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Genetic variation of silver fir (*Abies alba* Mill.) preserved in the Katowice Forest District

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Abstract. Environmental pollution greatly decreases a tree's health and results in dieback of forest stands. Owing to increasing industrial activity in the 20th century, silver fir became almost totally extinct in the Katowice Forest District. Only 19 individuals have survived to this day. The aim of the present study is to analyse growth characteristics and polymorphisms of 25 inter-simple sequence repeats (ISSR) of the preserved trees.

The mean height of the inventoried silver firs was 19 m with a diameter at breast height (DBH) of 29 cm. Flowers were observed on few trees only. However, all trees were of high vitality without signs of fungal pathogen infections or insect outbreaks. Parameters of genetic variability, including mean effective number of alleles per locus and expected heterozygosity, were higher than that described in the literature so far and they amounted to 1.659 and 0.396, respectively.

Keywords: genetic diversity, ISSR markers, growth traits, industrial pollution

1. Introduction

During 200 years (from the mid-17th century to the mid-19th century), silver fir (*Abies alba* Mill.) share in Upper Silesian forests decreased from 41% to 32% (Nyrek 1975). The main reason for this was forest management practice at that time, that is, clearcut logging, planting monoculture pine and spruce stands and forming the so-called 'forest of age classes'. In the mid of 1800s, in the region of Silesia, there was noted *A. alba* dieback for unknown reasons (Korpel, Vinš 1965). Further silver fir decline in the region was due to extensive environmental pollution that stimulated a spiral disease development (Bernadzki 1983; Zientarski et al. 1994; Jaworski 1995). Nowadays, as a result of long-lasting environmental changes, Silesian forests under administration of the Katowice Regional Directorate of State Forests (RDLP Katowice) comprise mainly coniferous species (78%), and silver fir proportion is no more than 1.3%. The total share of silver fir in Poland's forests administered by the State Forests is 2.2% (Wyniki aktualizacji stanu powierzchni leśnej 2013 – *Status of Forest Area in the State Forests – Update 2013*).

In recent times, a noteworthy silver fir proportion has been observed in forests administered by the Katowice Forest District. Since 2007, the species has been gradually introduced into reforestation areas, anywhere with suitable site and light conditions. Forest breeding material has been obtained from known sources, such as managed seed orchards (1st-category stands), located in the Forest District Kłobuck (654, region of origin) and the Forest District Limanowa (851). On the other hand, it would be preeminent to breed native plant material which is best adapted to local environmental conditions, as sustainability of forest ecosystems relies on their adaptation capability handed down from generation to generation (Degen 1995). In view of considerable silver fir dispersion (no cross-breeding) within the area of the Katowice Forest District, preserved genotypes cannot be used as forest breeding material for population restitution. In this instance, there should be established 'clone genetic archives' or else 'safeguarding seed plantations', which through accumulation of trees within a certain area would allow to include chosen specimens into cross-breeding processes (Burzdajn 2000). The return of silver fir into Silesian forests can also be based on the introduction of foreign provenances. In that case, the populations introduced should reflect variability of those local and also even better support fulfilment of silviculture tasks in managed stands (Barzdajn 2006).

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The aim of the present study is to list silver fir trees preserved within the area of the Katowice Forest District, to assess their growth parameters (height and diameter at breast height (DBH)) as well as to evaluate fir genetic variation using inter-simple sequence repeats (ISSR) markers. The results of research undertaken will allow for genotype appraisal in preserved fir trees with the purpose of using them in future fir reintroduction programs.

2. Materials and Methods

Study material

The inventory and assessment of tree growth parameters were carried out on silver firs preserved within the area of the Forest District Katowice. Tree height was measured using a Suunto clinometer (to the nearest 1 m) and DBH (at 1.3 m) – with a forestry caliper (to the nearest 0.5 cm). Additionally, in each tree, there were evaluated levels of insect infestation and fungi colonisation as well as the presence of cones. Information on the locality of fir trees within the area of the Katowice Forest District and their characteristics is presented in Table 1.

