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Genetic Analysis of Isoenzyme Variation in Mediterranean Cypress (Cupressus sempervirens L.)

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Abstract

Our inability to separate the haploid megagametophytic tissue from the diploid, maternal perisperm in the seeds of Mediterranean cypress was the reason for using single tree progenies from open pollination for the genetic analysis of isoenzyme phenotypes. Isoenzyme patterns from seed perisperms (maternal tissue) and seed embryos (progeny tissue) were compared and tested qualitatively and quantitatively for the mode of inheritance. In total, 8 variable proposed gene loci coding for 7 enzyme systems could be identified. The data of genetic analysis supported the intuitive genetic interpretation of the isoenzyme patterns.

Key words: *Cupressus sempervirens*, perisperm, enzyme gene loci, segregation analysis.

1. Introduction

Previous studies dealing with enzyme systems in Mediterranean cypress were limited to the application of isoenzyme phenotypes (zymograms) to distinguish cypress clones (Raddi et al., 1990). Their inability to isolate the haploid endosperm from other seed tissues was the major reason for not performing haploid segregation analysis. The endosperm is connected with the perisperm, a diploid tissue which surrounds the endosperm and originates from the parent tree (Raddi et al., 1990). However, the isoenzyme analysis of this tissue combination gives a clear zymogram that differs from the embryonic one and is identical to the parent tree zymogram. The genetic analysis described in this paper is simultaneously a test for the use of perisperm to obtain parent tree isoenzyme genotypes. This paper presents the results of the genetic analysis of 7 polymorphic enzyme systems in order to obtain biochemical genetic markers for population genetic studies. Due to lack of controlled cross progenies, the genetic analysis is based on single tree progenies derived from open pollination (Gillet and Hattemer, 1989).

2. Material and Methods

2.1 Material

The material used consisted of perisperm and embryo of single seeds. In most conifers, seeds consist of 2 tissue types: Seed endoperm (haploid megagametophytic tissue) and seed embryo (diploid progeny tissue). Seed perisperm is a tissue that surrounds the endosperm and in most conifers takes the form of a dry brown layer lacking enzyme activity. Thus, the analysis of the seed material after removing the embryo gives the zymograms of the haploid female gamete.

In our first experiments with cypress seeds, the zymograms obtained from the analysis of seed material after removing the embryo appear to present diploid patterns. These diploid zymograms were usually different from the embryonic ones. For each tree, the non-embryonic seed zymograms for all seeds were always identical, yet they differed from tree to tree. The nonembryonic and embryonic zymograms of each seed always had at least one isoenzyme variant in common. These results clearly suggest that the isoenzyme patterns obtained originate from the seed perisperm. Raddi et al. (1990) also reported that the perisperm is strongly connected with the seed endosperm, this being the major problem of obtaining isoenzyme patterns from haploid megagametophytic tissue. The perisperm was identified as diploid tissue, genetically identical to the maternal tree. It seems that in the case

of cypress, the perisperm is not dry and has enzymatic activity. Nevertheless, since the perisperm has a larger mass and enzyme activity than the endosperm and the genes of the endosperm are also present in the perisperm of the same seed, enzyme activity zones attributable to the perisperm tend to mask those of the endosperm in their common zymogram. Thus, we can obtain two isoenzyme patterns from each cypress seed: The diploid pattern of the seed parent (perisperm) and the diploid pattern of the progeny (embryo). Different perisperms from the same tree always yield the same isoenzyme pattern, therefore several seed perisperms could be mixed for one analysis to increase the band activity and to improve the whole pattern. The seeds were collected from various trees located in natural stands of Crete, Samos, Rhodos, Symi and Kos.

2.2 Electrophoresis

Starch gel electrophoresis was used to analyze the isoenzyme patterns of seven enzyme systems in seed perisperms and embryos: Glutamate dehydrogenase (GDH, 1.4.1.2), malate dehydrogenase (MDH, 1.1.1.37), menadione reductase (MNR, 1.6.99.2), NADH dehydrogenase (NDH, 1.6.99.3), 6-phosphogluconate dehydrogenase (6PGDH, 1.1.1.44), phosphoglucose isomerase (PGI, 5.3.1.9), and phosphoglucomutase (PGM, 2.7.5.1). The electrophoretic procedures were those described by BERGMANN (1974), CHELIAK and PITEL (1984), and WENDEL and WEEDEN (1989). The following zones of these systems were assumed to be controlled by single gene loci: GDH-A, MDH-B, MNR-B, NDH-A, 6PGDH-B, PGI-C, PGM-A, and PGM-C.

