11.1	I and II	2
MALATE DEHYDROGENASE	MDH	1.1.1.37	I	2
MALIC ENZYME	ME	1.1.1.40	II	2
PHOSPHOGLUCOMUTASE	PGM	2.7.5.1	II and III	2
6 PHOSPHOGLUCONIC DEHYDROGENASE	6PGD	1.1.1.44	I	2
PHOSPHOGLUCOSE ISOMERASE	PGI	5.3.1.9	II	2
SHIKIMATE DEHYDROGENASE	SKDH	1.1.1.25	II	2

Table 3. — Segregation of allozymes into megagametophytes of heterozygous trees and χ^2 test for goodness of fit to 1:1 ratio and heterogeneity among trees.

Locus	Phenotypes	No.phenotypes	Chi-square tests			
			pooled		among trees	
			χ^2(1df)	P	χ^2(df)	P
Lap-1	2/3	44/36	0.800	0.371	7.2(12)	0.845
	1/2	17/22	0.641	0.424	4.47(7)	0.725
	1/0	10/14	0.667	0.414	2.0(2)	0.368
Lap-2	1/2	146/134	0.514	0.474	27.16(45)	0.984
Idh-1	1/2	30/32	0.064	0.801	7.93(9)	0.542
G6pd	1/2	90/88	0.022	0.883	15.05(29)	0.984
Pgi-2	1/2	7/9	0.250	0.618	2.75(3)	0.432
	1/3	16/16	0.000	1.000	6.73(4)	0.151
Mdh-1	1/2	15/13	0.143	0.706	1.34(4)	0.855
6Pgd-2	1/2	12/10	0.182	0.670	1.49(2)	0.474
Pgm-1	1/2	10/13	0.390	0.533	2.21(1)	0.137
Dia-4	1/2	41/37	0.205	0.650	11.79(12)	0.463

ACPH

Gels stained for ACPH have 2 zones of activity that are clearly distinguished by the staining intensity (*Fig. 1*). The faster zone, ACPH-1, always appears more faintly stained in both seed tissues and has a single band coded by the Acph1 locus. The ACPH-2 zone stains very intensely and shows a single band in megagametophytes and embryos, but the band stained in the embryos is slightly more rapid than the megagametophyte band. This may be explained by the fact that the genes are differentially expressed or modified in the two tissues, assuming that megagametophytes and embryos are coded by different monomorphic loci, Acph2 and Acph2* respectively. Thus, the 2 invariant zones are interpreted as being coded by 3 monomorphic loci.

One polymorphic locus had been reported in some species of *Abies* (Jacobs et al., 1984) and 2 polymorphic zones of activity have been observed in other conifer species (Adams and Joly, 1980; Cheliak et al., 1984).

