INHERITANCE AND SEGREGATION OF ALLOZYMES IN EMPLOYING VIABLE FEMALE GAMETOPHYTES

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Summary

Enzyme varjants of GDH, GOT, IDH, LAP, MDH, 6-PGDH, PGM, SKDH, and SOD were investigated in blue pine (*Pinus wallichiana* A.B. Jackson). Analysis was carried out using female gametophytes and corresponding embryos of viable seeds. On the average the electrophoretical detectable variation was low. GDH, IDH, PGM, and SOD were monomorphic, whereas moderate electrophoretical variation was observed in the remaining enzymes. Analysis of female gametophytes of 29 putative heterozygous trees showed that the one-to-one segregation was significantly distorted. Single trees segregation for SKDH zones often was in accordance with the Mendelian expectation while for the other putative loci as well as pooled data, it deviated significantly from 1:1 segregation. In most cases segregation heterogeneity was significant for certain putative loci as well as for certain provenances.

Key words: *Pinus wallichiana*, isozymes, segregation distortion, inheritance pattern

Introduction

Pinus wallichiana is found in various ranges of Indo-Pakistan and extends to eastern Afghanistan, Nepal, Buthan, China, and Burma (Critchfield and Little, 1966). This species reveals an extensive ecological adaptation. In Pakistan it occurs between 31-37° N latitude

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and 69-76° E longitude both in dry and wet temperate forests at an altitude of 1475-3300 m, where annual rainfall varies from 200 to 1500 mm. Generally blue pine forms mixed stands with others conifers but pure stands can also be found .

The application of isozymes as genetic markers is an effective tool in forest genetics (Adams 1983).

As prerequiste for genetic markers the inheritance pattern was studied intensively, especially in conifers (Rudin, 1986) but isozymes as genetic markers were not employed to study the inheritance and segregation pattern in blue pine. Due to pronounced different environmental conditions, ecotypes of this forest tree species show considerable phenotypical variation in several characters (Shams, 1979), therefore high genetic variation at isozyme gene loci was expected.

The objectives of the present study are to describe 9 enzyme systems and to give some results concerning the mode of inheritance of enzyme polymorphisms of the female gametophytes in *Pinus wallichiana*. Special emphasis is given on the segregation of enzyme variants of putative heterozygous trees.

Materials and Methods

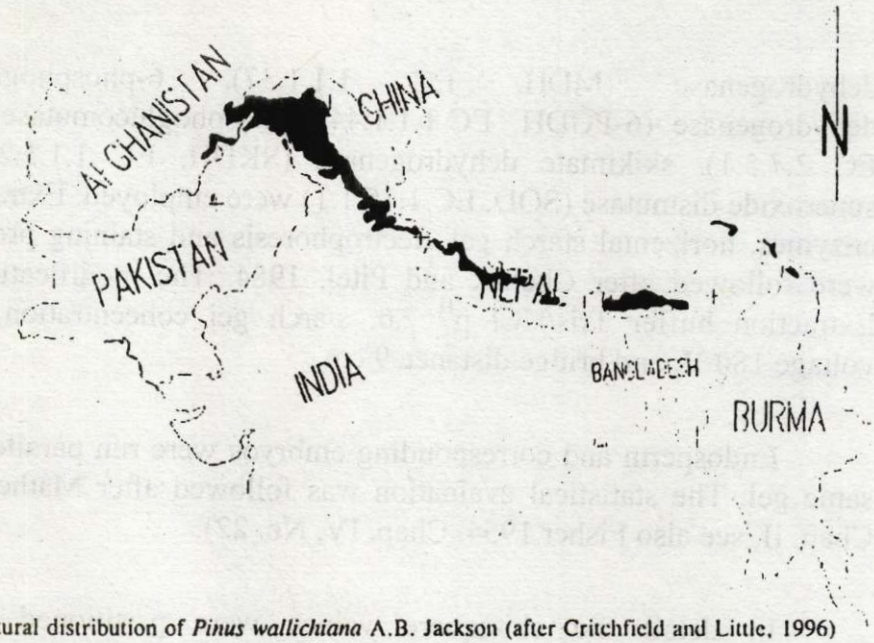
The seed originated from 29 single trees of natural stands of *Pinus wallichiana* growing in the Himalayan and Karakurum Ranges in Pakistan (Fig. 1). These 29 trees were selected out of 120 trees from which single tree seed analysis via electrophoresis show phenotypical variation. The sampling of the seed was performed in 1986, 1987, as well as in 1988.