Analysis of inter-simple sequence repeat (ISSR) loci

Genomic DNA was extracted following the methodology by Rogers and Bendich (1988). Each amplification was conducted in 10 µl mixture of water, 10× concentrated reaction buffer, 31 mM magnesium chloride, 40 mM dNTP, 50 mM primer, 0.46 U Taq polymerase and 40 ng genomic DNA. The sequences of ISSR markers used are presented in Table 2. The polymerase chain reaction (PCR) was carried out in a Biometra Thermocycler (T-Personal) at 38 amplification cycles, which included denaturation (95°C for 30 sec), primer annealing (45 sec in first three cycles at 54°C, subsequent 3 cycles at 53°C, and then at 52°C) and DNA amplification (at 72ºC for 2 min). The cycles described were preceded by 7-min initial denaturation at 95ºC and finished with 7-min elongation of amplification products obtained at 72ºC. After each reaction, electrophoresis in 2% agarose gel was carried out and next the results achieved were analysed under UV light and then archived.

Data analysis

The mean numbers of allele per locus (N_a) and allele frequencies were calculated. Additionally, the effective number of

Table 1. Location of firs preserved within the area of the Forest District Katowice

Tree No.	Forest Division	Management unit	Forest habitat type	Geographical coordinates	
				Longitude	Latitude
1	Murcki	123b	Lw	19.048676	50.179996
2		97d	LMśw	19.064944	50.186631
3		96i	Lw	19.067455	50.184023
4		95a	LMw	19.071771	50.190207
5	Czułów	178h	LMw	19.046023	50.161480
6	Podlesie	107j	LMśw	19.014022	50.174835
7	Wesoła	41g	LMśw	19.099448	50.219373
8	Śmiłowice	79Ak	LMw	18.869625	50.211539
9		87Ag	LMw	18.880393	50.207601
10		87Am	LMśw	18.880679	50.206542
11	Górki	163Ac	LMw	19.115206	50.134240
12		163Bc	LMw	19.111439	50.113409
13		168Bg	LMśw	19.116242	50.099856
14		159g	LMśw	19.135601	50.117411
15	Panewnik	38k	BMśw	18.905517	50.238181
16		38k	BMśw	18.905234	50.238061
17		38k	BMśw	18.905273	50.238116
18		38k	BMśw	18.905283	50.238073
19		38k	BMśw	18.909523	50.238076

alleles per locus (N_e) was estimated so as to determine the number of alleles that can be passed to the next generation (Bergmann, Gregorius 1979). The expected heterozygosity (H_e) that would exist in the population in Hardy–Weinberg equilibrium was assessed (Nei, Roychoundry 1974). Genetic similarity amongst observed specimens was evaluated by means of NTSys version 5.1 software (Rohlf 2001), and then a tree diagram of genetic distance (dendrogram) was prepared.

3. Results

The highest number of fir trees was recorded within the area of the Panewniki Forest Division, and the lowest in the Czułów, Podlesie and Wesoła Forest Divisions. Within the entire area of the Katowice Forest District, preserved fir trees showed on an average 19 m height and 29 cm DBH. The lowest values of growth parameters were observed in the trees numbered 17 and 18 in the Forest Division Panewnik. The specimens with the highest values of growth parameters were found in the Forest Divisions: Murcki (No. 2) and Czułów (No. 5). All the fir trees showed high vitality and no signs of either fungal pathogen colonisation or insect feeding. Most of the trees had no cones except for those growing in the Forest Divisions: Czułów (No. 5), Podlesie (No. 6) and Śmiłowice (tree No. 9) (Table 3).

Table 2. Sequence of ISSR markers and the number of amplification products

No.	Sequence (5′→3′)	Number of amplification products	Number of polymorphic products
1	$(AG)_8G$	9	5
2	$(GT)_8C$	7	5
3	$(AC)_8G$	7	6
4	$(GA)_8C$	7	4
5	$(GA)_8YC$	10	9
6	$(CA)_8G$	4	2
7	$(CA)_8GC$	7	6
8	$(TC)_8G$	7	7
9	$(TG)_8G$	4	4
10	$(AG)_8YT$	7	3
11	$(AC)_8C$	5	3
12	$(AG)_7A$	9	7
13	$(CA)_8A$	9	3
14	$(TC)_9A$	5	4
15	$(CT)_9T$	7	5
16	$(CTC)_6G$	6	5
17	$(ACC)_6T$	4	2
18	$(AC)_9G$	5	4
19	$(ATG)_6G$	3	2
20	$(ATG)_8C$	5	2
21	$(ATG)_8AC$	5	3
22	$(ATG)_8T$	2	0
23	$(AC)_8T$	11	8
24	$(AC)_8G$	12	9
25	$(AC)_{8Y}G$	8	7

Y– specifies T or C

The highest values of the effective allele number (N_e) and the highest expected heterozygosity (H_e) showed the following silver fir trees: No. 9 in Śmiłowice and 17 in Panewnik. The lowest values were observed in the trees: No. 9 in Śmiłowice and No. 17 in Panewnik. The mean number of alleles (N_a) per tree was 1.000. In the population of 19 silver fir trees tested, the mean effective allele number was 1.659 and the expected heterozygosity was 0. 396.