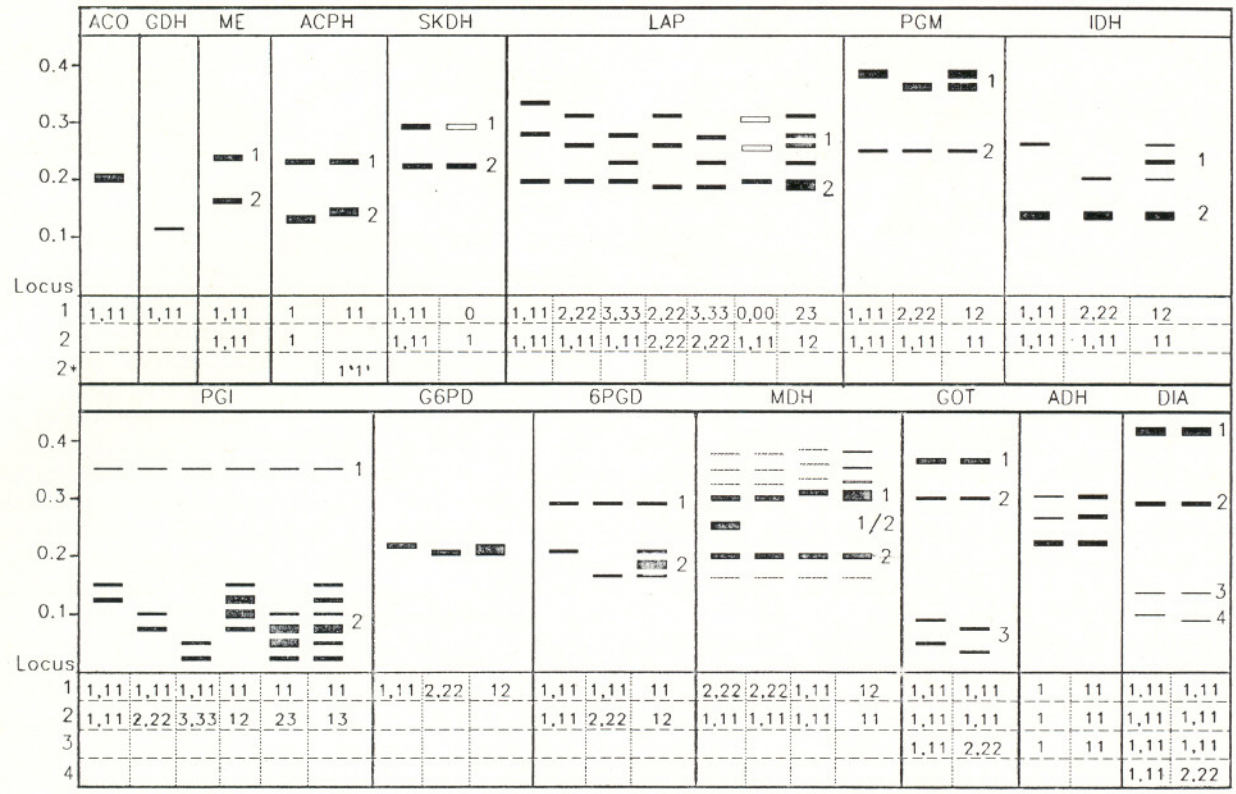

Figure 1. — Schematic illustrations of enzyme phenotypes in megagametophytes and embryos for enzyme systems analysed in *Abies pinsapo*. The open symbols on LAP-1 and SKDH-1 indicate zones interpreted as being null alleles. The lightly dashed lines on MDH indicate additional bands. The megagametophyte (e.g. 1) and embryo (e. g. 11) genotypes are also indicated and separated by a comma.

ADH

Gels stained for ADH show invariant phenotypes with 3 bands in both seed tissues, presumably representing three zones of activity, ADH-1, ADH-2 and ADH-3. In megagametophytes the 2 faster zones both stain faintly and equally. The 3. zone stains more intensely (*Fig. 1*). This same pattern appears in embryos, but it is more highly stained. This result may be interpreted as 3 single-banded monomorphic loci for ADH.

Enzyme systems with at least one polymorphic locus

G6PD

G6PD showed 1 zone of activity and exhibited 2 variants with closely migrating bands in megagametophytes (*Fig. 1*). The homozygous embryos show 1 single band, as do the megagametophytes. In the heterozygous embryos on the other hand, a very thick single band covers the 2 adjacent variants. (*Fig. 1*).

One locus has been reported to code for G6PD in other conifer species (El-Kassaby et al., 1987; Harry, 1986).

SKDH

Gels stained for this monomeric enzyme reveal two zones of activity, presumably representing the expression of two loci Skdh1 and Skdh2 (*Fig. 1*). Two variants in megagametophytes, 1 with a single band and the other lacking activity, representing a null allele, were observed in the faster migrating SKDH-1 zone. The null allele was present only in one heterozygous tree. The 18 megagametophytes analyses from this tree segregated into 14 Skdh1-0 and 4 Skdh1-1 and do not behave as alleles at a single locus. For this zone the embryos always showed one single band. The SKDH-2 zone was invariant, appearing as a single band (*Fig. 1*). This zone is interpreted to reflect a monomorphic locus (Skdh2).

A single monomorphic zone of activity has been reported in *Abies balsamea* (Jacobs et al., 1984), and a single polymorphic zone in *Picea mariana* (Boyle and Morgenstern, 1985). Two zones of activity exist in *Calocedrus decurrens* (Harry, 1986).

