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Investigations on the Genetic Variation of Beech (*Fagus sylvatica* L.) in Bavaria

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Summary

Using isozyme analysis the microgeographic genetic variation of beech in Bavaria is evaluated. Justification, goals and methods of this study are presented. For 20 populations (old beech stands) allele frequencies, genetic multiplicity and diversity values and genetic differentiation values are presented. The mean number of alleles per locus was 2.51, the gene pool diversity, v, varied from 1.27 to 1.35. The genetic variation *within* the stands is quite large and the variation *between* populations low. Less than 2% of the total diversity can be attributed to genetic differences among populations of beech. The vast majority of allelic variation (98%) resides within individual stands.

Key words: Beech (*Fagus sylvatica* L.), Bavaria, isozyme analysis, genetic variation.

FDC: 165.3; 165.5; 176.1 *Fagus sylvatica;* (430).

Introduction

Polymorphic isoenzymes, whose genetic control is known, are increasingly being used to describe and quantify the genetic variation of forest tree populations (BERGMANN, 1991; MÜLLER-STARCK, 1991; MÜLLER-STARCK et al., 1992). This is also true for beech where the genetic control of numerous enzyme systems has been clarified (MÜLLER-STARCK and STARKE, 1993) and which can be implemented for a genetic inventory.

Up to now little differentiation *between* beech stands in central Europe has been found, however there is considerable variation *within* stands (see summary in MÜLLER-STARCK et al., 1992; PAULE, 1992; HATTEMER et al., 1994). In the southern distribution range of beech in Europe COMPS et al. (1991a and b) found a higher degree of differentiation than in the northern part. The authors suggested that this was due to more heterogeneous ecological conditions and an older age of the investigated stands. At 2 gene loci selection was found to correlate to climatic factors. Further correlation between genetic structure and provenance location could not be found. Selection processes, gene flow, mating system, historical factors are suggested as possible causes for the differentiation, although it was not possible to rank this factors according to their significance (COMPS et al., 1991a; PAULE, 1992). Little information on the pattern of variation within smaller, but heterogeneous regions is available. An inventory study on the variation of

beech stands in Nordrhein-Westfalen showed that the indigenous stands differentiated only slightly one from another but are clearly different from artificial, planted stands (TUROK, 1994). The conclusions drawn regarding the degree of genetic differentiation of beech are partially contradictory since some studies are based on only a few and not always the same gene loci (HATTEMER et al., 1994).

In this paper a study of the genetic variation of beech in Bavaria (South Germany) is presented.

Justification and Goals of the Study

Beech – *Fagus sylvatica* L. – is the most common broadleaved tree species in Bavaria, comprising 10% of the tree species composition. Beech grows here on a wide variety of sites from the lower elevation up to the higher elevation of the Bavarian/Bohemian Forest and the Alps. It is found as a dominant species as well as a subordinate species in mixture with conifers (RUETZ, 1994). As far as possible beech is regenerated naturally in Bavaria. In order to establish more natural, site adapted forests (away from pure coniferous forests) beech is increasingly planted on these conversion sites (SEITSCHEK, 1993). The choice of the correct, best adapted but also genetically variable (great adaptive potential) provenance is important for the stability of the future forests.

The beech population in Bavaria is largely indigenous, the phenotype however is not uniform. Beech from the „Steigerwald" (Northern Bavaria) is characterized by above average growth and straight stems (PREUHSLER and REBHAN, 1991). In the young provenance trials there is great differentiation in the growth rate, stem and branch form as well as in their phenological response (RUETZ, pers. communication). It is not known whether these provenances can be distinguished from another on the basis of their genetic structure.

According to the German Law on Forest Seeds and Plants there are nine different provenance regions for beech in Bavaria. The provenance regions were delineated primarily on site and ecological characteristics (soil and climate). Seed from stands within the regions may be mixed together in the respective year of harvest, a compromise with practicability for seeds and nursery man. It is not known yet, however, if there are genetic differences between stands from different provenance regions.

