

Genetic variability and health of Norway spruce stands in the Regional Directorate of the State Forests in Krosno

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Abstract. The study was conducted in 2015 in six spruce stands situated in different forest districts administratively belonging to the Regional Directorate of State Forests in Krosno. Each spruce population was represented by 30 trees and assessed in terms of their current health status. Genetic analyses were performed on shoot samples from each tree using nine nuclear DNA markers and one mitochondrial DNA marker (*nad1*). The health status of the trees was described according to the classification developed by Szczepkowski and Tarasiuk (2005) and the correlation between health classes and the level of genetic variability was computed with STATISTICA ($\alpha = 0.05$).

Nuclear DNA analyses revealed a low level of genetic variability among spruce populations (only 3% of the total genetic variation ($F_{ST} = 0.028$) and a high variability within populations (97%). The total heterozygosity in all stands (H_T) was calculated as 0.646. Based on UPGMA analysis, the most genetically similar populations are spruce stands in the Bieszczady National Park and the Ustrzyki Dolne Forest District, which have the smallest genetic divergence of all populations ($D_N = 0.0165$).

Our analysis of the mitochondrial gene *nad1* revealed the presence of six different haplotypes ‘a’, ‘a1’, ‘b’, ‘c’, ‘d’ and ‘d1’. Comprising 56% of all haplotypes, ‘a’ was the most common showing a predominant impact on spruce migration from the Carpathian area. The analysis based on mitochondrial markers (by Nei) revealed a heterozygosity of 0.525.

Based on the observations of disease symptoms, 29% of the trees belong to health class 1, 30% to class 2, 28% to class 3 and class 4 contains 13% of trees. The comparison between health status and the level of genetic variation in the analysed stands showed a positive correlation. Spruce stands with better health were also characterised by a greater degree of genetic variability.

Since most of the investigated spruce populations shared the mitochondrial haplotype ‘a’, we have ascertained their Hercynian–Carpathian origin. Only one stand (Cisna) had a high frequency (43.3%) of the Nordic haplotype ‘c’ suggesting that this provenance is derived from the Baltic post-glacial refugium of *P. abies* in Europe.

Keywords: *Picea abies*, Norway spruce, health state, SSR markers, mtDNA, genetic diversity

1. Introduction

Forest trees are characterised by high levels of genetic variation within the population and a low degree of genetic variation between populations. This is, amongst others, due to the intensive gene flow in this group of plants (Hamrick et al. 1992; Działuk, Burczyk 2005). Based on genetic studies of forest tree populations, it is possible to assume that poor gene pool stands are less resistant to environmental changes and more likely

to be affected by unwanted factors (Nowakowska, Konecka 2013). Thus, genetic diversity of forest trees plays a key role in the stability and dynamics of forest ecosystems (Gregorius 1991, Wojnicka-Półtorak et al., 2014). Interspecific variation of species with a wide and continuous range of occurrence, for example, spruce, is often below 3% (Müller-Starck et al. 1992). This fact is conducive to high variability in intra-populations and multidirectional ecological, geographical, morphological and physiological variability (Boratyński et al. 1998).

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According to the results of the monitoring of forest damage in Poland (2013), the spruce (*Picea abies* L. Karst.) is a highly damaged species (26.96% of all damaged trees) (Wawrzoniak et al. 2014) and its health condition influences a number of features (e.g. shaping the timber quality, implies durability and longevity of stands) reflecting the stability and resilience of forest ecosystems to biotic and abiotic stressors and damaging factors. It may also affect forest preservation as a plant formation (Sierota 1998) and ensure continuity of basic ecological processes, sustainability of ecosystems, maintenance of the natural resilience of forests and communities and preservation of genetic resources for future generations (Matras 2013).

The aim of the study was to analyse the diversity and genetic variability of mature spruce stands in the Krosno Regional Forests (RDLP) based on the molecular markers, both nuclear DNA (microsatellite loci) and mitochondrial DNA (fragment of *nad1* gene), and comparison of genetic variability parameters with parameters determining the health status of the analysed trees.

2. Material and methods

Six populations of spruce (*P. abies*) were located in the area of RDSF in Krosno in the districts of Lutowska, Stuposiany, Cisna, Ustrzyki Dolne and Komańcza and in the Bieszczady National Park (BPN) (Table 1). From each stand, one-year shoots or increment cores were collected from 30 randomly chosen trees of 60 (Lutowska and Stuposiany) to 117 years (BPN) of age. For each tree, diameter of breast height (DBH), height (H) and crown length (H_k) were measured.

