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Inheritance of catalase multiple forms in Scots pine (*Pinus silvestris* L.) endosperm*

INTRODUCTION

In the last decade the monogenic mode of inheritance of various enzyme systems occurring in forest trees has been well documented. Only in *Pinus silvestris* about 19 enzyme loci are already described (Tab. 1), although there are still some disagreements among the authors

Table 1

Enzymatic gene markers of *Pinus silvestris* L.

Enzyme	Number of loci	Tissue	Reference
APh	3	endosperm	Krzakowa et al., 1977
APh	1	endosperm	Mejnartowicz, 1978
Est	3	endosperm, needles	Rudin and Rasmuson, 1973
LAP	2	endosperm	Bialobok et al., 1976
RNase	4	endosperm	Mejnartowicz and Bergmann, 1977
ADh	4	endosperm	Krzakowa et al., 1977
GOT	3	endosperm	Krzakowa et al., 1977
GOT	2	endosperm, needles	Rudin, 1975

Abbreviations: APh – acid phosphatase, Est – esterase, LAP – leucine aminopeptidase, RNase – ribonuclease, ADh – alcohol dehydrogenase, GOT – glutamate oxalate transaminase.

concerning the number of loci determining a particular enzyme. Substantial differentiation in enzyme patterns has been found among populations of Scots pine, Norway spruce and other forest tree species (Feret, 1974; Lundkvist and Rudin, 1977; Mejnartowicz, 1976; Rudin et al., 1974). Isozyme analysis has been also used in studies of mating systems in plant populations as well as in investigations of their reaction to natural selection (Bergmann, 1978; Brown et al., 1975; Grant and Mitton, 1977; Mitton et al., 1977). The present trends in plant population genetic studies have been recently rewieved by Brown (1978).

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The present study is an attempt to contribute to this general topic by an investigation of a new enzyme system occurring in Scots pine endosperm tissue. Catalase is an iron-containing enzyme widely distributed in various aerobic organisms including plants. Its major physiological role is the destruction of hydrogen peroxide involved in plant metabolism (Deisseroth and Dounce, 1970). Data on the genetic basis of plant catalase multiplicity have been reported most convincingly by Beckman et al. (1964) and Scandalios (1964). They described three different types of maize catalase coded by a single locus. So far, few reports have been published concerning catalase multiplicity in forest trees. Conkle (1971) described the occurrence of catalase bands in germinating seeds of *Pinus attenuata* and Hamaker and Snyder (1973) postulated, that catalase occurring in *Pinus taeda* and *Pinus palustris* needles may be potentially useful in pine genetics studies.

MATERIALS AND METHODS

Seed material was collected from 155 single trees of *Pinus silvestris* in two consecutive years 1976 and 1977 and stored at -3°. The investigated trees were from the following provenances: Goleniów (Go), Babki (Ba), Lubin (Lu), Świerklaniec (Św), Duszniki (Du), Włoszczowa (Wł), Strzałowo (St), and Maskulińskie (Ma). From 10 to 60 seeds were analysed for each individual tree.

Catalase polymorphism was investigated in the female gametophyte (endosperm) tissue isolated from dormant seeds. Homogenization of each individual endosperm was performed in 0.1 ml of phosphate buffer pH 7.5. The obtained homogenate was then separated using horizontal starch gel electrophoresis in a discontinuous buffer system (Poulik, 1957). The catalase bands were visualized by the method of Brewbaker et al. (1968), however a somewhat lower than original concentration of hydrogen peroxide was used. The starch gels were dipped in 0.75% H_2O_2 containing 0.06 M sodium thiosulphate for 30 s, washed with distilled water and then stained with 0.09 M potassium iodide acidified with glacial acetic acid for 1 - 3 min. In order to check whether the observed distribution of catalase phenotypes in the seed sample of an assumed heterozygous tree are in agreement with the expected 1 : 1 ratio for the one-locus/two-alleles system, the Chi-square goodness-of-fit test was employed.

RESULTS

A total of 13 catalase bands of different electrophoretic mobility have been identified after separation of more than 2000 homogenates of individual endosperms. However, since the most cathodal band was too blurry

and not repeatable enough it was excluded from further analysis. The remaining 12 bands could be grouped into 7 distinct combinations — — catalase variants designated as C-1, C-2, C-3, C-4, C-5, C-6, and C-7 respectively, consisting of from one to five single bands Fig. 1. Only one or two of these variants occurred per mother tree.

