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

— Inheritance of Glutamate-Oxalate Transaminase and Leucine Aminopeptidase, and Linkage Relationship among Allozyme Loci —

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소나무의 몇가지 多形的 同位酵素의 遺傳分析(I)[1]

— Glutamate-Oxalate Transaminase와 Leucine Aminopeptidase의 遺傳과 同位酵素 遺傳子座 間의 連關關係 —

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ABSTRACT

Megagametophyte and embryo tissue of *Pinus densiflora* were subjected to study the inheritance of glutamate-oxalate transaminase (GOT) and leucine aminopeptidase (LAP), and linkage relationship among isozyme loci coding both enzymes by starch gel zone-electrophoresis. Four zones of activity were observed for GOT. No variation was found in the fastest migrating zone (GOT-A). Electrophoretic phenotypes of the other two zones (GOT-B and GOT-C) showed 1:1 segregation ratio, suggesting that each zone is controlled by a single locus. Four and three alleles were identified at both loci, respectively. The isozyme pattern of the fourth zone (GOT-D), migrated cathodally, coincided precisely with that of GOT-C. Whether the two zones are controlled by the same locus or by two tightly linked loci remained unknown. In all three variant GOT zones, heterozygous embryos produced triple band patterns, indicating that GOT isozyme in *Pinus densiflora* is a dimer. Two zones of activity stained for LAP were found. The segregation of the two zones (LAP-A and LAP-B) suggested that two loci control each of both isozymes. Two and three alleles were identified at both loci. GOT-B and LAP-B were found to be tightly linked, showing an average recombination frequency of 12.5 percent. Slight deviation from independent assortment was observed between GOT-B and GOT-C, with recombination frequency of 41 percent.

Key words: isozyme loci; linkage; Pinus densiflora; megagametophyte.

要 約

소나무의 glutamate-oxalate transaminase(GOT)와 leucine aminopeptidase(LAP)의 遺傳樣式과 同位酵素 遺傳子座 간의 連關關係의 구명을 목적으로 胚乳 및 胚組織을 감자전분 전기영동법에 의하여 分析하였다. 4개의 GOT 地域(GOT-A~GOT-D)이 分離되었으며 가장 移動이 빠른 GOT-A 地域에서는 두 개의밴드를 보이는 表現型으로 變異가 發見되지 않았다. GOT-B와 GOT-C 두 地域의 同位酵素 表現型들은 공

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히 1 : 1의 分離比를 보여 각각 하나의 遺傳子座에 의해 支配됨이 推定되었으며 兩遺傳子座에서 각각 4개와 3개의 對立 遺傳子가 확인되었다. 陰極으로 移動한 GOT-D 地域의 表現型은 GOT-C 地域과 동일하게 分離되었다. 이 두 地域이 한 개의 동일한 遺傳子座의 支配를 받는지 또는 강하게 連關되어 있는 두 개의 遺傳子座에 의한 것인지 단정할 수 없었다. 變異를 보인 세 개의 GOT 地域에서 異型接合性의 胚組織은 세 개의 밴드를 보이는 表現型을 보여 소나무의 GOT 同位酵素가 二量體임을 제시하였다. LAP 同位酵素는 2개의 地域으로 分離되었다. 각 地域의 同位酵素 表現型은 각각 1 : 1의 分離比를 보여 LAP 同位酵素가 2개의 遺傳子座에 의해 支配됨이 확인되었고 두 遺傳子座에서 2개와 3개의 對立遺傳子가 각각 확인되었다. GOT-B와 LAP-B는 강하게 連關되어 있음이 밝혀졌고, 이들 간의 平均 再組合 比率은 12.5 %였다. 또 다른 한쌍의 遺傳子座, GOT-B와 GOT-C 간에도 再組合 比率이 41%로 나타나 連關의 可能性이 제시되었다.