LAP

Gels stained for LAP show two zones of activity, LAP-1 and LAP-2 (*Fig. 1*). Four variants in megagametophytes occur in the faster migrating zone LAP-1, 3 of which show double bands and the 4. no visible band. The double-banded variants in this zone are most likely controlled by a single locus. Double-banded phenotypes are very frequent in different enzyme systems in conifers, and they probably represent post-translational modification products of a single allele (Finnerty and Johnson, 1979; Newton, 1979; Ernst et al., 1987). The absence of a band at LAP-1 when accompanied by activity at LAP-2 is interpreted to reflect a null allele at Lap1. The null allele is present in only 2 of the trees analysed, and segregation of haploid megagametophytes indicates that these trees ought to be heterozygous (*Table 3*). This segregation is consistent with the expexted 1:1 ratio and supports the conclusion that the null allele is allelic to the visible variants at this locus. The slower migrating zone, LAP-2, is controlled by a 2. locus with 2 alleles encoding single banded allozymes with very little migration difference in megagametophytes (*Fig. 1*).

The allozymes encoded by Lap1 and Lap2 are also active in embryos. The LAP-1 zone shows double-band variants present in megagametophytes for homozygous em-

bryos and 4 bands in heterozygous embryos sometimes poorly discernable due to difficulties in resolving the closely migrating bands (*Fig. 1*). The heterozygous embryos for Lap2 show a very thick band which covers the 2 existing variants present in megagametophytes and homozygous embryos (*Fig. 1*).

Two polymorphic loci have been reported in *Abies balsamea* (Neale and Adams, 1981; Jacobs et al., 1984) and *Abies alba* (Mejnartowicz, 1979). In other conifer species, 1 or 2 loci for LAP exist (Rudin, 1977; Guries and Ledig, 1978; O'Malley et al., 1979; Loukas et al., 1983).

PGM

Two zones of activity appear on gels stained for this monomeric enzyme. The faster migrating PGM-1 zone reveals 2 single-banded variants (*Fig. 1*) with very little migration difference in megagametophytes and homozygous embryos. The heterozygous embryos show 2 bands. This zone is therefore controlled by 1 locus with 2 alleles. The PGM-2 zone always stains more faintly and is invariant, appearing as a single band (*Fig. 1*) in both megagametophytes and embryos, presumably being controlled by a monomorphic locus.

Two Pgm loci appear to exist in the majority of conifer species (Guries and Ledig, 1978; Mitton et al., 1979; Adams and Joly, 1980; Eckert et al., 1981; Neale and Adams, 1981; Loukas et al., 1983; Stewart and Schoen, 1986).

IDH

Two zones of activity were observed on gels stained for IDH. The faster IDH-1 zone presents 2 lightly dyed variants in megagametophytes (*Fig. 1*). IDH embryos have 3 phenotypes, 2 with a single band in 1 of 2 alternate positions, which are interpreted as homozygous for different alleles ,and a 3., representing the heterozygous phenotype. The latter shows 3 bands the intermediate of which is very active and corresponds to a heterodimeric band characteristic of a typical dimeric enzyme. The slower IDH-2 zone always appeared intensively stained and was invariant for a single band (*Fig. 1*) in megagametophytes and embryos, probably being coded by a monomorphic locus.

Two highly polymorphic loci have been reported in *Abies alba* (Bergmann et al., 1990). A single zone of activity exists for most conifer species (Guries and Ledig, 1978; O'Malley et al., 1979; El-Kassaby et al., 1982; Loukas et al., 1983).

PGI

PGI had 2 zones of activity but only the slower PGI-2 zone was polymorphic. The faster migrating PGI-1 zone appears in both megagametophytes and embryos as an invariant band (*Fig. 1*), presumably coded by 1 monomorphic locus, Pgi1.

The 3 variants appearing in PGI-2 (*Fig. 1*) present double bands in megagametophytes and homozygous embryos. Although heterozygous embryos were rare, 2 different patterns existed with 4 or 6 bands. Heterozygous embryos for contiguous alleles showed four bands (*Fig. 1*), because of an overlap between the heterodimeric bands and 2 of the bands of the double-banded variants. The heterodimer possesses 2 bands with the same electrophoretic migration as the slow band of the double-banded fast variant and the fast band of the double-banded slow variant. Furthermore, they showed higher activity when they overlapped. Heterozygous embryos for noncontiguous alleles showed 6 bands (*Fig. 1*), with a heterodimer, which also presented a double band of intermediate mobility compared to the homodimers.