Visual assessment of the health status of the tree was made in the spring, using a 4-level scale of damage developed based on the methods of Szczepkowski and Tarasiuk (2005), in which trees were classified based on the degree of crown defoliation. In addition, other symptoms of tree damage, such as the presence of insect feeders, pathogenic fungi and other tree trunk damages, were observed. Class 1 indicates trees without damage or slightly damaged (showing <25% defoliation and the presence of individual insect feeders), class 2 slightly damaged trees (25–50% defoliation, minor damage of trunk, resins exudes and rare insect feeding), class 3 moderately damaged trees (50–70% defoliation, mechanical defects, trunk fractures, frequent insect feeding and resin cracks), class 4 severely damaged individuals (showing more than 70% defoliation of crowns; the presence of deep, open wounds with very frequent residual spills; frequent mechanical damage; flaking or falling bark flakes; and a very high incidence of insect borers feeding and fruit bodies of fungal pathogens).

Extraction of genomic DNA from plant material was carried out using the NucleoSpin Plant II DNA isolation kit (Macherey-Nagel®), whilst the amount of DNA obtained was analysed spectrophotometrically with the NanoDrop® ND-1000 (TK-Biotech) apparatus. Microsatellite sequence analysis (SSR) was performed for 180 trees using the following 9 microsatellite loci: EATC1B02, EATC1E3, EATC2G05, SPAGC1, SPAGG3, EATC1G2, EATC2B02, G2 and SPAGC2 (Pfeiffer et al. 1997; Scotti et al. 2002). For tree genotyping, Genetic Analyzer 3500 (Life Technologies™) sequencer and Data Collection Software 3500 were used.

The mitochondrial spruce DNA haplotypes were evaluated on the basis of amplification of the *nad1* gene fragment according to Sperisen et al. (2001). Amplification products were analysed on the basis of Bioanalyser®, according to the manufacturer's recommendations, using DNA-1000 chips and classified according to the size of alleles 'a', 'b', 'c' and 'd' as described by Sperisen et al. (2001). Haplotypes that had a different allele size than that showed by Sperisen et al. (2001) were described by the code 'a₁' – for the allele of 350 base pair (bp) – and 'd₁' for 280 bp.

Statistical calculations of basic genetic parameters for microsatellite markers and mitochondrial DNA including observed and effective alleles in locus, observed (H_o) and expected (H_e) heterozygosity, number of private alleles (A_{priv}) and the Shannon index were computed with GenAlEx 6.501 software (Peakall, Smuse 2012). The genetic similarity dendrogram was performed using the Mega 6.0.6 program (Tamura et al., 2013), based on the average connection method (unweighted pair-group method with arithmetic mean, UPGMA). The correlation between genetic parameters and tree health was compiled using the Spearman's rank correlation coefficient and software such as Microsoft Office Excel 2007 and STATISTICA 10 (for $\alpha = 0.05$).

3. Results

Studied trees were characterised by DBH, crown height (H_k) and height (H) (Table 2).

Spruce of all six populations was characterised by the largest share (30%) of the slightly damaged trees (health class 2). Approximately 29% of trees are classified as the health class 1 (trees without damage/trees that are slightly damaged) and 28% of trees showed moderate damage (health class 3). The smallest share of trees (13%) was represented by the class 4 of health. Forest District Cisna was characterised by the best spruce health (Figure 1), expressed in high (64%) percentage of trees without any damage and trees slightly damaged (class 1). Only 3% of trees of this population were assigned to the class 4 (severely damaged trees). The most frequent symptoms recorded in all of the

Table 1. Stand description of studied spruce populations in the Krosno Regional Directorate of State Forests (data after Forest Management Plans for analysed Forest Districts)

Forest district	Forest inspectorate	Forest subdistrict	Forest compartment	Seed region	Latitude [N]	Longitude [E]
Bieszczadzki Park Narodowy	BPN Górny San	Górny San	61g. 61c	806	49°04'99"	22°86'38"
Ustrzyki Dolne	Brzegi	Brzegi	125a. 113d	806	49°43'76"	22°64'00"
Stuposiany	Stuposiany	Czereszanka	10c	806	49°19'46"	22°71'71"
Lutowiska	Lutowiska	Lipie	2a	806	49°27'62"	22°71'92"
Komańcza	Komańcza	Turzańsk	8f	806	49°35'27"	22°08'23"
Cisna	Wetlina	Stare Siolo	82a. 82f	806	49°16'85" 49°16'66"	22°44'69" 22°44'55"