INTRODUCTION

Forest trees have been main subjects of many quantitative genetic investigations[18]. In most cases, traditional methods for assessing genetic variation have provided informations on metrical traits controlled by a large but unknown number of genes. Since early 1970, electrophoretic techniques have been used in population studies of forest trees, as well as in other fields in tree breeding programs (for review see Feret and Bergmann[6]; Kim[9]; Rudin[15])

These techniques offer a number of advantages over other biochemical or quantitative approaches[8]. First, inheritance pattern of electrophoretically detectable traits can be easily demonstrated; second, most alleles at isozyme loci are codominant and gene frequencies can be calculated without genetic crosses; third, estimates of genetic variation can be compared directly between populations, or between species. Determining the inheritance mode of isozymes, started with forest trees in 1971, is a prerequisite to further applications of these techniques.

Female gametophyte of conifer seeds provides an excellent 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 parent trees are analysed to determine whether observed phenotypes segregate according to single Mendelian ratios. A one-to-one segregation is a good evidence for allelism. Deviations from independent assortment can be detected by analysing seeds from trees heterozygous at two loci.

Using megagametophyte and embryo tissue of *Pinus densiflora*, we provide inheritance pattern of glutamate-oxalate transaminase (GOT:E.C.2.6.1.1) and leucine aminopeptidase (LAP:E.C.3.4.11.1), and the linkage relationship between isozyme loci coding both enzymes. Inheritance of the two isozymes in forest trees has been reported by many authors (for review see Chung[3], Ryu[17]).

MATERIALS AND METHODS

Open-pollinated seeds from 150 trees in five natural populations of *Pinus densiflora* were collected and stored in the cool temperature (4°C) after well drying. Starch gel zone-electrophoresis was performed using a modified discontinuous buffer system described by Poulik[13]. The extraction and staining procedures as well as other experimental methods were already described in detail[10]

A minimum of six gametophytes per tree were analysed. The probability of mis-identifying a heterozygous tree as a homozygote is $(1/2)^5$, or 0.03. Assuming random distribution of gametes after meiosis, a minimum of fifty megagametophytes per tree were analysed to test a one-to-one segregation at polymorphic loci. The statistical evaluation on the agreement between observed and expected frequencies was obtained from the X^2-test for 1 D.F. Linkage analysis was possible with

mother-trees heterozygous at two or more loci, in which the equality of complementary gametes and their independent assortment were examined with the X^2-test for 2 D.F. and 1 D.F., respectively. As the two isozyme systems were stained with slices from the same starch gel, comparisons could be made in pairwise fashion for the same set of megagametophytes.

RESULTS

Glutamate-oxalate transaminase (GOT) allozymes

Four zones of activity were present on gels stained for GOT, three of them (GOT-A, GOT-B and GOT-C) migrated anodally and one (GOT-D) cathodally from the origin (Fig. 1). The most anodal zone (GOT-A), consisting of two unequally staining bends, was invariant in our materials. Both GOT-B and GOT-C zones showed variability: On the basis of migration rate, four different isozyme phenotypes (B1~B4) of GOT-B and three isozyme phenotypes (C1~C3) of GOT-C were observed (Fig. 1). The isozyme variants of both GOT zones showed a 1:1 segregation pattern, suggesting that each zone is controlled by a single locus (Table 1). The segregation pattern of the fourth zone (GOT-D) was coincided precisely with that of GOT-C (Fig. 1).

Embryos of heterozygous mother-trees produced either a single (same as the megagametophytes)

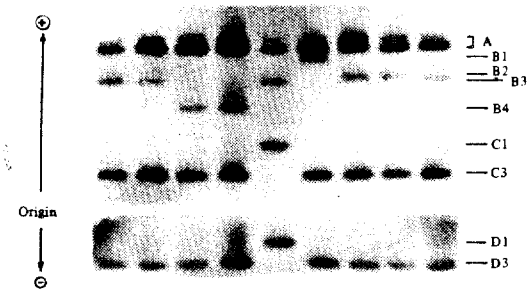

Fig. 1. Electrophoretic phenotypes of glutamate-oxalate transaminase found in megagametophytes of some mother-trees. All observed phenotypes are presented on one gel except for C2.

