Inheritance and Linkage of Allozymes in *Pinus strobus* L.

By E. M. CHAGALA

Kenya Forestry Research Institute, P.O. Box 20412, Nairobi, Kenya

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Summary

Needle and megagametophyte tissue were assayed using starch gel electrophoresis to determine the inheritance patterns and linkage relationships of 13 enzyme systems (ACO, AK, ALD, APH, DIA, GOT, IDH, LAP, MDH, PER, 6-PGD, PGM and SDH) encoded by 36 isozyme loci. One enzyme, 6-PGD, was observed to be monomorphic. Chi-square goodness of fit tests verified Mendelian control of segregating allozyme variants except for Ald-4 and Lap-3 where a segregation distortion was observed. All enzymes showed a monomeric pattern except MDH and 6-PGD whose banding patterns were dimeric in nature. Deviation from the 1:1:1:1 2 locus ratio of doubly heterozygous parents indicated linkage for the locus pair Mdh-2 and Got-3. The implication of these results in practical tree improvement is discussed.

Key words: Pinus strobus, starch gel electrophoresis, needle, megagametophyte, inheritance, linkage.

FDC: 165.3; 174.7 *Pinus strobus.*

Introduction

Eastern white pine (*Pinus strobus* L.), is one of the most important timber species, and the most widely distributed white pine in North America. It is also the only white pine native of the eastern part of North America. Because of its strong, attractive and easily worked wood, and its fast growth, it has been widely introduced in Europe and Asia (BAKSHI, 1972; GREMMEN, 1972; SAHO, 1972; SOERGAARD, 1972). These high qualities, plus the serious damage by white pine blister rust (caused by *Cronartium ribicola* J.C) to both natural and planted stands has resulted in initiation of *P. strobus* breeding programmes in North America (RIGHTER, 1945; BINGHAM *et al.* 1956; FOWLER and HEIMBURGER, 1958), Europe and Asia (BAKSHI, 1972; GREMMEN, 1972; SAHO, 1972; SOERGAARD, 1972). The main aim of these programmes has been to breed varieties resistant to white pine blister rust. However, with such a breeding programme, a thorough knowledge and understanding of genetic variation and diversity of *P. strobus* is of utmost importance, and isozyme analysis may be used to obtain such information.

Isozyme analysis has been widely applied in forest tree species for various genetic studies. Such studies include genetic variation within species (FINS and SEEB, 1986; MORAN and BELL, 1987; YEH, 1988), species and interspecific hybrid identification (ADAMS and COTINHO, 1977; TOBOLSKI and CONKLE, 1977; CHAGALA, 1990), certification of identity of parent trees and clones (ADAMS and JOLY, 1980a; CHELIAK and PITEL, 1984a), and genetic efficiency of seed orchards (e.g. MÜLLER-STARCK, 1979; FRIEDMAN and ADAMS, 1981).

Isozymes are expected to be inherited codominantly in accordance to Mendelian patterns. However, in some cases, allozymes may not show codominance due to modifiers or to lack of activity (null alleles). In addition, some enzymes may show developmental or environmental variation that mimic Mendelian segregation (HARRY, 1983; LEBHERZ, 1983; WOMACK, 1983). Lack of information on inheritance may therefore lead to problems such as an overestimate of heterozygosity, and biased estimates of allele frequencies. Knowledge of inheritance of isozymes is thus a prerequisite if allozyme variation is to be used for any genetic study.

Furthermore, the utility of isozyme analysis for genetic studies ideally requires knowledge of their linkage relationships (CHELIAK *et al.,* 1984). Knowledge of linkage of allozymes greatly increases the value of isozymes as biochemical markers, as it is important to know the distribution of identified loci in the genome. Linkage groups could be used for several purposes such as references for new genes to be identified. Linkage studies are also essential for answering some of the questions of evolution, and may serve as a basis for the genetic evaluation of a species, and for mapping and manipulation of genetically controlled traits (CLEGG *et al.,* 1972; CONKLE, 1979; KUMAR and GUPTA, 1988).

Inheritance and linkage of a limited number of isozymes have been reported for *P. strobus,* (ECKERT *et al.,* 1981; RYU, 1982). However, more information for additional enzyme systems is needed in order to have a thorough understanding of the genetics of this important species. This paper is on inheritance patterns of allozymes in *P. strobus* for 13 enzyme systems coded by 36 loci, and also on linkage relationships among these loci using megagametophyte and needle tissues.

