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A Short Note: Genetics of Enzyme Variants in *Austrocedrus chilensis* (ENGL.) FLORIN & BOUTELJE

By

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With 1 Figure

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Summary

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Inheritance of glutamate dehydrogenase (GDH), glutamate-oxaloacetate transaminase (GOT), leucine aminopeptidase (LAP), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), and superoxide dismutase (SOD) was analyzed in *Austrocedrus chilensis* using megagametophytes of single trees. Single locus control is proposed each for IDH, LAP, SKDH, and SOD. GOT is controlled by at least three gene loci. Two gene loci probably code 6-PGDH. Genetic pattern of multiple-banded MDH remains still uncertain, since electromorphs were only found at one zone.

Zusammenfassung

GALLO, L. A. & GEBUREK, Th. 1994. Kurzmitteilung: Genetik von Enzymvarianten bei *Austrocedrus chilensis* (Engl.) FLORIN & BOUTELJE. – Phython (Horn, Austria) 34 (1): 103–107, mit 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Die Vererbung der Glutamatdehydrogenase (GDH), Glutamatoxaloacetattransaminase (GOT). Leucinaminopeptidase (LAP), Isocitratdehydrogenase (IDH), 6-Phosphogluconatdehydrogenase (6-PGDH), Phosphoglucomutase (PGM), Shikimatdehydrogenase (SKDH) und Superoxiddismutase (SOD) wurde bei *Austrocedrus chilensis* anhand von Megagametophyten einzelner Bäume analysiert. Die Isoenzyme

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IDH, LAP, SKDH und SOD werden durch einen Genlocus kontrolliert. GOT wird mindestens durch drei Genloci kodiert. Zwei Strukturgene bestimmen 6-PGDH. Die genetische Kontrolle von MDH, einem Enzym mit komplexem Bandenmuster, konnte noch nicht gänzlich geklärt werden, da Elektromorphe nur für eine Enzymzone gefunden wurden.

1. Introduction

Inheritance studies in most organisms require controlled crossings and investigations of the progeny. Sometimes inheritance of isozymes is simply concluded from the enzyme structure (homomeric or heteromeric) or Mendelian inheritance is inferred when the observed genotypic proportions in a population sample are consistent with Hardy-Weinberg-expectation. In any event, caution is warranted because enzyme variants are not exclusively controlled by structural genes but can also stem from post-translational or posttranscriptional modification. The inheritance study of enzyme variants is a *conditio sine qua non* for populations genetic studies.

In gymnosperms, the female gametophytes (megagametophytes) are multicellular structures and nutritious tissues for the developing embryos. Since the megagametophytes are haploid they facilitate the investigation about the inheritance pattern. Megagametophytes in gymnosperms of single trees putatively heterozygous are electrophoretically analyzed and the maternal genotypes are inferred from the enzyme phenotypes observed. Mendelian inheritance is then verified when the megagametophytes of individual plants segregate according to the 1:1 expectation.

Whereas genetic variation has been thoroughly analyzed in many coniferous species, genetic knowledge based on isozymes as gene markers is still very limited in *Cupressaceae*, especially in the subfamily *Calitroideae*. So far, *Calocedrus decurrens* (HARRY 1983, 1986) is the only species of this subfamily that has been electrophoretically analyzed. However, isozyme studies in *Chamaecyparis lawsoniana* (MILLAR & MARSHALL 1991), *Cupressus macrocarpa* (CONKLE 1987), and *Thuja plicata* (YEH 1988), that all belong to the subfamily *Cupressoideae*, have been reported.

Since the existance of topodemes of *Austrocedrus chilensis* is at its stake, studies about the genetic variation are highly desirable in this conifer to enhance operational means to preserve gene resources. Hence, genetic control of isozyme variants is reported in this note.

2. Materials and Methods

Twenty-four open-pollinated seed samples originating from eight different sites within the natural distribution area in Argentina were used. The material was collected during fall in 1988 and 1989, respectively, and was stored at –20° C until electrophoresis was performed. A small drop of extraction buffer (Tris/HCl pH 7.5) was added to the megagametophyte and the corresponding tiny embryo. Samples

were stored overnight at +4° C in plexiglass mortars and were homogenized during the following day.

