ACTA AGROBOTANICA
Vol. 64 (4): 159–170
2011

VARIABILITY OF MORPHOLOGICAL AND ANATOMICAL TRAITS IN NATURAL POPULATIONS OF *Festuca rubra* AND *F. nigrescens*

Agnieszka Dąbrowska

Botanical Garden, University of Maria Curie-Skłodowska in Lublin, Sławinkowska 3, 20-810 Lublin, Poland
e-mail: dabrowskaa@vp.pl

Received: 03.09.2011

A b s t r a c t

Morphological and anatomical variability in 24 sub-populations of two native species of *Festuca* occurring in Poland was studied. The principal component analysis and cluster analysis of morphometric data and DNA classified specimens or sub-populations of *Festuca* into two groups, corresponding to the two species. *F. rubra* and *F. nigrescens* are similar to each other in most morphological characters: the number of the florets, the length of the spikelet, the length of the lemma awn, the width and thickness of the leaf, the length of the cauline leaf, the length of hairs in the leaf, the number and diameter of vascular bundles in the leaf, the hairiness of branches of the panicle, the hairiness of the lemma. The key morphological features distinguishing both species include the number of ribs in the leaf, the width of the cauline leaf (second from the top), caespitose, presence of rhizomes and quantity of the sclerenchymatous tissue in the leaf blade. Apparent differences were detected between the single sub-populations. However, between the intraspecific sub-populations, diversity in terms of the following traits was observed: the length of the stems, number of florets in the spikelet, the length of the spikelet and lemma awn, the width of the cauline leaves, the number of the ribs and quantity of the sclerenchymatous tissue in the leaf blade.

Key words: ecotype, *Festuca rubra*, *F. nigrescens*, morphological, anatomical, variability

INTRODUCTION

The genus *Festuca* contains several hundred species. These were placed by H a c k e l (1882) in six sections. One of the sections, (sect. *Ovinae* Hackel., now sect. *Festuca*) is by far the largest (for example, 129 of the 170 species of *Festuca* recognized in Europe by M a r k g r a f - D a n n e n b e r d (1980) belong to it) and includes the great majority of fine-leaved species of the genus in North Temperate regions. H a c k e l (1882) recognized only eleven species in sect. *Festuca*, but two of these (*F. ovina* L. and *F. rubra* L.) were divided into a complex hierarchy of subspecies, varieties and lower rank taxa. Many of them are now recognized at the species level by modern workers. Of the 129 species recognized by M a r k g r a f - D a n n e n b e r d (1980), 91 are referred to as *F. ovina* sensu Hackel and 21 to *F. rubra* sensu Hackel. Fourteen of the latter 21 fit into our concept of the *F. rubra* aggregate (*nigrescens*, *rubra*, *richardsoni*, *pyrenaica*, *trichophylla*, *pseudotrichophylla*, *cyrnea*, *oelandica*, *junicifolia*, *diffusa*, *rivularis*, *rothmaleri*, *nevadensis*, *cretacea*), corresponding to H a c k e l ' s (1882) *F. rubra* subsp. *eu-rubra*, *pyrenaica*, *dumetorum* and *nevadensis*. Taxa of the *F. rubra* aggregate possess extravaginal non-flowering shoots (often as long rhizomes), and leaf-sheaths which are closed almost to the mouth and lack deeply infolded thin margins. They have been recorded as varying from diploid to decaploid (2n = 14, 28, 42, 56 and 70).

F. rubra L. and *F. nigrescens* Lam. are two widely distributed and ecologically important grasses that exemplify very variable species in the aggregate. In Flora Europaea, M a r k g r a f - D a n n e n b e r d (1980) distinguished seven lower rank taxa from *F. rubra* L.: subsp. *arenaria*, *rubra*, *litoralis*, *asperifolia*, *pruinosa*, *juncea*, *thessalica* and two from *F. nigrescens* Lam.: subsp. *nigrescens* and *microphylla*.

Up to date, there have been no detailed reports of *F. rubra* and *F. nigrescens* diversity in Poland. The primary aim of this study, which is a preliminary step for further investigations, is to determine the actual ranges of morphological diversity of *F. rubra* and *F. nigrescens*. The structure of the phenotypic variation in morphological characters is therefore analyzed to investigate the relationship between the species. In this study, a molecular system has been used with the objective of clarifying the taxonomic status of *F. rubra* and *F. nigrescens.*

MATERIAL AND METHODS

Plant material included samples of *Festuca rubra* L. from 14 sub-populations (N-252 individuals) and samples of *Festuca nigrescens* Lam. from 14 sub-populations (N-252 individuals) collected in Poland from different habitats (Fig. 1). 50% of the *F. rubra* sub-populations originated from grassland habitats while the others were from well-lighted pine forests. Eight *F. nigrescens* sub-populations were from dry meadow communities, five from sand dunes, whereas sub-population N-97, characterised by a considerably bigger number of ribs in the leaf, a broader cauline leaf and the longest stems, originated from half-shadow mixed forest. Selected specimens from the particular populations were sampled as vegetative rhizomes from their natural habitats and, to avoid mutual contact, planted on distant plots in Maria Curie-Skłodowska University Botanical Garden in Lublin (Fig. 2). Each accession consisted of 18 plants of one sub-population. The study was carried out in the years 2001-2003 and in 2007-2010.

The studies involved twenty three traits: 16 quantitative and 7 qualitative (Table 1 and 2) and consisted mainly in biometric measurements of traits that distinguish both species. Some observations such as the stem length and number, the length of panicle, presence of rhizomes and caespitose were carried out directly on living plant specimens on the experimental plots. The other characters which demanded precise observation of flower and leaf elements were counted or measured with a ruler or an Opta-Tech stereoscopic zoom microscope. The anatomical analysis was performed with the use of a Nikon light microscope.

For the phenetic analysis, univariate and multivariate methods were used. In the univariate methods, descriptive statistics of quantitative characters was calculated for each taxon, based on the entire data set. Box-and-whisker plots were used to display these data. Differences among the means of characters were tested using a one-way ANOVA.

Multivariate methods of analysis were carried out using version 7.1 of the STATISTICA programme (StatSoft Inc. 2007). The principle component analysis (PCA), and cluster analysis (CA) were performed (S o k a l and R o h l f, 1981). PCA was used to detect groups of specimens and to estimate the contribution of each variable to the analysis. PCA was based on correlation matrices of the whole set of quantitative characters (Table 1) and three qualitative characters (caespitose, presence of rhizomes, distribution of the sclerenchymatous tissue in the leaf blade), (Table 2). Cluster analysis was used to assess the groupings among *Festuca* sub-populations based on 16 quantitative characters and three qualitative traits (Table 1 and 2). Standardised data were used to compute the distance matrix based on average taxonomic distance, and this was subjected to the unweighted pair-group method, arithmetic average (UPGMA) clustering algorithm.