Materials and Methods

Seed collection and preparation

Seeds were obtained from the Ontario Forest Research Institute (OFRI, Maple, Ontario, Canada), Petawawa National Forest Institute (PNFI, Chalk River, Ontario), and from Dr. H. B. KRIEBEL, Ohio Agricultural and Research Development Centre, Wooster, Ohio, USA.

Seeds were harvested by mother trees, and parental identity was maintained throughout the study. At least 100 seeds were obtained per seed batch. After collection or on receipt, seeds were stored at 4 °C until needed. Thirteen trees and between 8 to 249 seeds per tree were analyzed. Details of sources, origins and number of seeds analyzed are given elsewhere (CHAGALA, 1991).

To overcome dormancy, all seeds were cold stratified as follows: Seeds were wrapped in cheese cloth, placed in moistened steam sterilized peat in a plastic bag and stored at 4 °C in a refrigerator for 2 months. They were then taken out of the refrigerator, washed with distilled water and placed on a wet filter paper disc in a petri dish and left to germinate at room temperature. When the radicle extended 3 mm to 5 mm beyond the seed coat, the seeds were placed in a refrigerator at 4 °C until needed.

Megagametophytes were individually homogenized in 0.5 mL microcentrifuge tubes after addition of 2 drops of distilled water (ECKERT *et al.,* 1981). Homogenization was done using a power driven Dieldrin (an acetal resin) pestle fitted in a Canlab Cafrano stirrer type RZRI homogenizer for 20 seconds. When all grinding was completed, the samples were centrifug-

ed for 5 minutes in an Eppendorf microcentrifuge Model 5414, at 15 000 rpm in a refrigerator. After centrifugation, the microcentrifuge tubes were placed in a freezer at –5 °C until samples were needed.

Needle collection and preparation

Needle samples were collected from clones and families established in arboreta at OFRI. Additional material was collected at Midhurst Nursery and Melancton plantation, both managed by the Ministry of Natural Resources, Ontario, Canada. Samples from a total of 125 trees were analyzed. Details of their origins are given elsewhere (CHAGALA, 1991).

Needle samples from the lower part of the crown, selecting from the previous year's growth were collected from families and clones in January and September 1987 to determine if there were seasonal differences. As there were no differences, additional samples were collected from the same clones and families in January 1988 and corresponding results were added to those obtained in 1987.

Immediately after collection, the samples were parked in polyethylene bags which were then placed in a cooler with ice and transported to the laboratory for extraction and storage. The extraction buffer which was found most suitable is described in *table 1.* Sample preparation was done according to MITTON *et al.* (1979) with some minor modifications.

Table 1. – Extraction buffer used for homogenization of needle tissue.

Chemical	Amount
0.2 M trizma base	2.42 g
0.2 M citric acid (monohydrate)	4.20 g
5% polyvinnylpyrrolidone (PVP-40)	5.00 g
5% polyethylene glycol compound	5.00 g
2% L-ascorbic acid	2.00 g
10 mM ethylenediaminetetraacetic acid	0.336 g
Distilled deionized water	100.0 mL
Adjust to pH 7.4 with Sodium hydroxide	

*) This buffer system was originally described by Dr. GEORGE BUCKERT of OFRI, Maple, Ontario (personal communication).

Enzyme systems investigated

Eighteen enzyme systems were investigated using different buffer systems, pH conditions, electrophoretic conditions (*Table 2*) starch proportions (*Table 3*) and histochemical stains (CHAGALA, 1991). Analyzed enzymes, their Enzyme Commission Number and the buffer system used are listed in *table 4*.

Data analysis

Eight seeds per tree were first analyzed in order to infer the genotype of the mother tree from allele frequency of the gametophytes. The probability of k analysed megagametophyte from each mother tree to carry k times the same allele is given by: $0.5^{(k-1)}$ (MATHER, 1951). In this case, the probability for an heterozygote to be misclassified as an homozygote genotype at a given locus was: $(0.5)^7$ or approximately 0.008. I selected trees heterozygous for 2 or more loci and assayed between 57 and 249 megagametophytes for each tree (*Table 5*). Data from these trees were used to determine inheritance patterns and linkage relationships.

Table 2. – Composition of gel and electrode buffers used for starch gel electrophoresis and electrophoretic conditions.

