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Genetic polymorphism of Polish strains of *Gremmeniella abietina* and *Brunchorstia pinea* var. *cembrae*

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Abstract: Thirty-three type A strains of *G. abietina* from diseased shoots or needles of *P. sylvestris*, *P. nigra* and *P. armandii* and three strains of *Brunchorstia pinea* var. *cembrae* from *P. mugo* were isolated from four regions of Poland differing with respect to climatic conditions. Genetic polymorphism of the mitochondrial small subunit rRNA (mtSSU rRNA), ribosomal RNA fragment including ITS1, 5.8S and ITS2 and glyceraldehyde phosphate dehydrogenase (GPD) gene was examined by the PCR-RFLP method. Genetic distance was ascertained with respect to *B. pinea* var. *cembrae* strains from *G. abietina* isolated from the examined pine species (average Nei coefficient 0.137). The smallest genetic distance occurred between the strain groups of *G. abietina* isolated from *P. nigra* and *P. armandii* (0.059) and *P. nigra* and *P. sylvestris* (0.061), whereas the highest occurred between the groups of strains deriving from *P. armandii* and *P. sylvestris* (0.096). The impact of geographic distance on genetic distance between groups of strains from individual regions has been shown. *G. abietina* strains originating from mountainous areas were more distanced genetically (on average 0.031) from populations from other regions (Nei genetic distance 0.023). The main factors influencing genetic differences of the pathogen were specificity with respect to the species of the host plant and climate conditions, whereas geographic distance had lesser significance.

Additional key words: scleroderris canker, host preferences, PCR-RFLP markers

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Introduction

Gremmeniella abietina (Lagerb.) Morelet is a pathogen varying morphologically, biochemically and genetically, causing shoot blight and cankers on branches and the main stem of more than 40 species of coniferous trees, including pine, spruce, fir, larch and juniper (Donaubauer 1974, Dusabenyagasani et al. 2002, Hantula and Müller 1997, Hellgren and Hogberg 1995, Lecours et al. 1994, Petrini et al. 1990). Particularly significant economic losses occur in natural pine stands, tree nurseries and in plantations in northern parts of Europe and Northern America and in eastern Asia (Bernier et al. 1994, Santamaria et al. 2005, Yokota et al. 1974).

The pathogen is divided into two varieties: var. *balsamea* Petrini et al. found locally in Canada on *Abies balsamea* (L.) Mill. and *Picea* spp. and var. *abietina* Petrini et al. occurring world-wide (Petrini et al. 1989). On the basis of morphology of conidia (Dorworth and Krywienczyk 1975, Petrini et al. 1989), serological research (Dorworth and Krywienczyk 1975), electrophoresis of soluble proteins (Lecours et al. 1994, Petrini et al. 1990), pectic enzymes and genetic markers (Dusabenyagasani et al. 2002, Hamelin et al. 1993, Hellgren and Hogberg

1995, Santamaria et al. 2005), the second variety was divided into three races: The North American race occurs in North America on *Pinus* spp., where it causes symptoms only in the lower part of the tree crown. The European race is present throughout Europe and northeastern North America on *Pinus* spp., also occasionally on *Picea, Larix* and other conifers. The symptoms which are caused by the European race can be observed on the whole tree crown. The Asian race occurs only in Japan, where it causes damages to *Abies sachalinensis* Mast (Laflamme 1993, Yokota 1975).

On the basis of genetic markers, three amplitypes of the European race of *G. abietina* were differentiated. The central amplitype was found throughout Europe and occurs from the Apennines in Italy northwards up to central Sweden. A cold-adapted amplitype (Alpine amplitype) has been recorded only in the Alps above 2,000 m elevation on *P. cembra* L., *P. mugo* Turra, *P. sylvestris* L. and *Larix lyalli* Parl. This biotype corresponds to *Brunchorstia pinea* var. *cembrae* M. Morelet (Morelet 1980). A third amplitype (northern amplitype) was found above the 66 degree latitude in Scandinavian countries in areas with deep, long-lasting snow cover on *P. sylvestris* and planted *P. contorta* Dougl. ex Loud. The European race in Fennoscandia is represented by two types: a) type A (large tree type = LTT) causes more severe damage in mature pines and is more widely distributed geographically and ecologically (Hantula and Müller 1997, Hellgren and Hogberg 1995, Santamaria et al. 2005, Uotila et al. 2006) and b) type B (small tree type = STT), which occurs most frequently at high latitudes. Symptoms caused by this biotype resemble those caused by the North American race (Karlman et al. 1994). Type A appears to be more pathogenic and causes damage to all sizes of pine. Type B kills seedlings smaller than 2 m in regions that have thick snow cover in winter.