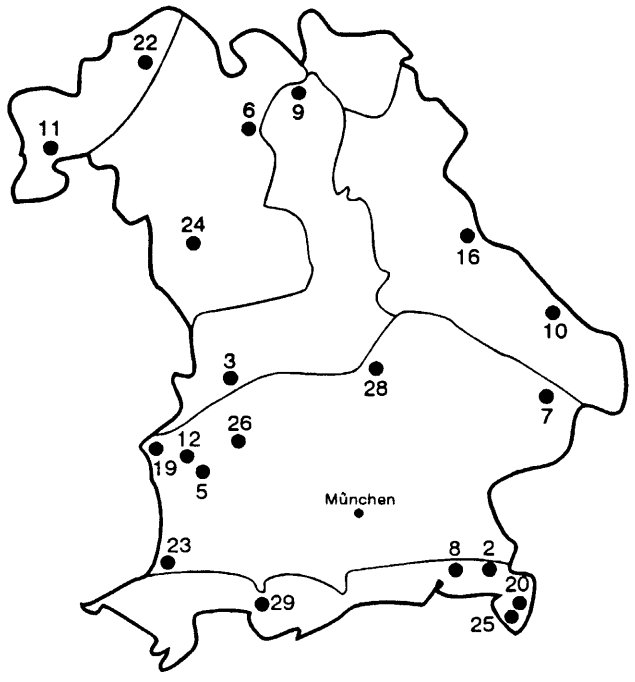

Fig. 1. – Location of stands in Bavaria.

Within the framework of this study we hope to obtain information on the genetic variation of beech in Bavaria through isoenzyme analysis with the following goals:

1) to characterize provenances of exceptional quality – so called „Sonderherkünfte" – and to find substitute provenances on a genetic basis;
2) to obtain data on the genetic structure of beech in natural forest reserves;
3) to provide basic data governing gene-conservation measures;
4) to obtain basic data for identification of forest reproductive material (beech) which would allow us to use isoenzyme analysis in control measures;
5) to find out from which refuges beech migrated into Bavaria following the last ice age;
6) to obtain more knowledge on the genetic variation of beech in Central Europe as a contribution to a uniform genetic inventory of beech in its entire native range.

Methods

Investigated material

Up to now 20 old beech stands, distributed throughout all regions in Bavaria were analysed. The location of these stands is shown in *figure 1*; the designation of stands (nr. in our evidence, name of forest district) is given in *table 2*. Of primary interest are the registered seed collection stands, including special and genetic-standard provenances (e.g. „Steigerwaldbuche").

Bud samples from 100 single trees per stand were taken. Until analysis the bud tissue was kept frozen at –75 °C.

Isoenzyme analysis

After extraction in a TRIS-HCl-buffer, pH = 7.2, the isozymes were separated by horizontal starch-gel electrophoresis. The technical procedure and the genetic interpretation of zymograms followed MÜLLER-STARCK and STARKE (1993) as well as MÜLLER-STARCK (1993). The enzyme systems and gene loci investigated are listed in *table 1*. On the basis of genotype and allele frequencies measures of genetic multiplicity, diversity and differentiation were computed in order to determine the extent of genetic variation within and between stands:

a) Multiplicitv and diversity measures

A/L = mean number of alleles per locus, including all studied loci;

v = gene pool allelic diversity (harmonic mean of single locus values, $v = (\Sigma_i p_i^2)^{-1}$,

v_{gam} = hypothetical gametic multilocus – diversity (product of single locus values of v),

δ_T = genetic differentiation (arithmetic mean of single locus values $\delta_T = N/(N-1) \cdot (1-\Sigma_i p_i^2)$ (for details see e.g. HATTEMER, 1991).

b) Differentiation among populations

D = allelic distance between pairs of populations according to GREGORIUS (1974),

G_{ST} = coefficient of gene differentiation among populations according to NEI (1977).

D_j,δ = differentiation measures of GREGORIUS and ROBERDS (1986).

Table 1. – Enzyme systems assayed, scored loci, number of alleles observed and separation buffer.