Isozymes were separated by (1) a continuous 0.135 M tris, 0.043 M citric acid system (electrode buffer, pH 7.0; gel buffer, 1:4 diluted electrode buffer) and (2) a discontinuous system (electrode buffer pH 8.2; gel buffer pH 8.7) according to POULIK (1957). Following isozymes were investigated (electrode gel buffer system in square brackets): glutamate dehydrogenase [2] (GDH, E.C.1.4.1.3), glutamate-oxaloacetate transaminase [2] (GOT, E.C. 2.6.1.1.), isocitrate dehydrogenase [1] (IDH, E.C.1.1.1.42), leucine aminopeptidase [2] (LAP, E.C.3.4.11.1), malate dehydrogenase [1] (MDH, E.C.1.1.1.37), 6-phosphogluconate dehydrogenase [1] (6PGDH, E.C.1.1.1.44), phosphoglucomutase [1] (PGM, E.C.2.7.5.1), shikimate dehydrogenase [1] (SKDH, E.C.1.1.1.25), and superoxide dismutase [1] (SOD, E.C. 1.15.1.1.). Gels (12% w/v) were prepared from starch hydrolyzed according to Smithies (Toronto-starch). Electrophoresis was carried out at a constant voltage of 15-20 V/cm with a bridge distance of 5.5 cm. Staining solutions were prepared according to CHELIAK & PITEL (1984).

Based on an initial scoring of megagametophytes, putative heterozygotes were identified. To verify Mendelian segregation, additional samples (up to 110 seeds for a single tree) were employed. Since the dissection of the tiny embryo in *Austrocedrus chilensis* is extremely difficult, megagametophytes including embryos of single seeds were homogenized. A simultaneous scoring of both tissues was possible since isozyme activity of the megagametophyte was higher than of the corresponding embryo.

Each isozyme zone (notation capitalized) was supposed to be controlled by a single gene locus (notation italicized). Mendelian segregation of gametes originating from putative heterozygous trees was assessed by χ^2-test. Heterogeneity among single trees and deviation from the expected 1:1 ratio were evaluated. Allozymes (italicized notation) were designated according to their migration distance (Rm-value) compared with that of the most common allozyme of the corresponding zone.

3. Results and Discussion

Electrophoretic enzyme pattern, designation of isozyme gene loci, and allozymes are shown by Fig. 1. IDH, LAP, SKDH, and SOD were each single-banded. Thus, single locus control was assumed for these enzymes. IDH and LAP were invariable. A polymorphic gene locus is proposed to control SKDH. This hypothesis is supported by single tree segregations and also by pooled data. Six trees out of 23 were heterozygous and had homogenous segregation ratios [heterogeneity χ^2 = 7.029, DF (degrees of freedom) = 6–1, not significant]; pooled segregation was not significantly different from the expected 1:1 ratio [deviation χ^2 = 0.801, DF (degrees of freedom) = 2–1] (Tab. 1). Putative homozygous trees (*Skdh-100/100, Skdh-94/94*) were also found. Two SOD allozymes [enzyme detectable (*Sod-100*), enzyme not detectable (*Sod-null*)] segregated according to the 1 : 1 expectation (Tab. 1). In contrast to *Calocedrus decurrens*, all above-mentioned enzymes, except SOD, are controlled by two gene loci (HARRY 1986).

