Allozyme Polymorphism in *Austrocedrus chilensis* (D. DON) FLORIN and BOUTELJE from Patagonia, Argentina

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(Received 15th May 1995)

Abstract

Genetic variability was estimated from allozyme electrophoretic patterns in 2 population samples of *Austrocedrus chilensis* ("Cordilleran cypress"), a native tree from the Andean Patagonian forest in Argentina. Peroxidase, esterase, leucine aminopeptidase and aspartate aminotransferase were analyzed in cotyledons of 7 day old seedlings which provided information on the allelic variation at 12 loci. The proportion of polymorphic loci, average number of alleles per locus and mean heterozygosity per locus were 41 %, 1.75 and 0.071 respectively. This last value is considerably lower than those reported for other species of conifers. The patchy distribution of the populations, the existence of natural barriers to gene flow and the low dispersion capacity of the seeds, may contribute to a reduced effective population size, which is compatible with the low level of polymorphism observed.

Key words: Austrocedrus chilensis, Cupressaceae, allozyme polymorphism, population genetics.

FDC: 165.3; 165.5; 174.4 *Austrocedrus chilensis;* (82).

Introduction

Austrocedrus chilensis or "Cordilleran cypress", is a Cupressaceae species native to Argentina and Chile. In Argentina it is found in the Andean-Patagonian forest between latitudes 39° 30' S. and 43° 35' S. (HUECK, 1978).

In addition to its commercial value, this species is of particular ecological interest because of its adaptability to extreme environmental conditions such as the xeric Patagonian climate. Moreover, studies are being conducted in order to evaluate its potential capacity for the reforestation of areas affected by fires (GOBBI and SANCHOLUZ, 1992; HAVRYLENKO et al., 1989). On account of its economic and ecological importance, information on the genetic resources of this species may be of interest.

In the field of forest genetics, levels of polymorphism in several wind pollinated species of conifers have been inferred from data of electrophoretic surveys. The species studied have relative large amount of genetic variability and low levels of interpopulation differentiation (LOVELESS and HAMRICK, 1984; HAMRICK et al., 1992; MÜLLER-STARCK et al., 1992; KRUTOVSKI and BERGMANN, 1995).

So far, the isozyme variability in the *Cypressaceae* family has been poorly analyzed. HARRY (1983, 1986) studied the inheritance and linkage of isozyme variants at 25 loci in incense-cedar (*Calocedrus decurrens*). Allozyme variation has also been investigated on other species of the family as *Cupressus macrocarpa* (CONKLE, 1987), *Thuja plicata* (YEH, 1988) and *Chamaecyparis lawsoniana* (MILLAR and MARSHALL, 1991).

In *Austrocedrus chilensis*, the only study utilizing isozymes as genetic markers was conducted by GALLO and GEBUREK (1994), who analyzed the genetic control in 6 polymorphic loci.

In this report we present the first estimation of the allozyme variability in a natural population of the "Cordilleran cypress" from the Andean Patagonian forest.

Materials and Methods

Seeds were collected at Esquel (Chubut province) during fall in 1990 and 1991. Populations samples collected at 2 sites 32 km apart (Futaleufú and Rio Percey) were studied.

Eight trees from each population were sampled. Sites of collection and number of seeds from each population analyzed are indicated in *table 1.*

Table 1. – Location of the studied populations.

Locality	Latitude	Longitude	Altitude	N. of indiv.
Futaleufú	43° 03'	71° 40'	400 m	120
Rio Percey	42° 51'	71° 25'	850 m	60

Different conditions for seed germination were tested in the laboratory. The highest percentage of germination was obtained by soaking the seeds in 1 % hydrogen peroxide solution for 10 min, storing at 4 °C for 14 days and then germinating on wet filter paper in Petri dishes at 26 °C with a 16:8 L/D photoperiod.

Cotyledons of 7 day old seedlings were used for the electrophoretic analysis of peroxidases (Px) and 14 or more days cotyledons, for the study of esterases (Es), leucine aminopeptidase (LAP) and aspartate aminotransferase (AAT).

A very low proportion of seeds from Rio Percey germinated and only peroxidases could be analyzed in this sample.

Electrophoretic techniques

The cotyledons from each seedling were homogenized with a drop of distilled water. The suspension was absorbed in an 8 mm x 3 mm piece of Whatman 3M paper and inserted in an 11% starch gel block where horizontal electrophoresis was carried out with 7 V/cm for 16 h at 4 °C.

