

Basing on previous years assessments and the ranking of species/provenances at age of 23 years (Table 4), it can be concluded that Mexican introductions of *Cupressus lindleyi* which are provenances C and D and *Cupressus benthamii* which is species E have shown no advantage over the Kenyan provenances of *Cupressus lusitanica*. This conclusion supports the observation by DYSON (1972) in BURLEY and NIKLES, (1973) that in East Africa, Kenyan races of *Cupressus* species/provenances were performing better than the Mexican ones. ZOBEL et al., (1987) also cite OWINO (1977) to have found that for *Cupressus lusitanica* in Kenya, the advanced "land race" selections were highly superior. He also found that selections from second to third generation exotic plantations were far superior to the best provenances newly introduced from the natural range of the species.

#### Recommendations

For an afforestation programme with exotics to be successful, sufficient seeds of good quality must be available from the desired seed source (ZOBEL et al., 1987). In this study, although the Kenyan races have proved to be superior, the differences are very small. Therefore, all species/provenances should be considered for afforestation to broaden the genetic base and to improve and allow other desirable traits to arise.

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#### Literature cited

- BURLEY, J. and NIKLES, O. G.: Tropical provenance and progeny research and international cooperation. Commonwealth Forestry Institute, Oxford. Based on papers submitted to a Symposium in Nairobi by IUFRO Working Parties. 597 pp. (1973). — LEMA, C. N., KITALI, M. M. and KLEM, G. S.: Basic density and its variation within and between trees of *Pinus patula* and *Cupressus lusitanica* in Meru Forest Project. University of Dar-es-Salaam, Division of Forestry Record No. 3. Morogoro, 11 pp. (1978). — MADOFFE, S. S. and CHAMSHAMA, S. A. O.: Tree improvement activities in Tanzania. Commonwealth Forestry Review 68(2), 101–107 (1989). — MALIMBWI, R. E.: Growth and yield studies of *Cupressus lusitanica* MILLER in Meru forest Project, Northern Tanzania. Unpublished M. Sc. (For.) thesis, University of Dar-es-Salaam, Morogoro (1984). — MALIMBWI, R. E., PERSSON, A., IDDI, S. CHAMSHAMA, S. A. O. and MWIHOKEKE, S. T.: Effect of spacing on yield and some wood properties of *Cupressus lusitanica* at Rongai, Northern Tanzania. Forestry 65(1), 73–82 (1992). — MASUNGA, E.: Basic density of wood of *Cupressus lusitanica* grown in Bunduki Forest Reserve, Morogoro. Unpublished Special Project, Sokoine University of Agriculture, Morogoro. 37 pp. (1989). — MATHU, W. J. and PHILLIP, M. S.: Growth and yield studies of *Cupressus lusitanica* in Kenya. Record No. 5. Division of Forestry, University of Dar-es-Salaam, Morogoro. 16 pp. (1979). — PAIVINEN, R. and MÄÄTTÄ, M.: Stand volume tables and taper curve functions for *Cupressus lusitanica* and *Pinus patula* in North East Tanzania softwood plantations. University of Helsinki, Department of Forest Mensuration and Management/Forest Division of Tanzania, Inventory Section. 24 pp. (1986). — PIKKAVAINEN, T.: Growth and yield tables for *Pinus patula* and *Cupressus lusitanica* in North East Tanzanian softwood plantations. University of Helsinki, Department of Forest Mensuration and Management/Forest Division of Tanzania, Inventory Section. 61 pp. (1986). — THOGO, S. and DYSON, W. G.: The growth of cypresses at Muguga arboretum. EAAFR Forestry Technical Note No. 30. 15pp. (1974). — ZOBEL, B. J., VAN WYK, G. and STAHL, P.: Growing exotic forests. A Wiley Interscience Publication, John Wiley and Sons. 508 pp. (1987).

