negative. It will not be difficult to select for fast growth with little or no dieback.

The expected genetic gain estimated for height from mass selection was the highest compared to family and combined selection. Since single-tree selection could result in many trees from relatively few families, this scheme was not recommended. On the other hand, combined selection will provide slightly less gain than mass selection, while providing a broader genetic base for future breeding programs (NEBGAN and LOWE, 1982). About 47% of the mean height can be gained from combined selection, and this is relatively high compared to gains estimated for other species (NEBGEN and LOWE, 1982; RINK, 1984; FARMER *et al.*, 1983; FOSTER and LESTER, 1983). Predicted genetic gains from combined and mass selection for length of thorns, bud-break, and leaf initiation were also high.

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Isozyme Polymorphisms in Silver Fir (Abies alba Mill.)

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Abstract

Data of isozyme analyses are reported for 4 enzyme systems in silver fir. The studies were based on single tree seed lots. Megagametophyte as well as embryo tissue was studied. SKDH is found to be monomorph. GDH shows two variants, two alleles may be involved. 6-PGD is under the control of two loci with two and two or three alleles respectively. IDH is coded for by two loci and five alleles as well. A further IDH-B allele is supposed. IDH-B enzymes seem to have a dimeric subunit structure. The IDH and 6-PGD loci act independently from each other, linkage tests gave no significant results. In bud tissue the same banding patterns as in seeds were found.

Key words: Abies alba, isozymes, linkage.

Zusammenfassung

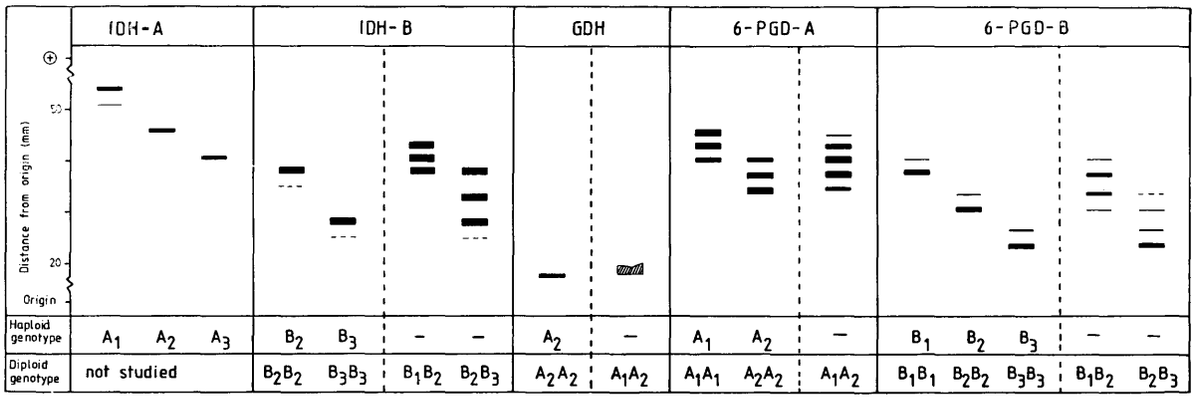
Die an vier Enzymsystemen der Weißtanne beobachteten genetischen Polymorphismen werden beschrieben. An Einzelbaumabsaaten wurde sowohl Megagametophyten- als auch Embryogewebe untersucht. SKDH zeigte keine Variation. Bei GDH wurden zwei Varianten beobachtet, möglicherweise liegen zwei Allele vor. 6-PGD wird von zwei Genorten mit je zwei bzw. zwei bis drei Allelen gesteuert. Auch bei IDH konnten zwei Loci mit insgesamt fünf Allelen nachgewiesen werden. Das Vorliegen eines weiteren IDH-B-Allels wird vermutet. Aktive Enzyme des Genorts IDH-B liegen als Dimere vor. Eine Kopplung zwischen den hier beschriebenen Genorten der Enzyme IDH und 6-PGD wurde nicht gefunden. Knospengewebe zeigte dieselben Bandenmuster wie Samengewebe.