2.3 Genetic analysis

The subsequent genetic analysis was done to test these hypotheses. The method specified by GILLET and HATTEMER (1989) was used. Three assumptions were made with regard to the mechanisms of meiosis and fertilization: 1. regular meiotic segregation during egg production; 2. random fertilization of the ovules by each pollen type; 3. absence of differential viability selection in the offspring prior to the investigation.

This method can be applied to test the hypothesis of single-locus codominant mode of inheritance. Under this hypothesis, each phenotype can be traced back to only one genotype which is thus identifiable. For each seed parent with the phenotype A_iA_j ($i \neq j$), the following quantitative and qualitative relations are expected to hold among its offspring from open pollination:

(i) Each offspring must possess at least either allele A_i or A_j

(ii) $N_{ij} = N_{ii} + N_{jj}$

(iii) $N_{ik} = N_{jk}$ ($k \neq i, j$)

where N_{xy} denotes the number of offspring that carry the phenotype A_xA_y. The qualitative tests prescribe the appearance of only such types that accord with the postulated genotype of the maternal tree. The relationships of equations (ii) and (iii) were quantitatively tested by the exact binomial goodness-of-fit test.

3. Results and Discussion

3.1 Isoenzyme phenotypes

The isoenzyme band patterns obtained from this material are described separately for each enzyme system:

PGI: This enzyme system shows 2 zones. However, only the slower-migrating zone (PGI-B) could be sufficiently resolved. For PGI-B 3 phenotypes were found, 2 single-band variants and a 3-band variant, which could be assumed to be encoded by the heterozygous genotype, since this system is generally dimeric.

GDH: This system reveals only 1 zone, which appears to be controlled by a single locus with 3 alleles. Three single-band variants and 3 multiband variants were detected. The system is generally tetrameric, so that five bands including three hybrid bands were expected in the putative heterozygous phenotypes.

6PGDH: This system was found to have 3 zones. The fastest and slowest zones do not show any variation. The intermediate zone (6PGDH-B) has 3 single-band and 3 triple-band isoenzyme variants. The latter phenotypes represent putative heterozygotes which produce 1 hybrid band because this system is usually dimeric.

MNR: This system consists of 4 activity zones. One zone appears with 3 isoenzyme variants of which 2 are single-bands and the third is a multi-band phenotype consisting of 5 bands. Since this enzyme system is generally tetrameric, the multi-band variant is assumed to be the phenotype of the heterozygote possessing 3 hybrid bands.

Table 1. — Genetic analysis of isoenzymes in *Cupressus sempervirens*. P. equals the level of significance under the exact binomial goodness-of-fit test, which represents the probability of equally or less likely samples. P_1 refers to tests of equations (ii), P_2 refers to tests of equations (iii). N is the size of the seed samples.

Type	tree	N	N_{11}	N_{22}	N_{33}	N_{12}	N_{13}	N_{23}	P_1	P_2
PGI-B_1B_2	U24	44	7	11		26			0.29	
B_1B_2	U3	38	10	10		18			0.87	
B_1B_2	D3	23	4	8		11			1.00	
B_1B_2	U29	25	5	6		14			0.69	
B_1B_2	Z3	39	1	17		21			0.75	
MDH-B_1B_2	Z6	28	0	14		14			1.00	
PGM-C_1C_2	N19	19	5	4		9			1.00	
C_1C_2	N16	53	11	24		29			0.53	
C_1C_2	N18	43	3	23		17			0.22	
MNR-B_1B_2	K12	13	5	3		5			0.58	
B_1B_2	N18	43	8	14		21			1.00	
NDH-A_1A_2	N16	47	3	7	0	10	12	15	1.00	0.70
A_1A_2	D3	15	2	4	0	7	1	1	1.00	1.00
A_1A_3	H4	13	2	0	2	3	2	4	1.00	1.00
A_2A_3	U24	19	0	7	2	1	2	7	0.80	1.00
A_2A_3	N6	68	0	21	7	11	9	20	0.31	0.82
6PGDH-B_1B_2	N18	14	4	2	0	5	2	1	1.00	1.00
B_1B_2	Z6	31	4	8	0	16	1	2	0.57	1.00
B_1B_2	D3	21	6	2	0	8	2	3	1.00	1.00
B_1B_3	N4	18	6	0	2	2	6	2	0.79	1.00
B_2B_3	U3	25	0	7	1	6	6	6	0.79	1.00
B_2B_3	N5	31	0	9	4	7	7	8	0.65	1.00
B_2B_3	U29	22	0	5	3	3	2	9	1.00	1.00
PGM-A_1A_2	N16	53	13	11	0	20	3	6	0.65	0.51
A_1A_2	K10	26	8	3	0	9	2	4	0.82	0.69
A_1A_2	N13	14	3	1	0	6	2	2	0.75	1.00
A_1A_3	N19	23	4	0	2	5	7	5	1.00	1.00
A_1A_3	N15	20	4	0	2	3	8	3	0.79	1.00
A_2A_3	N18	55	0	8	8	11	6	22	0.42	0.33
GDH-A_1A_2	N22	59	17	9	0	21	7	5	0.56	0.77
A_1A_3	N6	61	4	0	22	9	19	7	0.37	0.80
A_1A_3	N18	62	9	0	2	15	16	20	0.44	0.50
A_2A_3	H4	26	0	3	9	2	3	9	0.66	1.00
A_2A_3	Z6	23	0	4	6	0	2	11	1.00	0.50
A_2A_3	N3	58	0	7	21	4	6	20	0.31	0.75