## Morphometric and Electrophoretic Analysis of Two Populations of European Black Pine (*Pinus nigra* Arn.)

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#### Abstract

This paper examines genetic and phenotype variation of seeds from 2 populations of *Pinus nigra* ARNOLD *subsp. salzmanii* (DUNAL) FRANCO. Seeds size are compared with an image analyzer, showing significant differences between the 2 populations, which could be induced by observed environmental differences. The genetic distance is analysed, using the isozyme variation. The  $\chi^2$  test shows that the allele frequencies of the ACO-1 and GOT-1 isozymes are significantly different, which means that these isozymes would appear as genetic markers capable of differentiating the 2 populations studied.

**Key words:** *Pinus nigra*, isozymes, morphometric analysis, genetic markers, population origin.

FDC: 165.3; 165.5; 174.7 *Pinus nigra*.

#### Introduction

This paper presents methods to measure geographic variation in *Pinus nigra*: the conventional electrophoretic

analysis and the novel use of an image analysis to examine morphometric variation.

The remarkable increase of computerized image analyses in recent years has brought about a great improvement in morphometric characterization and a speeding up of the process of measuring the different characteristics. Not only are the measurements more accurate, but the number of data that can be handled by the computer is much greater (ORTIZ et al., 1990).

Electrophoresis techniques have come to be used routinely in the study of variations in enzyme systems, and they have been instrumental in determining the origin of populations of unknown ancestry (FALKENHAGEN, 1985). The allozyme variation expressed as the differences found in the allelic frequencies, is used to characterize the different populations under study.

*Pinus nigra* is found in 3 main locations in Spain: the Pyrenees, the Iberian Range and the Cazorla and Segura Mountain Ranges, in the Mediterranean area. The forest of the different zones exhibit great morphological differences with respect to the form of trunk, the size of crown and

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Table 1. — Morphometric analysis of seeds of *Pinus nigra*. Average values of 50 repeats/population.

Population	Length (mm)	Width (mm)	Wing scar (mm)
La Losilla	6.991 <sup>***</sup>	3.687 <sup>*</sup>	4.122 <sup>***</sup>
Cerro Gordo	6.699	3.550	3.586

(\* $p < 0.05$ ; \*\*\* $p < 0.001$ )

the tree's size, since the species is resilient and can thrive in widely varying conditions (GIL and ALIA, 1991). So within the area of distribution of *Pinus nigra* there are groups of populations that show differences in many characters related to their adaptation to different environmental conditions; it is these that we are trying to demonstrate by means of morphometric and isozyme variation analysis.

The real aim of this work is to find whether morphologic and/or genetic markers could distinguish 2 very valuable forest of *Pinus nigra* sp. *salzmanii* — "Cerro Gordo" and "La Losilla" — growing in the same forestal area of the Iberian Range, with a geographic separation of about 50 km. Their high value in reforestation makes their seeds characterization very interesting.

The identification of very close populations by quick and simple techniques, like morphometric measures and well established isozyme analyses, could be a useful approach to resolve existing problems in forestation work, not only for their application to existing populations but also in projects of reforestation or regeneration (PARDOS, 1989).

#### Material and Methods

The seeds were collected from 2 natural populations of *Pinus nigra* sp. *salzmanii* as a gene pool in 1990 by ICONA (Nature Conservation Institute of the Spanish Agriculture Ministry). The exact location was: "La Losilla" (Mountain number 132 in the National Ordenation Project from ICONA, with 1833 ha) and "Cerro Gordo" (Mountain number 108 with 2037 ha), both in the Cuenca province (Spain).

#### Morphometric study

The morphometric study was carried out with a "Summagraphics" MM1103 image scanner (Programme VIDSIII) on 50 seeds from each population. The parameters for the morphometric characterization of each seed were: length, width, and scar of wing insertion. The results are shown in table 1.

#### Electrophoretic study

The electrophoresis was carried out on starch gel, using protein extracts obtained exclusively from the megagametophytic tissue of each one of the seeds. According to EL-KASSABY (1991), a sample size of 40 to 60 individuals is needed in Conifers to obtain reliable estimates of allelic frequencies at the population level. In this study 40 seeds/population were used. The method of extraction and the methods used for horizontal electrophoresis of starch are described by CONKLE et al. (1982), specifically for seeds of conifers, and more recently by MURPHY et al. (1990). The Histidin-HCL at pH 7.0 was used exclusively for the 8 chosen enzyme systems:

Leucine aminopeptidase (LAP),  
Phosphoglucomutase (PGM),  
Alcohol dehydrogenase (ADH),  
Aconitase (ACO),  
Malate dehydrogenase (MDH),  
6-phosphogluconate dehydrogenase (6PDG),  
Glutamate oxalacetate transaminase (GOT),  
Isocitrate dehydrogenase (IDH).