The 2 zones of PGI activity observed are consistent with the activity reported in many other plant species (Adams and Joly, 1980; Neale and Adams, 1981; El-Kassaby et al., 1987).

6PGD

Gels stained for 6PGD present 2 zones of activity. The faster zone 6PGD-1 is invariant for a single band in both seed tissues (*Fig. 1*). The slower 6PGD-2 zone has 2 single-banded variants in megagametophytes (*Fig. 1*); homozygous trees have invariant megagametophytes for the fastest mobility and heterozygous trees segregate into megagametophytes for both the fast and slow variants. Embryos were either single-banded at the same position as in the megagametophytes or triple-banded with 2 bands migrating the same distance as the bands existing in megagametophytes. The 3. band occupying a position midway between them is a typical pattern for a dimeric enzyme.

Two zones of activity have been reported in *Abies alba* (Bergmann et al., 1990) and in other conifers (El-Kassaby et al., 1982; Boyle and Morgenstern, 1985).

MDH

For this enzyme system, marked differences existed between the Moroccan and Spanish populations in relation to the isozyme analyses in the MDH phenotype patterns.

In the Moroccan population the gels stained for MDH revealed 2 zones of activity with the appearance of additional faint bands in both zones (*Fig. 1*), originated probably by post-translational modifications and are not considered to represent MDH activity. Two variants in megagamethophytes with a single band and a very slight difference in mobility were observed in the faster migration zone MDH-1 (*Fig. 1*). Heterozygous embryos present a thick band due to overlapping of the 2 allelic variants and the heterodimeric band, typical for a dimeric enzyme such as the MDH. The MDH-2 zone was monomorphic for a single band in both seed tissues (*Fig. 1*).

The Spanish populations showed a different, invariant pattern, with the appearance of similar additional faint bands. However, there are 3 clearly separate zones of MDH activity present in both seed tissues, which we have designated in decreasing order of anodal mobility MDH-1, MDH-1/2 and MDH-2 (*Fig. 1*). The single bands, MDH-1 and MDH-2, in the Spanish populations are equal in migration mobility to the slower variant for the MDH-1 zone and the monomorphic MDH-2 zone from Morocco. The 3rd band, MDH-1/2, was always located midway between MDH-1 and MDH-2. Since it is expressed in haploid megagametophytes it is interpreted to be an interlocus heterodimer enzyme formed between the subunits coded by the monomorphic loci Mdh1 and Mdh2. A similar pattern with an interlocus heterodimer between Mdh2 and Mdh3 loci has been reported for *Picea glauca* (King and Dancik, 1983), *Picea mariana* (Boyle and Morgenstern, 1985) and other conifer species, although until now it has never been detected in other fir species (El-Kassaby, 1981; Neale and Adams, 1981; Neale et al., 1984). In all cases, 2 of the genes are apparently expressed in the same subcellular compartment and procedure a 3-band pattern in which the intermediate band is an interlocus heterodimer.

Two MDH zones of activity have been reported in megagametophytes of *Abies balsamea* (Neale and Adams, 1981; Jacobs *et al.*, 1984) and *Pinus taeda* (Adams and Joly, *1980*).

In other conifer species the dimeric enzyme MDH is coded by 3 (KING and DANCIK, 1983; BOYLE and MORGENSTERN, 1985) or 4 genes (O'MALLEY et al., 1979; EL-KASSABY, 1981).

GOT

Gels stained for GOT had 3 zones of activity in megagametophytes. The 2 faster migrating zones, GOT-1 and GOT-2, were monomorphic and appear on the gel as 2 invariant bands, GOT-1 and GOT-2, with different staining intensities (*Fig. 1*), probably controlled by monomorphic loci, Got1 and Got2, respectively. A similar patterns has been reported for GOT-1 and GOT-2 zones in *Pinus albicaulis* (FURNIER et al., 1986). Two variants were observed at GOT-3, each with a double-band phenotype (*Fig. 1*). The fast allele has only been found in 1 heterozygous tree in the Sierra de las Nieves population where 10 megagametophytes have been analysed and the 2 variants segregated at a 1:1 ratio (5:5). The activity of embryo isozymes is weaker in the positions corresponding to the GOT-2 and GOT-3 zones, disappearing completely in the GOT-3 zone when the radicle is very developed. Heterozygous embryos in the slower GOT-3 zone were too weak and diffuse for any band patterns to be discerned.