Enzyme system	E. C. No.	Scored loci	No. of alleles	Buffer system*)
Aconitase (ACO)	4.2.1.3	ACO-A ACO-B	2 3	A
Glutamate oxalacetate transaminase (GOT)	2.6.1.1	GOT-A GOT-B	2 3	B
Isocitrate dehydrogenase (IDH)	1.1.1.42	IDH-A	3	A
Leucine aminopeptidase (LAP)	3.4.11.1	LAP-A	5	C
Malate dehydrogenase (MDH)	1.1.1.37	MDH-B MDH-C	6 2	A
Menadione reductase (MNR)	1.6.99.2	MNR-A	4	C
NADH-dehydrogenase (NDH)	1.6.99.2	NDH-A	3	A
6-Phosphogluconate dehydrogenase (6PGDH)	1.1.1.43	6-PGDH-A 6-PGDH-B 6-PGDH-C	2 5 5	A
Phosphoglucoisomerase (PGI)	5.3.1.9	PGI-B	3	C
Phosphoglucomutase (PGM)	5.4.2.2	PGM-A	3	B
Shikimate dehydrogenase (SKDH)	1.1.1.25	SKDH-A	5	A

*) A = Tris-citric acid pH 7.0
B = ASHTON-system
C = POULIK-systen
(see MÜLLER-STARCK and STARKE, 1993)

Table 2. – Genetic variation within the 20 beech population

Population	Sample size N	Multiplicity A/L	Diversity v	Diversity v_{gam}	Differentation T
2 Siegsdorf	100	2,44	1,299	133,25	0,232
3 Kaisheim	102	2,44	1,350	235,31	0,261
5 Mindelheim	100	2,93	1,324	188,32	0,247
6 Burgebrach	103	2,63	1,404	140,46	0,221
7 Griesbach	118	2,75	1,357	259,71	0,265
8 Marquartstein	101	2,13	1,273	88,28	0,217
9 Lichtenfels	68	2,38	1,321	168,53	0,247
10 Zwiesel	106	2,56	1,349	249,32	0,261
11 Altenbuch	97	2,31	1,329	198,74	0,253
12 Krumbach	101	2,56	1,332	197,36	0,252
16 Waldmünchen	102	2,75	1,338	218,23	0,255
19 Weißenhorn	104	2,25	1,302	133,57	0,235
20 Berchtesgaden I	99	2,75	1,318	168,02	0,244
22 Bad Kissingen	101	2,56	1,333	197,72	0,252
23 Kempten	100	2,50	1,353	243,61	0,263
24 Ansbach	99	2,56	1,339	227,67	0,256
25 Berchtesgaden II	103	2,63	1,314	156,19	0,241
26 Biburg	111	2,25	1,331	177,24	0,251
28 Siegenburg	100	2,44	1,341	208,98	0,257
29 Füssen	100	2,63	1,348	232,27	0,261
Mean	-	**2,51**	**1,328**	-	**0,250**

Results

Genetic variation within populations

Of the 16 investigated gene loci 12 were polymorphic in all populations, namely ACO-B, GOT-A, GOT-B, IDH-A, LAP-A, MDH-B, MDH-C, MNR-A, 6-PGDH-A, 6-PGDH-B, 6-PGDH-C and PGM-A. The allele frequencies are given in Appendix 1. The gene locus NDH-A was polymorphic only in 2 populations, but the second allele variant (A_3 in pop. nr. 6 resp. A_1 in pop. nr. 22) appeared only with a frequency of 1%. Low variability was also shown by the gene locus PGI-B; eleven populations were fixed on the allele B_2, in the remaining populations a second allele (B_3) had a frequency of only 0.5% to 3.0%. For ACO-A and SKDH-A similar results were found with the main allele being present in frequencies of 96% to 100%. Well defined major polymorphisms were observed in all populations for the loci GOT-B, IDH-A, LAP-A, MDH-C and PGM-A.

In all a total of 57 allelic variants were found at the 16 loci. Of these however 20 had a frequency under 3%. Thus it is problematic to include these in a comparison between populations, since the probability of an allele to be discovered in a sample size of 100 is $\alpha = 0.056$ at 95% significance level (GREGORIUS, 1980).

The mean number of alleles per locus (values of A/L in *Tab. 2*) summed over all populations was 2.51. The values of gene pool diversity v vary from 1.273 to 1.357, (mean value = 1.328), values of hypothetical gametic multilocus-diversity v_{gam} between 88.28 and 259.71. The mean gene pool differentiation was 25%. The homogeneity of differentiation values was surprising; δ_T value varied between 22% and 26.5% for all populations, but 16 populations had values between 24% and 26% (*Tab. 2*).