GOT, MDH, and 6-PGDH were multiple banded. Each enzyme system is supposed to be controlled by at least two gene loci. In GOT, three zones

Table 1

Mendelian segregation of enzyme variants in *Austrocedrus chilensis*

Got-1		Got-3		Mdh-2		6-Pgdh-2		Skdh		Sod	
95/100	χ^2	50/100	χ^2	100/105	χ^2	77/100	χ^2	94/100	χ^2	100/null	χ^2
43:47	0.178	61:58	0.076	61:43	3.115	64:40	5.538	51:62	1.071	59:54	0.221
				3:6	1.000	2:7	2.778	63:42	4.200	50:55	0.238
				3:6	1.000	4:5	0.111	9:9	0.000	7:2	2.778
				17:10	1.815	4:5	0.111	12:15	0.333	13:14	0.037
				8:10	0.222	53:43	1.042	4:5	0.111	6:3	1.000
				2:7	2.778			4:5	0.111	13:5	3.556
				56:55	0.009			21:15	1.000		
				pooled:		pooled:		pooled:		pooled:	
				150:137	0.589	127:100	3.211	164:153	0.382	148:133	0.801

	χ^2	DF	Prob.		χ^2	DF	Prob.		χ^2	DF	Prob.		χ^2	DF	Prob.		χ^2	DF	Prob.
Dev.	0.178	1	>.50	Dev.	0.076	1	>.70	Dev.	0.589	1	>.30	Dev.	3.211	1	>.05	Dev.	0.382	1	>.50	Dev.	0.801	1	>.30
Het.	–	–	–	Het.	–	–	–	Het.	9.350	6	>.10	Het.	6.369	4	>.10	Het.	6.444	6	>.30	Het.	7.029	5	>.20

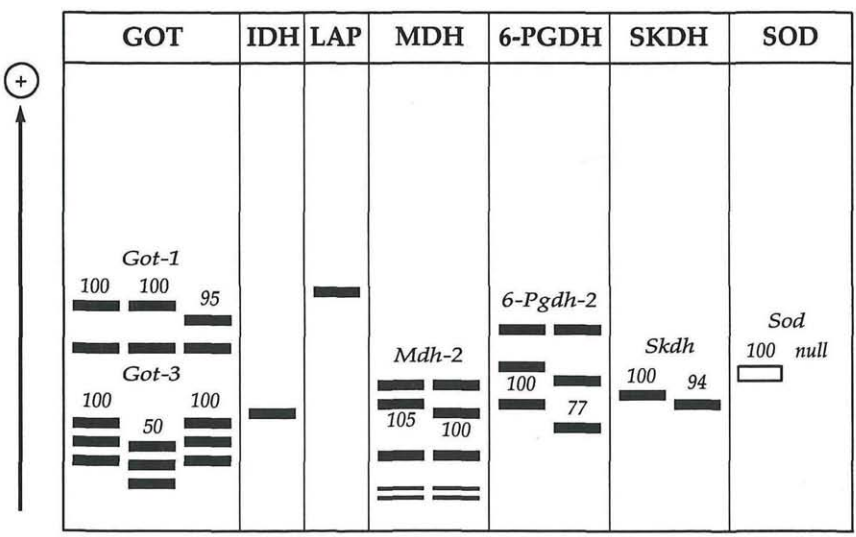

Fig. 1. Electrophoretic pattern found in *Austrocedrus chilensis*

were differentiated (Fig. 1). GOT-2 was monomorphic. Enzyme variants of GOT-1 and GOT-2 segregated according to Mendelian expectation in one tree, each. Triplebanded phenotypes of GOT-3 were also found in *Calocedrus decurrens* (HARRY 1986). If this zone were controlled by three closely linked gene loci (gene triplication), GOT would be coded by five loci. The proportion of trees heterozygous at these gene loci were small (4%). Two different phenotypes were found in MDH (Fig. 1). Isozyme variants at MDH-2 segregated according the 1:1 expectation (Tab. 1). Like in *Calocedrus decurrens* (HARRY 1986), two gene loci are proposed to control 6-PGDH (Fig. 1). A fast migrating isozyme was found in all samples. No electromorphs were found for this zone. Thus, genetic control of *6-Pgdh-1* remains putative. Enzyme variants controlled by *6-Pgdh-2* are inherited in a Mendelian pattern.

Genetic interpretations of varied enzyme zones are straightforward. Isozyme phenotypes were consistent in repeated assays, and embryo phenotypes were consistent with a single gene locus control. However, inheritance of monomorphic zones (GOT-2, IDH, LAP, MDH-1, -3, -4) remains putative.

4. Acknowledgements

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5. Literature

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