Biodiv. Res. Conserv. 37: 1-10, 2015

BRC
www.brc.amu.edu.pl

DOI 10.1515/biorc-2015-0005

Submitted 12.02.2015, Accepted 31.03.2015

Characterization of morphological traits and RAPD polymorphism in selected forms of Kentucky bluegrass (*Poa pratensis* L.)

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Abstract: Measurements were taken of seven morphological characteristics of various forms of *Poa pratensis,* including ecotypes (12), cultivars (4), and breeding lines (2). Statistically significant differences relating to the average number and length of vegetative shoots and the leaf width of the forms studied were determined using Tukey's HSD test. Evaluation of genetic polymorphism was carried out using the RAPD-PCR method. 18 primers were used for molecular analysis and 473 amplification products were obtained, in which high levels of polymorphism (84.2%) and specificity (63) were discovered. The mean value of Dice's coefficient, the genetic similarity indicator, was about 0.7. These results indicate a significant genetic similarity between most of the forms of *Poa pratensis* studied. A correlation was also observed between genetic variation and phenotypic diversity of the studied forms of *Poa pratensis*. Convergence was found to occur between phenotype and genetic similarity among the forms. Only the dos01 468 ecotype showed discrepancies in the assessment of phenotypic and genomic variability.

Key words: ecotypes, genetic polymorphism, morphological traits, *Poa pratensis,* RAPD analysis.

1. Introduction

Poa pratensis L. is a cosmopolitan species of grass with a wide ecological tolerance of most environmental factors (Meyer 1982; Thorne 1992; van Wijk 1997; Albertyni *et al*. 2004). In temperate climate zones, it is a cultivated plant. It can be easily cultivated both in fields and lawns. Most cultivars of *Poa pratensis* were originally derived from ecotypes, often from single plants selected directly from natural grasslands (van Dijk 1971; Hinzen & van Wijk 1985; Brede & Willard 1993; Carneiro *et al*. 2006) or from F1 generation (Brede *et al*. 1993). The various ecotypes of the species are characterized by high plasticity; this may be conditioned by the genetic structure of *Poa pratensis*. Hence, its capacity to grow in relatively diverse habitats and its significant resistance to both biotic and abiotic stresses. Habitats actively influence the formation of ecotypes, and this provides tremendous variation among individual species of plants which emerge in the natural environment; thus ecotypes originating from different eco-geographical conditions may be used as starting material either for creating new cultivars or for improving existing ones. In the case of *Poa pratensis,* since its dominant mode of reproduction is apomixis (Müntzing 1933; Huff & Bara 1993; Elgersma & van Wijk 1997), obtaining high variability in the starting material is difficult (Muller 1964; van Dijk 1971; Felsenstein 1974; Pamilo *et al*. 1987). Offsprings produced in this way will have the same genotype as the mother plant (Albertini *et al*. 2004; Carneiro *et al*. 2006). As it is difficult in the case of *Poa pratensis* to obtain cultivars with improved utility traits and with the preferred arrangement of genes, proper evaluation of the starting material for such cultivars is extremely important. Phenotypic evaluation based solely on biometric measurements is incomplete on its own because it does not allow hereditary characteristics to be distinguished from those resulting from the influence of the environment. Analysis of genetic variability at the DNA level is the only means of assessing properly the diversity of any plant population without also taking into account the complex relationships that exist between phenotypes, genotypes and the environment. One method often used to study the genetic diversity between different genotypes and species belonging to the *Poa* genus is random amplification of polymorphic DNA (RAPD) (Williams *et al*. 1990).

We wanted to investigate in this paper whether *Poa pratensis* forms chosen by us for the study were characterised by adequate genetic variability to constitute a potential starting material in the breeding of lawn or fodder cultivars. This is because the ecotypes play a major role in the breeding of this grass species and can be a valuable source of genes. We connected the evaluation of RAPD polymorphism of selected *Poa pratensis* forms with the characteristics of their morphological traits.

On this basis, an attempt was made to select, based on reference cultivars used in the study, forms (ecotypes and breeding lines) with the most favourable arrangement of traits for lawn-making or grazing use and with unique genotypes.

The presented work is a part of a doctoral thesis (Szenejko 2012).