NDH: One zone of the MNR zymograms with lowest migration rate proved to be another enzyme system, NDH. The reason for this conclusion is that this zone also appears when the gel is stained for NADH dehydrogenase. The NDH zone was found to reveal 6 variants of which 3 were single-band and 3 were multi-band phenotypes. Since the multi-band phenotypes consisted of 3 individual bands, it is assumed that this zone belongs to a dimeric enzyme.

PGM: This enzyme pattern consists of 3 zones. Variation could be found in 2 of these zones. The fastest migrating zone (PGM-A) shows in total 6 isoenzyme variants, 3 single-band and 3 double-band variants which were assumed to be heterozygous phenotypes, as this enzyme system is generally monomeric. The slowest migrating zone (PGM-C) appears to have 3 isoenzyme variants, 2 single-band and 1 double-band phenotypes, which again was regarded as the heterozygous expression of this monomeric enzyme.

MDH: This enzyme system shows 3 activity zones in zymograms, but only the intermediate zone (MDH-B) was found to exhibit variation. Two variants appear in this zone, a single-band variant and a triple-band variant, which was assumed to be the heterozygous phenotype of this dimeric enzyme system. A second single-band variant expressed by the other homozygous phenotype was not detected in our material.

3.2 Results of the genetic analysis and discussion

For each individual with a putative heterozygous seed perisperm isoenzyme pattern, a number of seed embryos (offspring genotypes) were analyzed. *Table 1* shows these individuals, grouped according to proposed heterozygous phenotype, together with the corresponding phenotypes of their progenies. The quantitative relations (ii) and, where possible, (iii) were tested using the exact binomial goodness-of-fit test, the exact levels of significance being given in *table 1*.

For all variable isoenzyme zones, the qualitative and quantitative conditions for a codominant mode of inheritance at single loci are fulfilled. Each offspring possesses at least one maternal allele, and none of the progeny phenotypic structures shows significant deviation from the ratios expected under equations (ii) and (iii). Equation (ii) was tested in all combinations of alleles recovered at a gene locus. Therefore, in all cases there is no apparent reason to reject the hypothesis of a single-locus, codominant mode of inheritance.

The conditions in cypress seed described in section 2.1 eliminate the necessity of sampling either buds or several endosperms for genotyping seed trees. The diploid genotype of the seed parent is expressed in the active seed perisperm, which can be assayed in 1 seed.

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Provenance Variation in Shoot Growth Components of Norway Spruce

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Abstract

The elongation of terminal shoots was measured during 2 growth seasons in a Norway spruce (*Picea abies* (L.) Karst.) provenance trial, comprising 36 provenances. Significant variation was found between provenance regions for traits that characterize the timing and duration of the growth period, but not for the rate of growth. Provenances from the Nordic countries terminated their growth 20 days earlier in the summer than the provenances from Eastern Poland which had the latest growth cessation. The duration of the growth period accounted for the larger part of the variation in shoot growth (80%) among provenances, while the rate of growth was an equally important factor within provenances. The day of growth cessation, the duration of the growth period and total annual shoot growth were closely correlated to altitude for provenances originating along an altitudinal gradient, but no such correlation was found between rate of growth and altitude. Within a provenance region in southern Poland the variation among provenances was exclusively in the timing and duration of the growth period. In another region in the Baltic countries, variation in the rate of growth was equally important as its duration for determining total shoot growth.

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Key words: Picea abies, provenances, shoot growth components, adaptation.