Genetic differentiation

With 2 exceptions at the gene locus LAP-A and 1 exception for GOT-B, the same allele shows the highest frequency in all populations. Specific is the allelic distribution at the gene locus LAP-A, where three alleles occur with approximately the same frequency in all populations.

Distinct differences in the allelic distribution are found between populations at the gene loci ACO-B (B_3 between 63.9% and 90.4%), GOT-B (B_3 between 49.5% and 74.8%) and PGM-A (A_3 between 47.6% and 75.5%).

The gene pool genetic distances between pairs of populations range from 3.1% to 8.7%, whereby more than half the values lie between 5% and 6%. These values suggest only slight differences between populations at the gene pool level. A correlation between "gene pool distances" and geographical proximity was not found. The values show a random distribution. No one of the populations has constantly higher genetic distances than the others.

The values calculated for the parameter Dj (Dj = differentiation among investigated stands) indicate a low differentiation level. Substantial variation exists among loci; As it is illus-

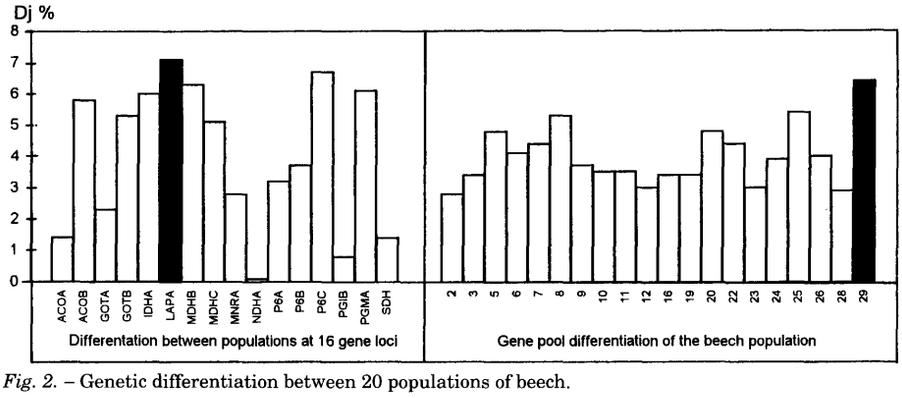

Fig. 2. – Genetic differentiation between 20 populations of beech.

trated in *figure 2* higher differentiation is found at the loci LAP-A, IDH-A, MDH-B, PGM-A and 6-PGDH-C. The mean gene pool differentiation in the whole investigated area is $\delta = 4.0\%$. The least differentiated populations over all loci are: pop. 2 Siegsdorf (Dj = 2.8%) and pop. 28 Siegenburg (Dj = 2.9%). Only 1 population, namely 29 Füssen situated at high elevation (1300 m) in the Alps, is clearly higher differentiated (Dj = 6.4%) (see *Fig. 2*).

Individual locus and average values of NEI's (1977) diversity statistics are listed in *table 3*. In this table H_T represents the total gene diversity over all populations based on average allele frequencies for each locus, H_S the average gene diversity within populations; $D_{ST} = H_T - H_S$. G_{ST} is the relative amount of gene diversity due to differences among populations ($G_{ST} = D_{ST}/H_T$). Seven of 16 loci (ACO-B, GOT-B, IDH-A, LAP-A, MDH-C, 6-PGDH-C and PGM-A) showed higher levels of diversity within populations, but the amounts were also very close to values shown by all populations considered together (H_T), resulting in diversities attributable to differences among populations (D_{ST}) which were not much larger than D_{ST} values for loci exhibiting moderate levels (MDH-B, MNR-A, 6-PGDH-A, 6-PGDH-A) or low levels (ACO-A, GOT-A) of diversity. The low level of differentiation is manifested in low G_{ST} values (from zero to 3.5%) and an average G_{ST} of 1.9%. This means that less than 2% of the total diversity detected in all the samples can be attributed to genetic differences among populations of beech. Thus, the vast majority of allelic variation (98%) resides within individual stands.

Table 3. – Gene diversity estimates for 16 loci averaged over 20 populations of bleech.