Px, ES, and LAP were separated using lithium borate 0.2 M buffer pH 8.3 (SCANDALIOS, 1969). Tris citric buffer pH 8.65 for gels and lithium borate pH 8.3 for the electrode cells (SELANDER et al., 1971) were used for the study of AAT. The staining methods proposed by SHAW and PRASSAD (1979) were used to reveal AAT, ES, and LAP. The staining of Px bands was performed according to SCANDALIOS (1969). After staining, the gels were fixed with methanol: water: acetic acid (5:5:1).

Nomenclature

The loci were numbered according to decreasing mobilities of the products toward the anode, eg: Est-1, Est-2. Alleles were assigned a small letter in alphabetical order, being *a* the one coding for the protein with fastest anodic migration.

Statistical analysis

Genotype frequencies were calculated according to the methods proposed by Levene for small samples (SPIESS, 1990).

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χ^2 test for heterogeneity was used in the evaluation of gene frequency data. Average heterozygosity (H) was estimated from allelic frequencies. An enzyme was considered polymorphic when the frequency of the most common allele was not higher than 0.99.

Results and Discussion

Peroxidases

Three anodic and 2 cathodic areas of activity were observed. The anodic areas showed either 1 or 2 bands, while the 2 cathodic zones of activity presented always 1 band each. The patterns are compatible whith the existence of 5 different loci, being *Px-1* and *Px-2* in the sample from Futaleufú and *Px-3* from Río Percey polymorphic, while *Px-4* and *Px-5* were monomorphic in the 2 samples (*Fig. 1*). Zymogram variants are consistent with a mendelian inheritance with 2 codominant alleles (*a* and *b*) at the *Px-3* locus and 3 alleles (*a, b,* and *c*) at the *Px-1* and *Px-2* loci.

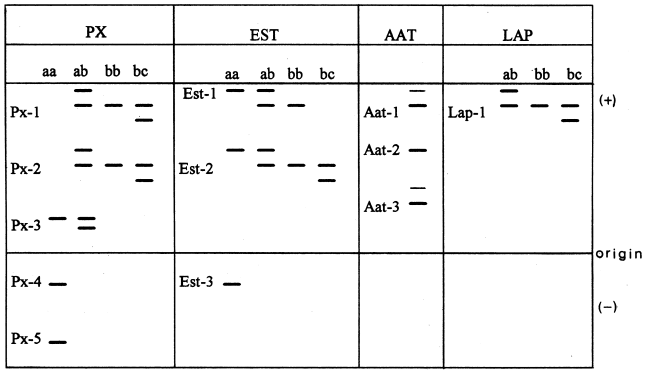

Figure 1. – Schematic representation of observed electrophoretic zymograms of peroxidases (PX), esterases (EST), aspartate-aminotransferase (AAT) and leucine-amino-peptidase (LAP) of *Austrocedrus chilensis* from Futaleufú and Río Percey populations.

Leucine-amino-peptidase

One zone of activity with 3 phenotypes (B, AB, and BC) were found, probably representing the products of 3 codominant alleles (*a, b, c*).

GALLO and GEBUREK (1994) found no polymorphism for this locus in a sample of *A. chilensis* from the Patagonia.

Patterns similar to the one described here were reported for *Picea abies* (KRUTOVSKI and BERGMANN, 1995), *Pinus leucodermis*, (BOSCHERINI et al., 1994), and *Callocedrus decurrens* (HARRY, 1986).

Esterases

Two zones of activity with anodic mobility and a zone of cathodic migration could be identified in the gels after staining for EST (*Fig. 1*).

The *Est-3* locus appears as monomorphic, while 3 and 4 different phenotypes were detected for *Est-1* and *Est-2* respectively, indicating the existence of 2 alleles at *Est-1* and 3 alleles at *Est-2*.

Studies in *Calocedrus decurrens* (HARRY, 1986), *Picea abies* (BRUNEL and RODOLPHE, 1985) and *Larix decidua* (LEWANDOWSKI and MEJNARTOWICZ, 1991) showed several bands with esterase activity under the control of 1 to 4 loci with 2 or 3 codominant alleles.

Aspartate-amino transferase

Anodic multiple banded phenotypes were obtained for this enzyme. There were 3 zones of staining, designated AAT-1, AAT-2 and AAT-3 in order of decreasing anodic mobility. AAT-1 showed 2 bands of unequal intensity. The band running in front was faintly stained in contrast with the dark second band. AAT-2 presented a single, well stained fraction, and AAT-3, 2 bands, the second being more intense than the first. The zone of light staining in front of AAT-1 and AAT-3 was not present in all cases, while the 3 dark bands were constant for all the individuals analyzed (*Fig. 1*).

