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Genetic variation of *Picea abies* in southern Germany as determined using isozyme and STS markers

Abstract: Over 50 populations of Norway spruce from Bavaria were analysed at 23 isozyme gene loci. The mean genetic distances between these populations were quite small. A geographical grouping could not be observed, and discrimination between provenances from high and low altitudes was not identifiable using this marker type, either. The only difference between spruce populations from South Bavaria and those from Northeast Bavaria is in the presence of some distinct rare alleles. The highest values for the genetic diversity were detected for spruce stands in Northeast Bavaria (Frankonian Forest). Using STS markers, further genes of the nuclear genome of *Picea abies* can be dealt with. The genetic differences found on the basis of ten STS markers between different *Picea abies* seed lots and/or seedling populations are generally 2–3 times greater than those found by means of isozyme gene markers. DNA markers turned out to be an appropriate and substantial addition or even more a suitable alternative to isozyme markers for analysing genetic variation and testing provenance identity. Their advantages consist in a markedly wider variation as well as in the enlarged genome segments investigated.

Additional key words: Norway spruce, genetic variation, isozymes, STS markers

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Introduction

One of the main objectives of the Bavarian Office for Forest Seeding and Planting in Teisendorf consists in determining the genetic variation of the main tree species in Bavaria (Southern Germany). Knowledge about the nature and frequency of genetic information in forests provides a basis for pursuing the research goals, e.g. for discriminating/classifying provenance regions, giving recommendations on provenances, ensuring proof of identity of forest reproductive material using genetic methods, as well as conserving genetic variation in commercial and natural forests in the long term.

Norway spruce (*Picea abies* (L.) Karst.) accounts for about 50% of the growing stock of the Bavarian state forests, thereof 10% in high mountains. Since the 15th century, large forest areas have been strongly overcut due to the saline industry, iron and glass production, alp cultivation, forests rights, and rafting operations. At the end of the 18th century, reconstruction of the overthinned forests was initiated, primarily by cultivating spruce. It can be supposed that seeds originating from lower regions were transferred to higher elevations, where the fructification of spruce was poorer. There is some evidence for the non-adaptability of those plants, e.g. strong snow breaks as well as the first results of genetic investigations (Schmidt-Vogt 1976; Ruetz et al. 1996).

As a consequence of former forestry practices, we have today in Bavaria a mixture of autochthonous and non-autochthonous, i.e. adapted and non-adapted,

spruce populations (e.g. Ruetz and Bergmann 1989). In the last centuries, with the increasing global warming, spruce as a tree species is becoming more and more at risk because of heavy infestations with pests (bark beetle) and a high storm hazard (e.g. Ammer 2007; Kölling 2007). Despite the considered reduction in the spruce-covered areas, the species still remains a basic element in the forests, especially in the mountainous mixed forests (e.g. Bentele 2007).

In 1995, investigations into the genetic variation of Norway spruce began at the Bavarian Office for Forest Seeding and Planting (ASP). First, isozyme gene markers were applied. When using this method, attention was focused on the genetic variation in the high-elevated regions of the Alps, among other things. In the development process of a new system for the proof of identity of forest reproductive material on the basis of genetic comparisons of reference samples, alternative genetic markers had to be found for Norway spruce that were cost-efficient and usable in standard applications. Thus, we examined nuclear STS-PCR markers, which are co-dominant inherited and refer to expressed DNA sequences.

Material and methods

Material

In order to study the genetic variation of Norway spruce in Bavaria, 34 populations from different sites and altitudes were selected in the Bavarian Alps (South), Bavarian Forest (East), and Franconian Forest (North) (for population designations and altitudes see Table 3). In these regions, Norway spruce is a native species. For each area, also closed neighbouring populations were deliberately chosen (indicated by different numbers with the same name in Table 3). The number of individuals sampled per population ranged between 50 and 100.

In order to detect the genetic variation in high-elevated populations, Norway spruce from six different stands in two transects situated in the Bavarian Alps was investigated (Table 1). In total, 620 dormant bud samples from adults and 731 bud samples from juvenile individuals were taken for the study.

Table 1. Norway spruce sampling: transects, plots, and sample size

Transect	Altitude (m)	Sample size	
		adult	juvenile
D1	1200	102	153
Oberammergau	1500	104	151
	1800	109	–
D2	1000	97	152
Berchtesgaden	1500	101	151
	1750	107	124

For the population analysis using isozyme markers versus STS markers, six seed lots originating from six seed stands were selected (see Table 5). One hundred seeds from each stand were germinated. The resulting seedlings provided enough plant material to isolate the enzymes as well as the DNA.