G. abietina also plays an important role in Poland. The occurrence of fungi was reported from the end of 19th century (Schwarz 1895). In the 1980's it appeared epidemically in the northern part of country in a 200,000 hectare stand of young *P. sylvestris* L. forests (Kowalski 2000, Sierota 1998) and in 1979 in the Upper Silesian Industrial Region. It also occurs with a low degree in other parts of the country. It is particularly virulent among *P. nigra* Arn. introduced into industrial regions and with a lower degree among *P. sylvestris* and *P. strobus* L. (Kowalski 1987, Kowalski and Domański 1983). In Poland, *G. abietina* infects seedlings in nurseries as well as mature trees. Among mature trees, symptoms occur either on the entire tree crown or sometimes on the lower part. Pycnidia, and considerably more rarely apothecia, are produced on dead shoots of pines. Both conidia and ascospores are characterized by wide variation with respect to size, shape and number of septa (Kowalski 1997).

Genetic variability and identification of races and ecological types of *G. abietina* was examined by different methods, including protein electrophoresis (Petrini et al. 1990), differences in profiles of fatty acids (Müller and Uotila 1997), isozymes (Lecours et al. 1994), restriction fragment length polymorphism (RFLP) (Bernier et al. 1994), random amplified polymorphic DNA (RAPD) (Hamelin et al. 1993) or random amplified microsatellite (RAMS) (Hamelin et al. 1998, Kraj and Kowalski 2008). The PCR-RFLP method was applied for examining the polymorphism of variable fragments of ribosomal (ITS1, ITS2, IGS), mitochondrial and genomic (GPD, β-tubulin) DNA in a number of fungi species, including *G. abietina* (Dusabenyagasani et al. 2002, Hantula and Müller 1997, Hellgren and Högberg 1995, Kaitera et al. 1998, Lecours et al. 1994, Petrini et al. 1989, Petrini et al. 1990).

The objective of the presented research was an evaluation of genetic polymorphism of Polish strains of *G. abietina* and *Brunchorstia pinea* var. *cembrae* isolated from infected shoots and needles of various pine species deriving from selected regions of Poland.

Materials and methods

Fungal strains

Thirty-three strains of *Gremmeniella abietina*, classified as the A biotype by the RAMS method (Kraj and Kowalski 2008), and three strains of *Brunchorstia pinea* var. *cembrae* isolated from *Pinus mugo* in the Tatra National Park in 1994 were used. The *G. abietina* strains were isolated from *P. sylvestris*, *P. nigra* and *Pinus armandii Franch.* in four regions of Poland (Table 1, Fig. 1) between 1980 and 2005. The regions differed in geographic location and elevation as well as in characteristic climatic features, such as average annual temperature, annual sum of precipitation, average annual humidity and average thickness and number of days of snow cover (Table 2). Data regarding the origin of strains is provided by Kraj and Kowalski (2008).

The cultures were isolated from diseased or recently killed shoots and needles which were discoloring from the base. From each sample, 6–12 fragments of shoots or the same amount of fragments from basal sections of needles were collected. The samples were surface sterilized with 96% ethanol for 1 minute, then in 4% sodium hypochlorite for 5 minutes and 96% ethanol for 30 seconds. After that, the surface layer of bark was removed. Shoot fragments with dimensions of 5 × 2 × 2 mm or 5 mm sections of needles were placed in Petri dishes with 2% malt extract (MEA, 20 g l^{-1} malt extract Difco, Sparks, MD, USA, 15 g l^{-1} agar Difco) supplemented with 100 mg l^{-1} streptomycin sulphate. From several colonies of *G. abietina*, one was randomly selected and subjected to molecular analysis.