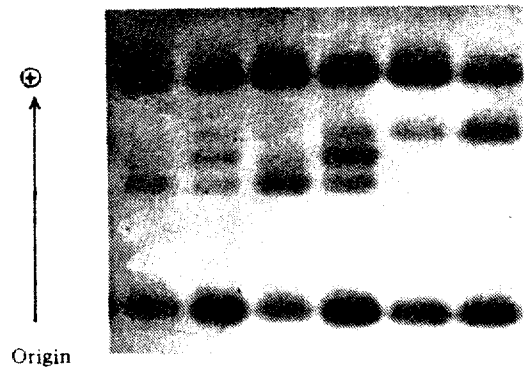

Fig. 2. Allozyme patterns of the GOT-B locus from three megagametophytes and the corresponding embryos of one heterozygous mother-tree (from the left to the right).

Table 1. Segregation analysis of glutamate-oxalate transaminase phenotypes found in megagametophytes of heterozygous trees.

Individual number	GOT-B				GOT-C			Total seed amount	$X^2(1)$	P
	B1	B2	B3	B4	C1	C2	C3			
KA-K 110	59		54					113	0.22	0.50-0.75
CN-S 1		52	51					103	0.01	>0.95
KA-CII 3		32	34					66	0.06	0.75-0.90
KB-UI 1			33	27				60	0.60	0.25-0.60
KB-UI 2	43		43					86	0	>0.99
KY-Y 11	34		38					72	0.22	0.50-0.75
KY-Y 25	48		50					98	0.04	0.75-0.90
KA-K 110					52		61	113	0.72	0.25-0.50
KA-CI 2					54		51	105	0.09	0.75-0.90
SE-A 10						28	23	51	0.49	0.25-0.50

or a triple band pattern in all three variant zones (Fig. 2 and Fig. 3). The presence of an intermediate band between the fast and slow bands indicated that the GOT enzyme in *Pinus densiflora* is a dimer. The co-variation of GOT-C and GOT-D was also maintained in embryos (Fig. 3).

From these results we can conclude that the GOT isozyme patterns are controlled by at least two polymorphic loci, one with four codominant alleles (B_1~B_4), and the other with three codominant alleles (C_1~C_3).

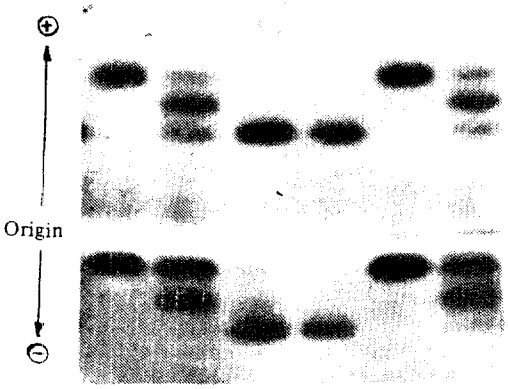

Fig. 3. Allozyme patterns of the two GOT loci, GOT-C and GOT-D, from three megagametophytes and the corresponding embryos of one heterozygous mother tree (from the left to the right).

Leucine aminopeptidase (LAP) allozymes

Clearly separated two zones could be identified in the gels after staining for LAP, designated as LAP-A and LAP-B (Fig. 4). Each zone showed remarkable variability with different migration rates in many trees. Two and three single banded electrophoretic phenotypes were observed in LAP-A and LAP-B, respectively (Fig. 4). The segregation of these phenotypes in heterozygous trees did not show any significant deviation from 1:1 segregation in both zones (Table 2). Therefore, the genetic control of LAP isozymes in *Pinus densiflora* seeds seems to be based on two gene loci (LAP-A and LAP-B) with two (A_1, A_2) and three (B_1, B_2 and B_3) codominant alleles, respectively. The two loci showed the pattern of a monomer; heterozygotes with two bands.