Buffer system		Buffer: Electrode	Buffer: Gel	
M	0.233 M tris	27.0 g	Electrode buffer	10.0 mL
	0.08615 M citric acid (monohydrate)		Deionized water	350.0 mL
	Deionized water	1000 mL		
	Adjust to pH 6.2 with NaoH, Current 70 mA			
P	0.04 M citric acid (monohydrate)	8.4 g	Electrode buffer	25.0 mL
			Deionized water	335.0 mL
	Adjust to pH 6.7 with N-(3-aminopropyl) morpholine, current 70 mA			
IA	0.0076 M citric acid (monohydrate)	1.6 g	IA electrode buffer	325.0 mL
			IB electrode buffer	35.0 mL
	0.0510 M tris	6.2 g		
	Deionized water	1000 mL		
	pH 8.0			
IB	0.028 M lithium hydroxide	1.2 g		
	0.190 M boric acid	11.8 g		
	Deionized water	1000 g		
	PH 8.3, current 80 mA			
B	0.065 M L-Histidine	10.1 g	Electrode buffer	75.0 mL
	0.02 M citric acid	4.125 g	Deionized water	425.0 mL
	pH 5.67, current 70 mA			

These buffer systems were originally described as follows:
M SHAW and PRASAD (1970)
P CLAYTON and TRETIAK (1972)
B CARDY *et. al.* (1980)
I SELANDER *et. al.* (1971)

Table 3. – Composition of starch used for different buffer systems.

Buffer system	Starch composition	Amount
M	Connaught starch	22.3 g
	Electrostarch	22.3 g
P	Connaught starch	29.8 g
	Electrostarch	14.9 g
I	Connaught starch	22.3 g
	Electrostarch	22.3 g
B	Connaught starch	47.52 g
	Electrostarch	23.76 g

Connaught starch is supplied and manufactured by Connaught Laboratories Ltd., Willowdale, Ontario.
Electrostarch is manufactured by Electrostarch Co. Ltd, Madison, Wisconsin, USA.

Table 4. – Enzyme systems with the best resolution, their abbreviations (abbrev.), enzyme commission numbers (E.C. No.) and the buffer systems used.

Enzyme	Abbrev.	E.C No.	Buffer systems
Acid phosphatase	APH	3.1.3.2	M
Adenylate kinase	AK	2.7.4.3	P
Aldolase	ALD	4.1.2.13	M
Aconitase	ACO	4.2.1.3	P
Diaphorase	DIA	1.6.4.3	M
Glutamate oxaloacetate transaminase	GOT	2.6.1.1	I
Isocitrate dehydrogenase	IDH	1.1.1.42	P
Leucine amino peptidase	LAP	3.4.11.1	I
Malate dehydrogenase	MDH	1.1.1.37	P
Peroxidase	PER	1.11.1.7	B
6-phosphogluconate dehydrogenase	6-PGD	1.1.1.44	P
Phosphoglucomutase	PGM	2.7.5.1	M
Shikimate dehydrogenase	SDH	1.1.1.25	M

Table 5. – Trees heterozygous for loci analyzed, their identification numbers, origin and the number of megagametophytes analysed.

Tree No.	Origin	No. of seeds analysed
WP1760	Algonquin Park, Stratton, Twp., Lot 12, Con 38 (their #4021), Ontario	249
WP1560	Simcoe District, Wingham Twp., Con. 11 received from Turkey Point, Ontario	179
2600	Londonville, Wooster, Ashland County Received from Ohio Agricutural and Research Development Centre. USA	150
WP1759	Algonquin Park, Stratton, Twp., Lot 12, Con 38 (their #4021), Ontario	57
7931220	Canadian Forces Base, Petawawa, received from Petawawa National Forest Institute, Ontario	103

For segregation analyses to determine inheritance at a given locus, data from trees showing the same genotype were pooled, and a chi-square was used to test the "goodness of fit" to the expected 1:1 ratio. To test linkage hypothesis, chi-square was used to test the "goodness of fit" of the pairwise recombinants to the expected 1:1:1:1 ratio. This chi-square was partitioned into 3 components: 2 for testing the 1:1 segregation ratio in each of the single locus of a pair and the third for testing the independence of loci (joint segregation) (ADAMS and JOLY, 1980b). For those 2-locus combinations from several trees, a heterogeneity chi-square was first performed to test for homogeneity of results over trees. This chi-square was partitioned in a similar manner as above.

One assumption of linkage analysis is that there is no disturbance of individual segregation ratios (BAILEY, 1961). However, BAILEY (1961) has also shown that both the chi-square for the detection of linkage and estimate of recombination are valid if only one and not both of the segregation ratios deviate significantly from the 1:1 segregation ratio. The two-locus combinations with only one but not both loci with significant deviation from the 1:1 ratio were therefore retained for further analysis.