Table 1. Origin and characteristic of *Brunchorstia pinea* var. *cembrae* (No. 1–3) and *Gremmeniella abietina* (No. 4–36) isolates examine

No.	Strain No.	Host	Origin			Age of tree, plant organ	Collection
			For. District	Region	Coordinates		
1	HMIPC 16040	*P. mugo*	TNP		49°18', 19°57'	dead shoot	9.12.1994
2	HMIPC 16041	*P. mugo*	TNP		49°18', 19°57'	dead shoot	9.12.1994
3	HMIPC 16043	*P. mugo*	TNP		49°18', 19°57'	dead shoot	9.12.1994
4	HMIPC 16612/1	*P. armandii*	Krynica		49°50', 20°57'	nursery, 2 years old, dead shoot	22.06.1999
5	HMIPC 16612/2	*P. armandii*	Krynica	I	49°50', 20°57'	nursery, 2 years old, dead shoot	22.06.1999
6	HMIPC 16612/3	*P. armandii*	Krynica	I	49°50', 20°57'	nursery, 2 years old, dead shoot	22.06.1999
7	HMIPC 17073	*P. nigra*	Miechow	II	50°21', 20°01'	26 years old, dead shoot	25.05.2002
8	HMIPC 17073/1	*P. nigra*	Miechow	II	50°21', 20°01'	27 years old, dead shoot	25.05.2002
9	HMIPC 17073/2	*P. nigra*	Miechow	II	50°21', 20°01'	26 years old, dead shoot	25.05.2002
10	HMIPC 17073/3	*P. nigra*	Miechow	II	50°21', 20°01'	26 years old, dead shoot	25.05.2002
11	HMIPC 17073/4	*P. nigra*	Miechow	II	50°21', 20°01'	26 years old, dead shoot	25.05.2002
12	HMIPC 17073/5	*P. nigra*	Miechow	II	50°21', 20°01'	26 years old, dead shoot	25.05.2002
13	HMIPC16616/1	*P. nigra*	Chelm	III	51°08', 23°29'	25 years old, dead shoot	22.06.1999
14	HMIPC 16616/2	*P. nigra*	Chelm	III	51°08', 23°29'	25 years old, dead shoot	22.06.1999
15	HMIPC 16616/3	*P. nigra*	Chelm	III	51°08', 23°29'	25 years old, dead shoot	22.06.1999
16	HMIPC 16616/4	*P. nigra*	Chelm	III	51°08', 23°29'	25 years old, dead shoot	22.06.1999
17	HMIPC 16616/4a	*P. nigra*	Chelm	III	51°08', 23°29'	25 years old, dead shoot	22.06.1999
18	HMIPC 16616/4b	*P. nigra*	Chelm	III	51°08', 23°29'	25 years old, dead shoot	22.06.1999
19	HMIPC 16549a	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	26 years old, dead shoot	29.07.1998
20	HMIPC 16676	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	15.03.2000
21	HMIPC 16678/1	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	80 years, 8 years, dead shoot	18.05.2000
22	HMIPC 16678/2	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	18.05.2000
23	HMIPC 16678/3	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	18.05.2000
24	HMIPC 16678/4	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	18.05.2000
25	HMIPC 16679/1	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	18.05.2000
26	HMIPC 16679/2	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	18.05.2000
27	HMIPC 16679/3	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	18.05.2000
28	HMIPC 16679/7	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	18.05.2000
29	HMIPC 16679/8	*P. sylvestris*	Swierklaniec	II	50°26', 18°55'	10 years old, dead needle with pycnidia	18.05.2000
30	HMIPC 16495/1	*P. sylvestris*	Kozienice	III	51°35', 21°32'	nursery , 2 years old, dead shoot	15.05.1996
31	HMIPC 16495/2	*P. sylvestris*	Kozienice	III	51°35', 21°32'	nursery, 2 years old, dead shoot	15.05.1996
32	HMIPC 16575A	*P. sylvestris*	Osie	IV	53°35', 18°20'	nursery, seedling 1 year old, dead shoot	27.06.1998
33	HMIPC 16594	*P. sylvestris*	Osie	IV	53°35', 18°20'	nursery, seedling 1-year old, conidia, multispore culture	30.06.1998
34	HMIPC 16343	*P. sylvestris*	Czarne	IV	53°41', 16°56'	30 years old, dead shoot	7.09.1996
35	HMIPC 16503	*P. sylvestris*	Biala	IV	53°02', 16°32'	2 years old, dead shoot, natural afforestation	16.05.1997
36	HMIPC 16338	*P. sylvestris*	Gwda	IV	53°44', 16°47'	30 years old, local necrose on 2 years shoot (formerly agricultural ground)	7.09.1996

