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The morphological traits of needles as markers of geographical differentiation in European *Pinus sylvestris* populations

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Abstract: To evaluate the geographic variability of *Pinus sylvestris* populations seven morphological traits of needles of pines from IUFRO 1982 provenance trial have been analyzed. The studied populations originated from northern ($>55^{\circ}\text{N}$ in Russia, Sweden and Latvia), central ($55\text{--}47^{\circ}\text{N}$ in Poland, Germany, Belgium, France, Slovakia) and southern ($<47^{\circ}\text{N}$ in Hungary, Bosnia, Montenegro and Turkey) European ranges of Scots pine. The analyzed provenance trial experimental areas were located in Kórnik (western Poland) and in Supraśl (north-eastern Poland). The greatest variation was found in needle length and number of stomata rows on the flat and convex side of a needle, whereas number of stomata per 2 mm of needle length on flat and convex side of a needle was stable, with minor interpopulational variation. Biometrical analyses revealed a significant population \times location interaction and a geographical pattern in interpopulational differentiation in both experimental sites, with the northern and southern European Scots pine groups of provenances differing significantly from the group of central origin. The results obtained are compatible with previous results of studies on provenance variability of the Scots pine from IUFRO 1982. In the light of available data, the influence of the Balkan glacial refugia of *Pinus sylvestris* on a present genetic diversity of this species in Europe and the reconstruction of Scots pine migration routes after the last glacial period are discussed.

Additional key words: interpopulational variability, migration routes, population \times location interaction, provenance trial

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Introduction

The Scots pine (*Pinus sylvestris* L.) is one of the most important forest-forming species in Europe and Asia. It is characterized by extremely vast distribution area (Meusel et al. 1965), in fact the biggest of all *Pinus* species. Scots pine can be found in lowland forests as well as in mountainous areas on altitudes up to 2500 m above sea level, with elevation generally increasing from north to south (Boratyński 1993). With

such a wide range of geographical and ecological distribution *P. sylvestris* reveals a considerable morphological and genetic diversity (Pravdin 1964, Ruby 1967, Mirov 1967).

Pinus sylvestris survived the glaciation in many possible refugial areas scattered throughout Europe (Birks 1989, Obidowicz 1996). Its early postglacial expansion is likely to have taken place both from remaining local populations as well as due to the northward expansion of southern refugial populations fol-

lowing the retreat of ice sheets (Ralska-Jasiewiczowa 2004). Palynological, molecular and phenotypical traits analysis has shown that the largest refugia of *Pinus sylvestris* were localized south of the Carpathians and in the Balkans, the Alps and the Iberian Peninsula and in the Russian Plains (Pravdin 1964, Huntley and Birks 1983, Bennet et al. 1991, Urbaniak 1998, Soranzo et al. 2000, Naydenov et al. 2007). An intensive silviculture, accompanied by frequent careless policy of forest stand regeneration (Ledig 1992), together with pollution impact (Oleksyn et al. 1994), had also a strong influence on present genetic diversity of Scots pine forests.

Morphological traits of the needles appeared to be a useful tool in estimating the intraspecific differentiation of the pine. These biometric features are partially affected by edaphic and climatic conditions (Irvine et al. 1998, Niinemets et al. 2001, Pensa et al. 2004). Nevertheless, similarly to the morphological traits of the cones (Staszkiwicz 1968), morphological traits of the needles proved their importance in the characterization of *Pinus sylvestris* population and description of the geographic differentiation of the species (Pravdin 1964, Zelawski and Niwiński 1966, Paule 1971, Urbaniak et al. 2003).

Provenance trials constitute convenient material for examining Scots pine variability. Such experimental systems not only provide information concerning plasticity and productivity of a population, but also enable an examination of a genetic structure and its geographical differentiation with the use of molecular and biochemical methods (Oleksyn 1988, Prus-Głowacki 1994, Prus-Głowacki and Bernard 1994, Stephan and Liesbach 1996). Moreover, in the analyses of morphological traits of Scots pine populations from provenance trial, the modifying influence of habitat and climate condition could be excluded. Hence, the observed morphological differentiation have the genetic background and can be considered as an expression of genetic variation of the species.

The aim of this study is to evaluate geographic diversity of European Scots pine populations, gathered in two IUFRO 1982 provenance trial plots in Kórnik and Supraśl, on the basis of morphological traits of needles. The value of certain morphological traits of needles in describing an interpopulational differentiation of the Scots pine will be also estimated. Moreover, the influence of different environmental conditions, stated for both provenance trial sites, on formation of analyzed morphological traits will be evaluated. On the basis of morphological data migration routes of *P. sylvestris* by which this species recolonized European continent after the last glacial period will be discussed.

Materials and methods

Two Polish locations (Kórnik and Supraśl) of IUFRO 1982 provenance trial with *Pinus sylvestris* were chosen for this study. The IUFRO 1982 provenance trial is comprised of 20 Scots pine populations (Table 1). In the following analysis IUFRO 1982 in Kórnik is represented only by 19 Scots pine populations because Bolewice provenance (9) dropped out the experiment and was not represented by any tree, when the needles were collected. Each population from both trial sites was represented by 15 individuals collected randomly from all available replicated plots. Only Turkish provenance Catacik (20) from IUFRO 1982 in Kórnik, consisted of six individual trees, the last which survived on this plot. Long shoots were cut from each individual and 10 two years old needles, each from a different shoot, were taken and used for biometrical analyses. The long shoots were cut from possibly the highest and the sunniest parts of each tree. A total of 5760 needles were analyzed.

IUFRO 1982 provenance trial in Kórnik is a permanent plot in the Institute of Dendrology in Kórnik experimental forest located in central Poland (52° 15' N and 17° 04' E). This trial was planned as a set of seven completely randomized blocks. Every provenance was planted in three to seven replicated plots (one per block) (Oleksyn 1988). The climate in this region is transitional from maritime to continental. The average annual precipitation is 526 mm and an average annual temperature 7.7°C, with a mean growing season length of 220 days, calculated as a number of days with an average temperature above 5°C (Oleksyn et al. 1992).

IUFRO 1982 provenance trial in Supraśl is an experimental area located in the south-eastern part of Knyszyńska Forest (53° 11' N and 23° 18' E), supervised by Department of Silviculture of Poznań University of Life Science (former Agricultural University in Poznań). This site was planned as a set of four completely randomized blocks with each provenance in two or four replicated plots (one per block) (Barzdajn 2000). The humid climate of the region is characterized by the clear influence of continental climate. An average annual precipitation is 617 mm and an average temperature is 7.0°C (Rzeźnik 1991). The mean growing season length for this part of Poland is about 202 days (Kožuchowski and Degirmendžić 2005).