Recombination frequency, r, was determined by the binomial estimator $r = k/n$, where k is the smaller sum of the repulsion or the coupling recombinants, and n the total number of observations. The standard error was: $[r(1-r)/n]^{0.5}$. For those locus combinations calculated from results of more than one tree, a chi-square was also used to test the homogeneity of recombination frequencies estimated from the data of x clones (ADAMS and JOLY, 1980b).

Map distances were calculated using the KOSAMBI's formula (1944) which has tried to take the problem of chiasma interference into consideration:

$$X = 25 \ln \frac{1+2y}{1-2y}$$

where y = recombination frequency.

Results

Enzyme description and inheritance

Monomorphic enzymes

Of the thirteen enzymes studied, 6-PGD was the only enzyme found to be monomorphic for the 2 zones observed.

Polymorphic enzymes

Banding patterns of polymorphic enzymes are shown in *figure 1*, while the results of the chi-square test for allele segregation at single loci are shown in *table 6*. Twelve enzyme systems were found to be polymorphic:

Aconitase (ACO)

This is the first report on inheritance of ACO in *P. strobus*, and 2 zones of activity were observed both in needles and in megagametophytes. The first zone of activity was inconsistent and therefore was not scored, while *Aco-2* had 2 alleles that segregated in accordance with the Mendelian pattern (*Table 6*). Needle samples showed individuals to be either single or double-banded. Banding patterns for ACO are not shown in *figure 1* due to poor resolution in needles.

Adenylate kinas (AK)

Adenylate kinase was analyzed for the first time in *P. strobus*, and 2 zones of activity were present in both needles and megagametophytes. Needle samples showed individuals with either single or double-bands. *Ak-1* had 2 alleles, the slower of which was detected only in needle samples, while *Ak-2* was not scored as it was inconsistent.

Aldolase (ALD)

Aldolase was analyzed for the first time in *P. strobus* and the gels showed 4 zones of activity in both megagametophytes and needles. For needles, only zone 3 was scored as the other zones were inconsistent, as well as *Ald-2* in megagametophytes. *Ald-1* was invariant, *Ald-3* had 2 alleles, the slower of which was detected only in the needle samples, while *Ald-4* had 2 alleles which did not segregate in accordance with 1:1 ratio (*Table 6*). Individuals in needles were either single or double-banded.

Acid phosphatase (APH)

Four zones of activity were observed for APH for both needles and megagametophytes, but zones 2 and 3 in needles were inconsistent and therefore were not scored. Variants in zone 1 were triple-banded in megagametophytes, while those in needles were single or double-banded for all zones. *Aph-1* had 3 alleles that segregated in a 1:1 ratio (*Table 6*), while *Aph-4* had 2 alleles, the slowest of which was found only in needle samples. *Aph-2* and *3* were monomorphic.

Diaphorase (DIA)

Four zones of activity were present for DIA, *Dia-2* and *Dia-4* in megagametophytes having 2 alleles each that segregated in a 1:1 ratio (*Table 6*), while *Dia-3* was monomorphic. Diffuse staining was observed for *Dia-2*. Activity for the first locus was too low to be scored in megagametophytes, while in needles only zone 3 was scored. Individuals were observed to be either single or double banded.

Glutamate oxaloacetate (GOT)

There were 4 zones of activity on GOT gels, 3 of which were anodally migrating, while the fourth zone, which had banding patterns identical with the third zone, was cathodal. *Got-1* and *Got-3* had 2 alleles each, while *Got-2* had 3. The second allele for *Got-1* and the second and third alleles for *Got-2* were only detected in needles, and individuals in both tissues were single or triple-banded for either variant. Segregation at *Got-3* was in accordance with 1:1 ratio (*Table 6*).

Isocitrate dehydrogenase (IDH)

A single zone of activity was detected for IDH. Two alleles were observed, but the first allele was very rare and could be observed only in one tree on megagametophytes (tree number wp-1772 from Raglan Township, Pembroke District, Canada). This is one of the few studies where variation for IDH has been observed and it is the first in white pines. Bands for needle samples were either single or double.

Leucine aminopeptidase (LAP)

Three zones of activity were detected for LAP, the first of which was faint and inconsistent and therefore was not scored. Single or double bands were observed in needles. Two and 3 alleles were detected for *Lap-2* and *Lap-3* respectively. The alleles segregated in a 1:1 ratio for *Lap-2* but not for *Lap-3* (*Table 6*).