TNP – Tatra National Park

Molecular Analyses

Genomic DNA was extracted as described by Carlson and others (1991) with modification by Kraj and Kowalski (2005). Genetic polymorphism of strains was determined using the PCR-RFLP method. Three DNA fragments (mitochondrial small subunit rDNA genes – mtSSU rDNA, ribosomal RNA fragment including: ITS1 (Internal Transcribed Spacer 1), 5.8S and ITS2 (Internal Transcribed Spacer 2) and the glyceraldehyde phosphate dehydrogenase gene – GPD) were amplified using the following primer pairs: MS1-MS2 (Glass and Donaldson 1995), ITS1-ITS2 (Innis et al. 1990) and GPD3'in-GPD5'ex2 (Dusabenyagasani et al. 2002). The amplification reaction was performed in 50 μl of a reaction mixture,

Fig. 1. Schematic map of Poland showing *Brunchorstia pinea* var. *cembrae* and *G. abietina* origin regions, I–IV – region sign

whose composition was as follows: PCR buffer, $MgCl_2$ 2.5 mM (in the case of GPD 3.5 mM), dNTP 200 μM (Fermentas), Q solution (Qiagen) in a concentration recommended by the producer, primers: in the case of mtSSU rDNA 1 μM, ITS 2 μM, GPD 0.5 μM, Taq DNA polymerase 2 U (in the case of GPD 4 U) (Qiagen), DNA 20 ng. The PCR reaction was conducted in a Biometra T3 thermocycler programmed as follows: an initial denaturation at 94°C for 5 min. followed by 36 cycles of denaturation at 94°C for 1 min., annealing for 120 sec. at variable temperatures, depending on the DNA fragment (mtSSU rDNA – 50°C, ITS1-5.8S-ITS2 – 56°C, GPD – 62°C), elongation at 72°C for 3 min., extended to 7 minutes in the final cycle. The amplification results and the length of products were checked by electrophoresis in a 1.5% agarose gel (Prona).

The DNA fragments were purified by precipitation: 10% 3 M acetate buffer (pH 5.2) was added followed by two volumes of 96% ethanol at –20°C. After 24 hours at –20°C the DNA was centrifuged (15 minutes at 18,000 rpm, 4°C), the pellet was rinsed with 70% ethanol, dried at 37°C for 15 minutes and dissolved in a 20 µl of TE buffer.

For digestion of DNA fragments, eight restrictive enzymes were used: AluI, Bsp143I, Hin6I, HinfI, SsiI, TaiI, TaqI, Tru1I (Fermentas). Digestion of DNA was conducted in 5 µl of reaction mixture consisting of: 1 µg DNA, 1U of a restriction enzyme and buffer for 2 hours under conditions recommended by the manufacturer. Restriction fragments were resolved electrophoretically in the Biometra MultiGel Long apparatus in 8% polyacrylamide gel at 3 $V \times cm^{-1}$ for 4 hours. pBR322 DNA/BsuRI (Fermentas) was used as a length marker. Gel was stained in an ethidium bromide solution at a concentration of 1 µg/ml for 10 minutes, the bands visualized under UV and their length determined using the BIO-1D++ (Vilber Lourmat) programme.

Data Analysis

Biallelic PCR-RFLP loci were scored on the presence or absence of restriction sites. χ^2 tests for homogeneity of allele frequency distributions across the regions were then performed. Jaccard's coefficient of genetic similarity was calculated for pairs of strains and, on their basis, a matrix of genetic similarity was prepared. Using the GeneAlEx 6.0 programme (Excoffier et al. 1992, Peakall and Smouse 2006), a separate analysis of molecular variance (AMOVA) was conducted with the use of 1,000 permutations for strain groups of *G. abietina*: a) deriving from the examined regions and b) isolated from various pine species (Table 1). This allowed for estimation of the variance components among and within groups. Distances among populations of *G. abietina* deriving from individual regions and groups of strains of *G. abietina* and *Brunchorstia pinea* var. *cembrae* isolated from the examined pine species were calculated with the use of the standard coefficient of Nei genetic distance (Hedrick 2000, Nei 1972).