Semi-specific PCR were used for evaluation of genetic variability among *F. rubra* (R=14) and *F. nigrescens* (N=14) individuals. The sequences of primers were based on the consensus sequences of the intron-exon junction, 7 and 9 bases in length, common for plants and necessary for effective splicing (B r o w n, 1986). The additional bases were added at random to extend the length of the primers (R a f a l s k i et al. 1997). Verification of classification of the specimens into the particular similarity groups was based on the analysis of 680 DNA fragments. The results obtained facilitated computing the indices of genetic distance between the specimens, performing the cluster analysis and estimation of compatibility of the molecular analysis results with the origin of the ecotypes examined. The UPGMA cluster analysis was performed using version 7.1 of the STATISTICA programme (StatSoft Inc. 2007).

Sampled sub-populations of *Festuca rubra* (R) and *F. nigrescens* (N) were collected by A. Dąbrowska.

F. rubra

R-120: Giżycko vicinity, Pojezierze Mazurskie, Węgorzewo, grassland, N 54°13', E 21°45'; **R-95:** Giżycko vicinity, Wydminy, pine forest between Białe Lake and Czarne Lake, N 53°58', E 22°04'; **R-118:** Kolno vicinity, Wincenta, grassland (Molinio-Arrhenatheretea), N 53°28', E 21°53'; **R-119:** Siedlce vicinity, Łosice, grassland near the road to Białystok, N 52°13', E 22°44'; **R-46:** Biała Podlaska vicinity, Leszczanka, grassland near roadside, N 51°56', E 23°03'; **R-121:** Łuków vicinity, Wojcieszków, grassland, N 51°46', E 22°19'; **R-93:** Włodawa vicinity, Sobibur, pine forest, N 51°29', E 23°39'; **R-94:** Lubartów vicinity, Bratnik, pine forest edge, N 51°26', E 22°28'; **R-47:** Łęczna vicinity, Piaseczno, roadside in pine forest N 51°23', E 23°02'; **R-100:** Puławy vicinity, Janowiec, pine forest, N 51°19', E 21°54'; **R-125:** Kraśnik vicinity, Janów Lubelski, pine forest, N 50°43', E 22°22'; **R-123:** Hrubieszów vicinity, Kryłów, grassland in the valley of the Bug River, N 50°41', E 24°04'; **R-14:** Biłgoraj vicinity, Hedwiżyn, pine forest edge, N 50°35', E 22°48'; **R-124:** Przemyśl vicinity, Cisowa, grassland near the Cisowa River, N 49°42', E 22°36'.

F. nigrescens

N-107: Gołdap vicinity, Stańczyki, grassland (Festuco-Cynosuretum), N 54°17', E 22°44'; **N-103:** Augustów vicinity, Płaska, sand dunes, N 53°54', E 23°17'; **N-106:** Mikołajki vicinity, Pojezierze Mazurskie, Bartlewo, steep escarpment alongside the Brłdany Lake, N 53°45', E 21°36'; **N-105**: Olsztyn vicinity, Kurki, pine forest edge, N 53°31', E 20°29'; **N-109:** Nowogród vicinity, Czantoria, semi-natural dry grassland, N 53°11', E 21°47'; **N-102:** Białstok vicinity, Janowo, escarp-

ment alongside the Narew River, N 52°54', E 23°24'; **N-122:** Białystok vicinity, Białowieski National Park, Białowieża, dry grassland, N 52°41', E 23°50'; **N-101**: Siemiatycze vicinity, Kózki, steep escarpment alongside the Bug River, N 52°22', E 22°52'; **N-11:** Łęczna vicinity, Poleski National Park, Wólka Wytycka, grassland (Meo-Festucetum), N 51°27', E 23°12'; **N-12:** Lubartów vicinity, Annobór, roadside, N 51°26', E 22°35'; **N-89:** Sandomierz vicinity, Góry Piepszowe, calcareous xerothermic grassland, N 50°40', E 21°45'; **N-88:** Zamość vicinity, Roztoczański National Park, Zwierzyniec, Piaskowa Góra, dry grassland, N 50°36', E 22°58'; **N-15:** Tomaszów Lubelski vicinity, Lubycza Królewska, grassland near roadside, N 50°20', E 23°32'; **N-97:** Sanok vicinity, Bieszczadzki National Park, Dwerniczek, mixed forest in the valley of the San River, N 49°13', E 38°76'.

RESULTS

Quantitative characters

The results of the ANOVA analysis showed a significant variation ($p<0.05$) between *Festuca* species for the morphological characters (Table 1). However, the most useful characteristic for discriminating between the studied species of *Festuca* is the number of ribs in the leaf and the width of cauline leaf (second from the top). On the upper surface of the leaf blade of *F. rubra* there were not more than 6 triangular-shaped ribs, while in *F. nigrescens* there were from 6.6 to 9 ribs, slightly rounded at the top (Fig 2). The cauline leafs of *F. rubra* were flat with the average width of 2-3 mm; in *F. nigrescens* they were often compound and rarely exceeded 2 mm. The remaining quantitative characters overlapped between the species of *Festuca*.

There was no apparent difference between the two ecotypes of *F. rubra* from grassland and woodland. Examination of variation in the traits for these two ecotypes revealed that plants from grassland habitats produced more florets in the spikelet and had a longer second spikelet and a longer lemma awn (measured at the first floret in the second spikelet) in comparison with the ecotypes from woodland habitats (Fig. 3).

The UPGMA dendrogram for the *Festuca* accessions (Fig. 4) shows two major clusters. The first major cluster contains four sub-clusters of *F. rubra* accessions. Three of these contain accessions of *F. rubra* from woodland habitats (R-W) and the other and most numerous contains accessions of *F. rubra* from grassland (R-G). Both ecotypes of *F. rubra* appear to be very similar and are placed very close to each other in a single cluster. The second cluster contains accessions of *F. nigrescens* at some distance. It interesting to note that the accession of *F. nigrescens* (N-97) from the half-shadow mixed forest reserve is distinct. Examination of the trait ranges for this accession revealed that N-97 is unusual, compared with the other *F. nigrescens* sub-populations, in having a bigger number of ribs, wider cauline leaves (second from the top) and longer stems (Table 1). The outlying values of these traits are likely to have resulted in separation of this population into a unique cluster.

There are some morphological differences between plants of different habitats within *F. rubra* those differences, however, are too inconsistent and imprecise to allow recognition of two intraspecific taxa within *F. rubra*. One would expect that, under such circumstances, a significant degree of morphological diversification would have occurred, making it possible to visually distinguish plants of different habitats.