GALLO and GEBUREK (1994) also reported 3 zones of activity designated GOT-1, GOT-2 and GOT-3. The last one was a 3-banded phenotype whose genetic control was not elucidated.

On the basis of our zymograms we propose that the faint, unconstant bands in front of AAT-1 and AAT-3 are satellite fractions, produced by post-translational modification of the main corresponding band. A similar pattern was reported for others species, where it has been proved that the satellite band results from deamidation of the principal fraction (JOHN and JONES, 1974).

Our zymograms for AAT-3 showed only 2 fractions corresponding to the 3-banded zone described by GALLO and GEBUREK (1994). The third band reported by these authors could also be the result of epigenetic modifications.

AAT-1, AAT-2 and AAT-3 were monomorphic in all the samples.

Allele frequencies

The allele frequencies for the Futaleufú and Río Percey populations are presented in *table 2.* The observed genotype frequencies in polymorphic loci of both populations are in agreement with the expected frequencies for a HARDY WEINBERG equilibrium. The *Px-3* locus was monomorphic in the Futaleufú sample and was highly polymorphic in that from Río Percey.

Table 2. – Allelic frequencies in the analyzed loci of both populations.

Allele	Futaleufú	Río Percey
Px-1 a	0.027	0.14
Px-1 b	0.944	0.81
Px-1 c	0.027	0.05
Px-2 a	0.017	0.07
Px-2 b	0.975	0.93
Px-2 c	0.006	0.00
Px-3 a	0.000	0.3158
Px-3 b	1.000	0.6842
Px-4	monomorphic	monomorphic
Px-5	monomorphic	monomorphic
Lap-1 a	0.008	not analyzed
Lap-1 b	0.974	not analyzed
Lap-1 c	0.017	not analyzed
AAT-1	monomorphic	not analyzed
AAT-2	monomorphic	not analyzed
AAT-3	monomorphic	not analyzed
Est-1 a	0.439	not analyzed
Est-1 b	0.56	not analyzed
Est-1 c	0.000	not analyzed
Est-2 a	0.077	not analyzed
Est-2 b	0.915	not analyzed
Est-2 c	0.006	not analyzed
Est-3	monomorphic	not analyzed

Table 3. – Genotype frequencies of polymorphic loci in populations from Futaleufú and Río Percey.

		Futaleufú					Río Percey				
genotype frequency		aa	ab	bb	bc	χ^2	aa	ab	bb	bc	χ^2
PX-1	O	–	3	48	3	0.08	–	6	13	2	0.4
	E	–	2.75	48	2.7	N.S.	–	4.7	13.7	1.7	N.S.
PX-2	O	–	5	137	2	0.04	–	2	13	–	0.029
	E	–	4.85	137	1.9	N.S.	–	1.8	13	–	N.S.
PX-3	O	–	–	100	–	–	–	1	10	8	0.811
	E	–	–	–	–	–	–	1.9	9.4	8.8	N.S.
Est-1	O	15	35	24	–	0.118	–	–	–	–	–
	E	14.2	36.3	23.2	–	N.S.	–	–	–	–	–
Est-2	O	1	9	60	1	0.923	–	–	–	–	–
	E	0.92	10	59.5	0.9	N.S.	–	–	–	–	–
Lap-1	O	–	1	55	2	0.04	–	–	–	–	–
	E	–	0.97	55.03	1.9	N.S.	–	–	–	–	–

Polymorphism

Five of the 12 enzyme loci studied (*Px-4, Px-5, Lap-1, Est-1* and *Est-2*) were found to be polymorphic in the natural population of Futaleufú (P = 41 %). The average number of alleles per locus was 1.75.

The mean heterozygosity per locus (H) estimated for the Futaleufú population was 0.071. This value is considerably lower than those obtained by other authors for different species of *Abies* (H = 0.145), *Picea* (H = 0.219), *Pinus* (H = 0.157) and *Pseudotsuga* (H = 0.201), (HAMRICK et al., 1992).

Factors like high fecundity rate, an outcrossing model of reproduction, wind pollination, and the lack of effective barriers to gene flow between subpopulations have been proposed to explain the important polymorphism found in conifers (LOVELESS and HAMRICK, 1984; HAMRICK and GODT, 1989; HAMRICK et al., 1992).

Although *Austrocedrus chilensis* has a wide distribution in the Andean Patagonian forest, it is mainly found in the dry eastern zones, being one of the few arborean species which colonizes these areas (GOBBI and SANCHOLUZ, 1992).