Locus	Total (H_T)	Within population (H_S)	Among population (D_{ST})	G_{ST}
ACOA	0,030	0,029	0,001	0,033
ACOB	0,334	0,325	0,009	0,027
GOTA	0,087	0,086	0,001	0,011
GOTB	0,465	0,457	0,008	0,017
IDHA	0,411	0,402	0,009	0,022
LAPA	0,672	0,664	0,008	0,012
MDHB	0,263	0,258	0,005	0,019
MDHC	0,407	0,400	0,007	0,017
MNRA	0,132	0,130	0,002	0,015
NDHA	0,001	0,001	0,000	0,000
6PGDHA	0,176	0,173	0,003	0,017
6PGDHB	0,114	0,110	0,004	0,035
6PGDHC	0,442	0,435	0,006	0,014
PGIA	0,013	0,013	0,000	0,000
PGMA	0,453	0,443	0,009	0,021
SKDHA	0,024	0,024	0,000	0,000
Mean	**0,252**	**0,247**	**0,005**	**0,019**

Discussion

The goal of our study is to determine the degree of genetic variation within and between the indigenous beech populations from Bavaria. Having analyzed 20 populations we have found that the genetic variation *within* the stands is quite large and the variation *between* populations low. Similar results have been found also by other investigations for beech (TUROK, 1994; HATTEMER et al., 1994, review in MÜLLER-STARCK et al., 1992).

In beech stands of Nordrhein-Westfalen (Western Germany) (TUROK, 1994) and Rheinland-Pfalz (Western Germany) (STARKE et al., 1995) for example only slightly higher values of variability (e.g. A/L = 2.63) and diversity (e.g. v = 1.45) than those found here were reported; however the authors considered only a part of the gene loci included in our study.

In complete agreement with the results of the mentioned studies is the low value of differentiation among populations. Referring to the same gene loci, the beech (*Fagus sylvatica*) in Bavaria is less differentiated as for example the fir (*Abies alba*) (Dj = 6.0%, KONNERT, 1994) or the spruce (*Picea abies*) (Dj = 5.3%, KONNERT, unpublished). Only 2% of the genetic variation of beech is due to differences between populations. Within larger regions and especially in the southern part of the natural range of beech there appears to be a stronger differentiation between populations (PAULE, 1992; COMPS et al., 1991a).

Practical forestry would like to know if the valuable beech provenances – by their phenotypical appearance (as for example „Steigerwaldbuche") – have a characteristic genetic structure which can be distinguished by isoenzyme gene markers. To answer this question and to complete the data on genetic variation of beech in Bavaria further stands will be evaluated, including the provenances from Bavaria in the international beech provenance trial initiated in 1994 (MUHS and VON WÜHLISCH, 1995). Four forest natural reserves will also bee studied. In the montainous regions of the Northeastern Bavaria and the Bavarian Alps altitudinal gradients will be sampled to test for clinal variation.

The described project represents a detailed study on a relatively small portion of the natural range of beech. To provide an overall picture of genetic differentiation of beech (*Fagus sylvatica* L.), it is mandatory to link it with other studies on beech in its native range. Only then we can look into the history of migration of the species after the last ice-age and hopefully develop a practical means of identification to serve in commercial seed and plant transfer.

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Appendix 1. – Allele frequencies at 16 gene loci for 20 beech population from Bavaria.