Each needle was analyzed separately with regard to the following seven morphological traits: 1) needle length (NL), 2) number of stomatal rows on the flat side of a needle (NRF), 3) number of stomata per 2 mm of needle length on the flat side (NSF), 4) number of stomatal rows on the convex side of a needle (NRC), 5) number of stomata per 2 mm of needle length on the convex side (NSC), 6) number of

Table 1. The origin of Scots pine (*Pinus sylvestris* L.) provenances used in the study. Provenances are ordered and grouped by latitude of origin

Region	Provenance	Country	Latitude	Longitude	Altitude
North (>55°N)	1. Roshtshinsaya Datsha	Russia	60° 15'	29° 54'	80
	15. Sumpberget	Sweden	60° 11'	15° 52'	185
	2. Kondezhscoe	Russia	59° 58'	33° 30'	70
	3. Serebyanskoe	Russia	58° 50'	29° 07'	80
Central (55–47°N)	4. Silene	Latvia	55° 45'	26° 40'	165
	5. Miłomłyn	Poland	53° 34'	20° 00'	110
	6. Supraśl	Poland	53° 12'	23° 22'	160
	10. Neuhaus	Germany	53° 02'	13° 54'	40
	11. Betzhorn	Germany	52° 30'	10° 30'	65
	9. Bolewice	Poland	52° 24'	16° 03'	90
	7. Spała	Poland	51° 37'	20° 12'	160
	8. Rychtal	Poland	51° 08'	17° 55'	190
	13. Ardennes	Belgium	50° 46'	04° 26'	110
	12. Lampertheim	Germany	50° 00'	10° 00'	95–100
	14. Haguenau	France	48° 49'	07° 46'	130–180
South (<47°N)	16. Zahorie	Slovakia	48° 46'	17° 03'	160
	17. Pornóapáti	Hungary	47° 20'	16° 28'	–
	19. Prusačka Rijeka	Bosnia	44° 06'	17° 21'	800–970
	18. Maočnica	Montenegro	43° 10'	19° 30'	1200
	20. Catacik	Turkey	40° 00'	31° 30'	1380–1420

serrations per 2 mm of needle length on the left edge of a needle (NSL) and 7) number of serrations per 2 mm of needle length on the right edge of a needle (NSR). These traits were recorded under a binocular microscope at magnification of 40× on material preserved in 70% alcohol. Biometrical analysis of traits 2–7 were conducted in the middle part of each needle. The data were analyzed statistically. The minimal and maximal values of these traits were found and arithmetic means, standard deviation and variation coefficient were estimated. The LSD (Fisher's Least Significant Difference) test was used for comparing all studied populations (Zar 1984). A discriminant analysis was used to present the scattering of the multitrait populations in the space of the first three discriminant variables. Mahalanobis distances for all population pairs were calculated and the shortest dendrite was constructed to examine possible relationships. Differences between three distinguished groups of populations were evaluated by contrasts (comparison of two groups of objects by the means of F-test with one degree of freedom) (Zar 1984). Moreover, two-factor multivariate analysis of variance was used (Morrison 1976) to estimate the influence of provenance trial location on the form of morphological traits of needles – Bolewice provenance (9) was excluded from that analyze because it was represented

only in IUFRO 1982 in Supraśl. All statistical calculations were made using STATISTICA 8.0 (StatSoft Polska).

Results

The analysis of variance showed that all morphological traits of needles discriminate significantly compared populations with the highest F-ratios for NL, NRC and NRF (Table 2, Appendix1).

The Scots pine populations from IUFRO 1982 in Kórnik appeared to be more variable than populations from provenance trial in Supraśl, with variation coefficient higher for most traits (Table 3, 4). The most variable traits in populations from IUFRO 1982 in Kórnik were NRF and NRC. These traits also reached high values of variation coefficient in populations from IUFRO 1982 in Supraśl but the most variable trait there was NL. NSF and NSC obtained the lowest values of variation coefficient in populations from IUFRO provenance trial in Supraśl and Kórnik as well.

For all traits and all combinations of provenances from both IUFRO 1982 experimental sites LSD test was calculated (Table 3, 4). All morphological traits showed significant differences between analyzed provenances. The highest interpopulational differen-

Table 2. The results of two-way analysis of variance

Source of variation	Degrees of freedom	Mean squares for trait							F – statistic for all traits
		NL	NRF	NSF	NRC	NSC	NSL	NSR	
Locations (L)	1	69658.11**	203.17**	98.88**	327.11**	78.11**	101.90**	121.62**	170.74**
Populations (P)	18	626.96**	11.36**	4.15**	19.04**	3.51*	2.95**	2.32**	3.80**
L x P	18	188.51**	4.60**	2.13	4.88	1.70	1.46*	1.25	1.78**
Error	514	77.58	2.24	1.60	3.03	1.81	0.78	0.81	
Total	551								

* difference significant at $p < 0.05$; ** difference significant at $p < 0.01$

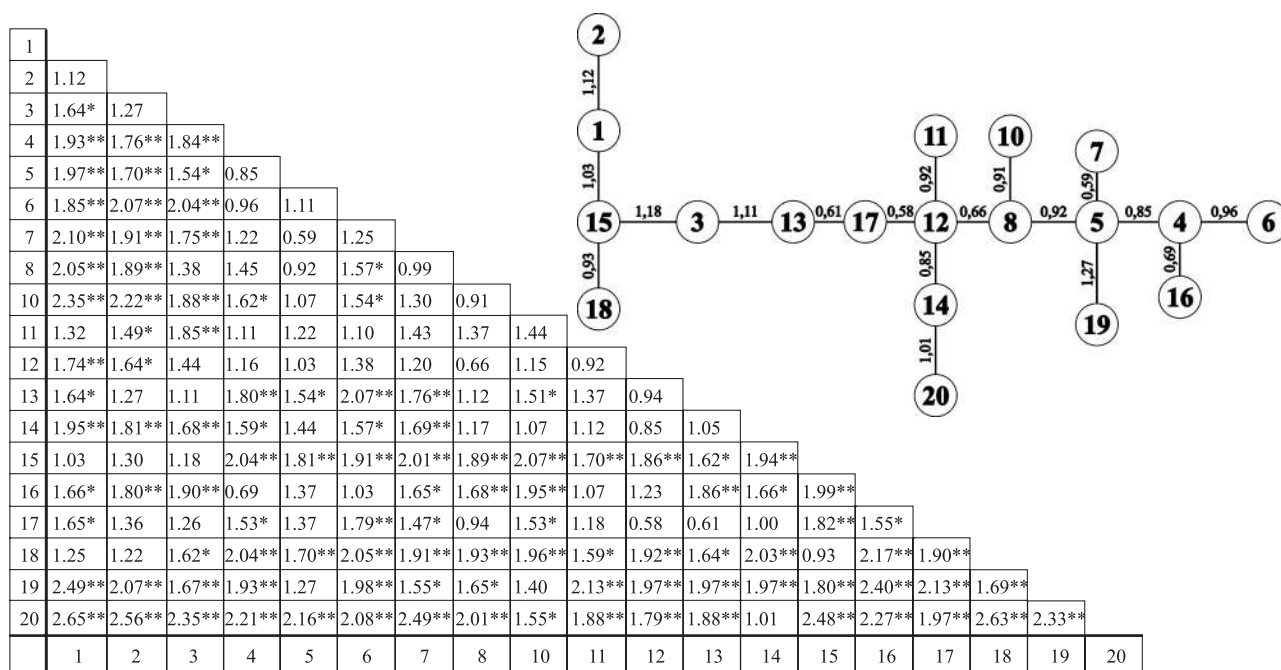
NL, needle length; NRF, number of stomatal rows on the flat side of a needle; NSF, number of stomata per 2 mm of needle length on the flat side; NRC, number of stomatal rows on the convex side of a needle; NSC, number of stomata per 2 mm of needle length on the convex side; NSL, number of serrations per 2 mm of needle length on the left edge of a needle; NSR, number of serrations per 2 mm of needle length on the right edge of a needle.

tiations were found in NL, NRF and NRC and in case of IUFRO 1982 from Supraśl also in NSL and NSR. NSC differentiated *P. sylvestris* provenances in a lesser extent in both trial sites, whereas NSF showed fewer differences among Scots pine populations from IUFRO 1982 in Supraśl.