Due to the agricultural and cattle breeding activities and the forestation with exotic species, which are very common in this region, the distribution of the populations occurs in "patches" of variable density (from a few to 250 trees/ha) (HUECK, 1978). The individuals analyzed in this study belong to groups of low density.

This species has abundant, light winged seeds. However, they have a poor dispersion capacity (GOBBI and SANCHOLUZ, 1992) which could favour the mating among neighbours. One evidence supporting this assumption is the difference in the allelic frequencies found in the *Px-3* locus between the Futaulefú (monomorphic) and Río Percey (highly polymorphic), localities distant 32 km from each other. The presence of mountain ranges perpendicular to the prevailing wind direction (SW to NE) could also act as natural barriers for the gene flow between individuals from different patches. These features, which may reduce the effective population size, as well as the restrictive geographic distribution of the species, are compatible whith the low level of polymorphism found by us.

However, the levels of genetic variability observed as well as the allelic distribution could be due to sampling errors caused by small sample size. Further studies increasing the number of loci and populations investigated are needed to asses with greater accuracy indices of gene diversity in this important forest species.

Acknowledgements

We thank Dr. A. BLANCO for his advice and critical revision of the manuscript, to G. CORTÉS, E. ANDESMATTEN and G. BASIL for their assistance in seed collection and to M. HAVRYLENKO, M. GOBBI and E. SÁNCHEZ who provided us valuable information.

Literature

BOSCHERINI, G., MORGANTE, M., ROSSI, P. and VENDRAMIN, G. G.: Allozyme and chloroplast DNA variation in Italian and Greek populations of *Pinus leucodermis*. Heredity **73**, 284–290 (1994). — BRUNELL, D. and RODOLPHE, F.: Genetic neighbourhood structure in a population of *Picea abies* L. Theor. Appl. Genet. **71**, 101–110 (1985), — CONKLE, M. T.: Electrophoretic variation in native monterey cypress (*Cupressus macrocarpa* HARTW.). In: T. S. ELIAS (ed.): Conservation and management of rare and endangered plants. Proceedings of the Conference, Sacramento, CA. CA Native Plant Society, Sacramento, CA. pp. 249–256 (1987). — GALLO, L. A. and GEBUREK, TH.: A Short Note: Genetics of enzyme variants in *Austrocedrus chilensis* (ENGL.) FLORIN and BOUTELGE. Phyton. **34**, 103–107 (1994). — GOBBI, M. and SANCHOLUZ, L.: Regeneración post incendio del ciprés de la cordillera (*Austrocedrus chilensis*) en los primeros años. Bosque **13** (2), 25–32 (1992). — HAMRICK, J. L.: Isozymes and the analysis of genetic structure in plant populations. pp. 87–105. In: Isozymes in Plant Biology. SOLTIS, B. E. and SOLTIS, P. S. (eds.). Advances in Plant Sciences Series, vol. 4. DUDLEY, T. R. (gen. ed.). Dioscorides Press, Portland, Oregon (1981). — HAMRICK, J. L. and GODT, M. J.: Allozyme diversity in plant species. In: BROWN, A. H. D., CLEGG, M. T., KAHLER, A. L. and WEIR, B. S. (eds.): Plant Population Genetics, Breeding and Genetic

Resources. Sinauer Associates, Sunderland, M. A., 43–63. — HAMRICK, J. L., GODT, M. J. and SERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forest **6**, 95–124 (1992). — HARRY, D. E.: Identification of a locus modifying the electrophoretic mobility of malate dehydrogenase isozymes in incense-cedar (*Calocedrus decurrens*), and its implications for population studies. Biochem. Genet. **21**, 417–434 (1983). — HARRY, D. E.: Inheritance and linkage of isoenzyme variants in incense-cedar. J. Hered. **77**, 261–266 (1986). — HAVRYLENKO, M. ROSSO, P. A. and FONTENLA, S. B.: *Austrocedrus chilensis*: contribución al estudio de su mortalidad en Argentina. Bosque **10**, 29–36 (1989). — HUECK, K.: Los bosques de Sudamérica. Soc. Alemana de Cooperación Técnica GTZ, Eschborn. pp. 474 (1978). — JOHN, R. and JONES, R.: The nature of the multiple forms of cytoplasmatic aspartate aminotransferasa from pig and sheep heart. Biochemical J. **141**, 401–406 (1974). — KRUTOVSKII, K. V. and BERGMANN, F.: Introgressive hybridization and phylogenetic relationships between Norway, *Picea abies* (L.) KARST., and Siberian, *P. obovata* LEDEB., spruce species studied by isozyme loci. Heredity **74**, 464–480 (1995). — LEWANDOWSKI, A. and MEJNARTOWICZ, A. L.: Levels and patterns of allozyme variation in some European larch (*Larix decidua*) populations. Hereditas **115**, 221–226 (1991). — LOVELESS, M. D. and HAMRICK, J. L.: Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. **15**, 65–95 (1984). — MILLAR, C. I. and MARSHALL, K. A.: Allozyme variation of Port-Orford-cedar (*Chamaecyparis lawsoniana*): implications for genetic conservation. For. Sci. **37**, 1060–1077 (1991). — MÜLLER-STARCK, G., BARADAT, PH. and BERGMANN, F.: Genetic variation within European tree species. New Forests **6**, 23–47 (1992). — SCANDALIOS, J.: Genetic control of multiple molecular forms of enzymes in plants: A review. Biochem. Genet. **3**, 37–79 (1969). — SELANDER, R. K., SMITH, M. H., YANG, S. Y., JOHNSON, W. E. and GENTRY, G. B.: Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Univ. Tex. Publ. 7103, 49–90 (1971). — SHAW, C. R. and PRASAD, R.: Starch gel electrophoresis of enzymes – A compilation of recipes. Biochem. Genet. **4**, 297–320 (1979). — SPIESS, E. B.: Genes in Populations. John Wiley and Sons, New York (1990). — YEH, F. C.: Isozyme variation of *Thuja plicata* (CUPRESSACEAE) in British Columbia. Biochem. Syst. and Ecol. **16**, 373–377 (1988).