Glutamate dehydrogenase (GDH, EC 1.4.1.3), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), leucine aminopeptidase (LAP, EC 3.4.11.1), malate

dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44), phosphoglucomutase (PGM, EC 2.7.5.1), skikimate dehydrogenase (SKDH, EC 1.1.1.25), and superoxide dismutase (SOD, EC 1.15.1.1) were employed. Extraction of enzymes, horizental starch gel electrophoresis and staining procedures were followed after Cheliak and Pitel, 1984. The modifications are: Extraction buffer Tris/HCl p^H 7.6, starch gel concentration: 12 %, voltage 180 V, and bridge distance 9 cm.

Endosperm and corresponding embryos were run parallel on the same gel. The statistical evaluation was followed after Mather (1951, Chap. II, see also Fisher 1954, Chap. IV, No. 22).

In detail the chi-square values were partitioned for the segregation ratio into different components. Assuming a one-to-one segregation, at first the chi-square was calculated for the deviation from the expected ratio and heterogeneity of segregation among the employed putative heterozygous trees. The total chi-squqre (χ^2-TOT) resulted from summing the chi-square for the deviation from one-to-one segregation for single trees (χ^2-DEV) and the chi-square for heterogeneity was calculated by subtracting χ^2-DEV from χ^2-TOT for the pooled data. If pooled segregation deviated significantly from the expectation, computing the heterogeneity chi-square on a one-to-one basis does not seem appropriate. Thus the chi-square for heterogeneity was calculated on the basis of observed total segregation. By doing this, one degree of freedom was lost. The chi-square for heterogeneity was partitioned into two components 1. heterogeneity among different localities (number of localities reduced by one) and 2. heterogeneity among single trees in a certain locality (number of trees which belong to a locality reduced by one).



Natural distribution of *Pinus wallichiana* A.B. Jackson (after Critchfield and Little, 1996)