Mahalanobis distances and minimum spanning trees (dendrites) constructed on the shortest Mahalanobis' distances showed geographical pattern of differentiation of Scots pine. Taking into consideration IUFRO 1982 provenance trial in Kórnik, the statistically significant values of Mahalanobis distances and appropriate dendrite (Fig. 1) allowed to distinguish Scots pine from north Russia (Roshtshinsaya Datsha (1), Kondezhscoe (2), Serebyanskoe (3)) and Sweden (Sumpberget (15)) and also three southernmost provenances: Maočnica from Montenegro (18), Prusačka

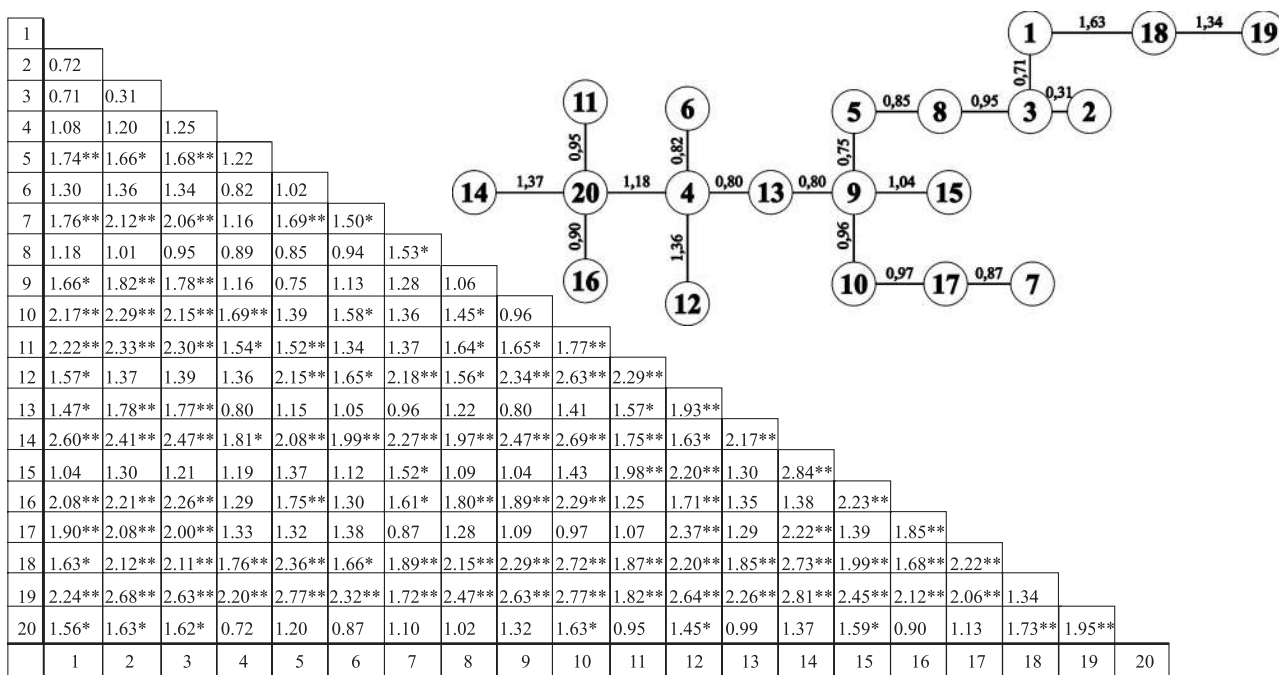
Rijeka from Bosnia (19) and Turkish population Catacik (20). The shortest Mahalanobis distance unites populations Lampertheim (12) and Pornóapáti (17), while the largest and statistically significant distances divide the provenance Catacik (20) from Roshtshinsaya Datsha (1), Maočnica (18) and Kondezhscoe (2).

In the case of IUFRO 1982 in Supraśl, a unique character was demonstrated by both Balkan populations (18 and 19) which differ considerably from all others populations (Fig. 2). Moreover, north Russian populations: Roshtshinsaya Datsha (1), Kondezhscoe (2) and Serebyanskoe (3) as well as Lampertheim (12) from Germany, Haguenau (14) from France and Zahorie (16) from Slovakia turned out to be significantly different from most of the populations. The shortest Mahalanobis distance was



* – statistically significant value for $p < 0.05$; ** – statistically significant value for $p < 0.01$

Fig. 1. Mahalanobis distances and dendrite constructed on the shortest Mahalanobis' distances describing similarity between 19 Scots pine populations from provenance trial IUFRO 1982 in Kórnik



* – statistically significant value for $p < 0.05$; ** – statistically significant value for $p < 0.01$

Fig. 2. Mahalanobis distances and dendrite constructed on the shortest Mahalanobis' distances describing similarity between 20 Scots pine populations from provenance trial IUFRO 1982 in Supraśl

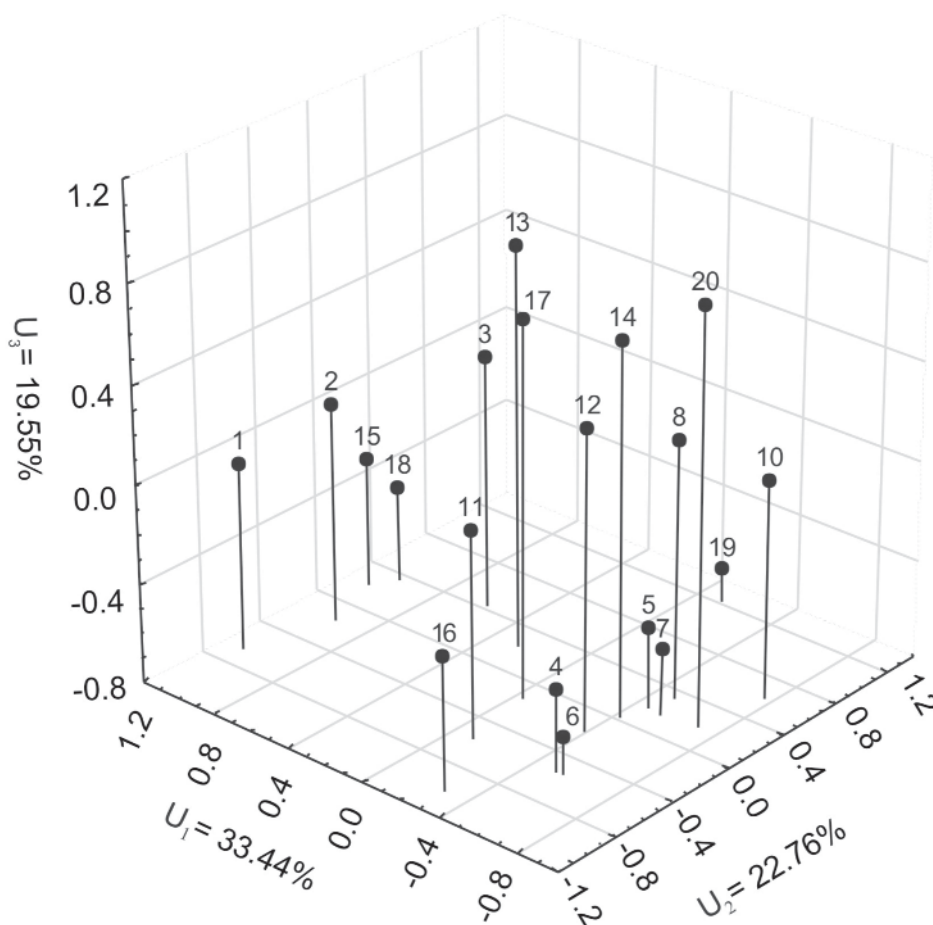


Fig. 3. Results of discriminant analysis based on seven needle morphological traits of *P. sylvestris* provenances from IUFRO 1982 in Kórnik plotted along three first discriminant variables U_1 , U_2 and U_3 accounted for 75.75% of total variation

Table 3. Mean values of analyzed morphological traits with its standard deviation [SD] and variation coefficient [C.V.] calculated for Scots pine (*Pinus sylvestris* L.) populations from IUFRO 1982 in Kórnik. Provenances are ordered and grouped by latitude of origin