The genetic base of the loci appearing as polymorphic were interpreted in the light of previous descriptions of those same enzymes in the same or in similar species (BONNET-MASIMBERT and BIKAY-BIKAY, 1978; EL-KASABY, 1981; NIKOLIC and TUCIC, 1983). Thus, in the genetic interpretation of the enzyme MDH, the most complex from the point of view of its resolution, 4 loci were observed (as in other conifer species), 2 of which (MDH-2 and MDH-3) regularly formed heterodimeric products (see Fig. 1).

In the zymograms obtained, 3 of which are shown in figure 1 the isozymes were numbered in order of decreasing mobility from the anode; the locus that specifies the isoenzyme with the least anodic migration is labelled as No. 1, the next as 2, etc. At each locus, the alleles of the different allozymes were designated according to their relative mobility from the bromophenol (1.00). The frequencies of each allozyme are shown in table 2, together with their statistical evaluation. The genetic distance between the 2 populations was calculated according to the method of NEI (1972).

#### Results and Discussion

Table 1 shows that the differences were statistically significant or greatly significant when the average values for the parameters used in the morphometric characterization of the seeds from the 2 populations are compared. Even though the parameters in this case may be considered interdependent to a certain extent, the technique would appear to be useful in the characterization of different populations. A possible extension of this study, to include other morphometric parameters that are not interrelated, as well as other populations geographically further apart, might provide more reliable data about those phenotype differences in relation to dissimilar environmental conditions.

The gametophytic tissue of the seeds of conifers is an ideal material to evaluate the genotype of these trees; the fact that it is haploid material makes it very easy to identify the different isoenzymatic forms and the allele frequencies in those loci. The 8 enzymatic systems chosen provided at total of 17 isoenzymes, 17 distinct loci, all of them anodic except the GOT-1 which showed mobility towards the cathode. The allele frequencies calculated for