The mean genetic distance was 0.163. Generally, no considerable genetic similarity was observed in silver fir trees growing on the area of a given forest division. In the Katowice Forest District taken as a whole, silver fir trees growing far away from each other, for example, No. 6 in Podlesie and No. 14 in Górki, were grouped in one cluster. On the other hand, quite large genetic distance was observed amongst fir trees growing in the Forest Division Murcki, for example, No. 1 in Murcki and No. 2 in Murcki. Fir tree No. 13 in Górki was genetically most distant (placed in a separate cluster) (Fig. 1).

4. Discussion

Former forest management activities, that is, formation of monoculture spruce stands, clearcut logging as well as considerable air pollution resulted in a substantial decrease in silver fir populations in Poland's forests. Now, consistent with the results of *Status of Forest Area in the State Forests – Update 2013*, silver fir proportion is estimated to 2.5%. In the Katowice Forest District, only 19 silver fir specimens with unknown source of origin are preserved. The preserved trees are roughly 50–65 years old; they apparently endured the period of hard-hitting industrial emissions in the 1980s.

The study presents the results of silver fir inventory carried out in the Katowice Forest District as well as the analysis of genetic variation performed with the use of the ISSR-PCR method (Ziętkiewicz et al. 1994). Initially, the method was used to examine agriculture plant species (Wolfe, Liston 1998), and soon it was implemented in research on population genetics (Qian et al. 2001; Smissen et al. 2003; Pérez-Collazos, Catalán 2007; Dantas et al. 2015). Yet, not many studies has been conducted on evaluation of genetic diversity of forest tree populations with ISSR markers (Tani et al. 1998; Mehes et al. 2007; Rubio-Moraga et al. 2012) and hardly any have been focused on the genus

Table 3. Growth characteristics and genetic variability parameters of firs preserved within the area of the Katowice Forest District

Tree No.	Forest Division	Height [m]	DBH [cm]	Presence of cones	N_e	H_e
1	Murcki	22	34		1.645	0.392
2		31	65		1.634	0.388
3		26	47		1.667	0.400
4		28	42		1.645	0.392
5	Czułów	35	52	yes	1.706	0.414
6	Podlesie	21	32	yes	1.592	0.372
7	Wesoła	12	28		1.613	0.380
8	Śmiłowice	18	28		1.634	0.388
9		28	38	yes	1.524	0.344
10		23	39		1.626	0.385
11	Górki	17	30		1.639	0.390
12		24	31		1.730	0.422
13		15	23		1.869	0.465
14		15	17		1.701	0.412
15	Panewnik	14	20		1.660	0.398
16		7	8		1.718	0.418
17		6	7		1.587	0.370
18		5,5	6		1.695	0.410
19		11	10		1.639	0.390
	Mean	19	29		1.659	0.396