Double-banded GOT allozymes have been reported in *Abies balsamea* (NEALE and ADAMS, 1981), *Pinus albicaulis* (FURNIER et al., 1986), *Pinus rigida* (GURIES and LEDIG, 1978), *Pinus strobus* (ECKERT et al., 1981), *Pinus silvestris* (RUDIN and EKBERG, 1978) and *Pseudotsuga menziesii* (EL-KASSABY et al., 1982).

Three Got loci have also been reported to code for these dimeric enzymes in *Abies alba* (BERGMANN et al., 1990) and several other conifers (RUDIN and EKBERG, 1978; O'MALLEY et al., 1979; ECKERT et al., 1981; NEALE and ADAMS, 1981; EL-KASSABY et al., 1982; FURNIER et al., 1986; ERNST et al., 1987). The electrophoretic patterns observed in our study confirm the existence of three loci.

DIA

Four zones of activity appeared on DIA gels, clearly distinguished by the staining intensity. The 3 faster migrating zones, DIA-1, DIA-2, and DIA-3 did not present variation and possibly represent 3 monomorphic loci with single-banded phenotypes. Two single-banded variants were observed at DIA-4 zone in megagametophytes and homozygous embryos (*Fig. 1*). Heterozygous trees segregated in the megagametophytes for these 2 allozyme variants but heterozygous embryos appeared inconsistently resolved and diffuse. Several zones of activity have been observed in many other conifer species for this monomeric enzyme (KING and DANCIK, 1983; NEALE et al., 1984).

In conclusion, the analysis of 15 enzyme systems in megagametophytes and embryos from four populations of *Abies pinsapo* yielded clearly interpretable results and revealed 22 monomorphic loci throughout all the populations (Aco, Gdh, Me1, Me2, Acph1, Acph2, Acph2*, Adh1, Adh2, Adh3, Skdh2, Pgm2, Idh2, Pgi1, G6pd2, 6Pgd1, Mdh2, Got1, Got2, Dia1, Dia2 and Dia3) and 11 polymorphic loci (Skdh1, Lap1, Lap2, Pgm1, Idh1, Pgi2, G6pd1, 6Pgd2, Mdh1, Got3 and Dia4). In all but one case (ACPH-2) the same genes were expressed in both seed tissues.

Evidence exists for the genetic control of the polymorphic loci. The confirmation of a 1:1 segregation ratio in heterozygous trees supports the idea that these allelic variants exhibit Mendelian inheritance. Deviations from a 1:1 ratio for 9 polymorphic loci were non-significant, nor was there any significant heterogeneity among heterozygous trees (*Table 3*). For the SKDH-1 zone, the segregation ratio differed significantly from 1:1 in the tree with the genotype carrying the null allele, favouring the null allele. Such deviation may result from various causes such as: meiotic drive; selection between meiosis; or linkage to a deleterious allele at another locus. Only 1 tree was heterozygous for this genotype; however, sampling error may well account for the deviation from the expected ratios (ADAMS and JOLY, 1980; HARRY, 1986; STRAUSS and CONKLE, 1986).

The genetic control of enzymes that were monomorphic in *Abies pinsapo* populations was postulated from comparisons of banding patterns in other conifer species where single-locus Mendelian segregation has been reported.

All populations analysed clearly differ from each other, and most loci with allelic variants show unusual variation patterns in that different alleles predominate in the different populations (PASCUAL et al., unpublished). The results of MDH analysis show that the Moroccan population putative var. *marocana*, is clearly different from the Spanish populations because of the presence of a heterodimeric band between the Mdh loci in the latter. The cause of these triple-banded phenotypes could reflect closely linked duplicate loci which would explain the fixed heterozygosity observed for MDH in the Spanish populations. Such differences may result from genetic divergence between the Spanish and Moroccan populations.

These results contribute to an understanding of the inheritance of *Abies pinsapo* and provide preliminary data for future studies on the genetic population structure of this species. Furthermore, the MDH enzyme provides an insight and important information in regard to the systematics of *Abies pinsapo*.