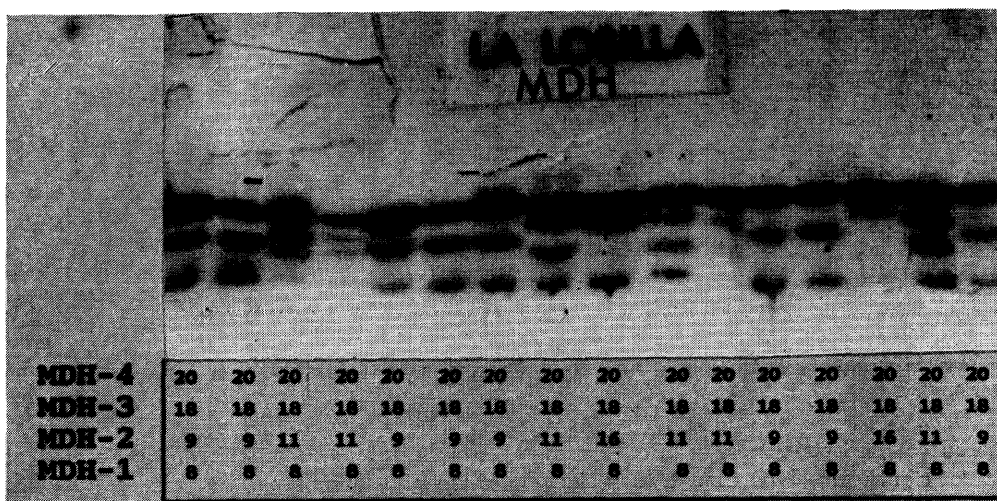
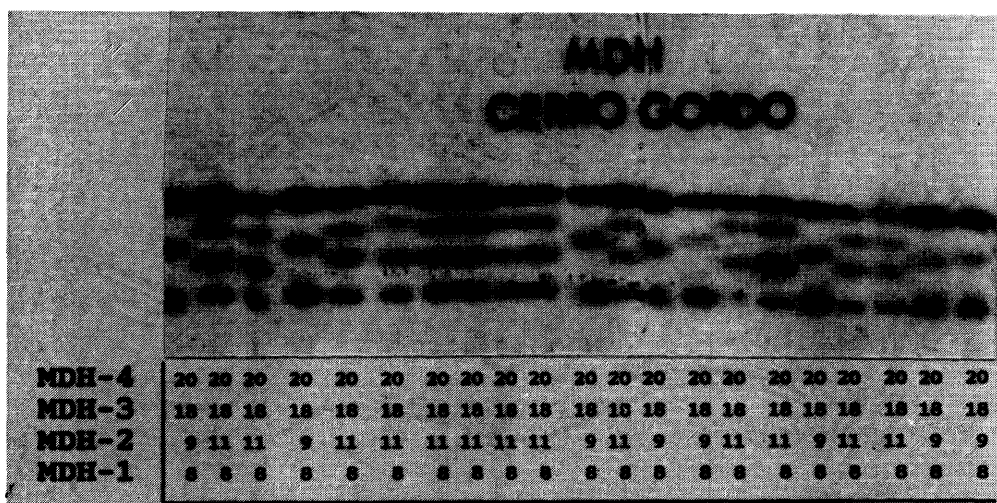
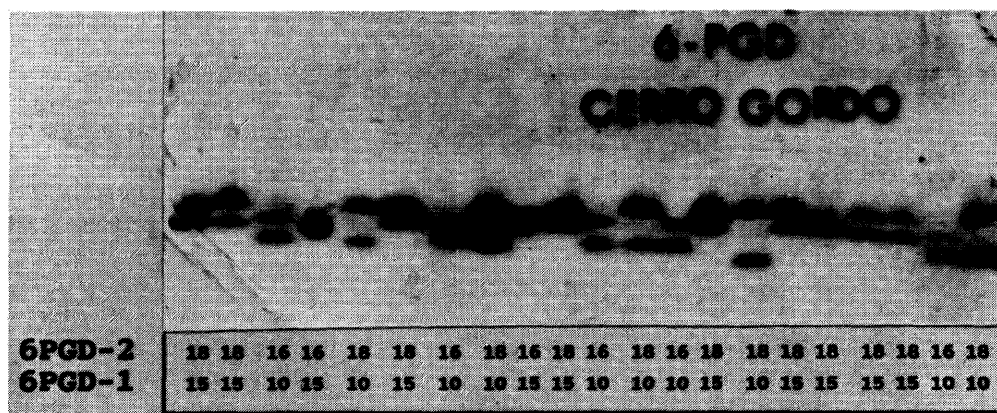


Fig. 1. — Three zymograms showing isoenzyme phenotypes for 6PGD and MDH in *Pinus nigra* endosperm.

these 17 loci in the 2 populations under study showed few major variations in most cases, which was not surprising in view of the proximity of the 2 (Table 2). Some of the isoenzymes (LAP-1, PGM-2, MDH-4, GOT-3) appeared as monomorphic in all individuals studied, so there was no

variation between the 2 populations. Both MDH-1 and MDH-3 had 1 allele each, the respective mobilities being 0.09 and 0.13. This was infrequent in the "Cerro Gordo" population, but did not occur at all in that of "La Losilla" (Table 2). The variations detected among the allele fre-

Table 2. — Allelic frequencies observed at 17 enzyme loci in 2 populations of *Pinus nigra* and significance level of  $\chi^2$  test for the heterogeneity of the allele frequencies (\*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