Malate dehydrogenase (MDH)

There were 4 zones of activity on gels stained for MDH but the fourth zone was not scored in needles as it was inconsistent. *Mdh-1* was monomorphic, while *Mdh-2* and *3* had 4 and 3 alleles respectively. For *Mdh-4*, needle samples stained inconsistently and therefore were not scored, while this locus was invariable in megagametophytes. Alleles for *Mdh-2* and *3*

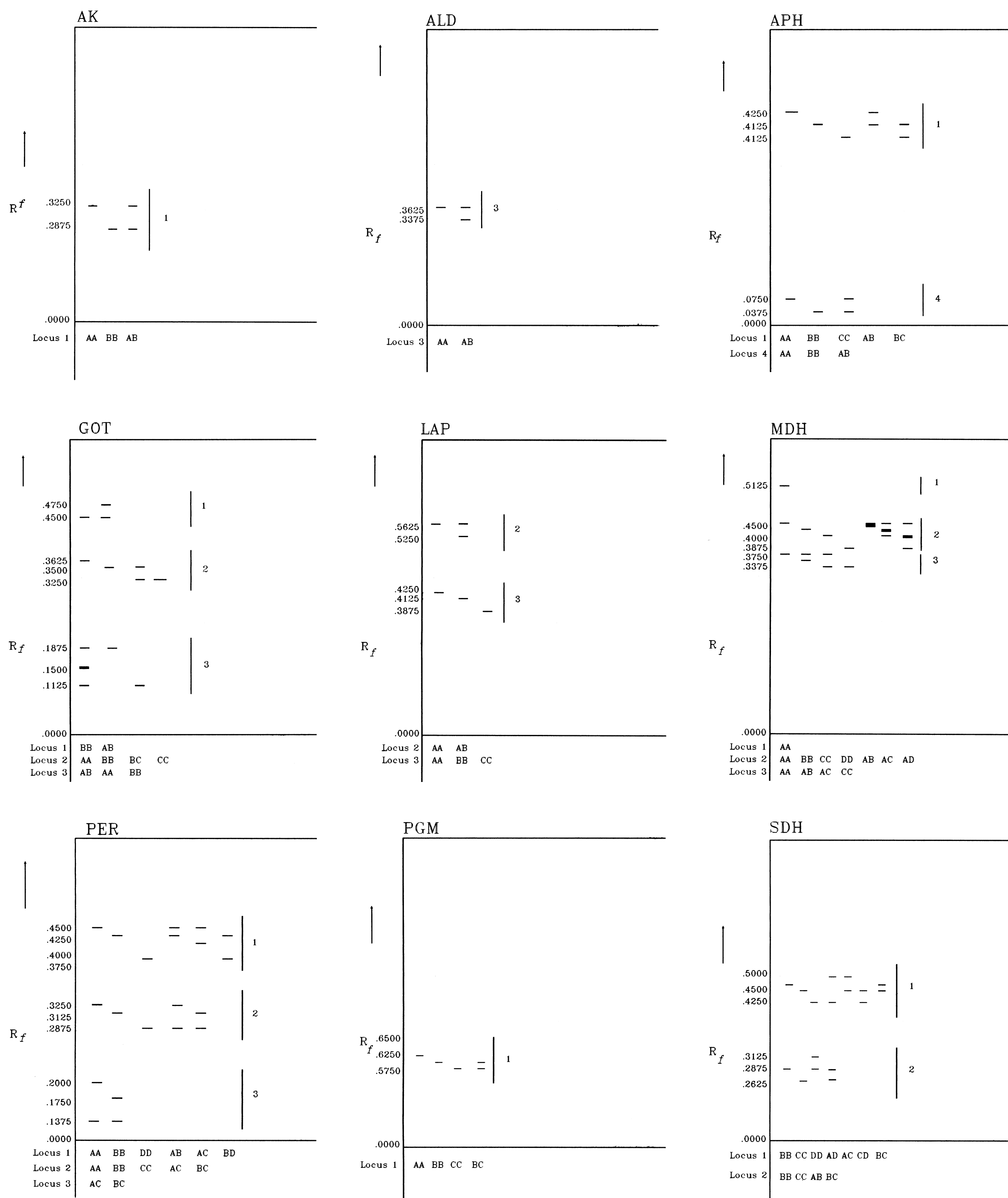

Figure 1. – Observed banding patterns and allelic designation for the enzyme systems analysed.

Table 6. – Chi-square test for allele segregation at single loci.

Locus	No. of megagametophytes per allele				
	Allele A	Allele B	Sum	χ^2	P
ACO2	250	237	487	0.347	>.900
ALD4	105	74	179	5.368	.01
APH1	25	25	50	0.000	.995
DIA2	216	212	428	0.037	>.975
DIA4	151	145	296	0.121	>.995
GOT3	260	225	485	2.525	.1
LAP2	122	127	249	0.100	.5
LAP3	78	128	206	12.130	<.001
MDH2	170	125	295	6.864	.1
MDH3	30	27	57	0.157	.5

segregated in accordance to 1:1 ratio (*Table 6*). Bands in needles were either double or triple, while in megagametophytes, the fastest migrating variant was double-banded.