Provenances	Morphological traits of needle													
	NL [cm]±SD	C.v. (%)	NRF [n]±SD	C.v. (%)	NSF [n]±SD	C.v. (%)	NRC [n]±SD	C.v. (%)	NSC [n]±SD	C.v. (%)	NSL [n]±SD	C.v. (%)	NSR [n]±SD	C.v. (%)
1. Roshshinsaya Datsha	70.6±6.89 ^a	9.75	12.5±1.37 ^{abcde}	10.99	22.4±1.55 ^{ab}	6.90	13.3±1.62 ^{bd}	12.11	22.6±1.55 ^{ab}	6.86	6.9±0.91 ^{ab}	13.19	6.7±0.90 ^a	13.36
15. Sumpberget	70.5±8.26 ^a	11.71	11.6±1.36 ^{abc}	11.69	22.9±1.45 ^{abcd}	6.32	12.5±1.90 ^{ab}	15.18	22.6±1.43 ^{ab}	6.34	7.4±0.86 ^{abcd}	11.71	7.2±0.89 ^{abc}	12.38
2. Kondezhskoe	70.9±8.71 ^a	12.29	12.6±1.35 ^{bde}	10.78	23.5±1.32 ^{def}	5.63	13.4±1.78 ^{bd}	13.30	24.0±1.73 ^d	7.24	7.3±0.77 ^{abcd}	10.51	7.2±0.89 ^{abc}	12.43
3. Serebyanskoe	72.8±9.61 ^{ab}	13.20	12.1±1.18 ^{abcd}	9.74	23.9±1.32 ^f	5.51	13.2±1.22 ^{abc}	9.24	23.4±1.45 ^{bd}	6.21	7.3±0.54 ^{abcd}	7.44	7.3±0.62 ^{abc}	8.46
4. Silene	82.4±7.06 ^{def}	8.57	14.0±1.98 ^{le}	14.11	23.1±1.42 ^{hdef}	6.16	14.8±2.21 ^{ef}	14.91	23.3±1.57 ^{bd}	6.75	7.7±0.97 ^{ab}	12.52	7.6±0.92 ^{cd}	12.11
Mean	73.4±9.17	12.49	13.0±1.6	13.08	23.2±1.47	6.33	13.0±1.9	14.01	23.2±1.6	6.90	7.3±0.8	11.48	7.2±0.9	12.12
5. Miliomyln	83.4±4.03 ^{def}	4.83	12.8±1.55 ^{cde}	11.98	23.1±1.26 ^{hdef}	5.45	13.5±1.53 ^{bde}	11.33	23.3±1.09 ^{bd}	4.69	7.6±0.91 ^{cde}	11.87	7.6±0.90 ^{cd}	11.83
6. Supraśl	83.7±9.29 ^{def}	11.10	13.4±1.78 ^{ef}	13.26	22.1±1.48 ^a	6.68	14.6±2.14 ^{def}	14.68	22.3±1.33 ^a	5.97	7.6±0.93 ^{cde}	12.22	7.5±0.99 ^{cd}	13.15
10. Neuhaus	88.8±12.04 ^f	13.56	12.0±1.65 ^{abcd}	13.80	23.2±1.20 ^{hdef}	5.19	13.2±1.99 ^{abcd}	15.09	23.1±1.17 ^{abcd}	5.06	7.4±0.84 ^{abcd}	11.38	7.2±0.62 ^{abc}	8.49
11. Betzhorn	82.1±11.19 ^{def}	13.62	13.1±2.92 ^{def}	22.34	22.6±1.30 ^{abc}	5.76	14.1±3.20 ^{def}	22.65	22.9±1.67 ^{abc}	7.27	7.1±0.80 ^{abcd}	11.26	6.9±0.68 ^{ab}	9.85
9. Bolewice	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7. Spala	84.2±7.38 ^{def}	8.76	12.5±1.28 ^{bde}	10.25	22.7±1.07 ^{abc}	4.73	13.2±1.72 ^{abc}	13.06	23.0±1.17 ^{abcd}	5.08	7.4±1.14 ^{abcd}	15.44	7.5±1.14 ^{bd}	15.24
8. Rychtal	84.5±8.74 ^{def}	10.33	12.3±1.68 ^{abcde}	13.68	23.3±1.05 ^{hdef}	4.52	13.2±1.90 ^{bd}	14.35	23.1±1.08 ^{abcd}	4.68	7.0±0.93 ^{abc}	13.35	7.0±0.89 ^{abc}	12.67
13. Ardennes	78.6±9.12 ^{bd}	11.61	12.2±1.34 ^{abcd}	11.03	23.8±1.54 ^{ef}	6.45	13.4±1.90 ^{bd}	14.17	23.7±1.41 ^{ef}	5.92	6.8±0.68 ^{ab}	9.97	6.7±0.69 ^a	10.30
12. Lampertheim	83.5±12.42 ^{def}	14.88	13.0±1.65 ^{def}	12.7	23.2±1.21 ^{hdef}	5.23	14.1±1.46 ^{cdef}	10.37	23.2±1.49 ^{abcd}	6.42	6.9±1.05 ^{ab}	15.14	6.9±1.08 ^{ab}	15.66
14. Hagenau	84.9±11.35 ^{def}	13.37	12.7±1.58 ^{cde}	12.50	23.3±1.04 ^{hdef}	4.45	14.4±1.88 ^{cdef}	13.05	23.4±1.12 ^{bd}	4.81	7.1±0.81 ^{abcd}	11.46	7.0±0.74 ^{abc}	10.53
16. Zahorie	80.0±11.23 ^{cde}	14.05	14.4±1.96 ^f	13.65	22.8±0.57 ^{abcd}	2.49	15.3±2.39 ^f	15.67	22.8±0.70 ^{abc}	3.07	7.4±0.76 ^{bd}	10.26	7.2±0.70 ^{abc}	9.69
Mean	83.4±10.03	12.03	12.8±1.86	14.49	23.0±1.25	5.43	13.9±2.12	15.22	23.1±1.26	5.47	7.2±0.9	12.56	7.2±0.9	12.33
17. Pomopáti	80.2±9.28 ^{de}	11.57	12.8±0.93 ^{cde}	7.28	23.5±1.08 ^{cdef}	4.61	13.9±1.21 ^{cdef}	8.68	23.5±1.22 ^{bd}	5.19	6.7±0.64 ^a	9.51	6.7±0.83 ^a	12.25
19. Prusačka Rijeka	81.1±12.13 ^{de}	14.96	11.4±1.44 ^{ab}	12.63	23.7±1.76 ^{def}	7.45	12.3±1.73 ^{ab}	14.10	23.7±1.99 ^{cd}	8.40	8.2±1.09 ^e	13.27	8.1±0.99 ^d	12.27
18. Maočnica	73.2±7.89 ^{abc}	10.78	11.3±1.67 ^a	14.71	22.9±1.36 ^{abcde}	5.91	11.9±1.75 ^a	14.79	23.2±1.40 ^{abcd}	6.02	7.4±1.26 ^{bcd}	17.01	7.1±1.20 ^{abc}	16.73
20. Catatic	87.9±10.88 ^{ef}	12.38	12.7±0.90 ^{cde}	7.11	23.5±1.63 ^{cdef}	6.93	15.1±1.91 ^{ef}	12.64	23.4±1.35 ^{bd}	5.79	7.5±0.54 ^{bcde}	7.24	7.2±0.74 ^{abc}	10.20
Mean	79.3±10.8	13.56	11.9±1.5	12.28	23.4±1.4	6.16	13.0±2.0	15.07	23.4±1.5	6.45	7.5±1.1	14.89	7.3±1.1	15.06
Total mean	79.9±10.78	13.49	12.6±1.76	13.98	23.1±1.35	5.83	13.6±2.05	15.07	23.2±1.41	6.08	7.3±0.93	12.77	7.2±0.92	12.81

The same letters means lack of statistically significant differences (LSD test, p<0.05); Abbreviations describing analysed traits was explained in Table 2.