Qualitative characters

F. rubra specimens formed lax tufts and rhizomes. The sclerenchymatous cells in the leaf blades were distributed in 5-8 small groups (3-,4-layered). All *F. nigrescens* individuals were densely caespitose and did not form rhizomes. Sclerenchymatous cells formed 4-, 6- or even 8-layered groups located under the lower epidermis opposite the vascular bundles and at the edge of the leaf blade. In cross-section, *F. nigrescens* leaf blades were wedge-shaped, with concave sides, whereas in *F. rubra* they were triangular (Table 2 and Fig. 2).

F. rubra individuals from grasslands had shorter rhizomes, which in the woodland specimens were – considerably longer. The sclerenchymatous tissue in the grassland specimens was distributed in remarkably bigger groups, compared with the woodland individuals.

Fourteen of the 18 individuals of *F. nigrescens* (N-97) that accessed from half-shadow mixed forest had hairy branches of the panicle; almost all individuals had hairy lemma and leaf-sheaths.

The panicle branches of most *F. rubra* individuals were usually hairy (73%), whereas only 52% of *F. nigrescens* plants had the same attribute. Most of the *Festuca* plants examined had hairy lemma and leaf-sheaths (Table 2). As regards hairiness, this character is not species-specific, since both species have the same attribute.

Quantitative and qualitative characters

The output from the PCA (Fig. 5) showed the pattern of morphological variation of Polish *Festuca* accessions based on 19 morphological characters: 16 quantitative and 3 qualitative characters (Table 1 and 2). The first coordinate axis presents the main directions of the species variation in the analysed group. The left side of the ordinate plane contains *F. nigrescens* specimens, and the right side – individuals of *F. rubra.* The gradient represented by the second axis

is connected with habitat preferences which result in internal variability within species. The upper and central parts of the diagram show *F. rubra* ecotypes from grassland habitats (R-G), whereas the lower part presents ecotypes from the woodland habitats (R-W). The vector length indicates relative significance in formation of species variation. The longest vector of the intensity of changes represents the traits: caespitose, the number of ribs in the leaf, presence of rhizomes, quantity of the sclerenchymatous tissue and the number of stems. The first four traits are most correlated with the second coordinate axis, whereas the latter character is most correlated with the first axis. The other vectors are considerably shorter, which testifies to their lower importance in species variation. The directions of most vectors point to the upper diagram plane.

Table 1
The range for the 16 quantitative characters and the results of ANOVA.
Differences between species were considered significant at the level of p<0.05

Character	Abbreviation	*F* (p<0.05)	*F. rubra* range	*F. nigrescens* (for N-97) range
number of ribs in the leaf	NR	93.51	5.0-6.0	6.6-9.0 (7.0-11.0)
*width of cauline leaf (mm)	WCL	52.38	1.9-3.1	1.4-2.3 (2.5-4.0)
number of florets in the second spikelet	NFSS	22.10	4.4 -5.7	3.9-5.1
length of stem (mm)	LS	21.34	510.8-672.6	445.0-602.5 (544.0-877.0)
**length of lemma awn (mm)	LLA	20.66	1.2-3.1	1.5-2.5
length of second spikelet (mm)	LSS	16.88	8.4-10.9	7.5-10.7
width of leaf (mm)	WL	13.87	2.9-4.8	3.1-5.2
number of spikelets in the panicle	NSP	9.02	22.7-36.6	22.4-32.1
number of vascular bundles in the leaf	NVB	6.80	5.0-7.0	5.0-10.0
* length of cauline leaf (mm)	LCL	4.64	60.9-110.2	70.1-121.9
weight of 1000 grains (g)	WG	3.65	0.8-1.1	0.8-1.1
thickness of leaf (mm)	TL	2.41	0.6-0.8	0.4-0.7
diameter of vascular bundle in the leaf (mm)	DVB	1.93	0.1-0.2	0.1-0.2
length of hair in the leaf (mm)	LHL	1.88	0.0-0.1	0.0-0.1
length of panicle (mm)	LP	0.25	92.8-134.9	91.6-134.1
number of stems	NS	0.01	158.8-457.2	207.0-399.8

*penultimate = second from the top
**measured at the first floret in the second spikelet

Table 2
Descriptions, abbreviations and attributes of qualitative characters and their frequencies in *Festuca rubra* and *F. nigrescens*.
[p] = characters used in PCA

Descriptions of character	Abbreviation	Abbreviation of character [attributes]	Frequency	
			F. rubra (N=252)	*F. nigrescens* (N=252)
caespitose [p]	C	[densely/laxly]	0/252	252/0
rhizomes [p]	S	[present/absent]	252/0	0/252
sclerenchyma [p]	SC	[small/bigger groups]	252/0	0/252
branches of the panicle	BP	[glabrous/hairy]	67/185	120/132
*lemma: hairiness, upper part	LH	[glabrous/hairy]	28/224	49/203
uppermost cauline leaf: hairiness of leaf-sheaths	UHL-S	[glabrous/hairy]	52/200	31/221
penultimate cauline leaf: hairiness of leaf-sheaths	SHL-S	[glabrous/hairy]	65/187	47/205

*estimate at the first floret in second spikelet

Table 3.
Nei's coefficients of genetic dissimilarity between sub-populations *Festuca* calculated from an analysis of 680 DNA fragments.

