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Inheritance of Isoenzymes in Root Tips of Trembling Aspen (*Populus tremuloides* Michx.)[1]

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北美사시나무 trembling aspen (*Populus tremuloides* Michx.) 根端組織內의 동위효소들의 유전[1]

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ABSTRACT

The inheritance of isoenzymes in young root tissues of trembling aspen (*Populus tremuloides* Michx.) was studied by electrophoretically analysing five parental clones and their full-sib progenies obtained by crossing one female clone to four male clones. The distal 2cm section of vigorous young roots of 70 seedlings per family were subjected to horizontal starch gel electrophoresis. The resulting gels were tested for activity of 7 enzyme systems. Evidences for the inheritance of isoenzymes observed indicated that the isoenzyme variants of every isoenzyme zone were under control of codominant alleles at a single locus. Chi-square test of joint segregation data of the two loci, 6-PGD-2 and PGI-2, indicated that the pair of loci was not linked.

Key words: Populus tremuloides; *inheritance; isoenzyme; linkage.*

要 約

北美 사시나무 trembling aspen의 根端組織內의 同位酵素의 遺傳樣相을 구명하기 위하여 母樹 1개체에 花粉樹 4개체를 교배하여 얻은 4개의 全兄妹家系의 종자를 온실內에서 파종, 7週後 家系當 70개체의 幼苗와 母樹와 花粉樹의 萌芽의 생장이 왕성한 뿌리의 根端部 2*cm*를 채취하여 수평식 전분젤 전기영동법으로 분석하였다. 7개의 同位酵素에 대하여 모두 11개의 밴드지역이 관찰되었으며, 각 지역에 대한 부모의 同位酵素表現型과 그 次代의 同位酵素表現型을 관찰, x^2-test로 그 分離比를 검정한 결과 모든 同位酵素 밴드지역은 각각 하나의 遺傳子座에 의하여 지배되고 있는 것으로 나타났다. 한편 6-PGD-2와 PGI-2와의 複合 分離比를 x^2-test로 검정한 결과 6-PGD-2와 PGI-2間에는 連關關係가 없는 것으로 추정되었다.

INTRODUCTION

Isoenzyme analysis using electrophoretic techniques has been widely adopted for estimating genetic variability and studying genetic architecture of natural populations of tree species (Lundkvist and Rudin 1977, Mitton et al. 1977, Bergmann 1978,

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Guries and Ledig 1977, Rudin et al. 1979). However, the advantages of isoenzymes for population studies can be fully realized only if the inheritance of isoenzyme band patterns are known.

Many investigators describe the inheritance of isoenzymes in conifers (Bartels 1971, Guries 1978, Conkle 1971, Bergmann 1974, Rudin and Ekberg 1978, Conkle 1979, Adams and Joly 1980). For hardwood species, Feret and Stairs (1971) reported the inheritance of peroxidase in Siberian elm, Valizadeh (1977) studied the inheritance of esterase and phosphatases in fig trees, and Hirano and Naganuma (1979) reported the inheritance of peroxidase in mulberry. The inheritance of leucine aminopeptidase and acid phosphatase in beech was reported by Kim (1979).

For *Populus* species, genetic variation studies based on isoenzyme analysis have been made by Cheliak (1980) and Weber and Stettler (1981), however, no reports of inheritance of isozymes have been dealt with species of *Populus*.

This reports deals with the inheritance of seven enzyme systems in root tip tissue of *Populus tremuloides*.

MATERIALS AND METHODS

In the spring of 1982, one female clone and four male clones were located in the natural stands near Barrie and Cambridge, Ontario, Canada and four full-sib families were produced by crossing the female clone (17-11) to the four male clones (14-1, 14-2, 14-4, and 14-6) in the greenhouse of Ontario Tree Improvement and Forest Biomass Institute at Maple, Ontario, Canada. By mind-June of 1983, seeds were sown in the tubelings filled with the mixture of peat and soil and placed in the greenhouse. When seedlings were 7 weeks old, the distal 2 cm section of vigorous young roots were sampled and homogenized in a cold extraction buffer (Weber 1980). Then, the homogenized samples were centrifuged for 5 minutes and stored at −60°c

Table 1. Enzyme systems assayed and running buffers for the study.

Enzyme	Abbreviation	Buffer[1] System
Aconitase	ACO	A
Glutamic dehydrogenase	GDH	B
Glutamate-oxaloacetate transaminase	GOT	B
Isocitrate dehydrogenase	IDH	C
6-Phosphogluconate dehydrogenase	6-PGD	A
Phosphoglucose isomerase	PGI	A
Phosphoglucomutase	PGM	A

1) The buffer systems used and running conditions are described in Table 2.

Table 2. Buffers and running conditions used.