LGśw**, fresh mountain coniferous forest; **LWYŻśw**, fresh upland broadleaved forest; *prz**, intermittent; **luź**, loose; **umiark**, middling

Table 2. Characteristics of studied Norway spruce populations according to health classes

Health state	Diameter at the breast height, DBH [cm]	Crown height, H_k [m]	Height, H [m]
1	48.94	4.54	26.42
2	42.62	6.01	25.87
3	39.00	5.43	25.14
4	38.50	5.45	25.55

analysed stands were as follows: residual spills (29.6%), insects – (*Adelgidae*) (27.2%), mechanical damage (22.8%) and defoliation (16%) (Figure 2). On single trees, other insects have been observed (3.7%).

The high proportion of trees in the health class 1 (47%) also included the Forest District Ustrzyki Dolne. Here trees from the class 2 of health (slightly damaged) accounted for 30%, and trees from the classes 3 and 4 accounted for only 13% and 10%, respectively (Figures 1 and 2).

The lowest proportion of trees without damage or slightly damaged (7%) was stated in Forest Stand Lutowiska (Figures 1 and 2). In this population, the majority of trees were assigned to the health classes 2 and 3, which means that the trees are slightly (43%) and moderately damaged (37%). Trees representing the class 4 of health (severely damaged) were assessed as 13%.

Based on the analysis of genetic structure (nuclear DNA), it was found that the most polymorphic locus was SPAGC2, which recorded 32 different DNA fragments of 83–181 bp,

of which 8 alleles were more frequent than 3%. The smallest number of alleles was recorded at the EATC1E3 locus (5 alleles), with an average effective allele number of 1.46. For all the analysed loci, the mean number of alleles in the locus was $A = 89.333$.

Populations that were most genetically diverse also had high values of the total number of alleles in the locus: $A = 97$ in Ustrzyki Dolne, $A = 94$ in Cisna and $A = 92$ in Komańcza. The highest number of alleles in the locus were represented in the spruces from Ustrzyki Dolne Forest District, $n_a = 10.778$ and $n_e = 5.412$, whilst the lowest values of these parameters were recorded in the population of Stuposiany Forest District $n_a = 9.111$ and $n_e = 4.786$, also with the smallest total number of alleles, $A = 82$ (Table 3).

All tested trees showed the high genetic diversity parameters: $A = 89.333$, $n_a = 9.926$, $n_e = 5.068$, $H_o = 0.487$ and $H_T = 0.646$ (Table 3). Mean values of indexes, inbreeding (F_{IS}) and heterozygosity (F_{IT}), of the genotypes of the ex-

Number of samples	Area [ha]	Forest site type*	Species composition	Age [years]	Stocking	Stand density**	DBH [cm]	Altitude [asl]	Site index	Quality of soil
30	20.53	LGśw	5Jd.3Bk.2Św / 6Św. 4Ol.S	117	0.5	prz/prz. umiark	50	619	II	3
30	19.4	LWYŻśw	6Św.4Jd/7Jd. 2Jw.1Św	87- 65	0.5-0.9	luż/umiark	37	463	III	3
30	26.31	LGśw	5Jd. 3Jd. 2Św	60	0.5	prz/luż	34-50	593	II	2
30	9.47	LGśw	8Św.2Św	60	0.5	prz	32	619	II	3
30	22.7	LGśw	6Św. 4Jd	95	0.5	prz/luż	36	560	II	3
30	28.13	LGśw	8Św(103)2Św(81)	87	0.7 / 1.0	umiark/luż	52	600	II	2