Introduction

The species *Abies alba* MILL. is found to have its natural range throughout Central and Southern Europe. Being, besides Norway spruce, the most common conifer species in the south of Western Germany it is of considerable ecological and economic importance. However, knowledge regarding the genetic differentiation of this species to date is very poor (GÜRTH, 1982; LARSEN, 1986).

In view of this efforts should also concentrate on an elucidation of basic genetic features of this species. For this purpose single tree seed collections were utilized to study the genetic control of isozyme systems.

Figure 1. — Isozyme banding patterns found in haploid and diploid tissues of silver fir.



In this paper results concerning the genetic control of some enzyme systems will be presented. Data on the provenance variation and mating system parameters will be published elsewhere (SCHROEDER, 1989; SCHROEDER, in press).

Materials and Methods

The seed material originated predominantly from populations throughout the German natural range of silver fir. Single tree seed lots were available from 243 mother trees. In some cases in addition buds were collected from the same trees. Until use seeds were stored at -5^0 C.

Horizontal starch gel electrophoresis was carried out to detect isozyme polymorphisms of the enzyme systems IDH (EC 1.1.1.42), 6-PGD (EC 1.1.1.44), GDH (EC 1.4.1.2) and SKDH (EC 1.1.1.25). Gel and electrode buffers and staining mixtures were modified from SHAW and PRASAD (1970) and BERGMANN (pers. comm.). Staining for SKDH was modified from LINHART *et al.* (1981). Gels were prepared at 13% w/v starch (Sigma Chemie GmbH) and 2% w/v sucrose in a 0.02 M TRIS citrate buffer pH 8.0. Single endosperms or embryos were homogenized in 50 μl to 100 μl of cold TRIS buffer (0.1 M TRIS-HCl pH 7.0, 7 mM β-mercaptoethanol, 3% w/v PVP-40). Freshly prepared crude homogenates were absorbed onto filter paper wicks which were inserted into a cathodal positioned slice across the gel. Electrophoresis was carried out at 4^0 C applying 9 V/cm for 5 hours. After the first 5 minutes the wicks were removed. The electrode buffer was a 0.15 M TRIS citrate buffer at pH 8.0. After the run gels were sliced horizontally and incubated in the appropriate staining solution at 35^0 C in the dark for one hour. Staining solutions were based on 0.05 M TRIS HCl buffer pH 8.5 with 0.04 mM NBT and 0.01 mM PMS. Concentrations for substrates were 1.5 to 2.3 mM, for coenzymes were 0.08 to 0.15 mM, for $MgCl_2$ (IDH and 6-PGD) were 0.15% w/v and for ATP (GDH) were 0.18 mM.

The genetic control of isozymes was postulated from banding patterns observed in megagametophytes and embryos of wind-pollinated single tree seeds. Megagametophyte segregation data of putative heterozygote maternal trees were tested for confirmation to the expected 1:1 Mendelian ratio by chi-square analysis. Nomenclature follows the literature: Capital letters refer to gene loci; alleles are numbered according to decreasing electrophoretic mobility versus the anode.

Linkage between loci was tested using a binomial estimator for calculation of the recombination frequency among gametes of double heterozygote mother trees (RUDIN and EKBERG, 1978; NORDHEIM *et al.*, 1983). If a locus had more than two alleles they were pooled to two alleles in a way that a maximum level of heterozygosity was maintained.

Results

Figure 1 shows isozyme banding patterns. The same patterns were also found in buds.

Shikimate dehydrogenase

Among all single trees and populations studied the isozyme system SKDH was found to be monomorphic, presenting one single band in all tissues analyzed.

Glutamate dehydrogenase

GDH also seems to be a rather invariable system showing one very slowly migrating band on starch gels. However, a differing variant was also found: the enzyme zone is more broad and diffuse and less intensively stained. Up to now it only appeared in diploid tissues, so that the genetic background could not be elucidated. Preliminarily it is assumed that this type characterizes a heterozygote with a second faster allele being involved.