2. Material and methods

2.1. Plant material

The study involved 12 ecotypes, 4 cultivars and 2 breeding lines of *Poa pratensis*. The employed seed material was obtained from the national collection of the Institute of Plant Breeding and Acclimatization of the Botanical Garden in Bydgoszcz (Poland) from the 2005 crop (in the case of the ecotypes) and from the 2003 crop (cultivars and breeding lines). The caryopses of the ecotypes that were originally used to establish the collection were obtained during field trips organized by the Botanical Garden to typical areas of two different Polish provinces – Lower Silesia Voivodship and to Podlaskie Voivodship (Fig. 1). The main criterion for the harvested caryopses was the variety of plant materials and habitats. Samples were collected from wasteland, as well as from agricultural areas (meadows, pastures). A detailed list of the ecotypes of *Poa pratensis* used in the study is shown in Table 1. The second group of objects comprised cultivars which differed in the form of use. In this study, these cultivars were used as a reference in the assessment of other forms. They comprised two lawn cultivars (Polish 'Alicia' and German 'Limousine') and two Polish forage cultivars ('Eska 46' and 'Skiz'). Two breeding lines of *Poa pratensis* were also included in the study ('Chałupy' and 'Dresa', which derive from Polish ecotypes for lawn use).

The study was designed as a pot experiment, conducted in laboratory conditions, in which 6-week-old seedlings of *Poa pratensis* were planted out in batches of 10 into pots with drainage. This was carried out three times for each form used in the study (i.e. 54 pots in total were used each year).

2.2. Biometric measurements

Biometric measurements were carried out in 2007 and 2008, during the first half of the growing season

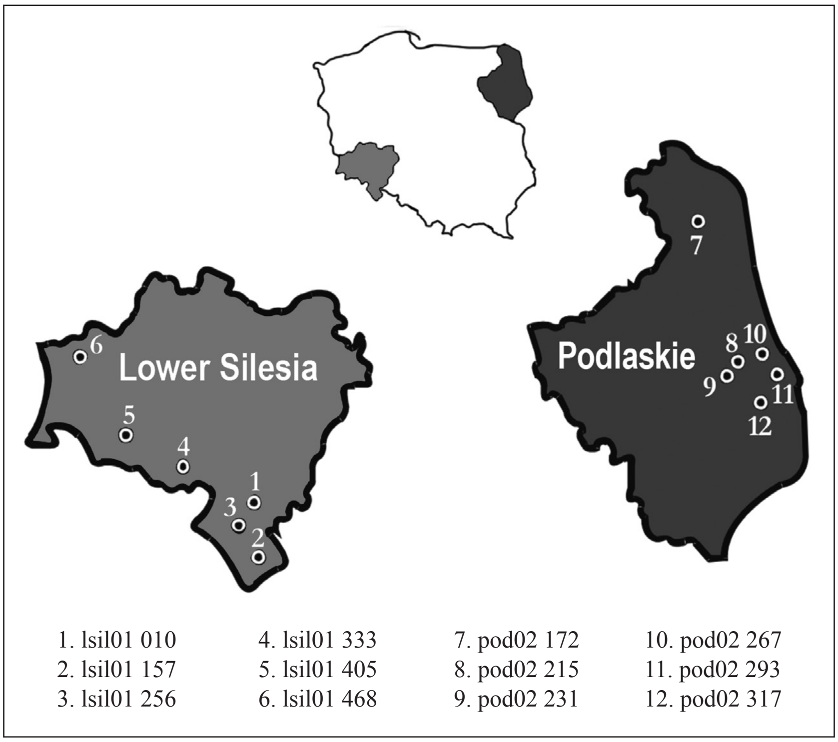

Fig.1. Sampling localities of caryopses of the studied ecotypes of *Poa pratensis* in the Low Silesia and Podlaskie Regions

Table 1. Ecotypes of *Poa pratensis* studied and locations where their caryopses were collected