NR	ACOA1	ACOA2	ACOA3	ACOB2	ACOB3	ACOB4	GOTA1	GOTA2	GOTB1	GOTB2	GOTB3	IDHA1	IDHA2	IDHA3	IDHA4	LAPA1	LAPA2	LAPA3	LAPA4	LAPA5
2	0.005	0.965	0.030	0.150	0.850	-	0.020	0.980	-	0.390	0.610	-	0.305	0.695	-	0.025	0.318	0.394	0.263	-
3	-	0.966	0.034	0.172	0.828	-	0.039	0.961	-	0.333	0.667	0.015	0.275	0.710	-	0.025	0.319	0.416	0.230	0.010
5	-	0.995	0.005	0.272	0.723	0.005	0.069	0.931	-	0.443	0.557	-	0.240	0.760	-	0.075	0.230	0.485	0.210	-
6	-	0.990	0.010	0.200	0.780	0.020	0.056	0.944	-	0.328	0.672	-	0.223	0.777	-	0.010	0.327	0.480	0.183	-
7	-	1.000	-	0.237	0.746	0.017	0.090	0.910	-	0.376	0.624	-	0.360	0.640	-	0.042	0.326	0.436	0.196	-
8	-	1.000	-	0.153	0.847	-	0.044	0.956	-	0.310	0.690	-	0.109	0.891	-	0.016	0.366	0.356	0.262	-
9	-	0.985	0.015	0.096	0.904	-	0.037	0.963	-	0.381	0.619	-	0.375	0.625	-	0.029	0.273	0.426	0.272	-
10	-	0.986	0.014	0.222	0.769	0.009	0.009	0.991	0.009	0.340	0.651	-	0.320	0.675	0.005	0.028	0.302	0.382	0.288	-
11	-	1.000	-	0.208	0.745	0.047	0.021	0.979	-	0.428	0.572	-	0.222	0.778	-	0.021	0.309	0.366	0.304	-
12	0.010	0.980	0.010	0.220	0.755	0.025	0.090	0.910	-	0.395	0.605	-	0.282	0.708	0.010	0.030	0.345	0.335	0.290	-
16	0.005	0.990	0.005	0.142	0.858	-	0.020	0.980	0.015	0.377	0.608	-	0.319	0.671	0.010	0.035	0.294	0.446	0.225	-
19	0.005	0.995	-	0.193	0.807	-	0.063	0.937	-	0.284	0.716	-	0.236	0.764	-	0.034	0.245	0.399	0.322	-
20	0.005	0.944	0.051	0.126	0.869	0.005	0.015	0.985	-	0.333	0.667	-	0.374	0.626	-	0.025	0.323	0.384	0.268	-
22	-	0.995	0.005	0.267	0.713	0.020	0.064	0.936	-	0.252	0.748	0.010	0.327	0.643	0.020	0.010	0.417	0.354	0.219	-
23	-	0.995	0.005	0.240	0.760	-	0.100	0.900	0.005	0.330	0.665	-	0.280	0.715	0.005	0.005	0.235	0.460	0.300	-
24	-	0.990	0.010	0.273	0.722	0.005	0.030	0.970	0.015	0.409	0.576	-	0.202	0.798	-	-	0.303	0.404	0.293	-
25	-	0.985	0.015	0.092	0.903	0.005	0.020	0.980	0.010	0.310	0.680	-	0.335	0.665	-	0.015	0.267	0.371	0.337	0.010
26	-	0.968	0.032	0.212	0.788	-	0.050	0.950	-	0.293	0.707	-	0.225	0.775	-	0.050	0.279	0.414	0.257	-
28	0.005	0.965	0.030	0.190	0.810	-	0.050	0.950	0.015	0.360	0.625	-	0.280	0.705	0.015	0.005	0.370	0.375	0.250	-
29	-	1.000	-	0.350	0.639	0.011	0.030	0.970	-	0.500	0.495	-	0.335	0.655	0.010	0.010	0.125	0.480	0.385	-