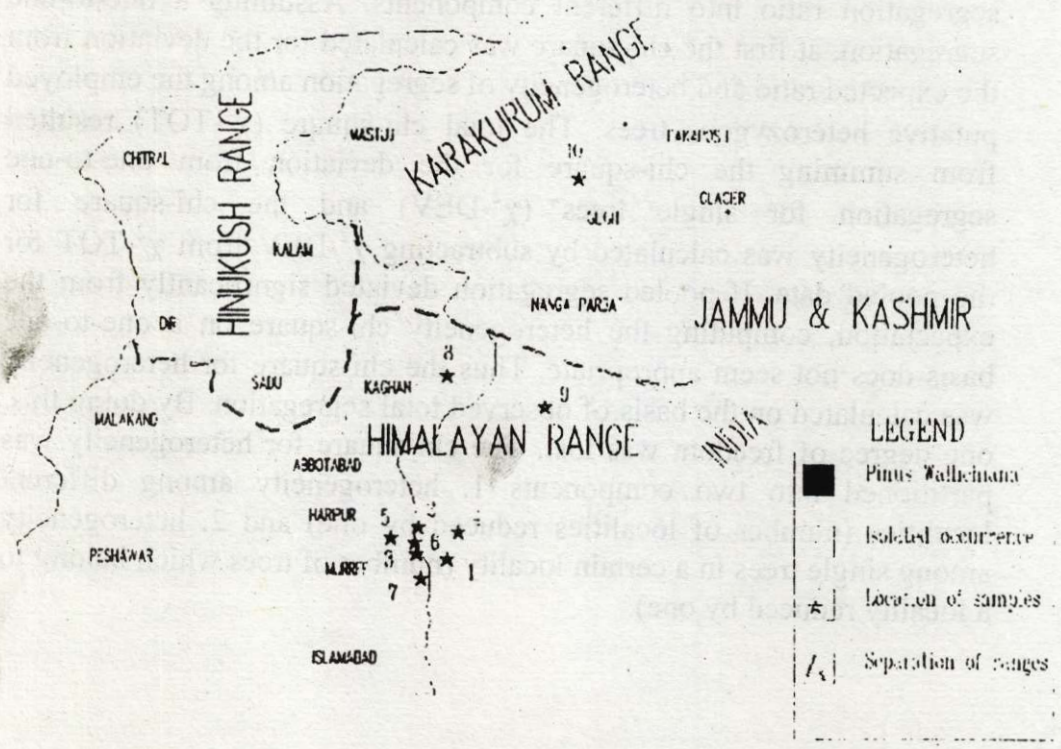


Fig.1 Distribution of seed samples of *Pinus wallichiana* in Pakistan and Azad Kashmir

Results

- Description of the enzyme variants

GDH, IDH, PGM, and SOD were sigle-banded and monomorphic in the employed material. The remaining enzymes were polymorphič. Whereas two enzyme variants were present at single zones of GOT, LAP, MDH, and SKDH, three enzymes variants could be differentiated for 6-PGDH. The banding-patterns of all enzymes, designation of the putative geneloci and designation of alleles are shown in Fig. 2. Either the banding pattern of the embryos and megagametophyte was the same or additional bands were found in putative heterozygous embryos.

Chraracteristic for dimeric enzymes double-banded phenotypes were observed for LAP and SKDH, where as triple-banded phenotypes occurred for all GOT zones. The embryonic enzyme variants of 6-PGDH and MDH did not differ from endospermic pattern.

SKDH. Two zones of activity were found in SKDH. SKDH-1 showed two enzyme variants in our material, whereas the slower zone was faint and was excluded for interpretation.

MDH. Five zones could be differentiated for MDH. MDH-2 was polymorphic in our material (Fig. 2). Employing endosperms as well as embryos sometimes additional bands of weak activity were present between MDH-2 and MDH-3. Double-banded phenotypes were observed at MDH-4 and MDH-5 zones.The double-banded MDH-5 could only be found in endosperm tissues.

6-PGDH. Three zones (6-PGDH-1, 6-PGDH-2, 6-PGDH-3) could be scored. 6-PGDH-2 and 6-PGDH-3 were monomorphic as well as single-banded in all female gametophytes. Triple-banded phenotypes occurred in some embryos at 6-PGDH-3. At 6-PGDH-1 double-or triple-banded phenotypes were found. These enzyme variants partly overlapped (Fig, 2).