Table 4. Mean values of analyzed morphological traits with its standard deviation [SD] and variation coefficient [C.V.] calculated for Scots pine (*Pinus sylvestris* L.) populations from IUFRO 1982 in Supraśl. Provenances are ordered and grouped by latitude of origin

Provenances	Morphological traits of needle													
	NL [cm]±SD	C.v. (%)	NRF [n]±SD	C.v. (%)	NSF [n]±SD	C.v. (%)	NRC [n]±SD	C.v. (%)	NSC [n]±SD	C.v. (%)	NSL [n]±SD	C.v. (%)	NSR [n]±SD	C.v. (%)
1. Roshtshinsaya Datsha	52.6±5.72 ^{abc}	10.89	11.1±1.59 ^{abcde}	14.34	22.7±1.25 ^{bcd}	5.53	11.6±1.96 ^{abc}	16.99	22.3±1.35 ^{abc}	6.06	7.8±0.90 ^{abc}	11.57	7.9±0.70 ^{abcde}	8.95
15. Sumpberget	56.3±5.66 ^{cde}	10.07	10.7±1.47 ^{abcd}	13.78	22.3±1.56 ^{abcd}	7.02	11.0±1.12 ^a	10.22	21.8±1.40 ^a	6.42	8.1±1.05 ^{abcde}	12.97	8.0±1.14 ^{abcde}	14.24
2. Kondezhskoe	56.7±8.55 ^{cdef}	15.09	11.6±1.53 ^{defg}	13.17	23.0±1.13 ^d	4.92	12.2±2.04 ^{bcd}	16.74	22.4±1.23 ^{abc}	5.48	7.7±0.60 ^a	7.78	7.7±0.61 ^{ab}	7.85
3. Serebyanskoe	56.5±5.98 ^{cdef}	10.59	11.5±1.06 ^{cdefg}	9.22	23.0±1.24 ^d	5.39	12.1±1.53 ^{abcd}	12.66	22.4±1.33 ^{abc}	5.93	7.9±0.87 ^{abc}	11.10	8.0±0.82 ^{abcde}	10.31
4. Silene	57.6±11.35 ^{cdef}	19.69	11.4±1.37 ^{bcdefg}	12.02	22.1±1.67 ^{abc}	7.56	12.2±1.34 ^{bcd}	10.93	22.1±1.97 ^{bc}	8.92	7.8±0.92 ^{abc}	11.81	7.7±0.86 ^b	11.17
Mean	55.9±7.8	13.90	11.2±1.4	12.60	22.6±1.4	6.20	11.8±1.7	14.10	22.2±1.5	6.50	7.9±0.9	11.10	7.9±0.8	10.60
5. Miliomyln	64.3±7.48 ^g	11.63	11.3±1.23 ^{bcdef}	10.89	22.3±0.90 ^{abcd}	4.01	11.7±1.14 ^{abc}	9.72	22.6±0.85 ^{abcd}	3.76	7.9±0.78 ^{abcd}	9.89	7.8±0.69 ^{abc}	8.81
6. Supraśl	60.5±9.80 ^{defg}	16.21	11.8±1.30 ^{gh}	11.01	22.0±1.20 ^{abc}	5.45	12.3±1.44 ^{bcd}	11.77	22.0±1.42 ^{ab}	6.46	8.1±0.79 ^{abcde}	9.76	8.0±0.70 ^{abcdef}	8.73
10. Neuhaus	62.4±7.30 ^{fg}	11.71	10.3±1.12 ^a	10.94	21.9±1.14 ^{ab}	5.24	11.0±1.53 ^a	13.88	21.8±1.31 ^a	5.98	8.4±0.55 ^{cdef}	6.52	8.5±0.53 ^{fg}	6.20
11. Betzhorn	61.7±10.31 ^{efg}	16.71	11.9±1.81 ^{fgh}	15.22	22.3±1.17 ^{abcd}	5.26	12.6±2.03 ^{abc}	16.10	22.9±1.12 ^{bcd}	4.88	8.8±0.65 ^{fg}	7.33	8.6±0.52 ^{fg}	6.02
9. Bolewice	60.9±7.49 ^{defg}	12.31	10.6±1.10 ^{abc}	10.40	21.9±1.40 ^{ab}	6.39	11.0±1.15 ^a	10.42	22.1±1.29 ^{ab}	5.84	7.9±0.69 ^{abcd}	8.68	7.9±0.75 ^{abcd}	9.54
7. Spała	55.1±6.41 ^{bcd}	11.65	10.4±1.29 ^{ab}	12.39	21.9±1.24 ^{ab}	5.68	11.4±1.57 ^{ab}	13.75	22.3±1.08 ^{abc}	4.85	8.4±1.38 ^{bcdef}	16.55	8.3±1.23 ^{bcdef}	14.85
8. Rychtal	60.8±9.23 ^{defg}	15.19	11.3±1.38 ^{abcdef}	12.21	22.8±1.38 ^{cd}	6.06	12.0±1.16 ^{abc}	9.71	22.6±1.49 ^{abcd}	6.60	8.0±0.96 ^{abcd}	11.99	8.0±0.85 ^{bcdef}	10.57
13. Ardennes	57.6±6.90 ^{cdef}	11.98	10.8±1.18 ^{abcde}	10.95	21.6±0.94 ^a	4.35	11.4±1.40 ^{ab}	12.22	22.0±1.00 ^{ab}	4.53	7.8±0.88 ^{ab}	11.37	7.8±0.80 ^{ab}	10.30
12. Lampertheim	57.7±8.13 ^{cdef}	14.10	12.3±1.60 ^{gh}	13.04	22.6±1.59 ^{bcd}	7.05	13.6±1.66 ^{ef}	12.19	22.4±1.47 ^{abc}	6.58	7.6±1.03 ^a	13.46	7.5±0.95 ^a	12.73
14. Hagenau	65.6±10.08 ^g	15.36	12.8±1.41 ^h	11.02	22.5±1.36 ^{bcd}	6.03	14.4±1.81 ^f	12.60	23.1±1.70 ^{cd}	7.34	8.0±0.72 ^{abcd}	9.04	8.0±0.64 ^{abcdef}	8.00
16. Zahorie	59.9±10.47 ^{defg}	17.48	12.3±1.74 ^{gh}	14.16	21.6±0.69 ^a	3.21	13.1±2.16 ^{de}	16.42	22.4±0.98 ^{abc}	4.36	8.0±0.82 ^{abcd}	10.18	8.0±0.72 ^{bcdef}	8.95
Mean	60.6±8.9	14.60	11.4±1.6	13.90	22.1±1.2	5.60	12.2±1.9	15.20	22.4±1.3	5.80	8.1±0.9	11.10	8.1±1.0	12.00
17. Pomóapáti	60.1±10.12 ^{defg}	16.84	10.7±0.98 ^{abcde}	9.13	22.3±1.09 ^{abcd}	4.88	11.6±1.23 ^{abc}	10.59	22.5±1.16 ^{abc}	5.17	8.7±1.03 ^{efg}	11.91	8.4±0.90 ^{def}	10.71
19. Prusačka Rijeka	48.0±5.12 ^a	10.66	11.1±1.27 ^{abcdef}	11.46	22.7±1.14 ^{bcd}	5.02	11.8±1.20 ^{abc}	10.15	23.5±1.08 ^d	4.61	9.2±0.80 ^f	8.68	9.0±0.78 ^f	8.63
18. Maočnica	49.9±7.05 ^{ab}	14.13	11.8±1.35 ^{fg}	11.41	22.1±0.80 ^{abc}	3.60	11.9±1.73 ^{abc}	14.48	22.7±1.12 ^{abcd}	4.96	8.5±0.82 ^{def}	9.63	8.4±1.00 ^{cdef}	11.82
20. Catatic	60.0±6.33 ^{defg}	10.56	11.7±1.21 ^{efg}	10.38	22.2±1.52 ^{abcd}	6.86	12.6±1.48 ^{cde}	11.70	22.6±1.45 ^{abcd}	6.44	8.2±0.85 ^{abcde}	10.34	8.2±0.85 ^{bcdef}	10.37
Mean	54.5±9.14	16.80	11.3±1.26	11.10	22.3±1.16	5.19	12.0±1.44	12.00	22.8±1.25	5.46	8.6±0.9	11.00	8.5±0.9	11.0
Total mean	58.2±9.03	15.52	11.4±1.48	13.02	22.3±1.28	5.74	12.1±1.74	14.38	22.4±1.33	5.96	8.1±0.93	11.46	8.1±0.95	11.70