Loci	Alleles (relative migration distance)	Populations		$\chi^2$
		La Losilla	Cerro Gordo	
LAP-1	0.35	1.00	1.00	
LAP-2	0.45	1.00	0.90	
PGM-1	0.25	0.38	0.31	
	0.30	0.62	0.68	
PGM-2	0.48	1.00	1.00	
ADH-1	0.13	0.15	0.23	
	0.17	0.85	0.77	
ADH-2	0.17	0.31	0.35	
	0.24	0.69	0.65	
ACO-1	0.15	0.25	0.45	**
	0.17	0.71	0.22	
	0.20	0.04	0.33	
MDH-1	0.08	1.00	0.93	
	0.09	0.00	0.07	
MDH-2	0.09	0.37	0.41	
	0.11	0.57	0.48	
	0.16	0.06	0.11	
MDH-3	0.13	0.00	0.03	
	0.18	1.00	0.97	
MDH-4	0.20	1.00	1.00	
6PGD-1	0.10	0.46	0.47	
	0.15	0.54	0.53	
6PGD-2	0.16	0.32	0.47	
	0.18	0.68	0.53	
GOT-1	-0.26	0.74	0.33	***
	-0.13	0.26	0.66	
GOT-2	0.03	0.08	0.06	
	0.07	0.70	0.61	
GOT-3	0.12	0.22	0.33	
	0.24	1.00	1.00	
IDH-2	0.20	0.13	0.05	
	0.22	0.87	0.95	

( $\chi^2$  = chi-square)

quencies of numerous other isoenzymes, codified by several alleles, were not significant, even though they showed a high rate of polymorphism within the populations (6PGD-1, 6PGD-2, MDH-2 or MDH-3). The ACO-1 and GOT-1 were found to be statistically significant in both populations. Therefore both enzymes appeared to be genetic markers capable of differentiating the populations of "La Losilla" and "Cerro Gordo". This preliminary result is interesting because of the proximity between both localities showing very similar adaptive characteristics. Nevertheless, "La Losilla" has an altitude of 1300 m on

a sunny area. "Cerro Gordo" is located at 1600 m on a shady and sunny area.

These results show that the geographic distribution of allele frequencies at 15 isozyme loci was rather homogeneous among the 2 populations. However, local differentiation at 2 loci was evident. Moreover, enzyme differentiation, when detected, discriminates between very close populations, as the case of the populations studied here, that could be connected by gene flow. However, the Conifers are considered as one of the most genetically variable groups of species (HAMRICK, 1979). Several studies have attributed this

high level of variation to the geographic range and distribution of the species and other life-history features (EL-KASSABY, 1991). For example, "La Losilla" and "Cerro Gordo" have not undergone an strong silvicultural management (Personal comm. J. A. GÓMEZ LORANCA).

Summarizing, the genetic difference between the 2 locations could be attributable to natural selection. Morphological features are more sensitive to selective pressures from the local environment, so the seed-size-difference among the studied populations could be induced environmentally.

The genetic identity (NEI, 1972), calculated on the basis of the allele frequencies obtained, gave a value of  $I = 0.967$ . This high rating is yet another index of the proximity of the 2 populations, but it suggests that the similarity would be lower if the study were widened to include populations of the same subspecies geographically further afield, and naturally to include other subspecies of *Pinus nigra* in the Mediterranean area.

### References

BONNET-MASIMBERET, M., BIKAY-BIKAY, V. and CAPELLI, P.: Variabilité intraspécifique des isozymes de la glutamate-oxalacetate-transaminase chez *Pinus nigra* ARNOLD. Intérêt pour la taxonomie des sous-espèces. *Silvae Genetica* 27: 71–79 (1978). — CONKLE, M. T., HODGSKISS, P. D., NUNNALLY, L. B. and HUNTER, S. C.: Starch gel