Peroxidase (PER)

Megagametophytes showed no activity for PER, while 3 zones of activity were observed in needles. Although inheritance patterns were not determined due to the inactivity of PER in megagametophytes, results from needle analysis and those of RYU (1982) on the same species suggest that PER is under control of 3 loci. *Per-1* had 4 alleles, while *Per-2* and had 3 alleles each. Individuals were either single or double-banded.

Phosphoglucomutase (PGM)

Although 2 zones of activity were observed for megagametophytes, the activity was very low and inconsistent and therefore were not scored. Needle samples also showed 2 zones of activity, the second of which was inconsistent and therefore not scored. Three alleles were observed for *Pgm-1* and individuals were either single or double-banded.

Table 7. – Two-locus segregation patterns and chi-square analysis for detection of linkage.

Locus combination	No. of megagametophytes per class					Segregation			
						Locus A	Locus B	Joint	
	AB	AB′	A′B	A′B′	SUM	χ^2	χ^2	χ^2	P
Data from single trees									
LocA:LocB									
ACO2:GOT3	57	67	59	66	249	0.00	1.16	0.04	NS
ACO2:LAP2	64	60	63	62	249	0.00	0.10	0.04	NS
ACO2:LAP3	62	41	66	37	206	0.00	12.14	0.31	NS
ALD4:GOT3	36	38	46	59	179	5.37	1.26	0.68	NS
APH1:ACO2	13	12	15	10	50	0.00	0.72	0.32	NS
APH1:GOT3	11	14	16	9	50	0.00	0.32	2.00	NS
APH1:LAP2	10	15	15	10	50	0.00	0.00	2.00	NS
APH1:LAP3	11	14	15	10	50	0.00	0.08	1.28	NS
DIA2:ACO2	60	70	64	55	249	0.49	0.00	1.45	NS
DIA2:ALD4	30	52	44	53	179	1.26	5.37	0.94	NS
DIA2:APH1	14	14	11	11	50	0.72	0.00	0.00	NS
DIA2:DIA4	14	10	11	8	43	0.58	1.14	0.02	NS
DIA2:LAP2	62	68	65	54	249	0.49	0.10	1.16	NS
DIA2:LAP3	58	46	70	32	206	0.02	12.14	3.28	NS
DIA4:GOT3	16	9	10	8	43	1.14	1.88	0.58	NS
DIA4:LAP2	11	14	10	8	43	1.14	0.02	0.58	NS
DIA4:LAP3	12	7	8	5	32	1.13	2.00	0.13	NS
DIA4:MDH2	32	29	33	56	150	5.23	2.67	4.51	**
DIA4:MDH3	27	32	17	27	103	2.18	2.18	0.24	NS
LAP2:GOT3	61	66	55	67	249	0.10	1.16	0.20	NS
LAP2:LAP3	69	38	59	40	206	0.31	12.14	0.70	NS
LAP2:GOT3	60	68	41	37	206	12.14	0.08	0.70	NS
MDH2:GOT3	21	5	6	25	57	0.44	0.16	21.49	***
MDH2:MDH3	11	15	16	15	57	0.44	0.16	0.44	NS
MDH3:GOT3	13	14	14	16	57	0.16	0.16	0.02	NS
Data from several trees									
ACO2:MDH2	27	32	17	27	103	2.18	2.18	0.24	
ACO2:MDH2	26	28	34	47	135	5.40	1.67	0.90	
Total	53	60	51	74	238	0.61	3.78	1.08	NS
DIA2:GOT3	41	41	41	56	179	1.26	1.26	1.26	
DIA2:GOT3	55	75	61	58	249	0.49	1.16	2.12	
Total	96	116	102	114	428	0.04	2.39	0.15	NS
DIA4:ACO2	36	23	23	21	103	2.18	2.18	1.17	
DIA4:ACO2	11	14	4	14	43	1.14	3.93	1.14	
Total	47	37	27	35	146	3.32	0.03	2.22	NS

) and *) designate significance at 1% and 0.1% probability level respectively, NS designates no significance, LocA and LocB are Locus A and Locus B respectively.

