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Genetic Analysis of Some Polymorphic Isozymes in *Pinus densiflora* (II)[1]

— Inheritance of acid phosphatase, alcohol dehydrogenase and catalase isozymes —

Z. S. Kim[2] · Y. P. Hong[2]

소나무의 몇가지 多形的 同位酵素의 遺傳分析(II)[1]

— Acid phosphatase, alcohol dehydrogenase와 catalase 同位酵素의 遺傳樣式 —

金 眞 水[2] · 洪 鎔 杓[2]

ABSTRACT

Megagametophyte tissues of *Pinus densiflora* were subjected to study the inheritance of acid phosphatase (ACP), alcohol dehydrogenase (ADH) and catalase (CAT) isozymes by starch gel zone-electrophoresis. At least three or four zones were segregated for ACP isozyme. However, as one isozyme of ACP-A zone was separated clearly, only that isozyme was analysed. Five isozyme phenotypes (A1 - A5), observed in ACP-A zone, were segregated to a simple Mendelian ratio, suggesting that these are controlled by five codominant alleles existed at ACP-A locus. Two zones of activity were segregated in the gels after staining for ADH, the more anodal zone (ADH-A) of the two was invariant in our materials. Three isozyme phenotypes (Bl-B3) were observed in ADH-B zone and these variants showed a 1:1 segregation pattern, suggesting that each variant is controlled by three codominant alleles at ADH-B locus. A total of five isozyme phenotypes, composed of multiple bands, were observed in CAT isozyme. The segregation of these phenotypes in heterozygous trees did not show any significant deviation from a 1:1 segregation. Therefore, the genetic control of CAT isozyme in *Pinus densiflora* seeds seems to be based on a single locus (CAT-A) with five codominant alleles (A_1-A_5).

Key words : Allozyme loci ; megagametophyte ; Pinus densiflora.

要　　約

소나무의 acid phosphatase(ACP), alcohol dehydrogenase(ADH)와 catalase(CAT) 同位酵素의 遺博樣式을 究明하기 위하여 胚乳組織을 수평식 감자전분 전기영동법에 의하여 分析하였다. ACT同位酵素는 최소한 3～4개의 地域으로 分離되었으나 분리가 잘된 ACP-A 地域의 同位酵素만이 分析되었다. ACP-A 地域에서 관찰된 5개(A1-A5)의 同位酵素 表現型들은 공히 Mendel의 分離比를 보여 이들이 각각 ACP-A 遺傳子座에 存在하는 5개의 對立遺傳子에 의해 지배받고 있음을 알 수 있었다. 2개의 ADH 地域이(AD

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[2] 高麗大學校 農科大學 College of Agriculture, Korea University, Seoul, Korea.

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H-A와 ADH-B) 分離되었으나, 陽極으로의 移動속도가 빠른 ADH-A 地域에서는 分析에 사용된 材料에서 變異가 發見되지 않았다. ADH-B 地域에서는 3개의 同位酵素 表現型(B1-B3)들이 관찰되었고 이들이 공히 1:1의 분리비를 보여 ADH-B 遺傳子座에 존재하는 3개의 對立遺傳子에 의해 지배됨이 추정되었다. 수개의 band로 구성된 5개의 同位酵素 表現型이 CAT에서 관찰되었으며, 異型接合性인 母樹에서 이들 表現型間의 分離가 1:1 分離比로부터 偏差를 보이지 않았으므로, 소나무에 있어서 CAT 同位酵素는 5개의 對立遺傳子가 存在하는 하나의 遺傳子座에 의해 지배되는 것으로 추정하였다.

INTRODUCTION

There has been an immense accumulation of data on electrophoretically detectable isozyme polymorphisms in haploid and diploid tissues of various coniferous tree species since the early 1970's.[3,6,8] Determining the inheritance mode of the various isozymes is a prerequiste to their use in population studies of forest trees and in other fields in tree breeding programs.[9,11,18] Megagametophyte of conifer seeds provides an advantageous research material for assessing isozyme variation. Each megagametophyte in seeds represents a single meiotic product, haploid nature regardless to embryo, a diploid tissue. A large number of seeds from some heterozygous mother-trees were analysed to determine whether isozyme variants segregated according to simple Mendelian ratio. An one-to-one segregation of isozyme variants is evidence for allelism.