electrophoresis of conifer seeds: a laboratory manual. USDA Pacific Southwest Forest and Range Experiment Station, pp 1–20 (1982). — EL-KASSABY, Y. A.: Genetic interpretation of malate dehydrogenase isoenzymes in some conifer species. *The Journal of Heredity* 72: 451–452 (1981). — EL-KASSABY, Y. A.: Genetic variation within and among conifer populations: review and evaluation of methods. In: "Biochemical markers in the population genetics of forest trees". pp 61–76. Ed. FINESCHI, S., MALVOLTI, M. E., CANNATA, F. and HATTEMER, H. H. SPB Academic Publishing, The Hague, The Netherlands (1991). — FALKENHAGEN, E. R.: Isozyme studies in provenance research of forest trees. *Theor. Appl. Genet.* 69: 335–347 (1985). — GIL, P., GALERA, R., MARTIN, S., AGUNDEZ, D. and ALIA, R.: Las regiones de procedencia de *Pinus nigra* ARNOLD, *subsp. salzmannii* (DUNAL) FRANCO. In "Regiones de procedencia de *Pinus sylvestris* L. *Pinus nigra* ARNOLD *subsp. salzmannii* (DUNAL) FRANCO". pp 21–31. Ed. CATALAN, G. ICONA-INIA, MAPA, Madrid, España (1991). — HAMRICK, J. L.: Genetic variation and longevity. In: SOLBRIG, O. T., JAIN, S. and RAVEN, P. H. (Eds). "Plant Population Biology". pp 84–113. Columbia University Press, N. Y. (1979). — MURPHY, R. W., SITES, J. W., BUTH, D. G. and HAUFLE, C. H.: Proteins I: Isoenzyme Electrophoresis. In "Molecular Systematics". pp 45–126. Ed. HILLIS, D. M. and MORITZ, C. Sinauer Associates, Sunderland, Mass., U. S. A. (1990). — NEI, M.: Genetic distance between populations. *Amer. Naturalist*. 106: 283–292 (1972). — NIKOLIC, D. and TUCIC, N.: Isoenzyme variation within and among populations of European Black Pine (*Pinus nigra* Arn). *Silvae Genetica* 32: 3–4 (1983). — ORTIZ, J., CABELLO, F., ALTUVE, H. and SOTES, V.: Multifactorial analysis of leaf parameters for identification of "Vitis" cultivars. *Rev. Vitic. Enol.* 1: 3–8 (1990). — PARDOS, J. A.: Variabilidad: sus clases y uso en la mejora forestal. En "Mejora genética de especies arbóreas forestales". pp 30–45. Ed. PARDOS, J. A. Fucovasa, Madrid, España (1988).

## Allozyme Variation and Mating System in Three Artificial Stands of Douglas-Fir (*Pseudotsuga menziesii* (Mirb.) Franco) Planted in Europe

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### Summary

Seed collected from some artificial stands of Douglas-fir produce trees having good adaptation to the local environment and substantial growth. The origin of these artificial stands is completely unknown and might be assessed by studying genetic variability and mating system, using isozymes as genetic markers.

Three European artificial stands (Au Charnay, Barlohe, En Argaud) and 1 natural stand (Bacon Creek) of Washington State were analysed in the present study. No significant variation of allelic frequency was observed among stands or between generations (mother trees and offsprings); nevertheless the gene diversity in the progenies was reduced. According to a multivariate analysis, the genetic variation, was mainly within open progenies, and weakly among provenances (about 2%); artificial provenances could not be differentiated.

The increase of homozygosity from mother trees to their progenies was general; it was higher than that expected from the observed outcrossing rate. This suggested pollination and crossing between related trees and neigh-

bourhood effect, that was also supported by the difference between multilocus and single locus outcrossing rate. Different levels of crossing between related trees were assessed (more than 45%) considering that fixation index resulted only from selfing and related mating. In 1 artificial provenance, the mother trees showed a weak and positive fixation index, and the level or related mating was relatively low. This artificial provenance (Au Charnay) may have resulted from a mixture of several progenies collected in different natural provenances, inducing a deficit of heterozygosity by structuring into subpopulations. Trees from seed collected in this stand produced a higher growth than those from En Argaud stand, which showed a presumed higher frequency of inbred mating.

*Key words:* allozyme variation, artificial provenance, genetic variation, genetic structure, mating system, provenance, *Pseudotsuga menziesii*.

*FDC:* 165.3; 165.53; 232.1; 174.7 *Pseudotsuga menziesii*; (4).

### Introduction

Plantations of Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO) have been developed in Europe and especially in France since its introduction during the last century. The

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