Ekotype	Collection date	Country	Town/place	Voivodship	Habitat
lsil01 010	22 Aug, 2001	Poland	Pilce	Lower Silesia	wasteland
lsil01 157	24 Aug, 2001	Poland	Kletno – Śnieżnik	Lower Silesia	the edge of forest and road
lsil01 256	26 Aug, 2001	Poland	Szalejów Górny	Lower Silesia	meadow
lsil01 333	27 Aug, 2001	Poland	Rybnica Leśna	Lower Silesia	pasture
lsil01 405	28 Aug, 2001	Poland	Dziwiszów – Góry Kaczawskie	Lower Silesia	meadow
lsil01 468	29 Aug, 2001	Poland	Oleszna Podgórska	Lower Silesia	meadow
pod02 172	22 Aug, 2002	Poland	Walne	Podlaskie	meadow
pod02 215	23 Aug, 2002	Poland	Lipina	Podlaskie	meadow/pasture
pod02 231	23 Aug, 2002	Poland	Studzianki	Podlaskie	wasteland
pod02 267	24 Aug, 2002	Poland	Mieszkieniki	Podlaskie	pasture
pod02 293	24 Aug, 2002	Poland	Sanniki	Podlaskie	meadow
pod02 317	24 Aug, 2002	Poland	Pieńki	Podlaskie	pasture

(April-June). At intervals of two weeks, one plant was harvested from each pot and then photographed. The photographs were then graphically processed using the ImageJ 1.38x public program. Using this method, mean values of the following seven morphological traits were determined: plant height (H), the number of vegetative shoots per plant (Nvs), the length of the vegetative shoot of the plant (Lvs), the number of leaves per plant (Nl), the length (Ll) and width (Wl) of the leaf, the area of the assimilation surface of the plant leaf (A).

2.3. DNA extraction and RAPD analysis

The genomic DNA of each form was isolated using the Wizard®Genomic DNA Purification Kit and following the instructions supplied by the company Promega. The material used was collected from a random sample of about 100 etiolated *Poa pratensis* seedlings. DNA was isolated in two repetitions from 40.0 mg of 4-week-old *Poa pratensis* plants used in the study. RAPD analysis was then carried out according to the modified method of Williams *et al*. (1990). The reaction mixture, whose total volume was 25.0 µl, contained 1x buffer for the reaction of PCR with Mg^{2+} (Novazym), 1.5 mM $MgCl_2$, 1.0 µM starter, 0.2 mM dNTP, 1.25 U REDAllegro *Taq* polymerase (Novazym) and 250 ng DNA. Out of 69 starters which were pretested on five genotypes to evaluate the selected RAPD polymorphism, 18 from Oligo IBB PAS, Poland were chosen. An amplification reaction was carried out twice in an MJ Mini Gradient Thermal Cycler (Bio-Rad) on two samples of DNA from each genotype, according to the thermal profile described by Rajasekar *et al*. (2005). The resulting amplification products were separated on 2.0% agarose gel with ethidium bromide (5 µg/ml, Sigma-Aldrich). To visualize, document and analyze the results obtained, a set of Gel Doc™ XR and Quantity One 4.6.5 software (Bio-Rad) were used. The genetic similarity of the studied forms of *Poa pratensis* was also determined using Similarity index, (S_i) according to Dice's formula (1945), as described by Nei & Li (1979). A specific genetic similarity matrix was used to construct the second UPGMA dendrogram using the FreeTree programs and TreeView 1.6.6. software (Pavlíček *et al*. 1999; Hampl *et al*. 2001).

3. Results

3.1. Morphological characterization

The most morphologically balanced traits were found to be the leaf width (9.7%), plant height (12.4%) and leaf length (14.2%). The value of the coefficient of variation for these parameters was the smallest. The average number of vegetative shoots and leaves on plants differed considerably (Table 2). The cultivars and breeding lines were discovered to be the most aligned forms, while the ecotypes exhibited higher coefficient of variation values, with the exception of those relating to the average number and length of vegetative shoots. The greatest differences, confirmed statistically, among all the forms of *Poa pratensis* studied were those related to the average number and length of vegetative shoots and to leaf width. Plants from breeding lines exhibited the weakest tendency to propagate (1.764 shoots per plant) and were also characterized by the narrowest leaves (0.188 cm). Plants in ecotype forms were observed to have a tendency to form significantly longer vegetative shoots (4.778 cm) (Table 2). The lawn cultivars, especially 'Limousine', were characterized by a greater alignment of morphological traits. In addition, these plants were shorter by about 5.0 cm on average than forage cultivars, and their vegetative shoots and leaves