Characterization and Variability of Seed Storage Protein Subunits in *Sophora japonica* (Fabaceae)

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(Received 14th June 1995)

Summary

The seed storage globulins of *Sophora japonica* correspond to 7 main groups of polypeptide monomeres – SP98, SP9*, SP74, SP7*, SP37, SP23 and SP22 – with molecular weights ranging from 98 kDa to 22 kDa that can be ascribed to 6 different loci (SP9* and SP7* polypeptydes may show different allelic forms of more than 90 kDa and 70 kDa respectively). SP9* form both disulphur-linked dimers and tetrameres, SP37 forms an heterodimer with SP23, and SP22 an homodimer. The polypeptides from 79 kDa to 71 kDa seem to be subject of some post-traductional partial modification.

Variability, in what molecular weight of monomere polypeptides was concerned, was found to be affected with loci *sp9*, sp7*, sp37*. Although this species was imported to Spain in the present century, 2 stands (populations) analysed show a high degree of variability (mean heterozygosity as 0.213 and polymorphic index as 0.288).

Key words: Sophora japonica, seed storage proteins, SDS-PAGE, polymorphism.

FDC: 165.53; 161.4; 161.6; 176.1 *Sophora japonica.*

Introduction

The pagoda tree, *Sophora japonica,* belongs to the subfamily Cæsalpinioideae and was originated from east Asia. During the last century *S. japonica* has been imported to Europe because it is well adapted in urban forestry and, at present, it is even frequent in natural or semi-natural woods. However, the facts that neither the populations of origin nor the existing levels of variability were known, the actual culture of this species may face problems such as inbreeding or to establish the best stands for use as seed source.

Legume seeds have been subject of intensive work due to their high protein content. These proteins are usually classified into 2 main groups, legumins and vicilins (DERBYSHIRE *et al.,* 1976). Most results about seed protein genetics refer to crops employed in human and animal feeding like the genera *Vicia, Glycine, Pisum* or *Phaseolus* (for review, see CASEY *et al.,* 1986) contrasting with the scarce data concerning tree species. In some cases, seed protein variation has been employed in taxonomic or evolutionary studies of certain tree groups (GIFFORD, 1988; JENSEN and BERTHOLD, 1989; JENSEN and LIXUE, 1991). Long generation periods make difficult traditional genetic studies, so that, for instance, a method designed for gene mapping in human families have been employed to establish linkage maps in *Pinus pinaster* (GERBER *et al.,* 1993). In *Cercis siliquastrum,* a cæsalpinoid tree, the genetic control of seed storage proteins and the linkage relationships of the correspondent loci have been recently described (GONZÁLEZ and HENRIQUES-GIL, 1994). It is important to keep in mind that many Fabaceae trees produce large amounts of edible legumes or seeds. Although this specific use as food is restricted to a few cases like the bean tree, *Ceratonia siliqua*, or some *Cassia* species (SÁNCHEZ-MONGE, 1991), legume seeds may have a stronger ecological importance as a protein source for a number of animals in natural conditions.

The levels of variability found in this type of proteins are usually high (CASEY *et al.,* 1986; TUCCI *et al.,* 1991) since their