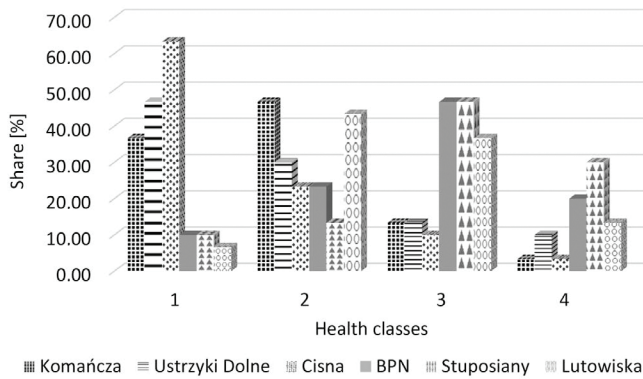


Figure 1. Share of health classes of studied spruce populations in the area of Krosno Regional Directorate of State Forests

mined populations were close to each other, $F_{IS} = 0.272$ and $F_{IT} = 0.292$, indicating the equilibrium of heterozygous and homozygous individuals in all populations. The average genetic variability (F_{ST}) for all populations tested is low $F_{ST} = 0.028$ and indicates greater genetic variability within the examined stands than that between them.

In all populations, private alleles were observed, with their highest number in Ustrzyki Dolne ($A_{priv} = 0.889$), whilst the smallest one was found in BPN ($A_{priv} = 0.333$). Tree No. 12 originating from the plot in Komańcza had as many as two private alleles – 110 bp in the SPAGC1 locus and 210 bp in the EATC1B02 locus.

Based on the analysis of the UPGMA grouping, two similar groups of populations were identified: group 1: BPN, Ustrzy-

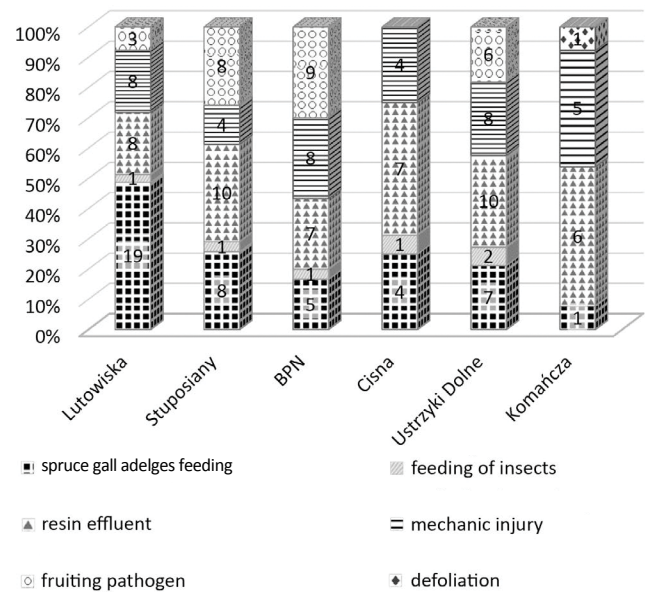


Figure 2. Share of damage symptoms in forest districts and Bieszczady NP

ki Dolne, Stuposiany and Lutowiska; and group 2: Komańcza and Cisna (Figure 3). The largest genetic distance based on the analysis of SSR loci placed itself between populations of Komańcza and Stuposiany ($D_N = 0.056$), and the smallest between populations of BPN and Ustrzyki Dolne ($D_N = 0.0165$). The geographic distribution of populations with similar genotypes shows a division onto the eastern part of region 806,

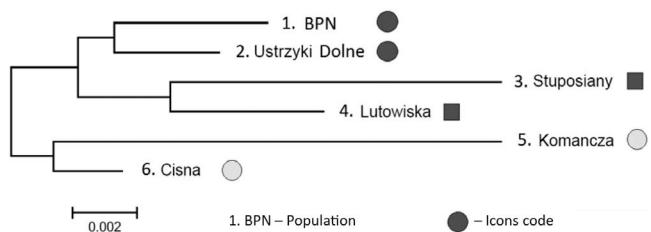
Table 3. Genetic diversity of studied spruce populations in the area of Krosno Regional Directorate of State Forests, based on the analysis of nuclear microsatellite loci of DNA

No	Population	A	n_a	n_c	A_{priv}	H_O	H_E
1.	BPN	85	9.444	5.08	0.333	0.467	0.631
2.	Ustrzyki Dolne	97	10.778	5.412	0.889	0.52	0.666
3.	Stuposiany	82	9.111	4.786	0.556	0.468	0.635
4.	Lutowiska	86	9.556	5.087	0.556	0.452	0.638
5.	Komańcza	92	10.222	4.7	0.667	0.509	0.668
6.	Cisna	94	10.444	5.344	0.778	0.507	0.637
Overall:		89.333	9.926	5.068	0.630	0.487	$H_T = 0.646$

A – total number of alleles,
 n_a – observed number of alleles per locus,
 n_c – expected number of alleles per locus,
 A_{priv} – number of private alleles,
 H_O – observed heterozygosity (Nei 1978),
 H_E – expected heterozygosity (Nei 1978),
 H_T – total mean heterozygosity per stand.

which includes the populations of the first dendrogram group, and the western part containing the two populations from the second dendrogram group (Figure 4).