Table 2. Mean values of the traits examined in *Poa pratensis* ecotypes, cultivars and breeding lines, 2007-2008 (April-June)

Traits	Mean	CV (%)	Ecotypes	CV (%)	Cultivars	CV (%)	Strains	CV (%)
H (cm)	17.800	12.4	18.159	12.8	17.018	11.5	17.267	10.6
Nvs per plant	2.718	22.0	2.785	20.4	2.900	25.9	1.764*	24.5
Lvs (cm)	4.585	14.6	4.778*	12.8	4.212	20.9	4.094	12.4
Nl per plant	10.665	19.4	10.833	19.5	11.026	20.7	9.028	13.0
Ll (cm)	13.327	14.2	13.529	15.0	12.873	11.8	13.075	15.6
Wl (cm)	0.216	9.7	0.218	9.9	0.223	8.5	0.188*	13.5
A (cm^2)	2.314	18.8	2.351	20.0	2.247	14.7	2.169	23.2

Explanations: CV – coefficient of variation (%), * p<0,05 (Tukey's HSD test), H – plant height, Nvs – the number of vegetative shoots per plant, Lvs – the length of vegetative shoots, Nl – the number of leaves per plant, Ll – the leaf length, Wl – the leaf width, A – the area of the leaf assimilation surface

were also significantly shorter. Taking into account mean values of the examined traits in various forms of *Poa pratensis* studied, a tree diagram was drawn up using the UPGMA method (Fig. 2). Two distinct main clusters were found (I and II). Only 5 forms, including two of the lawn cultivars and three of the ecotypes (pod02 267, dos01 333, dos01 157) were assigned to cluster I. These forms were characterized by the most abundant foliage (on average 13.456 leaves per plant), were not very tall (average – 11.947 cm) and had relatively long vegetative shoots (average – 4.797 cm). Cluster II contained majority of the examined forms – 13 in total, comprising two forage cultivars ('Skiz' and 'Eska 46'), two breeding lines ('Dresa' and 'Chałupy') and 9 ecotypes. Four of these, dos01 468, pod01 215, dos01 405, and pod02 317, were singled out as they nearly matched the forage cultivars. These forms produced the tallest plants (on average 19.145 cm), long vegetative shoots (average – 5.011 cm) and were characterized (except for dos01 405) by long, broad leaves with a large assimilation area. In addition, compared to the forage cultivars, in particular to 'Eska 46', they displayed lower coefficient of variation values, which indicated greater stability in relation to the analyzed traits.

3.2. RAPD polymorphism evaluation

As the result of the RAPD analysis, a number of amplification products (473) were obtained. These fragments showed high levels of polymorphism (84.2%) and specificity for the forms of *Poa pratensis* tested (13.3%) (Table 3). The selected primers generated amplification products ranging from 135 bp (in the case of B10) to 2971 bp (P08). On average, about 26 spectral lines (23 polymorphic) were obtained as a result of each reaction