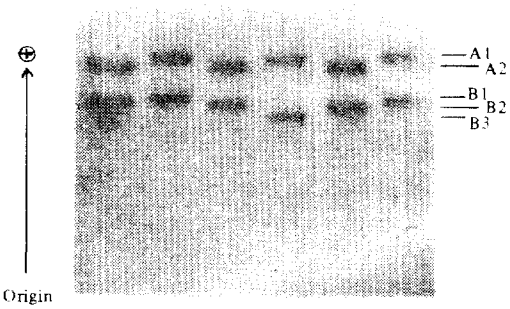

Fig. 4. Electrophoretic phenotypes of leucine aminopeptidase found in megagametophytes of some mother trees. All observed phenotypes are presented on one gel.

Linkage analysis

To test for linkage between the four polymor-

Table 2. Segreagation analysis of leucine aminopeptidase phenotypes found in megagametophytes of heterozygous trees.

Individual number	LAP-A		LAP-B			Total seed amount	$X^2_{(1)}$	P
	A1	A2	B1	B2	B3			
KA-CI 2	55	50				105	0.24	0.50-0.75
KA-CII 3	35	31				66	0.24	0.50-0.75
KB-UI 2	49	37				86	1.67	0.10-0.25
KY-Y 11	33	39				72	0.50	0.25-0.50
KY-Y 25	48	50				98	0.04	0.75-0.90
CN-S 1				49	54	103	0.24	0.50-0.75
KB-UI 2				42	44	86	0.05	0.75-0.90
SE-A 10				33	30	63	0.14	0.50-0.75
KY-Y 11				37	35	72	0.06	0.75-0.90
KY-Y 25			49	49		98	0	0.99

Table 3. Tests for linkage between four polymorphic loci.

Enzyme loci	Individual number	No. of gamete class				Total seed amount	Equality of complementary gametes		Test of Independent assortment		Recombination frequency
							$X^2(2)$	P	$X^2(1)$	P	
		B_1/C_1	B_1/C_3	B_3/C_1	B_3/C_3						
GOT-B:GOT-C	KA-K 110	22	37	30	24	113	0.82	0.50-0.75	3.90	<0.05	0.41
		B_2/A_1	B_2/A_2	B_3/A_1	B_3/A_2						
GOT-B:LAP-A	KA-CII 3	15	17	20	14	66	0.28	0.75-0.90	0.97	0.25-0.50	–
		B_1/A_1	B_1/A_2	B_3/A_1	B_3/A_2						
	KB-UI 2	24	19	25	18	86	1.68	0.25-0.50	0.05	0.75-0.90	–
	KY-Y 25	23	25	25	25	98	0.08	>0.95	0.04	0.75-0.90	–
	KY-Y 11	17	17	16	22	72	0.67	0.50-0.75	0.50	0.25-0.50	–
		B_1/B_2	B_1/B_3	B_3/B_2	B_3/B_3						
GOT-B:LAP-B	KB-UI 2	5	38	37	6	86	0.10	0.90-0.95	47.63	<0.01	0.13
	KY-Y 11	31	3	7	31	72	1.60	0.25-0.50	37.56	<0.01	0.14
		B_1/B_1	B_1/B_2	B_3/B_1	B_3/B_2						
	KY-Y 25	44	4	5	45	98	0.12	0.90-0.95	65.31	<0.01	0.09
		B_2/B_2	B_2/B_3	B_3/B_2	B_3/B_3						
	CN-S 1	6	46	43	8	103	0.39	0.75-0.90	54.61	<0.01	0.14
		C_1/A_1	C_1/A_2	C_3/A_1	C_3/A_2						
GOT-C:LAP-A	KA-CI 2	30	24	25	26	105	0.31	0.75-0.90	0.47	0.25-0.50	–
		C_2/B_2	C_2/B_3	C_3/B_2	C_3/B_3						
GOT-C:LAP-B	SE-A 10	16	12	14	9	51	2.13	0.25-0.50	0.02	0.75-0.90	–
		A_1/B_2	A_1/B_3	A_2/B_2	A_2/B_3						
LAP-A:LAP-B	KB-UI 2	24	25	18	19	86	1.72	0.25-0.50	0	>0.99	–
	KY-Y 11	20	13	17	22	72	0.63	0.50-0.75	2.00	0.10-0.25	–
		A_1/B_1	A_1/B_2	A_2/B_1	A_2/B_2						
	KY-Y 25	20	22	21	23	86	0.23	0.75-0.90	0	>0.99	–