Methods

Isozymes used as gene markers in determining the genetic structures of Norway spruce were extracted from the meristem of dormant buds or from seedlings, and separated by horizontal starch-gel electrophoresis using different enzyme-specific buffer systems (Konnert and Maurer 1995). For the general study of the genetic variation of Norway spruce in Bavaria (34 populations) as well as for the study of the genetic variation along altitudinal transects, genotypes were recorded at 23 polymorphic enzyme gene loci, namely: *Aco-A*, *Gdh-A*, *Got-A*, *-B*, *-C*, *Idh-A*, *-B*, *Lap-B*, *Mdh-A*, *-B*, *-C*, *Mnr-A*, *-B*, *-C*, *Nadh-A*, *-B*, *6Pgdh-A*, *-B*, *-C*, *Pgi-A*, *-B*, *Pgm-A*, and *Skdh-A*. For comparison with STS markers, the number of loci was reduced to 18 (*6Pgdh-A*, *Pgi-A*, *Nadh-A*, *-B*, and *Gdh-A* were excluded).

For genotyping nuclear STS markers, the total genomic DNA was extracted from seed embryos using the Qiagen MiniPlant Kit. The 10 co-dominant STS markers that were used are listed in Table 2.

Table 2. Investigated STS markers and number of alleles

Primer	Restriction enzyme	No. of alleles	References
Pa0034	without restriction	5	Schubert et al. 2001
Pa0066	without restriction	3	Schubert et al. 2001
Pa0043	without restriction	4	Schubert et al. 2001
Pa0055	DraI	2	Schubert et al. 2001
SB51	AluI	2	Perry and Bousquet 1998
SB58	AluI	2	Perry and Bousquet 1998
SB70	HaeIII	2	Perry and Bousquet 1998
SB72	AfaI	2	Perry and Bousquet 1998
SB72	AluI	2	Perry and Bousquet 1998
SB42	without restriction	2	Perry and Bousquet 1998

Genomic PCR, product separation and identification of alleles were performed according to Schubert et al. (2001) and personal communication (M. Mengl, BfW Vienna).

Results

Genetic variation of Norway spruce in Bavaria determined using isozyme markers – a general inventory

The values of the genetic multiplicity, diversity measures, and observed heterozygosity for the 34 investigated Norway spruce populations are shown in Table 3. They exhibit large differences between populations. Multiplicity ranges from 1.65 in Zwiesel (1) to 2.96 in Bad Steben. In the Franconian Forest populations, the number of alleles seems to be somewhat higher than in the populations of the other regions. The genetic diversity, n_e, varies between 1.21 (Zwiesel 2, Kreut 1) and 1.282 (Zwiesel 5). The highest hypothetical gametic multilocus diversity, v_{gam}, was found in Zwiesel 5 with a value of 1296.4. The observed heterozygosity values vary from 17.5%

Table 3. Inter-population variation in 34 Norway spruce populations from Bavaria

No.	Population	Geographic coordinates		Multiplicity A/L	Diversity		Heterozygosity obs. H_o
		longitude	latitude		n_e	v_{gam}	
	Bavarian Alps (South Bavaria)						
1	Sonthofen (1)	10°18’	47°31’	2.47	1.274	817.4	0.219
2	Oberammergau (1)	11°02’	47°35’	2.26	1.237	365.6	0.196
3	Fall (1)	11°33’	47°33’	1.91	1.211	200.3	0.194
4	Kreuth (1)	11°46’	47°39’	2.08	1.210	203.2	0.175
5	Schliersee	11°52’	47°44’	2.13	1.229	348.2	0.190
6	Fall (2)	11°32’	47°33’	2.13	1.254	683.2	0.207
7	Siegsdorf	12°39’	47°49’	2.21	1.218	268.4	0.187
8	Sonthofen (2)	10°13’	47°30’	2.26	1.235	425.9	0.181
9	Oberammergau (2)	11°02’	47°35’	2.34	1.223	321.7	0.197
10	Oberammergau (3)	11°05’	47°35’	2.09	1.214	205.2	0.195
11	Oberammergau (4)	11°05’	47°36’	2.26	1.224	229.9	0.186
12	Oberammergau (5)	11°03’	47°35’	2.17	1.233	323.8	0.199
13	Füssen	10°42’	47°34’	2.17	1.251	532.2	0.188
14	Kreuth (2)	11°42’	47°38’	2.34	1.248	513.2	0.206
15	Bad Reichenhall (1)	12°52’	47°42’	2.65	1.246	465.0	0.204
	Frankonian Forest (Northeast Bavaria)						
16	Bad Steben	11°32’	50°25’	2.95	1.264	791.2	0.214
17	Nordhalben	11°31’	50°22’	2.52	1.257	643.4	0.209
18	Rothenkirchen (1)	11°18’	50°22’	2.74	1.245	513.0	0.201
19	Rothenkirchen (2)	11°17’	50°23’	2.65	1.238	410.5	0.196
20	Weißenstadt	11°53’	50°07’	2.61	1.235	374.2	0.195
21	Wunsiedel	11°59’	50°02’	2.83	1.246	475.1	0.201
22	Fichtelberg (1)	11°52’	50°00’	2.74	1.254	549.5	0.207
23	Fichtelberg (2)	11°59’	49°59’	2.74	1.253	543.8	0.207
24	Fichtelberg (3)	11°52’	49°59’	2.70	1.225	310.4	0.188
25	Fichtelberg (4)	11°58’	49°59’	2.70	1.256	545.5	0.208
26	Goldkronach	11°44’	49°59’	2.43	1.235	423.4	0.195
	Bavarian Forest (East Bavaria)						
27	Zwiesel (1)	13°19’	49°00’	1.65	1.214	243.8	0.180
28	Zwiesel (1)	13°20’	49°03’	2.13	1.210	233.4	0.177
29	Zwiesel (1)	13°13’	49°03’	2.43	1.221	273.2	0.185
30	Zwiesel (1)	13°14’	49°01’	2.65	1.242	445.8	0.196
31	Zwiesel (1)	13°19’	48°55’	2.43	1.282	1296.4	0.228
32	Zwiesel (1)	13°15’	48°58’	2.43	1.242	475.8	0.201
33	Neureichenau	13°45’	48°45’	2.17	1.235	484.1	0.194
34	Bodenmais	13°13’	49°01’	2.34	1.246	529.4	0.203