Unweighted Pair Group Analysis with Arithmetic Averaging (UPGMA) was conducted with the use of the Statistica 7.0 (Statsoft Inc.) programme and Principal Coordinate Analysis (PCA) with the use of the GenAlEx 6.0 programme.

On the basis of the obtained Jaccard's coefficients for strain pairs, analysis of intrapopulation genetic variability among strains was conducted, depending on the affiliation of strains to regions and pine species from which they were isolated. In order to compare the variation of the listed strain groups, variation analysis was conducted with the use of the Statistica 7.0 programme.

Table 2. Climatic characteristics of strain origin regions of *G. abietina*

Region	Elevation [m]	Average annual temperature [°C]	Annual sum of precipitation [mm·m^{-2}]	Average annual humidity [%]	Average thickness of snow cover [cm]	Number of days with snow cover
Region I	590	5.4	1180	85	22.0	160
Region II	300	7.8	730	80	11.0	60
Region III	170	6.3	550	81	12.5	70
Region IV	75	7.5	670	79	8.0	65

Results

The lengths of the obtained DNA fragments: mtSSU rDNA, ITS1-5.8S-ITS2 and the GPD gene amounted to 714, 442 and 666 bp respectively. In total 108 PCR-RFLP markers for all three DNA fragments have been obtained. The number of polymorphic markers and values of Jaccard's coefficient for each of the examined fragments showed that the most variable was the ITS1-5.8S-ITS2 fragment. A smaller genetic polymorphism was displayed with respect to mtSSU rDNA and GPD gene (Table 3). No private alleles for strains isolated from individual species of host plants and the regions of their origin have been found.

The conducted χ^2 tests showed the homogeneity of allele frequency. It allowed for application of variation analysis (ANOVA) in order to show statistical differences with respect to intrapopulation variability among strain populations deriving from individual regions of Poland and groups of strains isolated from various pine species.

A slight correlation was found between the geographic and genetic distance of *G. abietina* strains. The Mantel test has not shown this dependency to be statistically significant. The group of strains deriving from region I was most distant genetically to the remaining ones. The genetic distance of strains deriving from this region was largest in comparison to region IV (most distant geographically), whereas it was smaller in comparison to regions II and III with a smaller geographic distance. The strain group deriving from region II was characterized by low Nei coefficients in comparison to strains from regions III and IV (0.017 and 0.018, respectively), whereas it had the highest coefficient in comparison to region I (0.031). The grouping of strains on the basis of the Nei distance shows that the *G. abietina* strains deriving from mountainous areas (region I) create a separate cluster, which is distant from the remaining strain groups deriving from regions II – IV (Fig. 2).

The analysis of Nei coefficients showed a relatively large genetic distance between *Brunchorstia pinea* var. *cembrae* and *G. abietina* strains deriving from different host species (from 0.124 to 0.146). The genetic distance coefficient among groups of *G. abietina* strains deriving from *P. nigra*, *P. sylvestris* and *P. armandii* was smaller and ranged from 0.059 to 0.096.

The UPGMA analysis (Fig. 3) showed that the isolates cluster according to species (variety) of pathogen and their host plant. The first cluster included strains of *Brunchorstia pinea* var. *cembrae* isolated from *P. mugo*, the second included *G. abietina* strains deriving from *P. nigra* and *P. armandii*, whereas the third included *G. abietina* strains isolated from *P. sylvestris*. The image obtained reflects genetic Nei distances among strain groups isolated from various species of host plants. Dendrogram shows a greater similarity within the scope of strain groups infecting individual pine species in comparison to the similarity resulting from their geographic origin. Similar results were obtained after the conduct of Principle Coordinate Analysis (PCA).