phic loci, deviations from independent assortment were tested from eight mother-trees heterozygous at two or more loci. One pair of loci, GOT-B and LAP-B, was found to be tightly linked (Table 3). The recombination frequencies estimated from four mother-trees were varied from 9 to 14 percent.

Slight deviation from independent assortment was observed between GOT-B and GOT-C (Table 3). The recombination frequency was estimated to be 41%. The other four pairs of loci, GOT-B: LAP-A, GOT-C: LAP-A, GOT-C: LAP-B and LAP-A: LAP-B, showed independent assortment, indicating each pair of loci is not linked.

DISCUSSION

Three zones of GOT activity have been observed in many conifer species.[7,10,12,16] Two zones of GOT activity were also reported in *Pseudotsuga menziesii*[20] and *Pinus virginiana*[19]. Four zones of GOT activity of *Pinus sylvestris* were reported[3]. The co-variation of two GOT zones was reported in *Pinus rigida*[7] and *Picea abies*[12]. On the basis of the three independently segregating GOT-loci in *Pinus sylvestris*[16], Lundkvist[12] gave a plausible hypothesis that the two GOT loci, GOT-B and GOT-C were still linked in Norway spruce. But he found no evidence of recombination between the two loci. On the other hand, Chung[3] reported four GOT loci in *Pinus sylvestris*, of which three (A,B, and C) were independently segregated and the allele D_1 of the locus GOT-D was linked to the allele C_1 of the locus GOT-C. Three indenpendently segregating GOT loci reported by Rudin and Ekberg[16] are supposed to correspond to A, B, and C identified by Chung[3]. Consequently, no appropriate interpretation is available at present, whether both zones are controlled by the same locus or they represent two tightly linked loci. GOT was reported as a dimeric[7,12,14] or a monomeric[20] system in conifers. Ryu[17] observed adimeric pattern in one zone of GOT but a monomeric one in the other two zones in needles of *Pinus strobus*.

Two loci for LAP have been reported for many conifers.[1,2,7,11] Three loci have been also reported for *Pinus attenuata*[4], *Pinus strobus*[17], and Pseudotsuga menziesii[20]. LAP is known to be a monomeric system in conifers studied to date. Frequently reported null alleles without enzyme activity was not observed in trees under this study.

Similar results to the linkage between two loci (GOT-B: LAP-B) detected in this study were reported in some *Pinus* species[5,16]. Conkle[5] reported that GOT-2 and LAP-2 were linked in four different pine species with varying map distances from 5.6 to 26.5 in centiMorgan(cM) units. Rudin and Ekberg[16] also reported similar linkage between the two loci, GOT-B and LAP-B,in Scots pine with an average map distance of 15.7 cM. GOT-B and GOT-C showed a weak evidence of linkage, but it was confirmed from one tree and the sample size was relatively small. It would be premature to consider them as linked loci without additional estimates from other trees. The use of allozymes detected in haploid gametophyte tissue can rapidly advance in mapping the conifer genome and in understanding the importance of multilocus combination of alleles in evolutionary processes.

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