Buffer Designation	Electrode Buffer Formulation	Gel Buffer Formulation	Running Conditions
A	0.040 M Citric acid monohydrate adjust to pH 6.7 with N-(3-aminopropyl) morpholine	1:20 dilution of electrode buffer	approximately 200 V at 75 m.a.
B	0.190 M Boric acid and 0.028 M Lithium hydroxide monohydrate buffer pH=8.1	0.0076 M Citric acid monohydrate and 0.051 M Tris buffer pH 8.3 gels were made using a 9:1 dilution of gel: electrode buffer	adjusted to 75 m.a.
C	0.223 Tris and 0.08615 M Citric acid monohydrate adjust to pH 6.2 with 1 M NaOH	1:35 dilution of electrode buffer	approximately 150 V at 75 m.a.

until used for electrophoresis.

Seven enzyme systems were assayed by horizontal starch-gel electrophoresis according to the procedure described by Cardy et al. (1980), using three different buffer systems (Table 1 and Table 2).

The genetic control of isoenzymes was postulated from the band patterns observed in root tips of full-sib progenies and their parents. To test these hypotheses of inheritance, 70 seedlings per family were assayed, and Chi-square values were calculated to determine the "goodness of fit" of segregating allozymes to the expected ratio. Test of linkage was performed by calculating Chi-square values.

RESULTS AND DISCUSSION

Inheritance of isoenzyme band patterns will be discussed separately for each enzyme along with the evidence for their genetic control. For enzymes with more than one zone of activity, the fastest migrating zone is labelled 1 and the slower zones 2,3 etc. Within a zone, the fastest migrating variant is designated as A and the slower variants B,C etc. Segregation data at each locus are summarized in Table 3.

Aconitase (ACO)

Two zones of activity were apparent on gels stained for ACO (Figure 1). ACO-1 segregated in the 1:1 ratio expected for single-locus control (Table 3). At the locus ACO-2, however, the band patterns were monomorphic since both parents were homozygous for same allele. This indicates that ACO-2 is also under control of single locus. The variants at both loci were single-banded. Adams and Joly (1980) also reported single-banded variants of ACO in loblolly pine.

Glutamate dehydrogenase (GDH)

Only one zone of activity was evidenced on gels stained for GDH (Figure 1). All the progenies produced from the cross between the female clone (17-11, phenotype of AA) and the male clone, (14-2, phenotype of BB) were heterozygotes (phenotype of AB) as expected. Thus, it appears that this zone is also under control of single locus. Single-banded variants were also reported in loblolly pine (Adams and Joly 1980).

Glutamate-oxaloacetate transaminase (GOT)

Gels stained for GOT had one zone of activity (Figure 1). The two variants in the GOT locus segregated in the 1:1 ratio expected for single-locus control (Table 3). The isoenzyme bands of heterozygotes were triple-banded indicating that the bands are of dimeric products, i.e. one additional hybrid band in heterozygotes (Figure 1). Rudin (1975) and Adams and Joly (1980) also reported dimeric variants of GOT in female gametophyte tissue of pine species.

Isocitrate dehydrogenase (IDH)

Two zones of activity were found in gels stained for IDH. For the zone IDH-1 all the progenies produced from the cross between clone 17-11 (homozygous for A allele) and clone 14-1 (homozygous for B allele) were heterozygotes (AB). This indicates that IDH-1 is controled by single locus. The variants observed were single banded. Single-banded variants in loblolly pine were also reported by Adams and Joly (1980). There was another band zone slower migrating than IDH-1, however, the band patterns in the zone were too weak to be analysed.

6-Phosphogluconate dehydrogenase (6-PGD)

Two zones of activity were found on gels stained for 6-PGD (Figure 1). 6-PGD-1 was monomorphic because both parents were homozygous for same allele and consequently all the progenies produced were also homozygous for the allele. At the 6-PGD-2 locus, however, variants were segregated in 1:1 ratio expected for single-locus control (Table 3). The heterozygotes were triple-banded indicating dimeric enzyme. Adams and Joly (1980) also reported dimeric 6-PGD in loblolly pine.

Phosphoglucose isomerase (PGI)

Gels stainned for PGI had two zones of activity

Table 3. Postulated genotypes of parents and progeny with the frequency of progeny phenotypes.

Locus	Cross	Postulated genotype		Number of progeny		Chi-square value (Probability)
		parents	progeny	expected	observed	
ACO1	17-11	A A	A A	35	37	
	x					0.29
	14- 6	A B	A B	35	37	(0.6-0.7)
GOT	17-11	A B	A A	35	38	
	x					0.51
	14- 2	AA	A B	35	32	(0.4-0.5)
6-PGD-2	17-11	A A	A A	35	30	
	x					1.43
	14- 1	A B	A B	35	40	(0.2-0.3)
	17-11	A A	A A	35	33	
	x					0.29
	14- 4	A B	A B	35	37	(0.6-0.7)
PGI-2	17-11	A A	A A	35	36	
	x					0.06
	14- 1	A B	A B	35	34	(0.8-0.9)
	17-11	A A	A A	35	35	
	x					0.0
	14- 4	A B	A B	35	35	(0.9-1.0)
PGM-2	17-11	A A	A A	35	38	
	x					0.51
	14- 2	A B	A B	35	32	(0.4-0.5)