The mitochondrial gene *nad1* showed the presence of six different haplotype variants: ‘a’, ‘a₁’, ‘b’, ‘c’, ‘d’ and ‘d₁’ (Figure 5). Amongst all studied populations, the most common was the ‘a’ haplotype (56%) followed by the ‘d’ haplotype (25%). The haplotypes ‘a₁’, ‘b’, ‘c’ and ‘d₁’ were less common (5%, 2%, 7% and 5%, respectively). Haplotype ‘c’ characteristic for northern spruce was recorded only in the stands of Cisna, with quite high frequency (43.3%). The stand of the Cisna Forest District was the most genetically variable. It contained the most (five) haplotype variants, ‘a’, ‘b’, ‘c’, ‘d’ and ‘d₁’; possessed the highest heterozygosity, h Nei = 0.618; and was the only one amongst all populations with private alleles. The lowest heterozygosity value was obtained for the population of Stuposiany Forest

**Figure 3.** Dendrogram of genetic similarity of Nei (1978) based on nDNA, between the studied populations of Norway spruce. Populations with similar genetic similarity was marked a different icon

District, h Nei = 0.382 (Table 4). The largest genetic distance, $D_N = 0.994$, calculated on the basis of mtDNA divided the populations of Ustrzyki Dolne and Lutowiska. The most genetically similar spruce populations occurred in BPN and Cisna stands, which shared the smallest distance, $D_N = 0.469$ (not illustrated).

Spearman’s rank correlation was statistically significant (significance level $\alpha = 0.05$) between crown height and breast height (0.405) and crown height and tree height (0.565). Comparison of health classes, DBH, height (H) and crown height (H_c) with respect to genetic parameters (Table 6) showed negative correlations between the health class and observed alleles at the locus (n_a) and Shannon’s variation index (I).

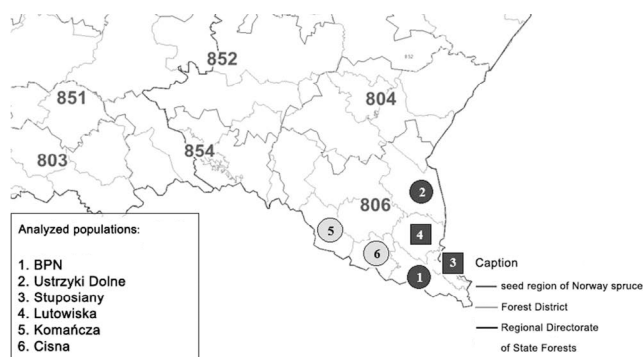
**Figure 4.** Geographical location of spruce population groups based on the microsatellite DNA analysis. Genetically similar groups of populations were marked by grouping UPGMA dendrogram of Figure 3

Table 4. Characteristics of studied spruce stands in the area of Krosno Regional Directorate of State Forests, based on analysis of mitochondrial gene *nad1*

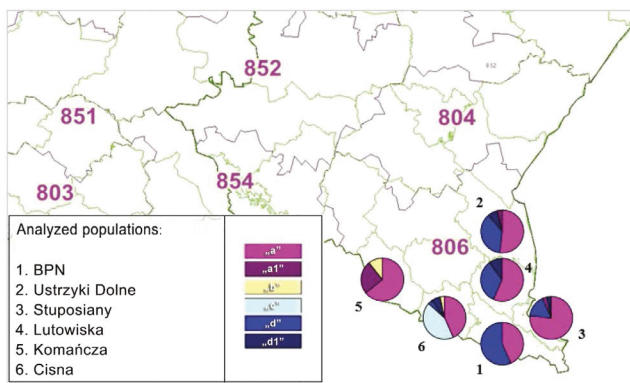
Population	n_a	n_e	A_{priv}	h Nei
BPN	2	1.965	0	0.491
Ustrzyki Dolne	4	2.422	0	0.587
Stuposiany	4	1.619	0	0.382
Lutowiska	3	2.261	0	0.558
Komańcza	3	2.052	0	0.513
Cisna	5	2.616	1	0.618
Mean	3.500	2.156	0.167	0.525

n_a – observed number of alleles per locus,

n_e – expected number of alleles per locus,

A_{priv} – number of private alleles,

h Nei – Nei (1978) heterozygosity.