N_e – effective number of alleles at locus, H_e – expected heterozygosity

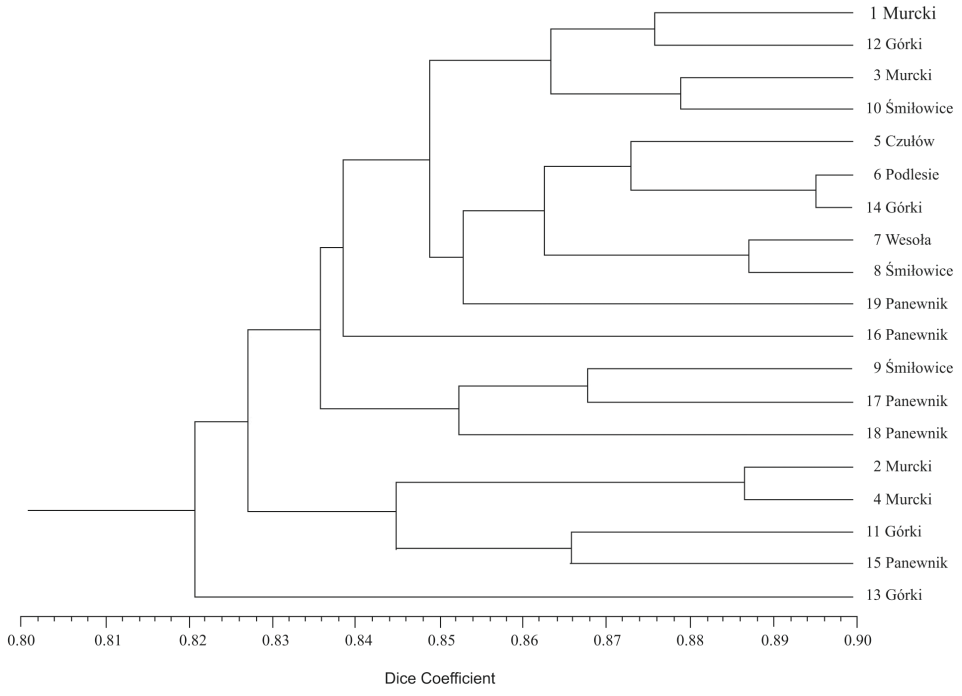

Figure 1. Dendrogram of genetic similarity among tested firs

Abies (Tsumura et al. 1996; Woo et al. 2008). The present study refers to the original results on genetic variation in silver fir obtained with the use of the ISSR method. It is worth stressing that the method combines the simplicity of RAPD markers with a possibility of obtaining a large number of products during an individual reaction. Just one primer is used in amplification – complementary to the microsatellite sequence. Microsatellite sequences commonly occur in eukaryotic organisms (Tautz, Renz 1984; Fang, Roose 1997); therefore, even several dozens of amplification products flanked by microsatellite genomic information can be obtained, which is generally more when compared to the reaction carried out with the use of random primers. Consequently, in a relatively short time and using lesser amounts of reagents, the results that adequately reflect a level of variability in a population can be achieved. Furthermore, ISSR primers are longer than RAPD and require higher hybridisation temperature, which enhances more specific primer binding to the template. As a result, the method is more reliable because of its repeatability and precision. On the other hand, however, the products obtained by means of this technique show dominant characteristics. In any case, many studies on genetic variability of forest trees are carried out with the use of dominant markers (Nkongolo et al. 2005; Vicario et al. 1995).

Silver fir trees preserved in the Katowice Forest District indicated a high level of genetic variation. Owing to the lack of information on polymorphism of ISSR loci in *A. alba,* it was not possible to compare the results obtained in this study with those of other authors. Then again, in view of the results of *A. alba* isoenzyme analyses, one can state that the fir trees from the Katowice Forest District clearly indicated higher genetic variation when compared with fir populations from Slovakia (H_e = 0.210–0.250) (Kormutak et al. 2008), Italy (H_e = 0.129–0.180) (Parducci et al. 1996), France (H_e = 0.139–0.161) (Fady et al. 1999) and the Balkans (H_e = 0.119–0.184) (Ballian et al. 2012). On the basis of isoenzyme markers, it was also observed that when compared to the trees investigated in the present study, fir populations from Italy, Bulgaria, Mecedonia and Romania as well as those from Eastern and Southern Carpathians showed lower expected heterozygosity values and lower effective allele numbers per locus (N_e=1.114–1.03. H_e=0.102–0.188) (Longauer et al. 2003). The expected heterozygosity (H_e = 0.416) which was similar to that obtained in this study was reported by Mejnartowicz (1980), who analysed just two isoenzyme loci in Polish silver fir populations.

Other studies carried out on different forest tree species also showed lower genetic variation when compared with polymorphism of the firs tested in the present study. Lower values of the effective allele numbers and observed heterozygosity were found in populations of Manchurian fir (*Abies nephrolepis*) (H_e = 0.240) (Woo et al. 2008), black pine (*Pinus nigra*) (N_e = 1.228–1.410, H_e = 0.123–0.242) (Rubio-Moraga 2012), Phoenicean juniper (*Juniperus phoenicea)* (H_e = 0.101–0.146) (Meloni et al. 2006) or else red spruce (*Picea rubens*) × black spruce (*Picea mariana*) hybrid populations (N_e = 1.22–1.34) (Narendrula, Nkongolo 2012).