Acknowledgements

We would like to express our thanks to the Institute of Forest Genetics and Plant Physiology, Umeå, Sweden, for their advice on forest genetics. We are also grateful to Agencia de Medio Ambiente de "La Junta de Andalucía" for helping us with the collection of seed.

Literature Cited

ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. J. Hered. **71**: 33—40 (1980). —— BERGMANN, F., GREGORIUS, H. R. and LARSEN, J. B.: Levels of genetic variation in European silver fir (*Abies alba*). Genetica **82**: 1—10 (1990). —— BOYLE, T. J. B. and MORGENSTERN, E. K.: Inheritance and linkage relationships of some isozymes of black spruce in New Brunswick. Can. J. For. Res. **15**: 992—996 (1985). —— CHELIAK, W. M., MORGAN, K., DANCIK, B. P., STROBECK, C. and YEH, F. C. H.: Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine, *Pinus banksiana* LAMB. Theor. Appl. Genet. **69**: 145—151 (1984). —— CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis on enzymes from forest trees species. Information Report PI- X- 42. Petawawa National Forestry Institute. Canadian Forestry Service (1984). —— ECKERT, R. T., JOLY, R. J. and NEALE, D. B.: Genetics of isozyme variants and linkage relationships among allozyme loci in 35 eastern white pine clones. Can. J. For. Res. **11**: 573—579 (1981). —— EL-KASSABY, Y. A.: Genetic interpretation of malate dehydrogenase isozymes in some conifer species. J. Hered. **72**, 451—452 (1981). —— EL-KASSABY, Y. A.: Associations between allozyme genotypes and quantitative traits in Douglas-fir (*Pseudotsuga menziesii* MIRB. FRANCO). Genetics **101**: 103—115 (1982). —— EL-KASSABY, Y. A., MEAGHER, M. D., PARKINSON, J. and PORTLOCK, F. T.: Allozyme inheritance, heterozygosity and outcrossing rate among *Pinus monticola* near Ladysmith, British Columbia. Heredity **58**: 173—181 (1987). —— EL-KASSABY, Y. A., YEA, F. C. and SZIKLAI, O.: Inheritance of allozyme variants in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Can. J. Genet. Cytol. **24**: 325—335 (1982). —— ERNST, S. G., KEATHLEY, D. E. and