Shikimate dehydrogenase (SDH)

Gels stained for SDH revealed 2 zones of activity that migrated to the same distance in both megagametophytes and needles. Sample size in megagametophytes was too small for segregation analysis. Staining for needle tissue was very intense for the first locus, while the second locus was lightly staining. Individuals had single or double-bands in needle tissues. *Sdh-1* and *Sdh-2* had 4 and 3 alleles respectively.

Table 8. – Two locus recombination frequencies (r), their standard errors (SE) and map distances.

Locus combination	r	SE	Map distance (centi-Morgans)
DIA4:MDH2	0.413	0.040	58.77
MDH2:GOT3	0.193	0.052	20.35

Linkage of isozymes

Two-locus segregation data are summarized in *table 7*. For the single trees, 25 locus pairs conformed to the hypothesis of independence of loci, except 2 pairs *Dia-4:Mdh-2* and *Mdh-2:Got-3* which showed significant deviation from 1:1:1:1 ratio for joint segregation ($P < 0.05$ and < 0.001 respectively). However, only one pair (*Mdh-2:Got-3*), had a map distance of < 50 centiMorgans (*Table 8*). Although the pair *Dia-4:Mdh-2* had a recombination frequency of < 0.5, the estimated map distance was > 50, indicating no linkage.

For the 2-locus combinations from several trees, heterogeneity chi-square revealed no significant difference among trees. In addition, none of the 2-locus combinations showed significant deviations from the expected 1:1:1:1 ratio.

Discussion

Allozyme segregation at most loci was in agreement with the expected Mendelian pattern, leading to acceptance of null hypothesis that these allozymes follow a single-gene inheritance with a codominant expression of alleles. Such simple inheritance confirms their genetic control.

Apart from AK and IDH which were under control of one locus, the other 11 enzyme systems analyzed were under multiple gene control. The 13 enzymes studied were coded by a total of 36 gene loci. 6-PGD was the only monomorphic enzyme, as has also been reported in the same species (ECKERT *et al.*, 1981; RYU, 1982).

Inheritance patterns for ACO, AK, ALD and SDH are reported for the first time in *P. strobus*. Their analysis is important as it increases the number of markers that may be used in genetic studies and practical tree improvement.

There were cases of significant deviation from the 1:1 ratio at *Ald-4* and *Lap-3* loci. Such lack of conformity to Mendelian inheritance has also been observed in other studies and it may be attributed to several factors such as 1) sampling error; 2) selection against (a) a particular allozyme (b) alleles of linked loci or (c) selection among meiotic products resulting in meiotic drive (ECKERT *et al.*, 1981; STRAUSS and CONKLE, 1986); and/or 3) stains with synthetic substrates that may affect enzyme expression or substrate specificity (GURIES and LEDIG, 1978; HARRY, 1986).

For most enzyme systems analyzed, the genetic models derived were in general agreement to those for the same species and in many other conifer studies. Among conifers, one locus has been reported in all studies on IDH, while 2 loci have been observed for PGM and 3 each for GOT, LAP and PER (e.g. ECKERT *et al.,* 1981; RYU, 1982; POULSEN *et al.,* 1983; STEINHOFF *et al.,* 1983; EL-KASSABY *et al.,* 1982, 1987). Two loci have also been observed for ACO in *Larix laricina* (CHELIAK and PITEL, 1985), while 4 each have been reported for APH in *Pinus attenuata* (STRAUSS and CONKLE, 1986), DIA in *Picea glauca* (KING and DANCIK, 1983) and MDH in various species (POULSEN *et al.,* 1983; STEINHOFF *et al.,* 1983; CHELIAK and PITEL, 1985; STRAUSS and CONKLE, 1986; EL-KASSABY *et al.,* 1987; PITEL *et al.,* 1987). As in the present study, ADAMS and JOLY (1980b) reported poor resolution for APH in *Pinus taeda* and EL-KASSABY *et al.* (1982) observed diffuse staining for DIA in *Pseudotsuga menziesii.*

For some enzyme systems, the interpretation of the number of loci has tended to vary. This is more so for PER where a single locus (MITTON *et al.,* 1977; COPES, 1979), and up to 42 loci were reported in *Thujopsis dolabrata* (SAKAI *et al.,* 1971). Such differences may result from laboratory conditions and/or from polymorphism of the studied populations. In the present study, it was observed that modification of pH led to different banding patterns. In addition, PER has been reported to be complex, and this leads to problems in interpretation (SAKAI *et al.,* 1971; SAKAI and MIYAZAKI, 1972; SNYDER and HAMAKER, 1978). Furthermore, as in the present study, tissue specificity has been reported by CONKLE (1971), as PER was not detected in seeds, but its activity was observed to increase with epicotyl elongation.