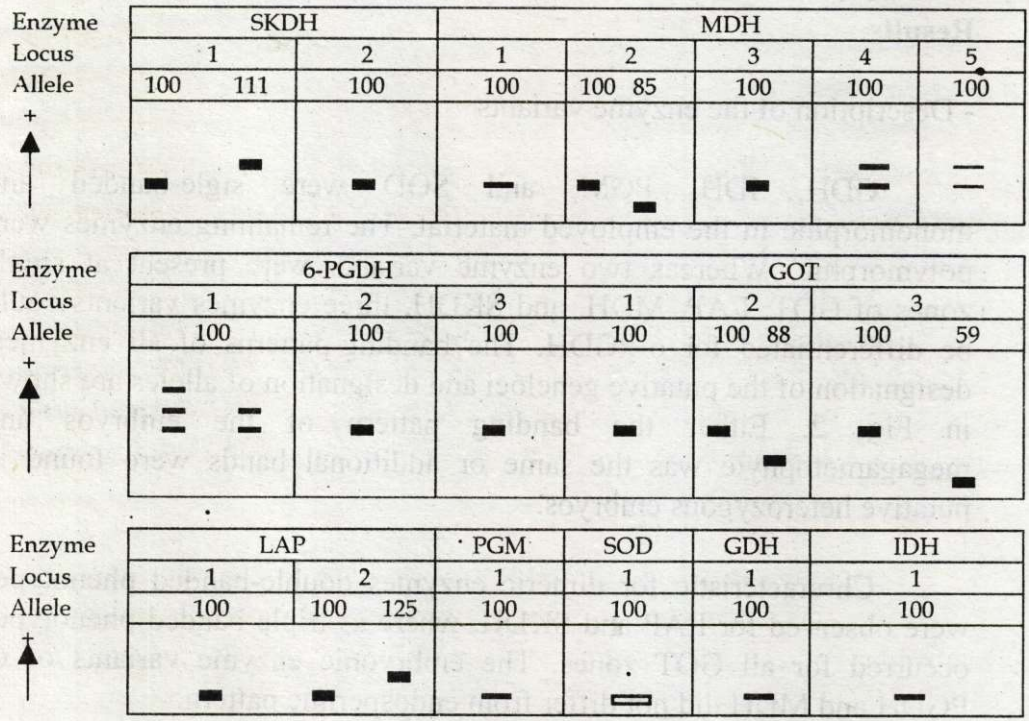


Fig. 2. Banding patterns and thier allelic designations for 9 Enzyme loci in *P. wallichiana*. Mobility is expressed relative to the common allele, whose mobility is set to 100.

GOT. These zone namely, GOT-1, GOT-2 and GOT-3 were scorable. With exception of GOT-1 at each of these zones two enzyme variants were presesnt using megagametophyte.The slow migrating GOT-3 band migrated cathodally (Fig. 2).

LAP. Gels stained for LAP resulted in two zones(LAP-1 and LAP-2) of enzyme activity. The faster migrating zone showed enzyme variants that could not always be clearly differentiated. Therefore records of LAP-1 enzyme variants are not given. At LAP-2 two enzyme variants were present in the material (Fig. 2).

- Segregation of different enzyme variants of putative heterozygous genotyes

At all designated zones a significant departure from the Mendelian expectation was present (Table. 1). Segregation ratios varied between 1:1.6 for SKDH and 1:18.2 for GOT-3. Single tree segregation was in accordance to the Mendelian expectation at 52% (SKDH), 14% (LAP-2), and 0% (GOT-2, GOT-3, 6-PGDH-1, MDH-2) of the employed single trees. Heterogeneity of the segregation ratios differed among provenances. Only for GOT-3 segregation ratio was homogenous (Table 2).

Discussion

The proposed genetic control of the enzyme polymorphisms in blue pine is similar to other electrophoretical studies in *pinaceae*. Thus nearly an identical GOT-banding pattern is observable in blue pine compared to *pinus sylvestris* (Chung, 1981) including the cathodally migrating enzyme variant at the third GOT zone (Rudin and Ekberg,1978).

Using female gametophytes, no enzyme variants were present at the GOT-1 zone in our material. But triple-banded GOT-1 phenotypes in embryonic tissues suggest that this zone in encoded by a polmorphic gene locus in *P. wallichiana*. In *Pinaceae* LAP and SKDH are often controlled by two - mostly polymorphic - gene loci (Lewandowski and Mejnartowicz, 1992). Presumably also two gene loci control these enzymes in blue pine. But due to blurred staining of SKDH-2 or similar migration rates of different enzyme variants of LAP-1, these enzymes

were excluded from our interpretation. The analysis of MDH and 6-PGDH polymorphisms were also aggravated. Our material did not allow the varification of hypothetical genetic control of these enzyme systems.

The non-codominant trait expression of certain alleles due to overlapping of allozymes at the putative 6-PGDH locus did not always enable a genotyping of diploid tissues. For the interpretation of the MDH polymorphism, it was disadvantageous that embryos showed the same banding pattern as it was found in the corresponding female gametophytes. Assuming the absence of null variants the embryos should be homozygous at all MDH gene loci. If embryos carry the rarer of the two enzyme variants of a certain zone and if this rare enzyme variant originated from the maternal genotype, the embryonic enzyme pattern should often differ from that of the corresponding mother tree due to the expected heterozygosity of the embryo. Similar to *Picea abies* (Poulsen *et al.*, 1983) or *cunninghamia laceolata* (Geburek and Wang, 1990). In blue pine endosperms a slow migrating zone of MDH was present which could not be found in diploid tissues.