**Figure 5.** Haplotype participation of the gene *nad1* in the studied populations of Norway spruce from the area of Krosno Regional Directorate of State Forests, based on the analysis of mitochondrial DNA

4. Discussion

Analyses of the health status of forest trees are a frequent subject of studies on the populations of various tree species occurring in Poland, both in the State Forests and in national parks, to compare the condition of our forests within a few decades (Zajączkowski 1993; Borecki et al. Wawrzoniak et al., 1996, 1998, 2000, 2003, 2014; Sierota 1998; Zwoliński 2003; Szczepkowski, Tarasiuk 2005; Nowakowska, Oszako 2008; Żółciak et al. 2009; Zmarz et al. 2012).

The health status of the six spruce populations surveyed in the RDSF in Krosno was characterised by the largest share (30%) of the slightly damaged and the smallest (13.33%) of the severely damaged. These results are confirmed by lon-

g-term monitoring studies conducted by Wawrzoniak et al. (1995, 1996, 1998, 2000, 2003, 2014). Despite the use of different classifications, the results of the spruce health state obtained in this work based on the sum of all factors assume the value of defoliation classes similar to those presented by Wawrzoniak et al. (2013), who recorded for the individuals over 60 years old 15.15% share of trees in defoliation class 0 (up to 10%), 72.73% in slight defoliation class and 12.12% trees over 25% defoliation and dead.

Visual assessment of the health status of trees in the analysed areas was made during multi-day rainfall in the spring of 2015, which could affect the masking of some of the symptoms, such as the colour and length of the needles, and the presence of fungal pathogens that appear in the fall, for example, honey fungus *Armillaria* (sensu lato).

Good health status of the analysed stands can be also determined genetically. Typically, it is a resultant of two types of resistance: vertical (directed to a specific pathogen) and horizontal (focussed on many damaging agents), for example, as a result of prolonged drought and weakness of trees because of an increased attack of *Armillaria* spp., *Phytophthora* and *Pythium* species, or secondary insects (Nowakowska, Oszako 2008). No recent analyses have been performed to identify the causative agents of lesions, based on sampling, isolation and culture of pathogens.

Differentiation of forest trees in terms of genetic features plays an important role in the stability and dynamics of forest ecosystems (Wojnicka-Półtorak et al., 2014), determines the value of raw material and determines the influence of negative biotic and abiotic factors (Matras 2006). SSR and STS molecular markers used in the work are one of the most accurate research tools in forest tree genetics; they are increasingly used to identify specimens and estimate genetic variability of populations and whole species (Lewandowski, Burczyk 2002, Maghuly et al.; Nowakowska 2006; Nowakowska 2011; Lewandowski 2013).

The average heterozygosity observed in all stands was 0.487. It is significantly lower than that obtained by the research conducted by Nowakowska (2009), who received $H_o = 0.729$ for spruce in Poland, but it was also significantly higher than that in other similar studies conducted using different molecular markers in Poland – $H_o = 0.129$ (Lewandowski et al. 1997), $H_o = 0.212$ (Modrzyński, Prus-Głowacki 1998), $H_o = 0.163$ (Lewandowski, Burczyk 2002), $H_o = 0.212$ (Prus-Głowacki, Modrzyński 2003), $H_o = 0.120$ (Sabor et al. 2013), $H_o = 0.237$ (Wojnicka-Półtorak et al. 2014), $H_o = 0.147$ (Masternak, Polak-Berecka 2014) – and in Europe – in Lithuania by Goncharenko et al. (1995) ($H_o = 0.187$) – and slightly lower than that in Austria $H_o = 0.495$ (Maghuly et al. 2006).