Pinus densiflora is one of the important native conifers in Korea which is also distributed in Japan and Manchuria. A large amount of geographic morphological variation has been recognized.[22] Through a series of researches, systematic classification among natural populations was performed based on morphological and anatomical characteristics.[21] The estimation of genetic variation in *Pinus densiflora*, based on isozyme analysis, was performed depending on the band number of peroxidase isozymes.[22]

For the purpose of population study in the future, the present paper contains a description of inheritance mode of some isozyme systems, acid phosphatase (ACP: E.C.3.1.3.2.), alcohol dehydrogenase (ADH: E.C.1.1.1.1.) and catalase (CAT: E.C.1.11.1.6.), which was ascertained from megagametophyte analysis.

MATERIALS AND METHODS

Open-pollinated seeds collected from 150 trees in five natural populations of *Pinus densiflora* were dried and stored in the cool temperature (4°C). Starch gel zone-electrophoresis was performed using two modified discontinuous buffer systems.[2,16] The extraction and electrophoresis procedures were already described in detail.[12,13]

After electrophoresis, the sliced gels were preincubated in each staining buffer solution for ACP and ADH, and in water for CAT for 15 minutes. The preincubated gel for CAT was treated in 0.03% H_2O_2 for 2 minutes in the dark. The isozyme phenotypes were made visible with following staining solutions; ACP (70mg Fast Garnet GBC salt, 80mg α-Naphtyl acid phosphate, 6ml 10% $MgCl_2$ solution, 100ml Acetate buffer pH 4.5), ADH (20mg NAD, 20mg MTT, 10mg PMS, 3ml 95% ethyl alcohol, 100ml Tris-HCl buffer pH 8.0), CAT (50ml 2% $FeCl_3$ solution, 50ml 2% $K_3Fe(CN)_6$ solution).

A minimum of six megagametophytes per tree were analysed, offering a 97% probability of detecting a heterozygote. The distribution of isozyme phenotypes in the seed sample of individual trees was examined with regard to a 1:1 segregation ratio assuming random distribution of gametes after meiosis in each case. The statistical evaluation on the agreement between observed and expected frequencies was obtained from X^2-test.

RESULTS AND DISCUSSION

Acid phosphatase (ACP)

There were at least three or four zones of acti-

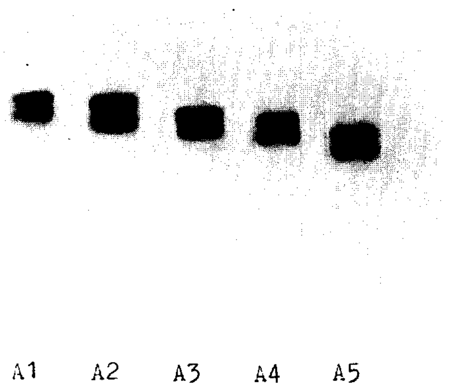

Fig. 1. Electrophoretic phenotypes of acid phosphatase found in megagametophytes of some mother-trees. All observed phenotypes are presented on one gel.

Table 1. Segregation analysis of acid phosphatase phenotypes found in megagametophytes of heterozygous trees.

Individual tree	ACP − A					Total seed amount	$X^2(1)$	P
	A1	A2	A3	A4	A5			
KB-UIII 2	25		26			51	0.02	0.90
KA-CII 1		30	42			72	2.00	0.10-0.25
KA-K 7		23	25			48	0.08	0.75-0.90
KB-UII 4		22	32			54	1.85	0.10-0.25
SE-A 18		31	35			66	0.24	0.50-0.75
KA-K 12		36		32		68	0.24	0.50-0.75
SE-S 15		48		56		104	0.62	0.25-0.50
KA-JY 1			25	25		50	0	>0.99
KA-K 22			45	53		98	0.65	0.25-0.50
KB-UI 5			32	40		72	0.89	0.25-0.50
SE-A 31				42	34	76	0.84	0.25-0.50

vity on gels stained for ACP, but only the most anodal zone (ACP-A) was separated clearly. On the basis of migration rate, five isozyme phenotypes (A1-A5), composed of equally stained double band, were observed (Fig. 1). Observed isozyme phenotypes in each heterozygous mother-tree were segregated to a simple Mendelian ratio (Tab. 1). Therefore, we can conclude that five isozyme phenotypes, observed in ACP-A zone, are controlled by a single locus with five codominant alleles.