The same letters means lack of statistically significant differences (LSD test, p<0.05); Abbreviations describing analysed traits was explained in Table 2.

Table 5. Determination coefficients between seven morphological traits of needles from IUFRO 1982 in Kórnik and the three first discriminant variables U_1 , U_2 and U_3

Traits	U_1 (33.44%)	U_2 (22.76%)	U_3 (19.55%)
NL	27.77	0.25	0.16
NRF	4.02	13.34	0.04
NSF	0.02	3.97	3.03
NRC	5.26	9.69	0.87
NSC	0.03	1.58	1.26
NSL	0.41	1.29	7.80
NSR	1.06	2.02	7.72

Abbreviations describing analysed traits was explained in Table 2.

found between populations Kondezhskoe (2) and Serebyanskoe (3), whereas the largest distances were found between population Haguenau (14) and two other Scots pine provenances Sumpberget (15) and Prusaèka Rijeka (19).

The distribution of populations in the space of the first three discriminant variables (U_1 , U_2 and U_3) emphasized the separation between the northernmost and southernmost provenances in both IUFRO 1982

Table 6. Determination coefficients between seven morphological traits of needles from IUFRO 1982 in Supraśl and three first discriminant variables U_1 , U_2 and U_3

Traits	U_1 (31.29%)	U_2 (28.53%)	U_3 (23.67%)
NL	0.51	3.07	15.97
NRF	3.02	7.69	4.40
NSF	0.69	2.78	0.06
NRC	4.74	7.10	9.01
NSC	3.30	0.51	0.00
NSL	5.74	2.66	3.14
NSR	3.70	0.79	1.70

Abbreviations describing analysed traits was explained in Table 2.

provenance trials and an individual character of the population Haguenau (14) from the experimental site in Supraśl (Fig. 3, 4). In case of IUFRO 1982 in Kórnik, NL had a decisive influence on the result of the analysis. Other important characters were successively NRF, NRC, NSL and NSR (Table 5). In Supraśl experimental site NSL together with NRF and NRC played a significant role in the analysis, slightly less important was NL (Table 6).

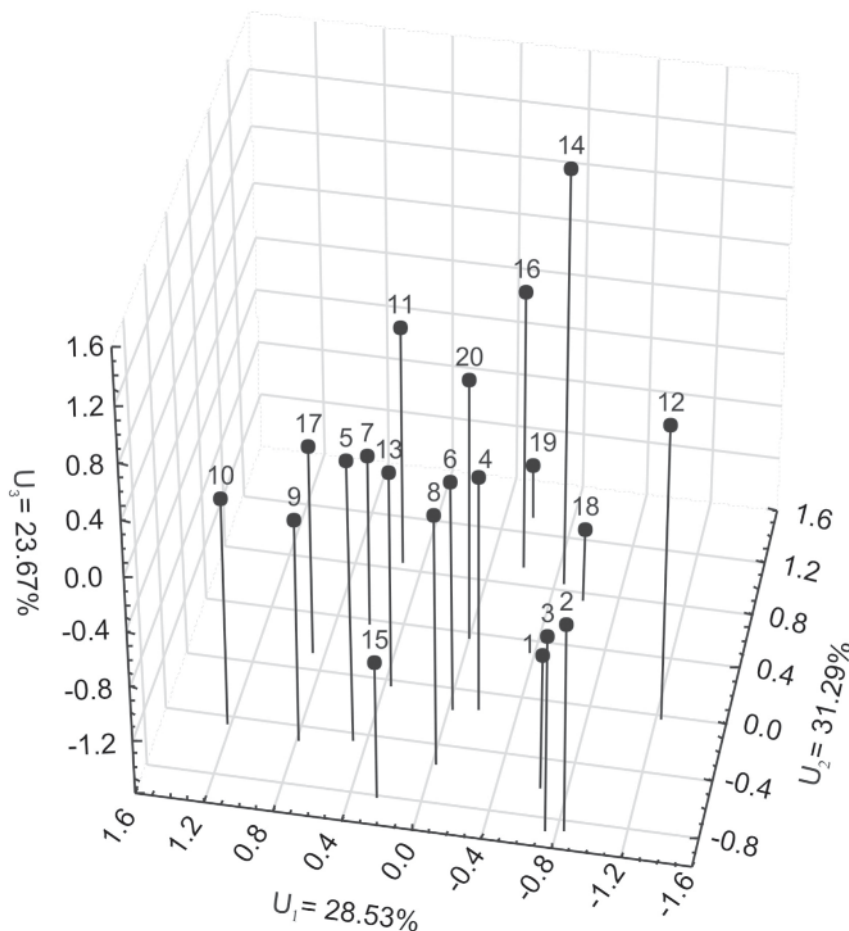


Fig. 4. Result of discriminant analysis based on seven needle morphological traits of *P. sylvestris* provenances from IUFRO 1982 in Supraśl plotted along three first discriminant variables U_1 , U_2 and U_3 accounted for 83.49% of total variation

Table 7. Estimates and results of testing contrasts between three groups of provenances from IUFRO 1982 provenance trial in Kórnik and Supraśl, respectively

Provenance trial location	Contrast	Values of contrast for the certain morphological traits of the needle							F statistic for all morphological traits
		NL	NRF	NSF	NRC	NSC	NSL	NSR	
IUFRO 1982 Kórnik	North-Centre	-9.93*	-0.27	0.13	-0.46	0.09	0.09	0.04	7.16
	Centre-South	4.05*	0.89*	-0.36	0.92*	-0.37	-0.22	-0.16	3.06
	North-South	-5.88*	0.63*	-0.23	0.46	-0.27	-0.13	-0.12	2.68
IUFRO 1982 Supraśl	North-Centre	-4.65*	-0.19	0.51*	-0.42	-0.18	-0.22	-0.23	8.32
	Centre-South	6.09*	0.09	-0.23	0.22	-0.41*	-0.54*	-0.43*	5.54
	North-South	1.44	-0.11	0.28	-0.20	-0.59*	-0.76*	-0.65*	8.21
F _{0.05}									2.64

*contrast is significant at 0.05 level; Abbreviations describing analysed traits was explained in Table 2.

The estimates and results of testing contrasts among three groups of Scots pine populations, which in IUFRO 1982 represent northern, central and southern part of distribution area of that species in Europe, were also studied.