MDH has also had varying interpretations. STEINHOFF *et al.* (1983) observed one locus in the same species, while 2 (ADAMS and JOLY, 1980b) and 3 loci (CHELIAK *et al.,* 1984) have also been observed. An interlocus heterodimer has been reported (EL-KASSABY *et al.,* 1982; KING and DANCIK, 1983; CHELIAK *et al.,* 1984; CHELIAK and PITEL, 1985), while a null allele has also been observed (EL-KASSABY *et al.,* 1982). MDH is also reported to be prone to segregation distortion (SIMONSEN and WELLENDORF, 1975; EL-KASSABY *et al.,* 1982; CHELIAK *et al.,* 1984; STRAUSS and CONKLE, 1986).

For the other enzymes, one locus each has been reported for ACO, APH, PGM and SDH (CONKLE and ADAMS, 1977; ADAMS and JOLY, 1980b; RYU, 1982; DANCIK and YEH, 1983; CHELIAK and PITEL, 1984b; PITEL *et al.,* 1987), 2 for APH, DIA, GOT, LAP (ADAMS and JOLY, 1980b; ECKERT *et al.,* 1981; EL-KASSABY *et al.,* 1982; STEINHOFF *et al.,* 1983; CHELIAK *et al.,* 1985; PITEL *et al.,* 1987), and 3 for AK (KING and DANCIK, 1983; CHELIAK *et al.,* 1984).

The observation of a cathodal locus in GOT is similar to that reported in other pines (O'MALLEY *et al.,* 1979; ADAMS and JOLY, 1980b; EL-KASSABY *et al.,* 1987). The occurrence of a cathodal locus with similar banding patterns as the third locus suggests that these loci are either closely linked or are controlled by the same locus (GURIES and LEDIG, 1978). As such close linkage cannot be observed, the latter reason is the more likely.

Among the 36 loci studied, 4 loci (*Ald-2, Aph-1, Got-3* and *Mdh-4*) had individuals that were double or triple-banded in megagametophytes. Such multiple banded individuals have also been observed in other studies (ADAMS and JOLY, 1980a; ECKERT *et al.,* 1981; KING and DANCIK, 1983). Their cause is not clear as they could arise from (closely linked) duplicated loci or they could be due to post-transcriptional modifications (HARRY, 1986).

The subunit structure of enzymes as determined from the needle tissue showed 6-PGD, MDH and GOT to have a dimeric structure, while all the other enzymes analysed (ACO, AK, ALD, APH, DIA, IDH, LAP, PER, PGM, and SDH) were monomeric. However, LUNDKVIST (1975) and LUNDKVIST and RUDIN (1977) reported a dimeric pattern for APH.

The pattern of linkage or non-linkage of isozyme loci was in general agreement to other reports on conifer species (CONKLE, 1979; ADAMS and JOLY, 1980c; ECKERT *et al.,* 1981; GURIES *et al.,* 1978; CHAGALA, 1991). Two loci: *Mdh-2* and *Got-3,* were clearly linked with an estimated recombination frequency of 0.193 and proposed map distance of 20.35 centiMorgans. These loci may serve as references on which new genes can be localized on chromosomes.

Although the recombination frequency for the locus pair *Dia-4:Mdh-2* was < 0.5 ($r = 0.413$), calculation of map distances indicated no linkage. This was probably due to the small sample size, as the recombination frequency indicates loose linkage which requires a large sample size to be detected. ECKERT *et al.* (1981) also reported for the same species loose linkage between *Aph1:Lap2* and *Aph1:Got3.* In the present study, no linkage was detected among these 2 locus combinations probably due to the small sample size.

The results in the present study on both inheritance and linkage have implications for practical tree improvement. The confirmation of genetic control of allozymes with simple Mendelian inheritance facilitates their use as gene markers in other studies. For example, *P. strobus* is reported to have a wide natural range (CRITCHFIELD and LITTLE, 1966). Furthermore, in its breeding programme, many inter- and intra-specific crosses have been made resulting in a large number of clones, half- and full-sib families and inter-specific hybrids. The allozymes studied could be used in determining the genetic variation and relationships within and between species, as well as for identification of species and interspecific hybrids. Such information is essential for the success of the *P. strobus* breeding programme.

The loci which have been confirmed to be linked may be used as markers in mapping and manipulation of important economic traits in *P. strobus,* particularly resistance to *Cronartium ribicola.* This disease has been shown to be under strong genetic control, and if detected to be closely linked to the marker loci, may lead to the early selection of this trait, resulting in a considerable reduction in the long breeding cycle.

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