The conducted analysis of intrapopulation variability of *G. abietina* strains showed that the values of

Table 3. Genetic variability for mtSSU, ITS and GPD fragments based on PCR-RFLP markers for strains of *G. abietina*

No.	DNA fragment	Number of markers		Jaccard coefficient
		total	polymorphic	
1	mtSSU rDNA	39	15	0.92
2	ITS1-5.8S-ITS2	48	18	0.77
3	GPD	21	2	0.99

Table 4. Characteristic of intrapopulation variation of *Brunchorstia pinea* var. *cembrae* (for *P. mugo*) and *Gremmeniella abietina* according to their provenience (region) and species of host tree

Strain provenience	Average of Jaccard Coefficient	Range of Jaccard coefficient	Variation coefficient [%]
Regions			
Region I	0.86	0.21	7.62
Region II	0.87	0.23	5.77
Region III	0.88	0.16	5.44
Region IV	0.90	0.20	6.36
Host			
Pinus mugo	0.82	0.03	2.13
Pinus armandii	0.94	0.05	2.92
Pinus nigra	0.91	0.15	4.07
Pinus sylvestris	0.90	0.22	5.50

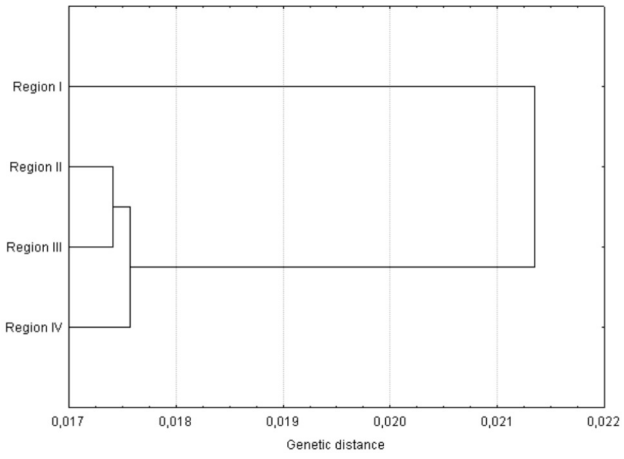

Fig. 2. A dendrogram illustrating genetic distance among *G. abietina* strains originating from different regions of Poland obtained through the unweighted pair group average (UPGMA) method