NR	MDHB1	MDHB2	MDHB3	MDHB4	MDHB5	MDHB6	MDHC1	MDHC3	MNRA1	MNRA2	MNRA3	MNRA4	NDHA1	NDHA2	NDHA3	6PGA1	6PGA2	6PGA3
2	0.085	0.010	-	-	0.855	0.050	0.278	0.722	0.005	0.945	0.015	0.035	-	1.000	-	-	0.950	0.050
3	0.031	0.064	-	-	0.789	0.113	0.304	0.696	0.005	0.912	-	0.083	-	1.000	-	-	0.956	0.044
5	0.015	0.010	0.060	-	0.850	0.065	0.330	0.670	-	0.960	-	0.040	-	1.000	-	-	0.944	0.056
6	0.068	0.015	0.028	0.010	0.874	0.005	0.180	0.820	-	0.873	0.034	0.093	-	0.990	0.010	-	0.908	0.092
7	0.083	0.004	0.017	-	0.896	-	0.263	0.737	0.004	0.890	0.017	0.089	-	1.000	-	-	0.852	0.148
8	-	-	-	-	0.980	0.020	0.347	0.653	-	0.866	0.045	0.089	-	1.000	-	-	0.926	0.074
9	0.125	0.007	-	0.007	0.816	0.044	0.221	0.779	-	0.941	-	0.059	-	1.000	-	-	0.853	0.147
10	0.057	-	-	0.014	0.872	0.057	0.307	0.693	-	0.925	0.005	0.070	-	1.000	-	-	0.825	0.175
11	0.052	0.015	-	0.005	0.825	0.103	0.263	0.737	-	0.900	-	0.100	-	1.000	-	-	0.918	0.082
12	0.075	0.010	-	-	0.875	0.040	0.250	0.750	-	0.960	-	0.040	-	1.000	-	-	0.930	0.070
16	0.088	0.025	0.010	-	0.833	0.044	0.238	0.762	-	0.926	0.015	0.059	-	1.000	-	-	0.892	0.108
19	0.067	-	-	0.005	0.909	0.019	0.212	0.788	-	0.942	-	0.058	-	1.000	-	-	0.894	0.106
20	0.071	0.025	-	0.020	0.884	-	0.335	0.665	-	0.949	-	0.051	-	1.000	-	-	0.929	0.071
22	0.054	0.025	-	-	0.812	0.109	0.257	0.743	-	0.970	-	0.030	0.005	0.995	-	-	0.950	0.050
23	0.025	0.056	-	-	0.828	0.091	0.265	0.735	-	0.945	-	0.055	-	1.000	-	-	0.900	0.100
24	0.086	0.043	-	0.005	0.844	0.022	0.278	0.722	0.015	0.960	-	0.025	-	1.000	-	-	0.894	0.106
25	0.010	0.024	-	-	0.820	0.146	0.404	0.596	-	0.917	0.010	0.073	-	1.000	-	-	0.845	0.155
26	0.081	0.018	-	-	0.838	0.063	0.221	0.779	-	0.937	-	0.063	-	1.000	-	-	0.897	0.103
28	0.075	0.015	-	-	0.820	0.090	0.336	0.664	-	0.930	-	0.070	-	1.000	-	0.005	0.875	0.120
29	0.010	0.030	0.005	-	0.865	0.090	0.390	0.610	-	0.945	0.015	0.040	-	1.000	-	-	0.915	0.085

NR	6PGB1	6PGB2	6PGB3	6PGB4	6PGB5	6PGC1	6PGC2	6PGC3	6PGC4	6PGC5	PGIB2	PGIB3	PGMA1	PGMA2	PGMA3	PGMA4	SKDA1	SKDA2	SKDA3	SKDA4	SKDA5
2	-	1.000	-	-	-	0.760	0.005	0.020	0.130	0.085	1.000	-	-	0.360	0.640	-	-	-	0.995	-	0.005
3	0.123	0.867	0.010	-	-	0.716	-	0.005	0.142	0.137	1.000	-	-	0.360	0.640	-	-	-	1.000	-	-
5	-	0.990	-	0.010	-	0.699	0.030	0.084	0.181	0.006	0.995	0.005	-	0.318	0.682	-	-	-	0.990	-	0.010
6	-	1.000	-	-	-	0.721	-	0.049	0.147	0.083	0.995	0.005	-	0.377	0.623	-	0.020	0.025	0.955	-	-
7	0.022	0.974	-	0.004	-	0.684	0.009	0.021	0.263	0.026	0.975	0.025	0.004	0.297	0.699	-	-	0.004	0.992	-	0.004
8	0.089	0.911	-	-	-	0.787	0.005	0.005	0.158	0.035	1.000	-	-	0.308	0.692	-	-	-	1.000	-	-
9	0.059	0.941	-	-	-	0.728	-	0.022	0.154	0.096	0.993	0.007	-	0.287	0.706	0.007	-	-	1.000	-	-
10	0.075	0.925	-	-	-	0.759	0.005	0.005	0.156	0.075	1.000	-	-	0.524	0.476	-	-	-	0.981	-	0.019
11	0.062	0.923	0.015	-	-	0.686	0.010	0.052	0.196	0.057	1.000	-	-	0.292	0.708	-	-	-	0.990	-	0.010
12	0.065	0.915	0.010	0.010	-	0.720	0.005	0.005	0.170	0.100	1.000	-	-	0.295	0.705	-	-	-	1.000	-	-
16	0.058	0.937	0.005	-	-	0.666	-	0.020	0.240	0.074	1.000	-	-	0.431	0.569	-	-	0.005	0.970	0.005	0.020
19	0.048	0.952	-	-	-	0.697	0.014	-	0.183	0.106	1.000	-	-	0.385	0.615	-	-	-	1.000	-	-
20	0.005	0.990	-	0.005	-	0.783	0.010	0.025	0.141	0.041	0.975	0.025	-	0.439	0.561	-	0.020	0.005	0.950	0.005	0.020
22	0.064	0.936	-	-	-	0.728	0.015	0.010	0.153	0.094	1.000	-	-	0.376	0.624	-	-	-	0.980	-	0.020
23	0.080	0.920	-	-	-	0.645	-	0.025	0.190	0.140	0.995	0.005	-	0.330	0.670	-	-	-	0.995	-	0.005
24	0.061	0.939	-	-	-	0.601	0.005	0.010	0.227	0.157	0.980	0.020	-	0.298	0.702	-	-	0.005	0.995	-	-
25	0.015	0.985	-	-	-	0.809	-	0.005	0.176	0.010	0.990	0.010	-	0.282	0.718	-	-	0.019	0.976	-	0.005
26	0.158	0.842	-	-	-	0.797	0.005	-	0.086	0.112	1.000	-	-	0.423	0.577	-	-	-	0.995	-	0.005
28	0.055	0.945	-	-	-	0.710	-	0.010	0.210	0.070	1.000	-	-	0.245	0.755	-	-	-	1.000	-	-
29	0.095	0.900	-	-	0.005	0.750	-	0.045	0.135	0.070	0.970	0.030	-	0.270	0.730	-	-	-	0.995	-	0.005