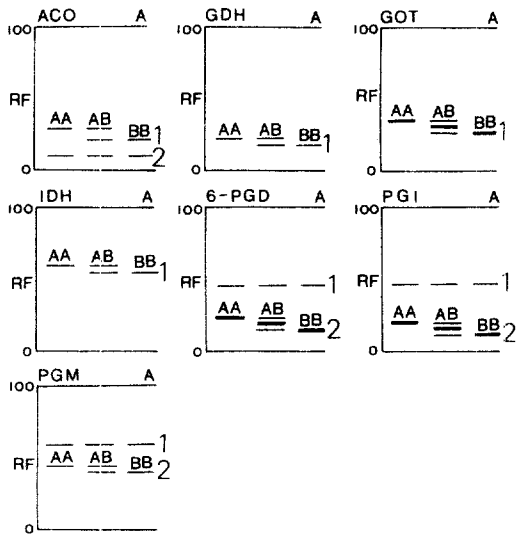

Fig. 1. Observed and expected zymogram patterns. Loci are numbered from the fastest migrating zone to slower migrating zones, and genotypes of polymorphic loci are labeled AA, AB, and BB. RF indicates relative migration rate and A indicates the anodal end.

(Figure 1). PGI-1 was monomorphic because the both parents were homozygous for the variant. Variants at the locus PGI-2, however, segregated in 1:1 ratio expected for single-locus control (Table 3). The heterozygotes had an additional hybrid band indicating that PGI enzyme in trembling aspen root is dimeric (Figure 1). However, Weber and Stettler (1981) reported single-banded variants of PGI in roots of black cottonwood.

Phosphoglucomutase (PGM)

Two zones of activity were observed on gels stained for PGM (Figure 1). PGM-1 was monomorphic because both parents were horozygous for same allele and thus all the progenies produced were homozygotes. On the other hand, PGM-2 segregated in 1:1 ratio expected for single-locus control (Table 3). Variants of PGM were appeared to be single-banded. Weber and Stettler (1981) also found two zone of activity with single-banded variants of PGM in root tissue of black cottonwood.

However, Cheliak (1980) reported only one zone of PGM activity in vegetative bud of trembling aspen. Adams and Joly (1980) also reported two zones of activity of PGM with single-banded variants in loblolly pine.

Linkage relationships

Only two male clones (14-1 and 14-4) were heterozygous at two loci, 6-PGD-2 and PGI-2, out of eleven loci detected. Combined segregating data of the two loci along with Chi-square test are presented in Table 4. In both crosses, 17-11 x 14-1 and 17-11 x 14-4, the pollen clones should produce the following four types of gametes with equal frequencies: 0.25 6-PGD-2 A/PGI-2 A, 0.25 6-PGD-2 A/PGI-2 B, 0.25 6-PGD-2 B/PGI-2 A, and 0.25 6-PGD-2 B/PGI-2 B; and the mother should produce only one type of gamete, 6-PGD-2 A/PGI-2 A. The combination of these gametes should produce four distinct genotype classes (Table 4). Assuming no differences in viability among gametes and no miotic drive, the frequency of the four different genotypic classes in this case should be equal (0.25). Chi-square tests indicated that the observed frequencies of genotypes were in agreement with the expected frequency (Table 4). The results from the two crosses indicates that the two loci, 6-PGD-2 and PGI-2, are not linked. In loblolly pine 6-PGD-2 was appeared to be weakly linked with PGI-2 (Conkle 1981).

Table 4. Test for linkage between 6-PGD-2 and PGI-2 loci.

Cross	Phenotypes of parents 6-PGD-2/PGI-2	Gametes of parents 6-PGD-2/PGI-2	Expected frequency	Genotypes of progeny 6-PGD-2/PGI-2	Number of progeny expected	observed	Chi-square value (Probability)
17-11 x 14- 1	A A / A A	A / A	1.0				
	A B / A B	A / A	0.25	A A / A A	17.5	23	
		A / B	0.25	A A / A B	17.5	14	0.5
		B / A	0.25	A B / A A	17.5	17	(0.5-0.4)
		B / B	0.25	A B / A B	17.5	15	
17-11 x 14- 4	A A / A A	A / A	1.0				
	A B / A B	A / A	0.25	A A / A A	17.5	16	
		A / B	0.25	A A / A B	17.5	17	
		B / A	0.25	A B / A A	17.5	19	0.29
		B / B	0.25	A B / A A	17.5	18	(0.9-1.0)

CONCLUSION

Eleven loci of seven enzyme systems examined in trembling aspen root tissue appeared under control of single-locus. All the segregating loci followed Mendelian inheritance pattern. Variants of GOT, 6-PGD and PGI were dimeric and variants of other enzyme systems were monomeric. There was no evidence of linkage between 6-PGD-2 locus and PGI-2 locus. For further investigation of linkage of trembling aspen, more doubly heterozygous individuals for various isoenzyme systems should be obtained and used in future.

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