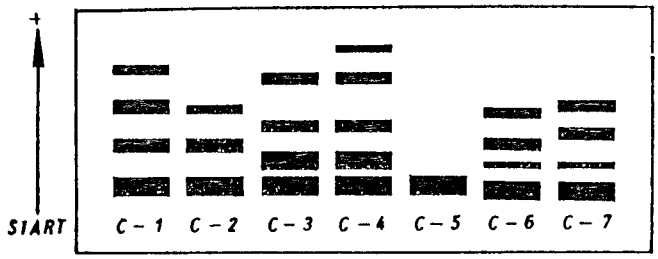

Fig. 1. Scheme of electrophoretic variants of catalase from *Pinus silvestris* endosperm

Out of the 155 single trees analysed in the present study, 83 were homomorphic i.e. possesed only one catalase variant, in most cases C-1 or C-5. The remaining 72 trees were heteromorphic with only two mutually exclusive catalase variants occurring alternately in the seed sample from any of these trees (e.g. C-1 : C-2, C-5 : C-7). Therefore it could be assumed, that each of these variants represents the phenotypic expression of an allele at one gene locus. The statistical evaluation of the agreement between the observed distribution of catalase variants in seed samples from several heteromorphic trees with those expected for a one-locus/ /two-alleles system has been presented in Table 2. A fairly good agree-

Table 2

Frequency distribution of different catalase variants in seed samples of individual *Pinus silvestris* trees

Tree No.	Segregation pattern of catalase phenotypes	Total seed amount	χ^2 1DF	P
Wl - 187	C-1 : C-2			
	32 : 28	60	0,267	50%
St - 096	C-1 : C-3			
	29 : 25	54	0,296	50%
Ba - 260	C-1 : C-4			
	31 : 27	58	0,276	50%
St - 081	C-1 : C-5			
	28 : 29	57	0,018	80%
St - 099	C-1 : C-6			
	22 : 23	45	0,022	80%
Wl - 177	C-1 : C-7			
	26 : 25	51	0,020	80%
St - 090	C-2 : C-5			
	17 - 18	35	0,029	80%
St - 095	C-2 : C-7			
	23 : 19	42	0,381	50%
Go - 005	C-5 : C-7			
	25 : 25	50	0,000	99%

ment with a 1 : 1 segregation ratio supported the above assumption, that electrophoretically detectable catalase variants occurring in Scots pine endosperm tissue represent the phenotypic expression of seven different alleles at one gene locus.

DISCUSSION

Similarily as other enzyme systems occuring in Scots pine endosperm, catalase exhibits a marked electrophoretically detectable polymorphism. Even in so restricted material, seven different catalase variants have been found. It seems therefore possible, that the real number of catalase multiple forms occurring in Scots pine endosperm may be greater. All investigated trees could be characterized by one or two clearly distinguishable catalase variants, independently of the place of collection or seed storage duration. The persistency of catalase electrophoretic patterns was also found by Conkle (1971) in *Pinus attenuata* dry and germinating embryos. He reported, that first changes of catalase electrophoretic patterns did not appear before hypocotyl emergence beyond the seed coat. Since there are only two catalase variants per heteromorphic tree and the observed frequencies of these variants do not deviate from a 1 : 1 ratio it can be concluded, that this enzyme is controlled by only one gene locus. The variants can serve as genetic markers in pine population genetics research. On the other hand, since most of the catalase variants detected in this study consisted of several bands, it is possible, that some of these bands arose from certain modifications of the native enzyme molecule (Holmes and Duley, 1975). However, since nothing is known on the chemical and physical properties of Scots pine catalase, it was not possible to determine whether or not all these bands represent true catalase enzymes.

SUMMARY

Starch gel electrophoresis was used to assay for catalase polymorphism in the haploid endosperm of dormant seeds of Scots pine (*Pinus silvestris* L.). Seven different catalase variants consisting of from one to five bands were detected. Only one or two mutually exclusive catalase variants could be found in seeds from each of 155 investigated trees. Statistical analysis of the catalase variants segregation patterns in the seed samples from individual heteromorphic trees revealed no deviations from the expected 1 : 1 segregation ration for a one-locus/two-alleles system. It could be postulated therefore, that catalase electrophoretic variants occurring in Scots pine endosperm are coded by seven alleles at one locus.

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Podstawy genetyczne polimorfizmu katalazy w endospermie sosny zwyczajnej (Pinus silvestris L.)

Streszczenie

Stosując elektroforezę na żelu skrobiowym, badano polimorfizm genetyczny katalazy w haploidalnej tkance makrogametofitu żeńskiego (endospermie) izolowanej z nasion sosny zwyczajnej. Stwierdzono występowanie 7 fenotypów enzymu, które oznaczono symbolami: C-1, C-2, C-3, C-4, C-5, C-6 oraz C-7. Analizując częstość występowania fenotypów katalazy w próbach nasion ze 155 pojedynczych drzew wykazano, że każde z badanych drzew posiadało tylko jeden lub dwa fenotypy katalazy. Statystyczna analiza częstości występowania tych fenotypów w próbkach nasion z pojedynczych drzew heteromorficznych nie wykazała istotnych odchyleń od stosunku 1 : 1, co pozwala sądzić, że stanowią one wynik działania jednego locus genu z siedmioma allelami.

АЛФРЕД Э. ШМИДТ

Генетический полиморфизм каталазы в семенах сосны обыкновенной (Pinus silvestris L.)

Резюме

Методом горизонтального электрофореза в крахмальном желе исследовано генетический полиморфизм каталазы в макрогаметофитах сосны обыкновенной. Обнаружено 7 фенотипов этого фермента: C-1, C-2, C-3, C-4, C-5, C-6, C-7. Частота появления фенотипов каталазы у гетероморфических деревьев не откланяется достоверно от простой реляции 1 : 1. Это дает возможность для вывода что для них генетический код базирован на одном локусе с 7 аллелями.