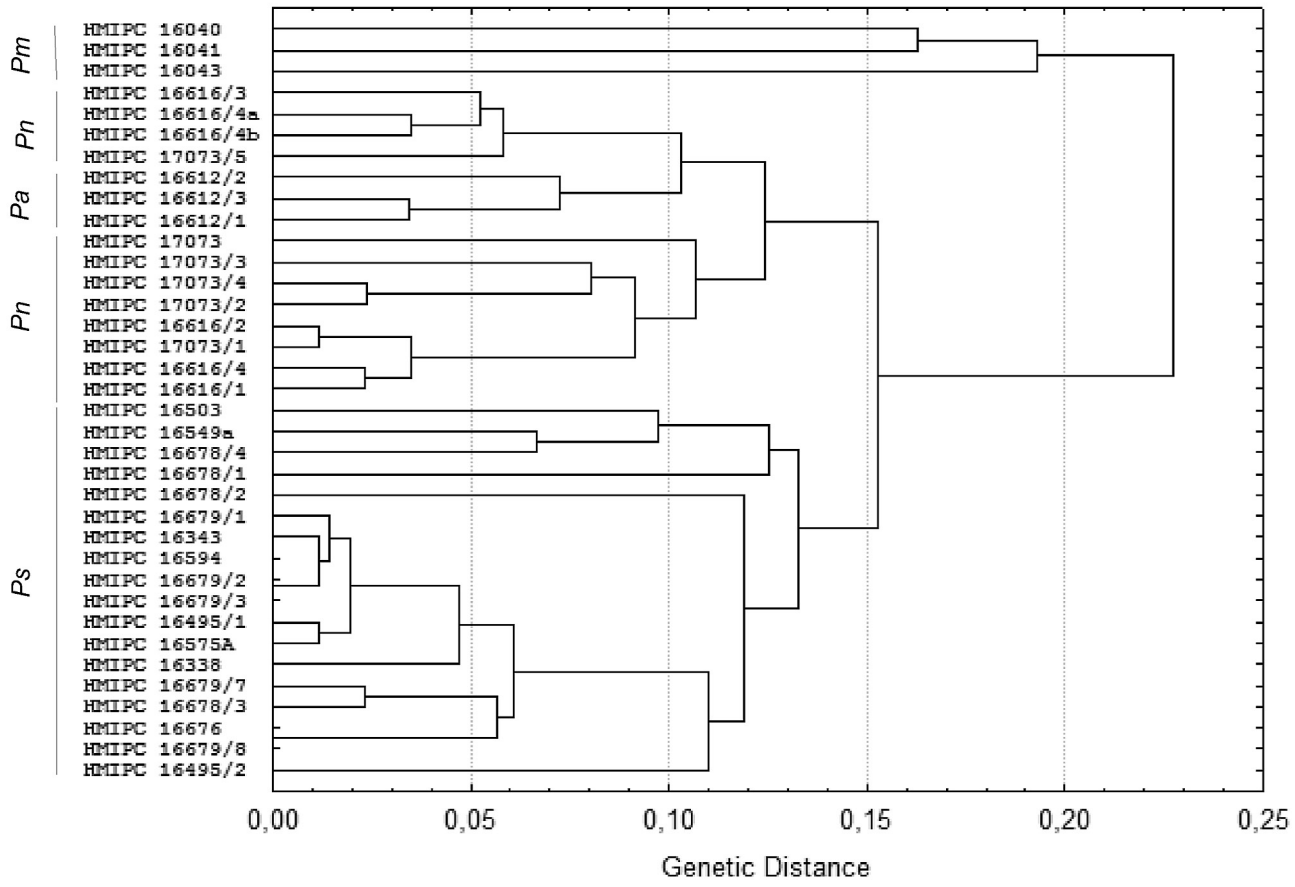

Fig. 3. A dendrogram illustrating genetic distance among strains of *Brunchorstia pinea* var. *cembrae* (for *P. mugo*) and *G. abietina* isolated from different host species obtained through the unweighted pair group average (UPGMA) method. *Ps – P. sylvestris, Pn – P. nigra, Pa – P. armandii, Pm – P. mugo*

Jaccard's coefficient changed depending on the regions and host plant species. The coefficient grew along with the shifting of the region of origin to the North and a drop in elevation above sea level, i.e. whilst moving towards milder climatic conditions (Table 2, 4). This dependency was confirmed by a high correlation coefficient (r = –0.91) between the elevation above sea level and the average value of Jaccard's coefficient among pairs of strains deriving from a given region. The differences among Jaccard's coefficients for strains inside the regions were slight, yet statistically significant at P = 0.05. It was shown that the values of this coefficient did not differ statistically among groups of strains deriving from various host plants.

Molecular analysis of variance (AMOVA) (Table 5) showed that the differences among regions amounted to 8% of the total variation (P = 0.022). The remaining variation (92%) was localized inside the regions. When the isolates were grouped according to their host plant, 39% of variation was related to pine species, thus confirming a higher impact of the host plant than that of geographic location.

Table 5. Analysis of molecular variance (AMOVA) for 36 strains of *Gremmeniella abietina* conducted on the basis of PCR-RFLP analysis of examined sequences

Source of variation	df	Sum of squares	Variance	% Total variance	Φ	P
Between groups of strains from particular regions	3	29.382	0.508	8%		
Within regions	32	177.757	5.555	92%	0.084	0.022
Total	35	207.139	6.062			
Between groups of strains from hosts investigated (*P. sylvestris*, *P. nigra* and *P. armandii*)	2	63.853	2.829	39%		
Within hosts	33	143.286	4.342	61%	0.395	0.001
Total	35	207.139	7.171			

Discussion

AMOVA has shown a high partition (39%) and significant differences among strains isolated from various pine species. A similar partition of variability among groups of strains deriving from Norway and Sweden occurring on *P. sylvestris* and *P. contorta* were found by Hellgren and Hogberg (1995) with the use of the AP-PCR method. Wang and others (1997) also confirmed a significant partition of genotypic variability resulting from various species of host plants.

Despite the use of various molecular markers and biochemical methods no monomorphic markers have been found for strains infecting specific tree species (Hamelin et al. 1996, Kraj and Kowalski 2008, Wang et al. 1997, Hansson et al. 1996, Hellgren 1995, Hellgren and Hogberg 1995). The present research indicates that genetic differences among strains of *G. abietina* and *Brunchorstia pinea* var. *cembrae* deriving from the examined pine species and regions resulted from differences in frequency of alleles creating a gene pool for strain groups. It confirms the lack of sufficiently large genetic differences among groups of *G. abietina* strains infecting various species of coniferous trees.