Isocitrate dehydrogenase

IDH activity was found in two different zones. In the faster migrating one (zone A) each megagametophyte exhibited one of 3 different variants, consisting of one (A_2, A_3) or two (A_1) bands. Among megagametophytes of trees which show two different variants a 1:1 segregation ratio was found. According to this segregation data a one-locus-three-alleles model is assumed. Unfortunately up to now this locus could not be studied in diploid tissue because the staining intensity was too weak and there were special problems in interpreting patterns of heterozygotes.

Zone B showed two different patterns in haploid tissue each consisting of one very strong and one weak band. The latter sometimes was missing. All trees with megagametophytes of both types showed a 1:1 segregation ratio among them indicating the presence of one locus with two alleles (B_2, B_3). Embryo tissue exhibited two further triple-banded variants, designated B_1B_2 and B_2B_3 in *Figure 1*. B_2B_3 is found among the progeny of each mother tree studied and shows both bands of the megagametophyte variants as well as one additional band occuring midway between them. These data led to the conclusion that there is one IDH-B locus with at least two condominant alleles. Owing to the presence of a hybrid band with intermediate electrophoretic mobility in diploid tissues the active enzyme seems to have a dimeric structure.

Table 1. — Megagametophyte segregation of double heterozygotes and linkage test for four enzyme loci in silver fir.

Enzyme loci	Tree no.	Endosperms observed				Segregation		Linkage			
		11	12	21	22	χ^2_I(1df)	χ^2_{II}(1df)	n	k	recombination frequency	χ^2_L(1df)
IDH-A : IDH-B	5	7	11	8	12	0.11	1.68	38	19	0.50	0.00
	7	12	7	10	4	0.76	3.67	33	16	0.49	0.03
	12	6	9	10	14	2.85	1.26	39	19	0.49	0.03
	14	9	10	11	4	1.06	1.06	34	13	0.38	1.88
	15	10	12	10	5	1.32	0.24	37	15	0.41	1.32
	19	8	9	10	8	0.03	0.03	35	16	0.46	0.26
	23	11	8	9	9	0.03	0.24	37	17	0.46	0.24
IDH-A : 6-PGD-A	14	9	10	8	7	1.06	0.00	34	16	0.47	0.12
	15	11	12	12	4	1.26	1.26	39	15	0.38	2.08
	20	8	9	9	10	0.11	0.11	36	18	0.50	0.00
IDH-A : 6-PGD-B	5	7	11	9	13	0.40	1.60	40	20	0.50	0.00
	6	12	8	9	7	0.44	1.00	36	17	0.47	0.11
	12	4	12	12	12	1.60	1.60	40	16	0.40	1.60
IDH-B : 6-PGD-A	14	10	18	12	14	0.22	0.02	41	20	0.49	0.02
	15	8	9	9	14	0.10	0.90	40	16	0.40	1.60
	17	11	11	10	9	0.07	1.85	54	24	0.44	0.67
	18	9	10	14	7	0.90	0.90	40	18	0.45	0.40
IDH-B : 6-PGD-B	5	7	10	9	14	0.90	1.60	40	21	0.48	0.10
	11	9	13	9	8	0.64	0.23	39	17	0.44	0.64
	12	5	11	11	13	1.60	1.60	40	18	0.45	0.40
6-PGD-A : 6-PGD-B	13	7	16	10	18	0.49	5.67+	51	25	0.49	0.02

n = number of gametes
k = number of observations in the smaller of the two classes 11 + 22 and 12 + 21
+ = significance at the 5% level

The other pattern of zone B was always combined with allele B_2 in the corresponding endosperm and was regularly triple-banded like B_2B_3. It is assumed that this is the phenotype of a further heterozygote B_1B_2 with allele B_1 being the fastest migrating band of this zone. However, this cannot be proved until allele B_1 will also be found in the endosperm.

6-Phosphogluconate dehydrogenase

Two overlapping zones of activity were found on gels stained for 6-PGD. In zone A megagametophytes banded with two triple-banded variants. Additionally in diploid tissue there was found a third pattern consisting of five bands in precisely the same positions as the single bands of the megagametophyte variants. The occurence of this pattern showed just the same characteristics as the IDH-B_2B_3 pattern. Together with a 1:1 segregation ratio among megagametophytes of single trees a diallelic locus 6-PGD-A is assumed.