sub-populations																												
	N-109	N-106	N-107	N-103	N-102	N-101	R-100	N-105	N-122	R-123	R-124	R-125	R-118	R-119	R-120	R-121	R-95	R-94	N-97	R-93	R-47	R-46	R-14	N-12	N-11	N-88	N-15	N-89
N-109	-	0.222	0.273	0.269	0.264	0.301	0.327	0.322	0.295	0.314	0.319	0.331	0.339	0.346	0.326	0.385	0.365	0.378	0.366	0.322	0.363	0.343	0.378	0.354	0.334	0.350	0.369	0.378
N-106			0.225	0.213	0.248	0.286	0.315	0.279	0.253	0.292	0.322	0.346	0.347	0.326	0.338	0.381	0.379	0.360	0.349	0.339	0.371	0.352	0.371	0.325	0.303	0.321	0.348	0.377
N-107				0.198	0.230	0.278	0.330	0.314	0.277	0.321	0.326	0.355	0.343	0.338	0.359	0.405	0.368	0.367	0.361	0.363	0.355	0.338	0.375	0.340	0.327	0.348	0.369	0.391
N-103					0.212	0.281	0.338	0.282	0.265	0.320	0.325	0.348	0.367	0.362	0.349	0.398	0.378	0.368	0.348	0.359	0.368	0.334	0.354	0.336	0.326	0.341	0.345	0.349
N-102						0.253	0.329	0.304	0.289	0.305	0.352	0.364	0.358	0.350	0.366	0.386	0.415	0.357	0.362	0.341	0.359	0.351	0.391	0.350	0.336	0.349	0.353	0.383
N-101							0.367	0.295	0.295	0.314	0.350	0.360	0.382	0.377	0.361	0.388	0.376	0.383	0.334	0.359	0.354	0.355	0.398	0.369	0.360	0.362	0.360	0.413
R-100								0.316	0.355	0.303	0.306	0.323	0.330	0.340	0.352	0.352	0.370	0.377	0.391	0.359	0.393	0.378	0.400	0.394	0.414	0.400	0.410	0.377
N-105									0.251	0.318	0.345	0.358	0.393	0.383	0.382	0.394	0.391	0.401	0.364	0.389	0.358	0.347	0.402	0.366	0.375	0.351	0.379	0.394
N-122										0.289	0.342	0.343	0.378	0.368	0.347	0.413	0.398	0.374	0.354	0.362	0.371	0.354	0.385	0.342	0.334	0.341	0.357	0.414
R-123											0.252	0.280	0.266	0.305	0.309	0.343	0.337	0.331	0.362	0.338	0.330	0.356	0.361	0.372	0.379	0.389	0.399	0.382
R-124												0.236	0.290	0.319	0.308	0.328	0.342	0.355	0.363	0.326	0.346	0.327	0.368	0.382	0.388	0.368	0.386	0.386
R-125													0.263	0.314	0.317	0.329	0.329	0.359	0.367	0.341	0.358	0.350	0.381	0.369	0.364	0.355	0.376	0.345
R-118														0.244	0.299	0.322	0.330	0.331	0.341	0.329	0.330	0.329	0.349	0.371	0.383	0.374	0.357	0.377
R-119															0.258	0.306	0.334	0.309	0.347	0.332	0.297	0.330	0.335	0.369	0.378	0.357	0.376	0.394
R-120																0.295	0.332	0.290	0.346	0.290	0.306	0.317	0.330	0.348	0.354	0.347	0.375	0.390
R-121																	0.329	0.296	0.392	0.333	0.340	0.361	0.351	0.383	0.415	0.392	0.411	0.403
R-95																		0.307	0.372	0.325	0.331	0.352	0.339	0.383	0.389	0.371	0.411	0.390
R-94																			0.348	0.283	0.301	0.303	0.308	0.373	0.354	0.378	0.388	0.416
N-97																				0.287	0.333	0.333	0.326	0.297	0.302	0.317	0.329	0.364
R-93																					0.262	0.283	0.294	0.343	0.324	0.336	0.384	0.362
R-47																						0.276	0.306	0.344	0.333	0.326	0.350	0.360
R-46																							0.279	0.326	0.342	0.349	0.359	0.354
R-14																								0.302	0.306	0.324	0.346	0.354
N-12																									0.202	0.250	0.284	0.379
N-11																										0.208	0.258	0.376
N-88																											0.230	0.346
N-15																												0.315
N-89																												-

Molecular analysis of genetic variation

The dendrogram of Euclidesian distances for the *Festuca* accession (Fig. 6) shows two major clusters, within which remarkable distances between *Festuca* species can be observed (Table 3). The first cluster containing accessions of *F. nigrescens* is divided into two sub-clusters, in which the specimens display mutual relatedness, possibly due to their place of origin. Eight specimens from the first sub-cluster were from northern sub-populations, and the other six of the second sub-cluster from southern ones (Fig. 1). Ecotype N-97 from the half-shadow mixed forest is a distinctly separate group characterised by a bigger number of ribs, a broader cauline leaf (the second from the top) and longer stems (Table 1). The highest coefficients of genetic distance were calculated for this ecotype and a majority of *F. nigrescens* specimens from the northern sub-populations (Table 3). The results of this analysis also confirm distinctness of this ecotype probably resulting from the place of its origin. A similarly distant place is held by N-101 from the northern sub-population, in comparison with the specimens from the southern sub-population.

The second cluster contains accessions of *F. rubra* from grassland (R-G) and accessions of *F. rubra* from woodland (R-W) habitats. Considerable Euclidesian distances indicating high variation are observed between the profiles (R-G and R-W). The genetic distance coefficients between the extremely located specimens from these profiles exceed 0.400 (Table 3). This confirms the validity of division into similarity groups (R-G and R-W) shown by the morphological analysis (Table 1, Fig. 3 and 4) and the habitat preferences revealed by the PCA analysis (Fig. 5).

The mean genetic similarity for the whole genotype set analyzed was 0.361. For the *F. rubra* and *F. nigrescens* species, it was 0.342 (0.256-0.425) and 0.341 (0.203-0.436), respectively (Tab. 3). The mean genetic distance between ecotype R-97 and the other *F. rubra* individuals was 0.356 (0.334 to 0.414).