The study shows different polymorphism of amplified DNA fragments. The partition of polymorphic markers for mtSSU rDNA and ITS region was similar whereas smaller for GPD gene. It is probably caused by different rate of evolution of examined fragments. Various studies have shown that the rate of nucleotide substitution is greater in mtDNA than in coding regions of nuclear DNA (Guarro et al. 1999). The low level of polymorphism of GPD gene in *G. abietina* was agreed with results of Dusabenyagasani and others (2002). The lack of variation in the conserved genomic regions of DNA indicates that mutations in *G. abietina* strains are too recent to be accumulated. On the other hand, it should be emphasized that in many species of fungi the GPD gene is more variable because of evidence for the presence of pseudogenes and horizontal gene transfer (Bruns et al. 1991).

Our analysis show dependency between the genetic distance of strains occurring in examined regions and infecting pine species. It allows for stating that the main factors differentiating the fungi species into races, ecotypes and groups of strains are pathogen specificity in relation to the host species and adjustment of strains to climatic conditions in areas inhabited by these tree species. The geographic origin of strains had small share in the process of genetic differentiation of a pathogen. This may occur, but the strains have to be physically distanced from each other. This is reflected in the genetic differences among the *G. abietina* var. *abietina* races occurring in Europe and Northern America and between European and Northern American strains of G. *laricina* Ettl (Bernier et al. 1994, Dusabenyagasani et al. 2002).

Current research confirmed the genetic distance of *B. pinea* var. *cembrae* from *G. abietina* strains. This is testified to both by the average genetic distance Nei coefficient in relation to groups of *G. abietina* strains colonizing *P. armandii, P. nigra* and *P. sylvestris,* as well as the conducted UPGMA and PCA analyses. Differences among strains of *B. pinea* var. *cembrae* and *G. abietina,* which are the basis for differentiating an Alpine amplitype adjusted to unfavorable climatic conditions occurring above 2,000 metres above sea level, was confirmed by Donaubauer (1972). Petrini and others (1990) showed a similarity of electrophoretic profiles of protein occurring in similar climatic conditions of strains of B ecotype from northern Finland and *B. pinea* var. *cembrae* from the Alps. At the same time, the authors showed significant genetic differences among B type strains and *B. pinea* var. *cembrae* and the remaining *G. abietina* strains from Europe occurring in a milder climate, e.g. in southern Sweden. The presented results of research on Polish strains of *G. abietina* and previous analyses with the use of the RAMS method (Kraj and Kowalski 2008) on these strains confirm the impact of climatic conditions on the genetic variability of the pathogen. It seems that the main factor genetically differentiating the pathogen strains (except for specificity with respect to the host plant) are climatic differences among the examined regions and the occurrence of individual pine species attacked by the fungus, which is related with these differences.

Research has shown differentiation of intrapopulation genetic variability among regions of origin of strains. The main factor deciding the value of the Jaccard's coefficient of similarity in individual regions was the elevation of the region above sea level and climatic conditions which occur in the place of origin of strains. This was testified to by the high value of correlation coefficient between genetic similarity and elevation above sea level. The dependencies obtained in the current research comply with the results obtained by Kraj and Kowalski (2008) with the use of RAMS markers.

The level of genetic differentiation of fungi populations depends on a number of factors, among which the most important are the mode of reproduction, the partition of sexual reproduction and speed and distance of dispersal of spores. The frequency of production of apothecia, which is a result of the sexual reproduction of fungus, and the higher level of intrapopulation variability, which is related to this, depends on climatic conditions. The conditions in region I, characterized by a harsher climate with lower temperatures, higher rainfall and longer lasting snow cover are most conducive to this process (Donaubauer 1974, Roll-Hansen and Roll-Hansen 1973, Wang et al. 1997). Undoubtedly, due to the fact that more variable populations have a better chance of adjust-

ment to unfavorable climatic conditions, this phenomenon is very favorable for fungus. Ascospore discharge is closely related to rainfall (Skilling 1969). Moreover, ascospore may be transferred over larger distances contributing to higher intrapopulation diversity (Skilling 1993), whereas conidia spread over small distances from their source. Skilling (1969) found no evidence of wind dissemination of conidia. These may be the cause of the ascertained higher similarity of strains (smaller genetic variability) when moving towards a milder climate.

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