(Kreuth 1) to 22.8% (Zwiesel 5), with most values lying between 19 and 20%.

The pair-wise gene pool distances range between 1.3 and 3.2%, whereas 75% of the values are around 2%. Only in a few cases were single-locus distances statistically significant at the 95% level. Geographical grouping could not be observed, and discrimination between provenances from high and low altitudes was not identifiable using this marker type. Differences between spruce populations from South Bavaria and those from Northeast Bavaria could only be detected due to the presence of some rare alleles. The allele *6-PGDH-B3* was found in all populations in the Franconian Forest, in five of nine populations from the Bavarian Forest, but in only one of 15 populations in the Bavarian Alps.

Genetic variation of Norway spruce along elevation transects in the Bavarian Alps

Within the two altitudinal transects, the mean number of alleles per locus is very similar among the populations, and no trend is evident due to altitudinal aspects. In adult populations, the mean number of alleles per locus ranges between 2.22 and 2.35, with the overall mean of 2.29. In juvenile populations, the A/L-values are more heterogeneous and vary between 2.13 and 2.56, with the overall mean of 2.36 (Table 4).

Populations of transect D2-Berchtesgaden have a clearly higher hypothetical gametic diversity (v_{gam} between 388 and 441) than populations of transect D1-Oberammergau (v_{gam} between 202 and 280). The same holds for the observed heterozygosity: in transect D2-Berchtesgaden, H_o is higher than in transect D1-Oberammergau.

The differences in the level of genetic variation between adult and juvenile stands are very low in transect D1, whereas in transect D2 the diversity of juvenile populations is clearly lower than in adult populations.

As with allelic multiplicity, a correlation between altitude and the diversity or heterozygosity of *Picea abies* populations could not be observed.

Table 4. Genetic variation of Norway spruce adult and juvenile populations along two altitudinal transects in Bavarian Alps

Transect	Altitude (m)	Multiplicity A/L		Diversity				Heterozygosity obs., H_o	
				n_e		v_{gam}			
		adult	juvenile	adult	juvenile	adult	juvenile	adult	juvenile
D1	1200	2.35	2.22	1.23	1.23	242.1	285.1	0.179	0.185
Oberammergau	1500	2.35	2.47	1.24	1.23	280.4	280.5	0.189	0.167
	1800	2.26	xx	1.22	xx	202.3	xx	0.167	xx
D2	1000	2.30	2.43	1.25	1.24	388.4	334.4	0.199	0.194
Berchtesgaden	1500	2.26	2.56	1.25	1.24	410.1	343.6	0.193	0.193
	1750	2.22	2.13	1.25	1.24	440.8	330.9	0.202	0.190

Table 5. Genetic variation of Norway spruce seed populations from Bavaria determined by means of isozyme and STS markers