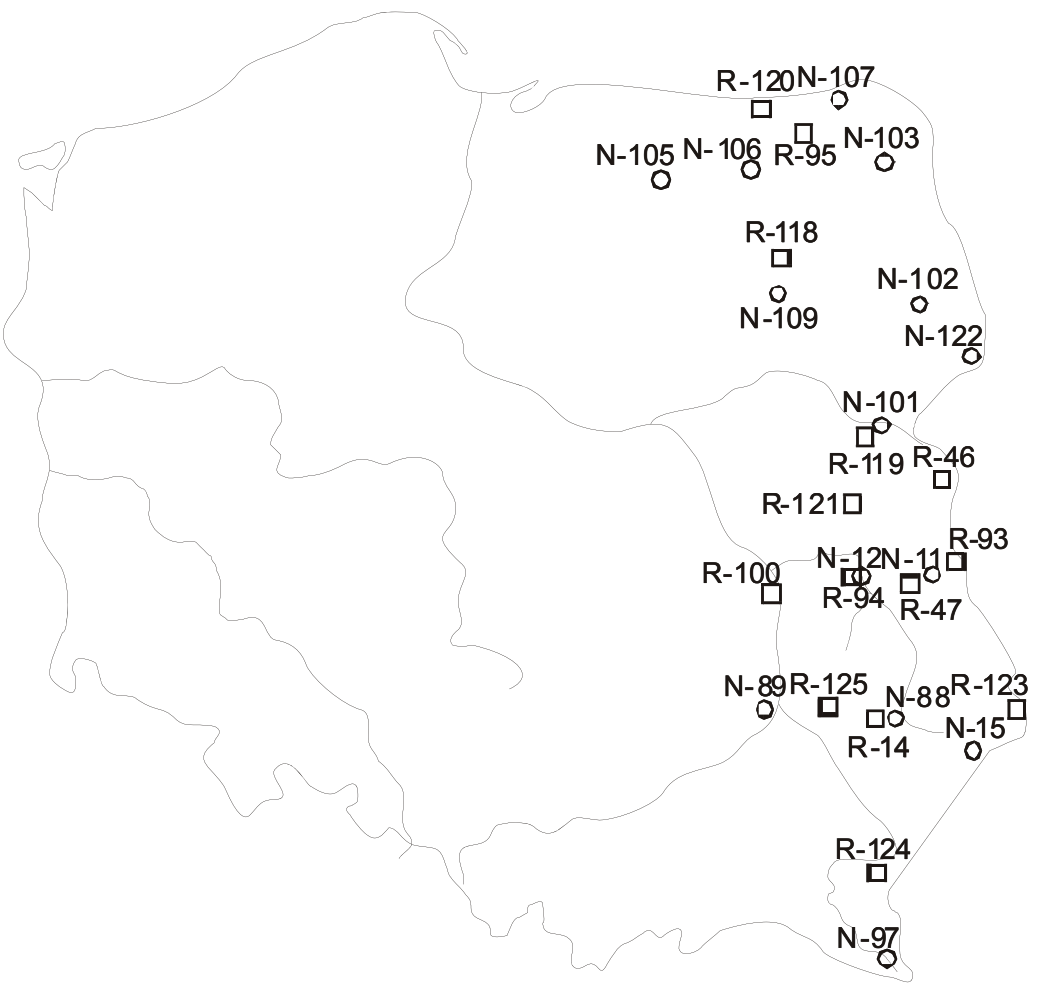

Fig. 1. Map of Poland with geographic locations of the sub-populations of *Festuca rubra* (square) and *F. nigrescens* (circle).

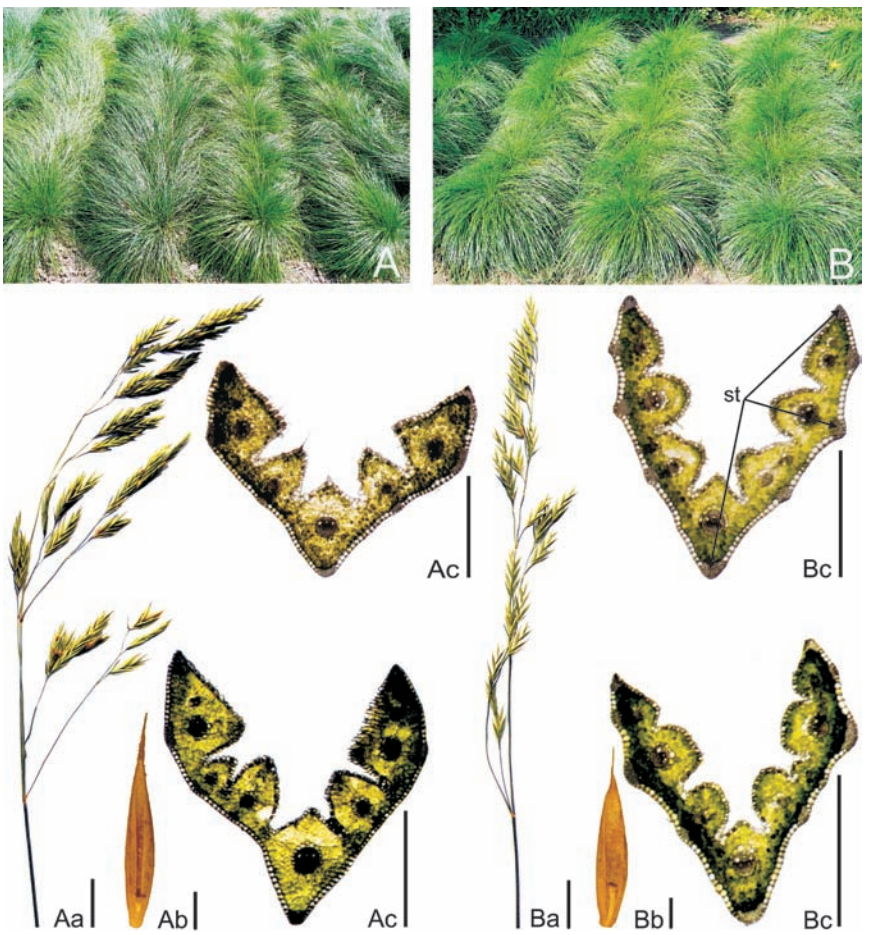

Fig. 2. Morphology and anatomy of *Festuca rubra* and *F. nigrescens*.
A – Habit of *Festuca rubra*; Aa – panicle, scale bar=10 mm; Ab – grain, scale bar=1 mm; Ac – cross of the leaf blade, scale bar=1 mm.
B – Habit of *Festuca nigrescens*; Ba – panicle, scale bar=10 mm; Bb – grain, scale bar=1 mm; Bc – cross of the leaf blade, scale bar =1 mm.
st – sclerenchymatous tissue.

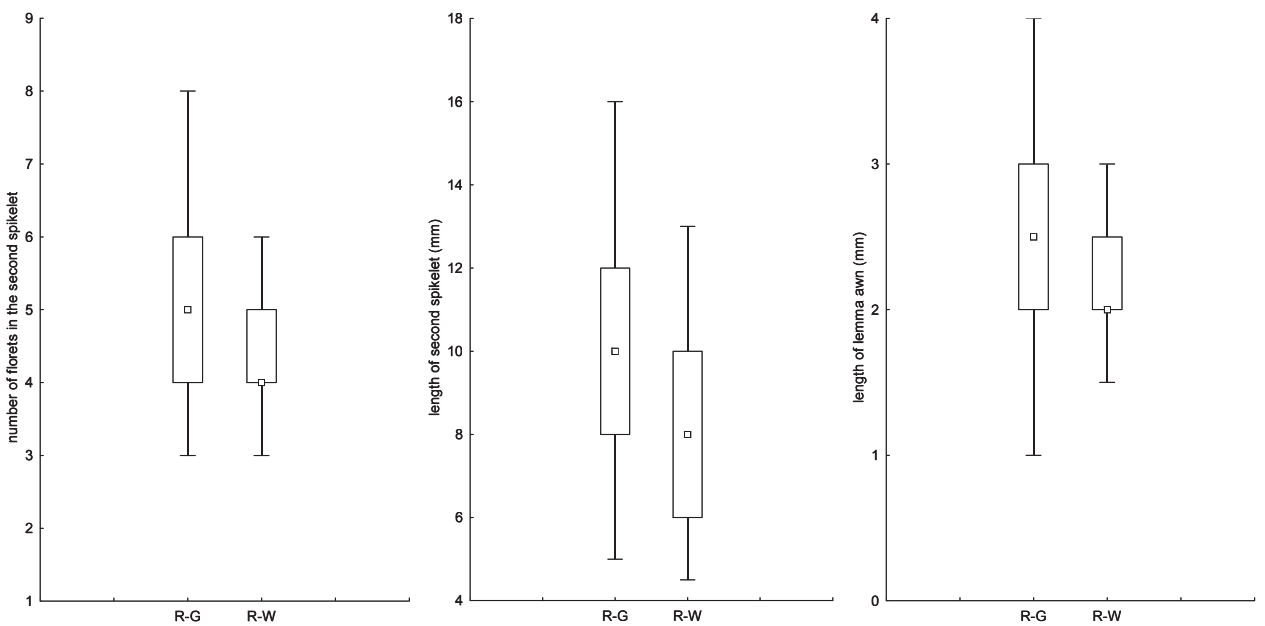

Fig. 3. Variation of morphological characters for two ecotypes of *F. rubra* from grassland (R-G) and woodland (R-W) habitats. The mean (dots), range of standard deviations (box), and minimum/maximum (whisker) indication.