The mean genetic variability (F_{ST}) between all populations tested was very low, $F_{ST} = 0.028$. This indicates that

Table 5. Genetic diversity parameters in all studied populations based on health class and type of analysed DNA (nuclear microsatellite loci nSSR and mitochondrial gene *nad1* – mtDNA)

Marker type	Health classes	n_a	n_e	I	H_o	H_E	F
nSSR	1	5.389	3.663	1.181	0.471	0.562	0.172
	2	5.852	3.971	1.289	0.495	0.584	0.194
	3	5.315	3.797	1.247	0.496	0.593	0.161
	4	3.333	2.668	0.854	0.492	0.455	-0.122
		n_a	n_e	I	N	h Nei	uh
mtDNA	1	2.667	2.064	0.745	8.333	0.459	0.604
	2	2.5	1.743	0.664	9	0.408	0.47
	3	2.333	1.886	0.707	8.167	0.468	0.59
	4	1.667	1.452	0.338	3.667	0.211	0.265

n_a – observed number of alleles per locus,

n_e – expected number of alleles per locus,

I – Shannon index after Lewontin (1972),

H_o – observed heterozygosity after Nei (1978),

H_E – expected heterozygosity after Nei (1978),

F – fixation index,

N – mean allele number,

h Nei – Nei (1978) heterozygosity,

uh – unbiased heterozygosity.

Table 6. Spearman's rank correlation matrix between health classes and parameters describing genetic variability

Features	Growth			Parameters of genetic variability					
	DBH	H	H_k	n_a	n_e	I	H_o	H_E	F
Health class	-0.264	0.237	-0.210	-0.469	-0.285	-0.447	-0.011	-0.248	-0.280
DBH	-	-0.286	0.405	0.122	0.127	0.073	-0.103	-0.008	0.002
H	-0.286	-	0.565	0.124	0.187	0.149	0.387	0.334	0.207
H_k	0.405	0.565	-	0.204	0.282	0.168	0.217	0.282	0.231

DBH – diameter of breast height,

H – height of the tree,

H_k – crown height,

n_a – observed number of alleles per locus,

n_e – expected number of alleles per locus,

I – Shannon index after Lewontin (1972),

H_o – observed heterozygosity after Nei (1978),

H_E – expected heterozygosity after Nei (1978),

F – genetic diversity coefficient (Nei 1978).

there is a greater genetic variability within the surveyed stands than between them, which is supported by the principle that most forest tree species have only a small percentage of variation on the interpopulation level, as a result of higher polymorphism consisting of the DNA of individual populations (Hamrick et al. 1992; Nowakowska 2007). The studied populations of spruce were characterised by small nuclear DNA differentiation in comparison with the results of research from Poland and Europe. Lewandowski et al. (1997) received a genetic variation of 0.059 for the Beskidy spruce population, Modrzyński and Prus-Głowacki (1998) got $F_{ST} = 0.063$ for the Polish population, Nowakowska (2009) got the highest F_{ST} score of 0.088, whilst Maghuly et al. (2006) in Austria recorded $F_{ST} = 0.014$. The identical result of the genetic variation coefficient, as presented in this paper ($F_{ST} = 0.028$), was recorded by Lewandowski and Burczyk (2002) for Polish spruce populations, analysed on the basis of isoenzymatic markers.

Considering the genetic distance between the studied populations, stated that BPN populations, Ustrzyki Dolne, Stuposiany and Lutowska belong to the first group of and are located closer to the eastern border of Poland and Ukraine, whilst spruce populations from the second group of dendrogram (Komańcza and Cisna) are located closer to the west. Such a grouping of forest tree populations with similar level of genetic variation in a given area is very rare and would require further research.

As the mitochondrial DNA marker, the *nad1* gene was used, whose effectiveness has been confirmed in many populations of spruce tested in Poland (Nowakowska 2009, Nowakowska 2011, Lewandowski 2013). The mitochondrial DNA markers (STS) were used for *Pinus sylvestris* and *P. abies* (Sperisen et al. 2001; Nowakowska 2007). Mitochondrial genes are often helpful in exploring the pathways of post-glacial migration of fauna and flora (Gugerli et al., 2001; Naydenov et al 2007; Pyhajarvi et al. 2008; Dering, Lewandowski 2009, Litkowiec et al. 2009).