Some investigators reported that two to four alleles were existed at ACP-A locus in some conifers.[1,4,5,7,15,17] Similar isozyme phenotypes, composed of double bands, were reported also in *Picea abies,*[4] *Pinus sylvestris*[17] and *Pinus taeda.*[1]

Alcohol dehydrogenase (ADH)

Two zones of activity were segregated in the gels after staining for ADH, designated as ADH-A and ADH-B based on migration rate(Fig. 2). The more anodal zone (ADH-A) of the two was invariant in analysed materials and showed superior staining reaction velocity and activity to ADH-B. Three isozyme phenotypes (B1-B3) were observed in ADH-B zone and they showed a 1:1 segregation

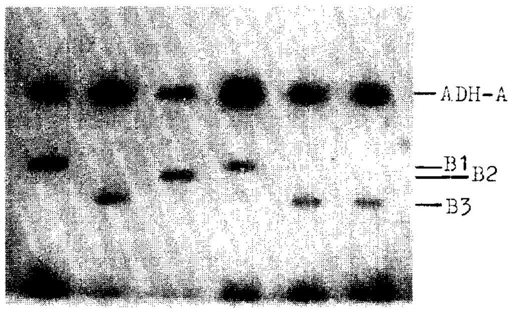

Fig. 2. Electrophoretic phenotypes of alcohol dehydrogenase found in megagametophytes of some mother-trees. All observed phenotypes are presented on one gel.

Table 2. Segregation analysis of alcohol dehydrogenase phenotypes found in megagametophytes of heterozygous trees.

Individual tree	ACP−A			Total seed amount	$X^2(1)$	P
	B1	B2	B3			
KA-K 110	33	39		72	0.50	0.25-0.50
SE-A 13	42	32		74	1.35	0.10-0.25
KA-K 7	21		27	48	0.75	0.25-0.50
KA-K 12	32		36	68	0.24	0.50-0.75
KA-K 22	42		56	98	2.00	0.10-0.25
KB-UII 4	26		28	54	0.07	0.75-0.90
SE-A 15	55		49	104	0.35	0.50-0.75
SE-A 18	38		28	66	1.52	0.10-0.25
SE-A 31	38		38	76	0	>0.99
CN-CK 1	65		86	151	2.92	0.05-0.10
CN-CK 5	74		76	150	0.03	0.75-0.90
KA-CII 1		35	37	72	0.06	0.75-0.90
KA-CII 5		36	41	77	0.32	0.50-0.75
KB-UIII 2		27	24	51	0.18	0.50-0.75

pattern, suggesting that ADH-B isozymes are controlled by a single locus with three codominant alleles (Tab. 2).

This result is in accordance with the data of Conkle,[6] and Rudin and Ekberg[17] who also reported that two zones of activity in *Pinus attenuata* and *Pinus sylvestris.* Two zones of activity were also reported by Conkle[7] for *Pinus contorta, Pinus taeda, Pinus jeffreyi, Pinus lambertiana* and *Pseudotsuga menziesii.* Two to six alleles at the second locus were also detected in these species.

Catalase (CAT)

A total of five variants, composed of multiple bands, were observed on gels stained for CAT which were designated as A1-A5 (Fig. 3). All investigated trees were characterized by one or two clearly distinguishable CAT variants and only two mutually exclusive CAT variants were observed alternately in the heterozygous parent trees. Consequently, we could presume that each variant, composed of multiple bands, represents the phenotypic expression of an allele at one gene locus. The 1:1 segregation ratio of these phenotypes in heterozygous trees supported this hypothesis. Therefore, the genetic control of CAT isozyme in *Pinus densiflora* megagametophytes seems to be based on a single locus with five codominant alleles (A_1-A_5) (Tab. 3).

The above-mentioned exclusive CAT isozymes were observed also in *Pinus rigida.*[14] Szimidt[19] observed seven variants, composed of multiple bands, in *Pinus sylvestris.* These variants showed a 1:1 segregation ratio. In conclusion, he reported that CAT isozyme was controlled by only one gene locus with seven alleles.

A2 A3 A1 A4 A1 A4 A1 A5

Fig. 3. Electrophoretic phenotypes of catalase found in megagametophytes of some mother-trees. All observed phenotypes are presented on one gel.

Table 3. Segregation analysis of catalase phenotypes found in megagametophytes of heterozygous trees.

Individual tree	CAT-A A1	A2	A3	A4	A5	Total seed amount	$X^2(1)$	P
KB-CII 1	37	35				72	0.06	0.75-0.90
SE-A 13	43	31				74	1.96	0.10-0.25
KA-K 22	47		51			98	0.16	0.59-0.75
KB-UI 5	38			34		72	0.22	0.50-0.75
KA-CIII 5	30				36	66	0.55	0.25-0.50
SE-A 5	43				39	82	0.20	0.50-0.75
SE-A 18	29				37	66	0.97	0.25-0.50
KA-CI 2				29	25	54	0.30	0.50-0.75

Isozyme phenotypes composed of multiple bands are observed often from haploid megagametophyte tissues and these phenotypes seemed to be due to certain modification of the native enzyme molecule.[3,10]

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