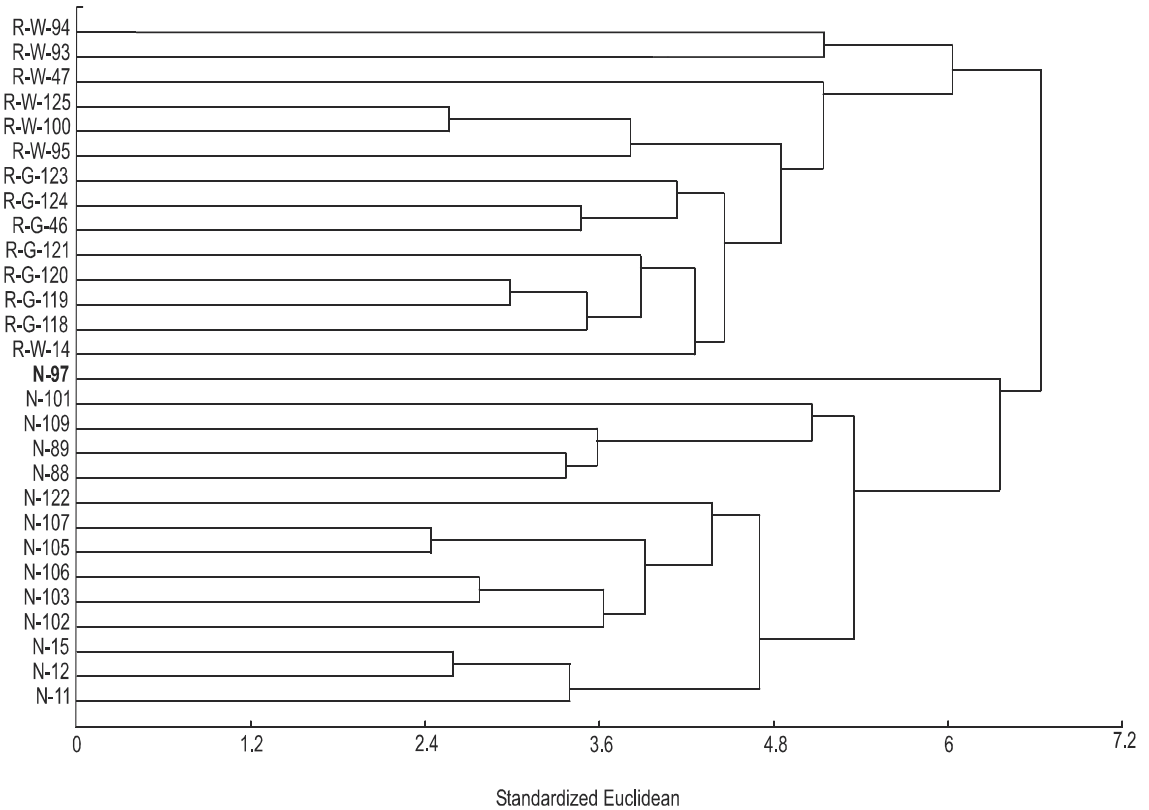

Fig. 4. UPGMA dendrogram of 28 accessions of *Festuca* species from Poland based on Euclidean distances computed among accession means of quantitative and qualitative morphological traits listed in Table 1 and 2.

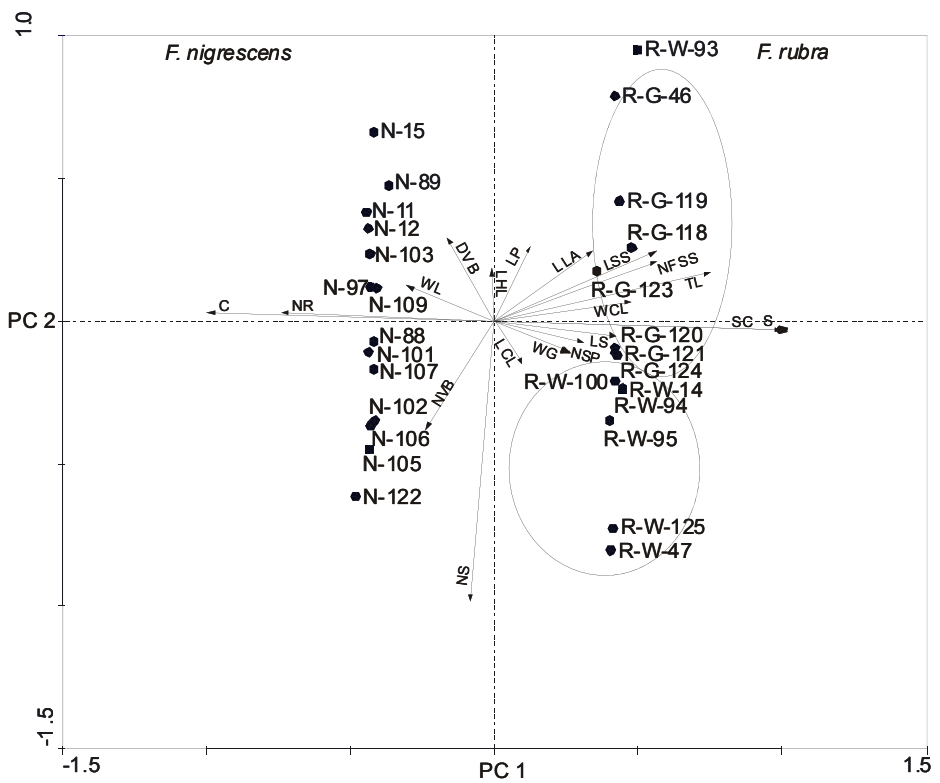

Fig. 5. Principle component analysis (PCA) – scatter diagram of sub-populations of *F. rubra* and *F. nigrescens* along PC 1 and PC 2, based on 16 quantitative characters (Table 1) and 3 qualitative characters (Table 2).