Considering the provenance trial in Kórnik the results of testing contrasts revealed a significant differ-

ence in NL between all three groups of provenances in questions (Table 7). The longest needles were observed in provenances from Central Europe, medium NL values were found in southern part of continent, whereas the shortest needles were characteristic for northern Russia, Latvia and Sweden. Significant values of contrast between following groups: Cen-

Appendix 1. Testing of the differences (results of F-test) between populations from IUFRO 1982 provenance trial in Kórnik and Supraśl

Population	Traits							F – statistic for all traits
	NL	NRF	NSF	NRC	NSC	NSL	NSR	
1. Roshtshinsaya Datsha	18.1**	1.41**	-0.24	1.79**	0.28	-0.89*	-1.13**	6.77**
2. Kondezhskoe	14.2**	0.97	0.46	1.19	1.56**	-0.40	-0.56	5.68*
3. Serebyanskoe	16.2**	0.68	0.83	1.08	1.05*	-0.57	-0.65*	5.33*
4. Silene	24.7**	2.65**	1.01*	2.57**	1.17*	-0.09	-0.15	11.26**
5. Miomlyn	19.1**	1.49**	0.79	1.80**	0.73	-0.29	-0.25	6.17**
6. Supraśl	23.2**	1.59**	0.13	2.38**	0.27	-0.47	-0.51	8.26**
7. Spała	29.2**	2.09**	0.85	1.73**	0.77	-1.05**	-0.80*	16.47**
8. Rychtal	23.8**	1.03	0.53	1.26**	0.47	-1.00**	-1.03**	9.88**
10. Neuhaus	26.4**	1.70**	1.33**	2.17**	1.27*	-1.05**	-1.23**	14.26**
11. Betzhorn	20.4**	1.23**	0.30	1.55*	-0.01	-1.73**	-1.71**	10.74**
12. Lampertheim	25.8**	0.69	0.64	0.54	0.81	-0.70*	-1.08**	12.02**
13. Ardennes	21.0**	1.42**	2.27**	1.96**	1.72**	-0.94**	-1.08**	13.14**
14. Haguenau	19.3**	-0.15	0.81	0.03	0.25	-0.87**	-1.02**	7.97**
15. Sumpberget	14.2**	0.95	0.61	1.54*	0.79	-0.75*	-0.77*	4.71*
16. Zahorie	20.1**	2.07**	1.23**	2.15**	0.35	-0.61	-0.77*	9.63**
17. Pornóapáti	20.1**	2.01**	1.16*	2.32**	1.05*	-1.91**	-1.68**	14.53**
18. Maočnica	23.4**	-0.47	0.82	-0.09	0.53	-1.07**	-1.27**	11.53**
19. Prusačka Rijeka	33.1**	0.29	0.97*	0.45	0.20	-0.97**	-0.92**	19.01**
20. Catacik	27.9**	1.03	1.33**	2.48**	0.79	-0.71*	-0.94**	15.52**
SD of mean difference	3.22	0.55	0.46	0.64	0.49	0.32	0.33	

* difference significant at $p < 0.05$; ** difference significant at $p < 0.01$

NL, needle length; NRF, number of stomatal rows on the flat side of a needle; NSF, number of stomata per 2 mm of needle length on the flat side; NRC, number of stomatal rows on the convex side of a needle; NSC, number of stomata per 2 mm of needle length on the convex side; NSL, number of serrations per 2 mm of needle length on the left edge of a needle; NSR, number of serrations per 2 mm of needle length on the right edge of a needle.

tre-South and North-South; observed for NRF, showed that Scots pine from Central and Northern Europe display a higher value of that trait than individuals of this species originating from Southern Europe. The distinct character of Scots pine provenances from Central Europe in comparison to those from Southern Europe is also shown by significant values of contrast for NRC: *P. sylvestris* from Central Europe reaches higher values than those from Southern Europe as far as NRC is concerned.

Results of testing the contrasts for provenance trial in Supraśl also proved that Scots pine from both North and South European provenances have significantly shorter needles (NL) than individuals of that species from Central Europe (Table 7). The value of contrast for NSF pointed that *P. sylvestris* from North European populations obtain higher values of this trait than those from central part of the continent. Moreover, statistically significant values of contrast for NSC, NSL and NSR indicated that both Central and North European Scots pine populations, in comparison to those from Southern part of continent, demonstrate higher average values for all three morphological traits.

The significant values of F statistic (Table 2) for population \times location interaction in the case of NL, NRF and NSL showed that in many cases Scots pine populations react differently to dissimilar environmental conditions stated for IUFRO 1982 provenance trial plots in Kórnik and in Supraśl. Moreover, comparison of table 2 with data included in Tables 3 and 4 clearly showed, that average values of the NL and traits describing a spatial arrangement and a number of stomata on the surface of a needle (NRF, NSF, NRC and NSC) noted for the Scots pine population from IUFRO 1982 in Supraśl are lower than analogous values observed in appropriate populations from IUFRO 1982 in Kórnik. Only provenances Roshtshinsaya Datsha (1), Haguenau (14) and Maočnica (18) were an exception, for which higher values of NRF and NSF were noted in the experimental area in Supraśl. In case of NSL and NSR an opposite situation can be observed – higher values were obtained for Scots pine from IUFRO 1982 provenance trial in Supraśl.

Discussion

Our results of biometrical analysis of Scots pine from both locations of provenance trial IUFRO 1982 clearly point out a geographical pattern of interpopulational differentiation of morphological traits of needles. On the one hand, a huge heterogeneous group of provenances from Central Europe is clearly visible, while on the other the distinct character of Scots pine populations from northern and southern edges of its European distribution area can be observed (Fig. 1–4).

Similar pattern of geographical variation of Scots pine populations can be found also in previous studies performed in different locations of IUFRO 1982 provenance trial. In comparison to the Scots pine from Central Europe, mentioned above *Pinus sylvestris* populations from northern Russia, Sweden, mountainous areas of the Balkans and Turkey reveal definitely weaker growth and productivity (Kocięcki, 1985; Oleksyn, 1988; Oleksyn and Rachwał, 1994; Oleksyn et al., 1999; Barzdajn, 2000, 2008), poorer survival rate and lower resistance to pine needle cast (*Lophodermium pinastri*) (Rzeźnik, 1991) or needle feeding beetle *Brachyderes incanus* (Stephan and Liesbah, 1996). The weaker growth of northern provenances planted in more southern locations is a consequence of differences in both growth rate (Oleksyn et al. 1998) and growth period with earlier growth cessation caused by higher values of accumulated heat sun and shorter photoperiod observed in lower latitudes (Oleksyn et al. 1992). In case of southern populations their inferior growth may be a result of reduction of their genetic variation and specialization to local climatic conditions (Pravdin 1964, Szmidt and Wang 1993).

The similarity of Scots pine populations from Central Europe, described in this paper, is also confirmed by the results of studies evaluating the root biomass production of *P. sylvestris* from IUFRO 1982 provenance trial in Kórnik (Oleksyn et al. 1999). A low level of interpopulational differentiation of *P. sylvestris* from lowlands of Central Europe was also pointed by e.g. Giertych (1979), Mejnartowicz (1993), Stephan and Liesbach (1996), Urbaniak (1998) and Sinclair et al. (1999). There are several reasons which can explain such an occurrence: the intensive gene exchange between neighbouring populations, the lack of sufficient physiographic barriers which may limit that process or intense, long-lasting silviculture.