Despite many similarities in enzyme polymorphisms compared to other conifers, the genetic control is still unclear in blue pine. Extreme segregation distortion at all putative gene loci put the putative genetic control into question. Significant departure from the Mendelian segregation for single tree and as well as partly for pooled data are relatively common in conifers. In *Pinus attenuata* the more commen allele of a locus were significantly in excess at LAP-1 and Alanine aminopeptidase (AAP-1) amounting to 16% and 91% respectively (Strauss and Conkle, 1986). 75% and 24% excess of the more commen enzyme variant was found by El-Kassaby *et al.* (1982) in *Pseudotsuga menziesii* at a Diaphorase (DIA) and Malate dehydrogenase (MDH) locus. Muona *et al.* (1987) reported significant deviation from the one-to-one segregation of enzyme variant in *Picea abies* at gene loci that control Acid phosphatase (APH), Phosphoglucose isomerase (PGI),

Leucine aminopeptidase (LAP), and Malate dehydrogenase (MDH). An excess of the more commen allele up to 70% were recorded in their study. In *Pinus banksiana* up to a two-fold excess of the more commen enzyme variants was reported for APH-2 and a Glucose-6- phosphate dehydrogenase (G6P) locus (Cheliak *et al.*, 1984). Several other studies exist that show significant deviation from the Mendelian expectation, but such extreme cases of segregation distortion, as found in the present investigation, have not been reported so far.

Several factors - experimental and genetical - can contribute to a real or a putative segregation distortion at isozyme gene loci using the female gametophyte of viable seed. Misinterpretation of the mode of inheritance, the tendency of scoring the most commen allozyme more often, linkage of isozyme gene loci to distorter or deterimental effects of the isozymes itselves as well as pre- and post-fertilization selection. But most of these factors can be excluded for our study.

In this paper results of those enzymes are reported that could be easily scored. Thus, as mentioned above the results of the LAP-1 and SKDH-2 are excluded. All employed allozymes differ to a greater extent in their migration rate and a misinterpretation of enzyme polymorphisms is highly unlikely. Mode of inheritance is sometimes difficult due to interlocus heterodimers and overlapping zones. But even if we assume a misinterpretation for the enzyme system - for example for MDH -, the fact still exists that two enzyme variants differed significantly from the 1:1 segregation ratio. Since only two enzyme variants were found, segregation distortion must be present independently irrespective of how many gene loci control the enzyme polymorphism.

Isozyme genes that are linked to lethal genes will be given to the offspring less frequent than other genes. But such linkage is not very probable in our material. Assumption of linkage would mean that some enzyme variants should be found in excess and in deficiency. All single tree segregation in this study shows a distortion in the same direction. Moreover the probability that all studied enzyme gene loci belong to the linkage group which include lethal loci seems to be low. Linkage could

not be studied in this investigation due to extreme segregation distortion but such studies in other conifers suggest that the gene loci belong to different groups (Conkle, 1981; Geburek and von Wuehlisch, 1989). Using endosperms, rare enzyme variants may occur, if the origin of the female gametophyte is polysporic. In such a case the endosperm is not derived as usually from one megaspore. However, such mosaic female gametophytes (O'Malley *et al.*, 1988) can not explain our data. With the exception of hybrid enzymes, the electrophoretical pattern of such mosaics were identical to those enzyme pattern which results from heterozygous loci in embryos, buds etc.

Even this factor that contributes to a putative segregation distortion at isozyme loci can be excluded for the present data, artefacts of isozymes as well as mutation can contribute to putative segregation distortion at isozyme gene loci. Despite these factors can not be excluded, the frequency of the rare enzyme is too high to be due to mutation and moreover only two different enzyme variants were observed at a certain zone.

Presumably the gametic selection may be contributing to segregation distortion in blue pine. Unfortunately our data do not allow a futher interpretation. However, pollination and fertilization are separated by approximately 13 months in *Pinus wallichiana* (Konar and Ramchandani, 1958), so there is the chance of ample gametic selection. Since only successful female gametes can be scored in seed, the question whether the selection is pre- or post-zygotic can not be ruled out. Futher studies analysing buds and pollen of putative heterozygous trees as well as the genotyping of parental trees including their corresponding offspring may help to clearify the unexpected finding in this pine.

Due to the distortion of the customary equality of enzyme variants of putative heterozygotes, population genetical studies in blue pine seems to be difficult.The frequencies of the female gametophytes represent only inadequately the population structure of an adult forest stand of this forest tree species. Thus, application of isozymes on female gametophytes alone does not clearify the genetical characterizations and

comparisons of different populations as long as the exact causes of the distorted segregation are not known.