Zone B consists of bands that differ in staining colour and that are not influenced by any segregation within zone A, both zones are overlapping. In megagametophytes three variants are found. Two of them (B_1 and B_2) are segregating in a 1:1 ratio. One tree however (*table 1*, no. 13) showed a significant deviation from this ratio. For B_3 being only found in bulked seed lots no segregation data are available. Therefore a second 6-PGD gene locus B with at least two alleles can be postulated with the probability of the presence of a further allele B_3.

Linkage studies

Four loci were compared. Results for 13 double heterozygote maternal trees are given in *table 1*. Values of recombination frequency ranged between 0.38 and 0.50 and did not indicate strong evidence for the existence of linkage at the given sample numbers. Nevertheless for each combination of loci separate x^2-tests for allele segregation as well as for the presence of linkage were done (Mather, 1951; Rudin and Ekberg, 1978). Only one tree showed a significant value for segregation but not for linkage. Therefore it can be assumed that there is no linkage between these four loci in *Abies alba*.

Discussion

Megagametophyte segregation analysis has often been used to postulate the genetic control of enzymes in conifers (Mejnartowicz and Bergmann, 1977; Rudin and Ekberg, 1978; Moran *et al.*, 1980; Neale and Adams, 1981; Cheliak and Pitel, 1985). However, isozyme data of *Abies alba* are scarcely found in the literature. Therefore the above presented data have to be compared to those of other conifer species.

In *A. alba* SKDH was found to be monomorph, whereas variation in this system is reported for several other tree

species (LINHART *et al.*, 1981; SZMIDT and YAZDANI, 1984; PITEL *et al.*, 1987).

GDH showed very little variation. This is in accordance with all other conifer species studied up to now. One diallelic GDH locus is also described for *Abies balsamea* (NEALE and ADAMS, 1981), *Picea spec.* and *Pinus spec.* (LUNDKVIST, 1979; MORAN *et al.*, 1980; YEH and EL-KASSABY,, 1980; WOODS *et al.*, 1983; CHELIAK and PITEL, 1984; CHELIAK, 1985; PITEL *et al.*, 1987).

The enzyme systems IDH as well as 6-PGD were found to be under the genetic control of two loci with two and three alleles each. MOLLER (1986) also found two IDH loci and 5 alleles in *A. alba*. There seems to be more variation in IDH than found in numerous other conifer species, e.g. *Picea, Pinus, Larix, Pseudotsuga*. In general there is reported only one locus with one to three alleles (GURIES and LEDIG, 1978; O'MALLEY *et al.*, 1979; NEALE and ADAMS, 1981; CHELIAK and PITEL, 1984; and others). The same authors also describe a dimeric IDH quaternary structure like it is supposed in this investigation for *Abies alba*. The variation of 6-PGD in *A. alba* is comparable to data reported for other conifer species: in Picea as well as in Pinus several authors have found two loci and 4 to 6 alleles usually.

No linkage was found between four loci of the enzyme systems IDH and 6-PGD. This is in agreement with studies on other conifer species, e.g. by O'MALLEY *et al.* (1979; ponderosa pine) and EL-KASSABY *et al.* (1982; Douglas-fir).

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Genetic Variances and Covariances in Freezing Tolerance of Lodgepole Pine During Early Winter Acclimation

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Summary

Nine-year-old *Pinus contorta* trees representing nine families from each of four seed sources were used to assess genetic variances in cold hardiness during mid-winter and to evaluate the covariance between cold hardiness and tree height. Leaves from about 20 trees within each family were collected in late autumn when acclimation was well advanced and were frozen to five temperatures in a laboratory freezing chamber. Differences in freezing tolerance of families were statistically significant, but additive genetic variances and heritabilities were low. Consequently, genetic gains from tree improvement would accumulate slowly. Although a relatively high genetic correlation (0.74) linked freezing injury and height at age 7, the coefficient of genetic prediction was extremely low, 0.04. These statistics suggest that strong selection for rapid growth would result in a correlated decrease in mid-winter freezing tolerance, but the size of the decrease would be negligible.

Key words: Tree breeding, quantitative genetics, cold acclimation.