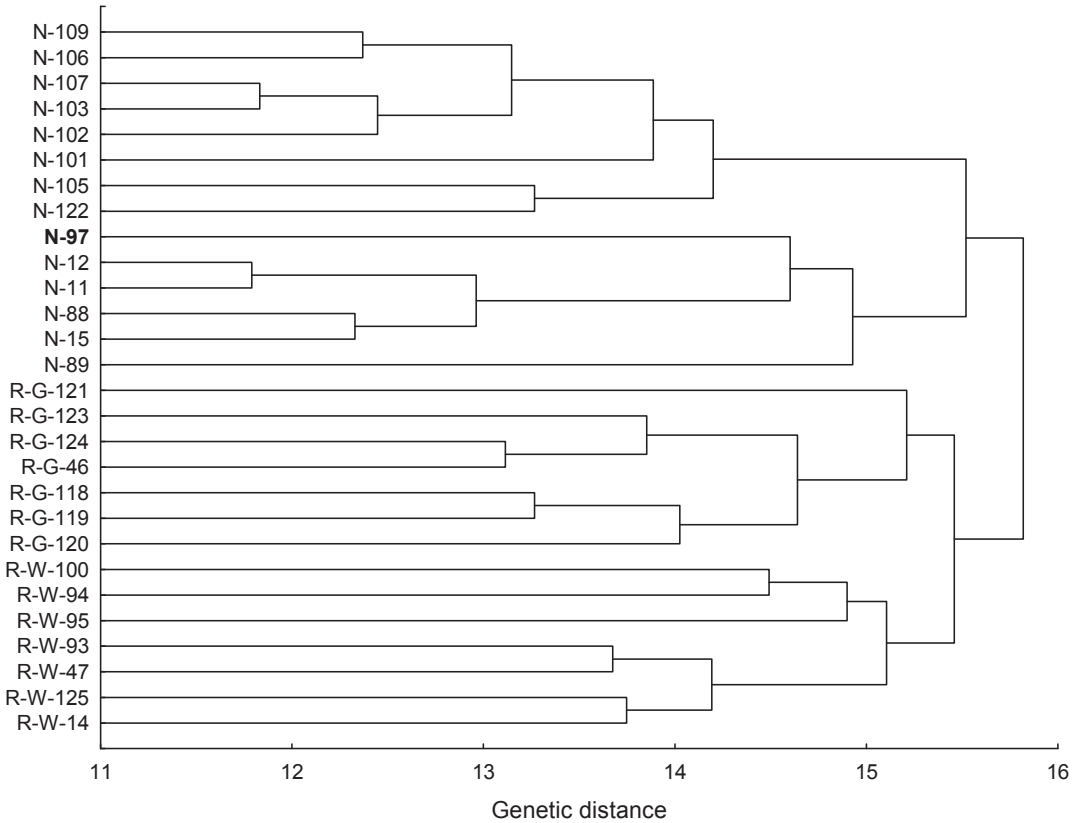

Fig. 6. Association among sub-populations *Festuca* revealed by UPGMA cluster analysis of 680 DNA fragments. R-G accessions of *F. rubra* from grassland, R-W accessions of *F. rubra* from woodland habitats.

DISCUSSION

The results obtained in the present study are partly consistent with investigation data reported by other authors (Markgraf-Dannenberd, 1980; Falkowski, 1982; Szafer et al. 1986; Conert, 1996), who regarded the presence of rhizomes w *F. rubra* and absence thereof in *F. nigrescens* as the most important trait differentiating the two species. However, the results of this study do not correspond to those obtained by Hubbard (1973), who found the hairless lemma and leaf-sheaths in *F. rubra* to be the significant difference between the two species. Nevertheless, according to my observations, the pattern of variation within both Polish *Festuca* species is very similar as regards the lemma and leaf-sheath hairiness. 89% and 80% of individuals of *F. rubra* and *F. nigrescens*, respectively, have a hairy lemma. Lemma hairiness, especially on the upper part, has a dominant character in both native species of *Festuca* in Poland. However, hairiness is a dominant characteristic of the leaf-sheaths; 79% and 88% of individuals of *F. rubra* and *F. nigrescens*, respectively, have hairy uppermost leaf-sheaths, 74% and 81% have hairy penultimate leaf-sheaths. Both studied species of *Festuca* consist of many forms as regards the hairiness of the lemma and leaf-sheaths, from almost glabrous types, through a multitude of intermediate forms, to abundantly hairy types. Almost glabrous types are rather rare within both species; most frequent are largely hairy individuals or plants of intermediate appearance. The above-mentioned statement has not been confirmed in the research conducted by Szafer (1919), Markgraf-Dannenberd (1980) and Falkowski (1982), who ascribe little value to hairiness as a trait of individuals from the genus *Festuca*. The disagreement between the results presented here and those obtained by the above-mentioned authors is explained by the fact that two different species were compared. Exact boundaries of morphological similarity between taxa in the *F. rubra* aggregate are difficult to establish. Hence, there are difficulties in unambiguous identification of a particular taxon. Numerous authors for example, Mirek et al. (2002), Frey (2003) or Rutkowski (2003) have their own concept of taxonomic divisions.

Both species of *Festuca* studied occur in the same plant communities, which renders hybridisation possible (Stace, 1975; Stace and Ainscough, 1984). Occurrence of this phenomenon between the two *F. rubra* and *F. nigrescens* populations under study is highly probable, since they exhibit many common inflorescence features, which according to

Conert (1996) are assigned to the individual species, i.e. in comparison to *F. nigrescens, F. rubra* should have a longer panicle with glabrous branches, a bigger number of flowers in the spikelet, and a longer lemma awn. No distinct boundaries of taxonomic distinctiveness related to the aforementioned traits were found in the present study. At present, it is difficult to suggest the taxonomic status of these taxons. *F. rubra* and *F. nigrescens* are cleistogamous and largely autogamous species; however, it hybridizes to an inconsiderable extent. The taxons in question may, therefore, be a product of hybridization. Hybrid zones may confound morphological characters alone; therefore, other markers – preferably chromosome count – would be needed in further assessment of these populations. Further investigation and confirmatory chromosome count in a region where both *Festuca* species sympatrically appear are needed to cast light on this interesting issue.

It is interesting that ecological segregation among accessions of *Festuca* was detected on the basis of morphological traits. The results of PCA and genetic analysis can only be interpreted to support the recognition of single sub-populations in *F. rubra* and *F. nigrescens.*

These observations suggest that characterisation of the floristic composition of the potential collection would be helpful in maximizing the genetic diversity among ecotype populations. A similar conclusion was drawn by Suzuki et al. (1999, 2006); Nova et al. (2006); Šmarda (2008); Šmarda et al. (2008); Armonienė et al. (2010); Yamada (2011).

CONCLUSIONS

The main characters for identifying the two species studied: the number of ribs in the leaf, width of the cauline leaf (second from the top), caespitose, presence of rhizomes, and quantity of the sclerenchymatous tissue in the leaf blade.