Table. 1. Segregation of different enzyme variants using the endosperm of putative heterozygous blue pine trees.

Provenance	Tree	Putative Gene Loci					
		GOT-2	GOT-3	LAP-2	MDH-2	6-PGDH- 1	SKDH-1
Banjosa	T-01	- : 50	6 : 44	14 : 28	-: 66	44 : 4	46 : 20
	T-04	9 : 91	8 : 88	15 : 81	-: 56	24 : -	46 : 18
	T-06	2 : 58	3 : 57	22 : 38	-: 60	58 : 2	30 : 30[ns 1)]
	T-07	- : 40	2 : 38	10 : 22	-: 56	43 : 5	44 : 12
	T-10	1 : 59	4 : 56	9 : 51			
Kalabagh	T-02	- : 60	6 : 54	19 : 41	-: 60	59 : 1	34 : 2[ns]
	T-05	- : 40	6 : 34	8 : 24	-: 64	48 : -	40 : 24
	T-10	- : 60	4 : 56	14 : 46	-: 60	59 : 1	30 : 30[ns]
Bhurban	T-01	- : 40	- : 40	11 : 21[ns]	9 : 55	48 : -	54 : 10
	T-05	- : 60	1 : 59	20 : 40	13 : 47	77 : 1	40 : 20
	T-07	1 : 47	- : 48	1 : 47			
	T-08	- : 81	- : 81	11 : 70	2 : 48	20 : 4	39 : 18
Ban	T-01	- : 62	3 : 59	24 : 28[ns]	- - : 62	62 : -	36 : 26[ns]
	T-02	- : 48	2 : 46	23 : 25[ns]			28 : 20[ns]
	T-07	- :100	8 : 92	27 : 73	3 : 57	23 : 1	37 : 31[ns]
Kuzagali	T-04	- : 60	5 : 55	17 : 43	2 : 58	59 : 1	34 : 26[ns]
	T-08	- : 80	- : 80	6 : 74			
	T-13	- :100	- :100	9 : 91	2 : 58	24 : -	43 : 25
Kuldana	T-02	- : 53	1 : 52	16 : 37	1 : 60	26 : 5	32 : 37[ns]
	T-03	- : 65	6 : 59	13 : 52	7 : 58		37 : 28[ns]
	T-06	- : 71	8 : 63	22 : 49	10 : 61	67 : 4	44 : 27
Patriata	T-04	- : 58	1 : 57	24 : 54	2 : 36		30 : 20[ns]
	T-05	- : 62	1 : 61	20 : 42	2 : 60	71 : 15	43 : 19
	T-06	- : 50	2 : 48	2 : 48			
	T-09	- : 80	8 : 72	17 : 63	4 : 71	73 : 3	46 : 50[ns]
Kamalband	T-03	2 : 90	9 : 83	16 : 76	-: 60	82 : 10	64 : 24
	T-08	- : 62	4 : 58	14 : 48	- -: 62	57 : 5	42 : 20
Sharda	T-04	- : 50	6 : 44	21 : 35[ns]	- -: 40		26 : 24[ns]
Naltar	T-01	11 : 55	11 : 66	12 : 53	- -: 50		42 : 24

1) indicate a ratio in accordance to the Mendelian expectation (all other deviate significantly at 5% level)

Table. 2. Heterogeneity among single-trees in certain localities as well as for provenances for different putative gene loci.

Localities	Putative Gene Loci					
	GOT-2	GOT-3	LAP-2	MDH-1	6PGDH-1	SKDH-1
Banjosa	n s	n s	**	-	n s	**
Kalabagh	-	n s	n s	-	n s	n s
Bhurban	-	-	***	**	**	*
Ban	-	n s	*	-	-	n s
Kuzagali	-	-	**	**	-	n s
Kuldana	-	n s	n s	n s	n s	n s
Patriata	-	n s	**	-	**	*
Kamalband	-	n s	n s	-	n s	n s
Provenances	***	n s	***	*	*	***

* = P< .05 / ** = P< .01 / *** = .001 / n s = not significant

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