A Methodical Study to Improve the Isozyme Analysis for Identification of Clones of Tilia (Linden syn. Lime Tree)[1])

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Abstract

The methodical results obtained for a first isozyme study on linden are presented. These include the choice of plant material, the preparation of enzyme extracts as well as their efficient storage over longer periods of time. Tried and tested buffer systems are given for a variety of enzymes to separate isozymic forms by using starch gel electrophoresis. Moreover, an efficient microwave method for the preparation of starch gels is described.

In first practical applications, the combined zymograms of the enzyme systems tested were used to individually define the *Tilia cordata* clones in a seed orchard as well as to detect the clonal character of linden trees growing in an avenue along a country road.

Key words: Tilia cordata, small-leaved linden, isozyme analysis, starch gel electrophoresis, microwave method, clone identification.

FDC: 165.3; 165.441; 176.1 *Tilia cordata.*

Zusammenfassung

Die methodischen Ergebnisse einer erstmalig an Linden durchgeführten isoenzymatischen Untersuchung werden bezüglich der Wahl des Pflanzenmaterials, der Herstellung von Enzymextrakten sowie deren aktivitätserhaltenden Lagerung über längere Zeit dargestellt. Zur elektrophoretischen Trennung der Isoenzyme mittels Stärkegelelektrophorese werden erprobte Puffersysteme für eine Reihe von Enzymen angegeben. Zudem wird eine effiziente Mikrowellenmethode für die Herstellung von Stärkegelen beschrieben.

In ersten prazisbezogenen Anwendungen kamen Kombinationen von Zymogrammen der untersuchten Enzymsysteme zur individuellen Bestimmung von *Tilia cordata*-Klonen einer Samenplantage sowie zur Aufdeckung des Kloncharakters von Bäumen einer Lindenallee zum Einsatz.

Schlagwörter: Tilia cordata, Winterlinde, Isoenzymanalyse, Stärkegelelektrophorese, Mikrowellenmethode, Klonidentifizierung.

Introduction

Among the estimated 400 tree and shrub species of the *Tiliaceae* family, taxonomically classified in 30 to 40 genera and mostly restricted to the tropics, 10 monoecious deciduous tree species have their natural range in the temperate zones of the northern hemisphere. Only 4 of these species occur naturally in Europe, *i.e.* Caucasian linden (*Tilia dasystyla* STEV.), silver linden (*Tilia tomentosa* MOENCH.), small-leaved linden (*Tilia cordata* MILL.) and broad-leaved linden (*Tilia platyphyllos* SCOP.) (KRÜSSMANN, 1978). The latter 2 species are indigenous

[1]) Dedicated to Dr. G. H. MELCHIOR on his 70th birthday.