Common characters of the species studied: the number of florets in the second spikelet, the length of the stem, the length of the lemma awn, the length of the second spikelet, the width and thickness of the leaf, the number of spikelets in the panicle, the number and diameter of vascular bundles in the leaf, the length of the cauline leaf, the weight of 1000 grains, the length of hairs in the leaf, the length of the panicle, the number of the stems, hairiness of the panicle branches, the hairiness of the lemma and leaf-sheaths.

Differences related to the area of plant origin: the number of florets in the second spikelet, the length of the second spikelet, the length of the lemma awn (measured at the first floret in the second spikelet), number of ribs in the leaf, width of the cauline leaf (second from the top), the length of the stem, the length of the rhizomes, quantity of the sclerenchymatous tissue in the leaf blade.

REFERENCES

Armonienė R., Stukonis V., Paplauskienė V., Brazauskas G., 2010. The Genetic diversity of fine-leaved fescue (*Festuca* L.) species in Lithuania 4. [In:] Sustainable use of genetic diversity in forage and turf breeding 2, C. Huyghe (ed.), Hardcover: 41-45.

Brown J.W., 1986. A catalogue of splice junction and putative branch point sequences from plant introns. Nucleic Acids Res. 14: 9549-9559.

Conert H.J., 1996. Festuca. [In:] Illustrierte Flora von Mitteleuropa 1(3) G. Hegi (ed.), Paul Parey, Berlin-Hamburg: 561-633.

Falkowski M., 1982. Trawy polskie. PWRiL, Warszawa (in Polish).

Frey L., 2003. Grass taxonomy in Poland. [In:] Problems of grass biology L. Frey (ed.), W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków: 27-49.

Hackel E., 1882. Monographia Festucarum Europaearum. Kassel, Berlin.

Hubbard C.E., 1973. Gräser – beschreibung, verbreitung, verwendung. Eugen Ulmer, Stuttgart. (in German)

Markgraf-Dannenberg I., 1980. Festuca L. [In:] Flora Europaea 5 T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb (eds), Cambridge University Press, Cambridge: 125-153.

Mirek Z., Piękoś-Mirkowa H., Zając A., Zając M., 2002. Flowering plants and pteridophytes of Poland a checklist 1, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Nova P.J., Cruz M., Monte J.V., Soler C., 2006. Genetic relationships within and among Iberian fescues (*Festuca* L.) based on PCR-amplified markers. Genome, 49: 1170-1183.

Rafalski A., Gidzińska M., Wiśniewska I., 1997. PCR-based system for evaluation of relationship among maize inbreds. [In:] Genetics, biotechnology and breeding of maize and sorghum, A.S. Tsaftaris (ed.), Royal Soc. Chem., Cambridge: 106-111.

Rutkowski L., 2003. Lowland grasses in Poland. [In:] Problems of grass biology L. Frey (ed.), W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków: 83-94.

Sokal R.S., Rohlf F.J., 1981. Biometry. 2nd edition. Freeman and Co., New York.

Stace C.A., 1975. Hybridization and the Flora of the British Isles. Academic Press, London and New York.

Stace C.A., Ainscough M.M., 1984. Continuing addition to the gene-pool of the *Festuca rubra* aggregate (Poaceae:Poeae). Pl. Syst. Evol. 147: 227-236.

Statsoft INC. 2007. Statistica (data analysis software system), version 7.0 www.statsoft.com.

Suzuki J-I., Herben T., Krahulec F., Hara T., 1999. Size and spatial pattern of *Festuca rubra* genets in a mountain grassland: its relevance to genet establishment and dynamics. J. Ecol. 87: 942-954.

Suzuki J-I., Herben T., Krahulec F., Štorchová H., Hara T., 2006. Effects of neighbourhood structure and tussock dynamics on genet demography of *Festuca rubra* in a mountain meadow. J. Ecol. 94: 66-76.

Szafer W., 1919. Rodzina Gramineae, Trawy. [In:] Flora polska. Rośliny naczyniowe Polski i ziem ościennych 1. Paprotniki, iglaste i jednoliścienne M., Raciborski W., Szafer (eds), Akademia umiejętności, Kraków: 329-340 (in Polish).

Szafer W., Kulczyński S., Pawłowski B., 1986. Rośliny polskie. Opisy i klucze do oznaczania wszystkich gatunków roślin naczyniowych rosnących w Polsce bądź dziko, bądź też zdziczałych lub częściej hodowlanych. Państwowe Wydawnictwo Naukowe, Warszawa (in Polish).

Šmarda P., 2008. DNA ploidy level variability of some fescues (*Festuca* subg. *Festuca*, Poaceae) from Central and Southern Europe measured in fresh plants and herbarium specimens. Biologia, 63(3): 349-367.

Šmarda P., Bureš P., Horová L., Foggi B., Rossi G., 2008. Genome size and GC content evolution of *Festuca*: Ancestral expansion and subsequent reduction. Ann. Bot. 101: 421-433.

Yamada T., 2011. *Festuca* 9. [In:] Wild crop relatives: Genomic and breeding resources, Millets and grasses, C. Kole (ed.), Berlin: 153-164.

Zmienność cech morfologicznych i anatomicznych naturalnych populacji *Festuca rubra* i *F. nigrescens*

Streszczenie

W pracy przedstawiono zmienność morfologiczną i anatomiczną dwóch gatunków *Festuca rubra* i *Festuca nigrescens* w 24 sub-populacjach występujących we wschodniej części Polski. Na podstawie wyników PCA oraz analizy skupień UPGMA wykonanej w oparciu o badania morfologiczne i DNA, zakwalifikowano badane osobniki *Festuca* do dwóch grup, odpowiadających dwóm gatunkom. *Festuca rubra* i *F. nigrescens* mają wiele cech wspólnych jak: liczba kwiatów w kłosku, długość kłoska, długość ości plewki dolnej, szerokość i grubość blaszki liściowej, długość liścia flagowego, długość włosków na blaszce liściowej, liczba i średnica wiązek przewodzących, owłosienie gałązek wiechy i plewki dolnej, przez co są bardzo do siebie podobne. Jednak liczba żeberek w liściu, szerokość liścia flagowego (drugiego od szczytu), zwartość kęp, obecność rozłogów i ilość tkanki sklerenchymatycznej w blaszkach liściowych to podstawowe cechy różnicujące oba gatunki. W obrębie pojedynczych sub-populacji nie stwierdzono znaczących fenotypowych różnic. Natomiast pomiędzy sub-populacjami wewnątrz badanych gatunków występowała zmienność pod względem następujących cech: długość pędu, liczba kwiatów w kłosku, długość kłoska i ości plewki dolnej, szerokość liścia flagowego, liczba żeberek w liściu i ilość tkanki sklerenchymatycznej w blaszce liściowej.