The frequency of the haplotype 'a' (56%) is significantly prevalent in four populations, which largely confirms the membership of the researched stands from RDSF Krosno to Hercynian–Carpathian prevalence, which is predominantly characterised by 'a' and 'a₁' haplotypes (Pfeiffer et al. Sperisen et al. 2001; Eriksson 2009; Nowakowska 2007). Of particular importance is the stand in the Cisna Forest District, with a high frequency of (up to 43%) the haplotype 'c', characteristic for northern (Nordic) spruce range in Poland. This fact can be attributed to the large mix of forest tree gene pools in the past, mainly as a result of forest management in recent centuries (Dering, Lewandowski 2009). The presence of the haplotype 'c' in the Cisna stand can also be attributed in part to the succession of the forest and to the national

afforestation programme after war losses. In the 1920s, the intensive exploitation of Carpathian stands took place in order to obtain the necessary building blocks for the reconstruction of villages and towns (Luboński 2006, Marshall 2011, Orłowski 2012). Forests of the Cisna Forest District in the 1920s were privately owned and focused on intensive wood production (Regional Directorate of State Forests in Krosno, 2015). After the tree felling, the spruce was restored with imported spruce seedlings (Orłowski 2012). In addition, the cuttings of stands in the mature stand caused considerable havoc in the Bieszczady forest resources (Luboński 2006). The robotic way of farming has become the main cause of the change in their species composition, for example, in place of fir and beech, spruce was introduced (Marszałek 2011).

The presented historical and economic relations confirm the consideration of the possibility of bringing in the past Nordic plantation materials to the present Cisna Forest District. The other haplotypes, 'b', 'd' and 'd₁' occur in both the southern and the northern so-called the natural range of spruce in Poland and reflect two major migration trends (boreal/Nordic and Hercynian–Carpathian range) of *P. abies* in Europe.

For tree parameters such as DBH, H and H_k , positive correlations were obtained between health classes and above measurable parameters, which indicates that the length of the crown increases in proportion to the height of the tree or the value of the breast. This is the result of the physiological construction of trees. Kowalczyk and Kahan (2010) have confirmed this dependence, which demonstrated strong positive phenotypic and genotypic correlations between breast, height and height increment, straightness of stem on phenotypic level was negatively correlated with the width of the crown but positively correlated on the genetic level.

Nowakowska and Oszako's (2010) studies on the comparison of the degree of genetic variation and the degree of damage by phytopathogens in beech and oak stands have shown that stands in better health stands exhibited a greater degree of genetic variation. Referring to the compilation of results from all populations, negative correlations between health status and genetic diversity coefficients were obtained in the second part of the matrix. This means that the higher the health class (worse health), the lower is the value of these indicators (less genetic variation). Thus, the results obtained by Nowakowska and Oszako (2008, 2010) confirm that the susceptibility of trees to various diseases or resistance to insect attacks has genetic background.

As stated by the mtDNA marker analysis, the spruces tested were not only locally sourced but were imported as seedlings or seeds from areas located in the northern spruce range. The phenotypic features that are taken into account

when evaluating reproductive material may not always coincide with the native range of the species in the area. The use of molecular techniques makes it possible to obtain more objective criteria for the selection of parent material for planting (Zawadzka 2011).

5. Conclusions

1. Good health condition of spruce tested in RDSF area in Krosno confirms results of long-term monitoring studies conducted in Poland.

2. The susceptibility of trees to disease and injury is genetically conditioned. It has been confirmed that there is a positive relationship between genetic diversity and the health status of forest trees: the greater the genetic variability, the better is the health status.

3. Observed low (2.8%) level of variability amongst spruce populations compared to the level of intra-spatial variability (97.2%) is consistent with a generally observed trend of genetic variability in forest tree populations.

4. The high frequency of haplotype ‘a’ mitochondrial locus *nad1* (56%) indicates that most of the spruce stands surveyed belong to the Hercynian–Carpathian range.

5. The genetic structure of spruces from the Cisna Forest District suggests a distinct, boreal origin of stands, probably derived from imported spruce seeds (or seedlings).

Conflict of interest

Authors declare no potential conflicts.

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Authors' contributions

J.G. – field material collection, laboratory analysis, elaboration of results, health assessment in the field, manuscript editing; J.A.N. – concept of work, substantive contribution, elaboration of results, editorial work on manuscript; M.B. – laboratory analysis; A.T. – genotyping DNA analysis; M.T. – statistical analysis; T.O. – concept of work, phytopathological consultations, manuscript editorial.