Genetic distance analyses gave no clear answer whether the observed trees originated from an individual *A. alba* provenance. This could be determined after comparative analyses of the larger number of provenances from different regions of Poland. Furthermore, taking into account the lack of information on derivation of the fir trees preserved in the Katowice Forest District, there is a possibility that the fir trees observed are the progeny of silver fir populations once growing within the region and now as individually preserved specimens, they represent genetic diversity amongst previous populations. If so, no relationships found between genetic distance and geographical distance could be explained.

Nevertheless, the reference to the results of other authors should be treated with caution. The comparison of the results obtained in this study with those reported by other researchers would be most trustworthy if genetic variation parameters were measured using equivalent genetic markers. Then again, in subject literature, there is no information available on silver fir genetic variation investigated with the use of the ISSR method. The reference to the results on silver fir obtained using different marker systems as well as to genetic variation levels observed in other forest tree species aims to pointing out that silver fir growing in the Katowice Forest District is characteristic of high genetic variation, which is a valuable feature in the view of tree adaptation potential (Gregorius 1989; Hattemer et al. 1993). The fir trees preserved on the territory of the forest district observed are probably the progeny of native provenances that endured natural and anthropogenic selection. Hence, these are valuable genotypes, especially taking into consideration the exploring mechanisms of tree resistance to industrial pollution and their relationships with tree genetic variation. The relationships of this kind have been so far observed using a small number of biochemical markers. The results of the studies carried out on tree stands growing in polluted areas showed amongst others, lower isoenzyme variability in specimens with visible damages when compared to healthy trees (Ziegenhagen et al. 1997). Correlation between fir defoliation levels and genotype frequency and isoenzyme loci alleles involved in metabolic pathways was proved by Konnert (1993). Genetic polymorphism of isoenzyme markers is also correlated with environmental stress resistance in Norway spruce (Bergmann, Scholz 1985) and beech (Müller-Starck 1989; Brus 1996). In view of the fact that protein variability relies on DNA sequence, the abovementioned results indicate that correlations are possible at a level of metabolic pathways and DNA as well.

Forest management practice commonly applied in the 1800s in Poland did not include introducing silver fir in reforestation areas, and for that reason, natural regeneration of local fir populations has not been possible (Barzdajn 2012).

The recommended average number of trees at one site in the Sudety Mts. is 15 specimens. Nonetheless, 75% of areas comprise less than 10 trees (Filipiak, Barzdajn 2004). In the Katowice Forest District, there are only 19 fir specimens. In order to restore this species, seed banks are created, and these are mainly clone archives that consist of genetic material of dispersed specimens. According to Poland's Program on Preservation of Forest Resources and Tree Breeding for the years 2011–2035, within the territory administered by the Katowice Regional Directorate of State Forests (RDLP Katowice), the area of selected seed fir stands will be increased from 76.69 to 96.69 ha, that of progeny plantations will be increased from 75.19 to 195.19 ha and the area of managed seed stands will be decreased from 584.05 to 560 ha. At the same time, the number of maternal trees will be increased from 30 to 70 specimens. Additionally, it is planned to establish second-generation stands on the area of 60 ha. On the area of 5 ha, there will be established seed plantations (which have not so far existed in RDLP Katowice). All these objects are to form the seed base for increasing a fir proportion in forests under administration of RDLP Katowice.

High genetic variation observed in fir trees preserved within the area of the Katowice Forest District indicates that they constitute most valuable source of genetic material that can be used in establishing clone plantations. The seeds obtained from such plantations could be used in reforestation carried out in the district. Silver fir was commonly present in the area of the Katowice Forest District until the 1950s; therefore, the reintroduction of this species is by all means beneficial. Appropriate sites and reduction of air pollution can certainly support the return of this valuable forest tree species back to Silesian forests.

Conflict of interests

No potential conflicts are declared by the authors.

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Author's contribution

K.M. – research conception, inventory works and collection of biological material, genetic analyses, result analyses, preparation of manuscript; B.N. – research conception, inventory works and collection of biological material, preparation of manuscript; K.G. – genetic analyses, result analyses, preparation of manuscript.

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