Hanover, J. W.: Inheritance of isozymes in seed and bud tissues of blue and engelmann spruce Genome **29**: 239—246 (1987) —— Farjon, A. and Rushforth. K. D.: A classification of *Abies* Miller Pinaceae. Notes from the Royal Botanic Garden Edinburgh **46**: 56—79 (1989). —— Finnerty, V. and Johnson, G.: Post-translational modification as a potential explanation of high levels of enzyme polymorphism, xanthine dehydrogenase and aldehyde oxidase in *Drosophila melangoaster*. Genetics **91**: 695—722 (1979). —— Franco, J. A.: Abetos. An. Inst. Super. Agron. 17. Lisbon, Portugal (1950). —— Furnier, G. R., Knowles, P., Alfersiuk, M. A. and Dancik, B. P.: Inheritance and linkage of allozymes in seed tissues of white-bark pine. Can. J. Genet. Cytol. **28**: 601—604 (1986). —— Guries, R. P. and Ledig, F. T.: Inheritance of some polymorphic isoenzymes in Pitch pine (*Pinus rigida* Mill.). Heredity **40**: 27—32 (1978). —— Guries, R. P. and Ledig, F. T.: Genetic diversity and population structure in Pitch pine (*Pinus rigida* Mill.). Evolution **36**: 387—402 (1982). —— Harry, D. E.: Inheritance and linkage of isozyme variants in incense-cedar. J. Hered. **77**: 261—266 (1986). —— Jacobs, B. F., Werth, C. R. and Guttman, S. I.: Genetic relationships in *Abies* (fir) of eastern United States, an electrophoretic study. Can. J. Bot. **62**: 609—616 (1984). —— King, J. N. and Dancik, B. P.: Inheritance and linkage of isozymes in white spruce (*Picea glauca*). Can. J. Genet. Cytol. **25**: 430—436 (1983). —— Ledig, F. T. and Conkle, M. T.: Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Perry ex Carr). Evolution **37**: 79—85 (1983). —— Liu, T. S.: A monograph of the genus Abies. Dep. For. Nat. Taiwan Univ., Taipei, Taiwan. (1971). —— Loukas, M., Vergini, Y. and Krimbas, B.: Isozyme variation and heterozygosity in *Pinus halepensis* L. Biochem. Genet. **21**: 497—509 (1983). —— Mejnartowicz, L.: Polymorphic of the LAP and GOT in *Abies alba* Mill. Bulletin Académie Polonaise Sciences. Serie des Sciences Biologiques XXVII **12**: 1063—1069 (1979). —— Millar, C. I.: Inheritance of allozyme variants in Bishop pine (*Pinus muricuta* D. Don). Biochem. Genet. **23**: 993—946 (1985). —— Mitton, J. B., Linhart, Y. B., Sturgeon, K. B. and Hamrick, J. L.: Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Hered. **70**: 86—89 (1979). —— Moran, G. F., Bell, J. C. and Matheson, A. C.: The genetic structure and levels of inbreeding in a *Pinus radiata* D. Don Seed Orchard. Silvae Genet. **29**: 5—6 (1980). —— Moretti, J., Broussier, G. and Jayle, M. F.: Réalization techniques et premières applications de l'electrophorèse sur gel d'amidon. Bull. Soc. Chim. Biol. **39**: 593—605 (1957). Neale, D. B. and Adams, W. T.: Inheritance of isozyme variants in seed tissues of balsam fir (*Abies balsamea*). Can. J. Bot. **59**: 1285—1291 (1981). —— Neale, D. B., Webwe, J. C. and Adams, T.: Inheritance of needle tissue isozymes in Douglas-fir. Can. J. Genet. Cytol. **26**: 459—468 (1984). —— Newton, K. J.: A gene which alters the electrophoretic mobilities of maize mitochondrial malate dehydrogenase isozymes. Genetics **91** (Suppl.): 88—89 (1979). —— O'malley, D. M., Allendorf, F. W. and Blake, G. M.: Inheritance of isozyme variation and heterozygosity in *Pinus ponderosa*. Biochem. Genet. **17**: 233—250 (1979). —— Pascual, L., Marquez, I. A. and Lopez-Alonso, D.: Evidence for the duplication of PGI genes in *Dipcadi serotinum* L. (Liliaceae). Heredity **60**: 247—252 (1988). —— Pitel, J. A. and Cheliak, W. M.: Effect of extraction buffers on characterization of isoenzymes from vegetative tissues of five conifer species. A user's manual. Information Report PI-X-34. Petawawa National Institute. Canadian Forestry Service (1984). —— Rudin, D.: Leucine-amino-peptidases (LAP) from needles and macrogametophytes of *Pinus sylvestris* L. Inheritance of allozymes. Hereditas **85**: 219—226 (1977). —— Rudin, D. and Ekberg, I.: Linkage studies in *Pinus sylvestris* L. using macro gametophyte allozymes. Silvae Genet. **27**: 1—12 (1978). —— Steinhoff, R. J., Joyce, D. G. and Fins, L.: Isozyme variation in *Pinus monticola*. Can. J. For. Res. **13**: 1122—1132 (1983). —— Stewart, S. C. and Schoen, D. J.: Segregation at enzyme loci in megagametophytes of white spruce, *Picea glauca*. Can. J. Genet. Cytol. **28**: 149—153 (1986). —— Strauss, S. H. and Conkle, M. T.: Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. **72**: 483—493 (1986). —— Vallejos, E.: Enzyme activity staining. In: Isozymes in Plant Genetics and Breeding. Part A. S. D. Tanksley and T. L. Orton (eds.). Elsevier, Amsterdam. pp 469—516 (1983). Wheeler, N. C. and Guries, R. P.: Populations structure, genetic diversity, and morphological variation in *Pinus contorta* Dougl. Can. J. For. Res. **12**: 595—606 (1982). —— Yeh, F. C. and El-Kassaby, Y. A.: Enzyme variation in natural populations of sitka spruce (*Picea sitchensis*). I. Genetic variation patterns among trees from IUFRO provenances. Can. J. For. Res. **10**: 415—422 (1980).