Population	Multiplicity, A/L		Diversity, n_e		Heterozygosity, H_o	
	isozymes	STS	isozymes	STS	isozymes	STS
Weißenhorn	2.5	2.7	1.23	1.56	17.5	33.9
Oberhamersbach	2.3	2.6	1.23	1.48	18.1	32.9
Bad Schussenried	2.4	2.8	1.23	1.51	19.0	33.6
Bodenmais	2.2	2.6	1.22	1.52	17.4	36.2
Altötting	2.2	2.9	1.22	1.47	17.7	33.7
Sonthofen	2.5	2.6	1.23	1.51	16.7	32.5

Table 6. Mean allelic distances between six Norway spruce populations from Bavaria. Above diagonal: distances determined by means of isozyme gene markers; below diagonal: distances determined by means of STS markers

Population	Weißenhorn	Oberhamersbach	Bad Schussenried	Bodenmais	Altötting	Sonthofen
Weißenhorn	xxx	2.4	3.2	2.7	2.6	3.2
Oberhamersbach	7.1	xxx	3.2	3.1	2.1	3.6
Bad Schussenried	8.7	7.9	xxx	2.5	2.4	4.1
Bodenmais	5.9	5.3	5.5	xxx	2.8	3.6
Altötting	8.4	5.3	8.2	7.4	xxx	2.6
Sonthofen	7.4	6.1	8.4	7.6	7.7	xxx

Genetic distances and genetic differentiation within the transects are low. For adult stands, the lowest distances (around 2.0%) in each transect are found between the low and the middle population. For juvenile populations, the genetic distances are more homogeneous, ranging from 2.2 to 2.7%. The genetic distances between the populations of different transects are somewhat larger: the mean values range between 3.3 and 5.0% for adult populations, and from 3.3 to 3.6% for juvenile populations.

Genetic differentiation is low, both for adult and juvenile populations. In transect D2, the D_j value is lowest (1.9%) for the adult population from the middle of the transect, and highest (3.5%) for that from its top. This indicates that the population situated in the middle has the largest proportion of common genetic information, while the population located highest displays the widest genetic differences.

The mean differentiation of the gene pool for all six populations is 2.5% for adult populations, and 2.4% for juvenile ones.

Analysis of genetic variation using isozyme markers versus STS markers

In Table 5, genetic parameters are surveyed separately for isozymes and STS markers for Norway spruce seed populations originating from six Bavarian seed stands.

All the values of genetic variation within populations are higher for STS markers, whereas the values of genetic multiplicity are only slightly higher (on average 2.7 vs. 2.35). The diversity values are about 25% higher if determined by STS markers (on average 1.51 vs. 1.23). STS markers reveal also much higher heterozygosity than isozyme markers do (on average 34% vs. 18%).

The differences found on the basis of ten STS markers between the six spruce seed lots are much wider than the differences based on isozyme analysis. This can be seen in Table 6 showing the genetic distance values as well as the differentiation values: 3.7% for isozymes and 5.6% for STS-markers.

The higher distance values obtained using STS markers result from the fact that all ten STS loci display variation, with only two loci (SB60 and SB72b+AluI) varying comparatively little (see Table 2). Regarding isozyme markers, null values at the monomorphic gene loci and the very low distance values at the loci with distinctly minor polymorphism are reflected in the mean values.

Discussion

The present study reveals that the major part of the allelic variation among populations of *Picea abies* in Bavaria resides within individual stands. Less than 3% of the total diversity detected in all samples is due to the genetic differences among populations. This finding corresponds with the results of investigations into Norway spruce in other areas of Central Europe (Tigerstedt 1973; Konnert 1991; Konnert and Franke 1991; Morgante and Vendramin 1991; Gömöry 1992). The differences in genetic variation between spruce stands in Bavaria are small. No trend corresponding with altitudinal aspects is observed for genetic multiplicity, diversity, or heterozygosity. This is remarkable since many of the today's stands have definitely been artificially established. Against this background, therefore, bigger differences were expected according to the source material.

As in other studies (Schubert et al. 2001), STS markers reveal much higher heterozygosity levels than isozyme markers do. This is a consequence of the marker-dependent frequency distribution of alleles. STS gene markers often have many alleles per locus (occurring with equal frequency per population), whereas isozyme gene markers show a clearly minor polymorphism for many loci in *Picea abies*. Thus, these markers may present a substantial addition or even an alternative to isozyme markers for the control of provenance identity (Konnert and Behm 2006), but also for other purposes such as genetic response to pollution stress (Riegel et al. 2001). The markedly higher variation and the enlarged genome segments investigated display their advantages. For *Picea abies*, there are about 22 isozyme gene loci available at present which can be interpreted confidentially; about 30% of them show little or no variation. It may be assumed that the potential of these markers is more or less exhausted. With STS markers, further genes of the nuclear genome of *Picea abies* can be dealt with.

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