The distinct character of Scots pine populations, which in IUFRO 1982 provenance trials represent both North and South European edges of its distribution range, was shown mainly by NL (Tables 3, 4, 7). The geographical pattern of interpopulational differentiation of the needle length of Scots pine from IUFRO 1982 provenance trial was also noted by Oleksyn and Rachwał (1994) on the experimental plot in Niepołomice and by Oleksyn et al. (1999) in the experimental area in Kórnik. In both studies Scots pine provenances from central part of its European distribution range are characterized by needles longer than *P. sylvestris* from northern and southern Europe. Moreover, Oleksyn et al. (1999) emphasizes the positive and statistically significant relation between the needle length and tree height. The observation made in IUFRO 1982 experimental area in Niepołomice, pointing on longer needles of Scots pines originated from West European part of its distribution area in

comparison to these from Central Europe (Oleksyn and Rachwał 1994), was not confirmed by our results neither for Kórnik nor for Supraśl experimental plots of IUFRO 1982 provenance trial.

In addition, the results showed that out of all traits describing the distribution of stomata on the surface of the Scots pine needle, NSF and NSC showed a minor interpopulational differentiation. Considerably more important in this respect appeared to be NRF and NRC. Some populations were distinguished in this way, however no correlation between geographical distribution of populations and average values of NRF and NRC was observed (Table 3, 4). The importance of NSF and NSC in describing interpopulational differentiation of *P. sylvestris* was also stressed by Urbaniak (1998), who with the use of these traits, for IUFRO 1938 provenance trial in Lubień, proved the distinct character of Scots pine from northern Europe, i.e. from Scandinavia and Scotland, which are characterized by lower number of stomata in contrast to *P. sylvestris* from continental Europe. The geographical pattern of interpopulational differentiation of Scots pine, concerning special arrangement and number of stomata on the surface of a needle was also described by Mercet (1967) and Paule (1971) who observed lower number of stomatas in populations from higher latitudes for Finnish and Swedish locations, respectively. However, in case of our data this pattern of morphological differentiation of Scots pine was not so obvious.

The result of discriminant analysis based on seven needle morphological traits of *P. sylvestris* provenances from IUFRO 1982 in Supraśl showed the statistically significant influence of NSL on a pattern concerning interpopulational differentiation. NSL had the most significant impact on the first discriminant variable U_1 , which describe 31.29% of total variation (Table 6). Also in analyze of variance and in analyzing of contrast between groups of provenances NSL and NSR proved to be good source of information about interpopulational differentiation of the species.

The variation of average values of needle morphological traits observed between Scots pine populations from particular location of IUFRO 1982 provenance trial undoubtedly reflected genetic regulation of interpopulational differentiation of *P. sylvestris*. Whereas the differences in average values of a certain trait, noted for each pair of populations of the same origin planted in different trial plots (Kórnik and Supraśl) indicated that the location of experimental area had also an influence on the observed variation.

The experimental area of IUFRO 1982 in Supraśl, in comparison to the analogous experimental plot in Kórnik, are characterized by more severe general weather conditions which are under a considerable influence of continental climate. This influence is visible in e.g. lower average temperature during the year

or a shorter vegetation period. Previous data showed that the needle length of Scots pine depends on genetic regulation only partially and to a considerable extent undergoes a modifying influence of the environment (Żelawski and Govin 1966, Żelawski and Niwiński 1966, Urbaniak et al. 2003). It is observed, that the more severe environmental conditions are, the shorter needles become. This was proved by the analysis of the *P. sylvestris* needle length variation in transect from the north to the south (Langlet 1959, Pravdin 1964), in the case of Scots pine populations from mountainous (Żelawski and Niwiński 1966) and nutrient-poor stands (Niinemets et al. 2001).

The lower number of stomatal rows on the surface of a needle, concerning the Scots pine provenances from IUFRO 1982 in Supraśl, can be explained in a similar way. The modifying influence of the environment, which has an impact on the length and width of a needle, indirectly affects the number of stomatal rows as well (Urbaniak 2003). Thames (1963) and Salazar (1983) claimed that the lower number of stomata on the surface of a needle could be a form of adaptation to xeric conditions. Scots pine from north European terrains is exposed to strong, intense drying winds and periodically undergo physiological drought. An adaptation to such environmental condition could be manifested by restraining transpiration surface in order to decrease the loss of water (Urbaniak 1998).

Taking into consideration NSL and NSR, there are very little data published describing the variation of these traits. Nevertheless, our data confirm previous reports which point out that the environmental conditions can influence these traits formation (Woźniak et al. 2005, Urbaniak et al. 2006).

The last glacial period, which played a significant role in shaping a present-day differentiation of *Pinus sylvestris*, became a subject of interest for many scientific investigations (Tobolski and Hanover 1971, Petit et al. 2003, Cheddadi et al. 2006, Pyhäjärvi et al. 2008). Climate changes together with the expansions of ice sheets restricted *Pinus sylvestris* distribution range to patchy, discontinuous and spatially isolated areas designated as glacial refugia (Birks and Line 1993, Ralska-Jasiewiczowa 2004), localized in ice free terrains of southern and central Europe and also farther to the west and even in Siberia (Willis and van Andel 2004). Bennet et al. (1991) suggested that *Pinus sylvestris* had its refugia south of the Carpathians, on Iberian Peninsula, in the Alps, in the Balkans and in the Russian Plains. Similar observations were also made by Prus-Głowacki and Bernard (1994), Prus-Głowacki and Stephan (1994), Obidowicz (1996), Sinclair et al. (1999) and Soranzo et al. (2000).

In this respect Scots pine population from south-eastern Europe, Pornóapáti (17) from Hun-

gary, Maočnica (18) from Montenegro and Prusačka Rijeka (19) from Bosnia, deserve special attention. The populations mentioned above, however, geographically not very distant from one another, appeared to be different (Figs 3, 4, Tables 3, 4). Considering provenance Catacik (20) from Turkey, its geographical isolation seemed to affect its morphological variation in lesser extent.

In the case of provenances Maočnica (18) and Prusačka Rijeka (19), which originated from nearby mountainous massifs of the Balkans, complex physiographic conditions of this area could limit the gene flow between adjacent populations, which caused independent changes in a genetic structure and form a new variation. After the retreat of ice sheets, glacial refugia could become starting points from which *Pinus sylvestris* populations recolonized Europe. The similarity of Scots pine from central Europe to provenances from Hungary (17), Montenegro (18) and Bosnia (19) shows that in these regions of Europe the glacial refugia were found (Figs 1, 2). From these refugia Scots pine could have migrated northward, along the Carpathian arch on the terrains of present-day Slovakia, Poland and Germany. Moreover, a similarity of the Balkans Scots pine provenances to these from Western Europe and Sweden, and also to the populations of the species from Baltic countries could suggest the presence of different pathways of Scots pine migration (Fig. 1). On one hand, *Pinus sylvestris* recolonization routes could led from Balkan refugia through Jutland Peninsula and Danish straits to Scandinavia, whereas on the other hand, through the lowlands of central and eastern Europe, along south coast of the Baltic Sea to the northern and northwestern edges of our continent. The results of the morphological analysis seem to confirm such hypothesis.

Similar observations were made by Cheddadi et al. (2006), who on the basis of paleobotanical data and the analysis of mitochondrial DNA, revealed the existence of numerous fronts of Scots pine migration to northern Europe from Apenin Peninsula, terrains of Hungarian Plains, Danube region and the Balkans. Moreover, they claimed that Scots pine refugia from the eastern Alps, Hungarian Plains and Danube region seem to have played a major role during the entire recolonization process which took place between 14 ka and 8 ka (1ka = 1000 ¹⁴C years BP).

In summary, our studies indicate that in spite of different environmental conditions which are observed for both provenance trial locations, the Scots pine populations showed similar geographical pattern of morphological differentiation. Moreover, all morphological traits of the needles proved their usefulness in analyzing the intrapopulational differentiation of the Scots pine and together with biochemical and molecular data can be used in discussion over the history of the species in Europe.

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