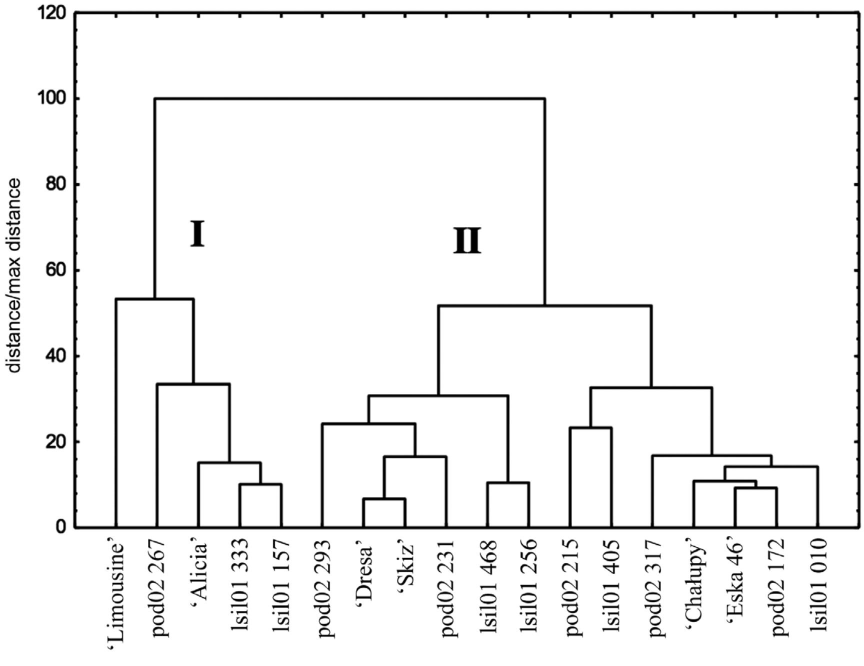

Fig. 2. UPGMA dendrogram of the mean values of the traits examined. Most forms of *Poa pratensis* represented a similar morphological type. Over 70% of them were assigned to the cluster II

Table 3. Primers employed with the number of RAPD markers obtained, their sequence, the size of fragments, their unique products and the percentage of polymorphic markers (P) for each primer

Name of primer	Sequence 5'-3'	Size range (bp)	Total number of products	Number of polymorphic products	P (%)	Number of unique products
A04	GCAGACTGAG	215-1493	20	14	70	1
B08	GTCCACACGG	221-1498	28	28	100	2
B10	CTGCTGGGAC	135-1807	34	30	88	8
B17	AGGGAACGAG	265-893	28	27	96	1
C05	GATGACCGCC	459-1495	18	12	67	3
C06	GAACGGACTC	213-1378	29	26	90	7
C08	TGGACCGGTG	215-1563	28	25	89	5
C16	CACACTCCAG	307-1656	35	31	89	4
D02	GGACCCAACC	251-1319	17	12	71	3
D11	AGCGCCATTG	230-1596	25	21	84	2
F05	CCGAATTCCC	321-1574	32	29	91	4
G19	GTCAGGGCAA	221-1912	31	29	94	5
M14	AGGGTCGTTC	250-1451	36	36	100	3
P08	ACATCGCCCA	221-2971	25	22	88	6
P13	GGAGTGCCTC	254-1443	22	13	59	4
P15	GGAAGCCAAC	290-1334	23	20	87	2
Y05	GGCTGCGACA	236-1223	19	12	63	2
Y06	AGCAGCGCAC	312-1824	23	21	91	1
	Total	-	473	408	-	63

with a single primer. A total of 63 specific amplification products were identified. These ranged in size from 258bp to 405bp in case of the dos01 405 ecotype in the presence of primer D11 to 2971 bp in the case of dos01 468 in reaction with P08. The largest number of amplification products was obtained with the use of the

Table 4. Dice's similarity matrix of *Poa pratensis* forms

Forms	lsil01 010	lsil01 157	lsil01 256	lsil01 333	lsil01 405	lsil01 468	pod02 172	pod02 215	pod02 231	pod02 267	pod02 293	pod02 317	'Alicia'	'Limousine'	'Eska 46'	'Skiz'	'Chałupy'	'Dresa'
lsil01 010	1																	
lsil01 157	0.73	1																
lsil01 256	0.72	0.70	1															
lsil01 333	0.68	0.73	0.73	1														
lsil01 405	0.66	0.67	0.70	0.68	1													
lsil01 468	0.64	0.65	0.66	0.69	0.62	1												
pod02 172	0.66	0.71	0.66	0.73	0.66	0.68	1											
pod02 215	0.65	0.72	0.67	0.73	0.66	0.66	0.74	1										
pod02 231	0.68	0.72	0.73	0.73	0.75	0.68	0.72	0.71	1									
pod02 267	0.68	0.73	0.70	0.83	0.70	0.70	0.78	0.76	0.78	1								
pod02 293	0.70	0.73	0.74	0.75	0.70	0.66	0.74	0.78	0.77	0.78	1							
pod02 317	0.68	0.69	0.66	0.70	0.64	0.65	0.70	0.73	0.70	0.70	0.71	1						
'Alicia’	0.72	0.76	0.68	0.79	0.68	0.70	0.71	0.74	0.75	0.86	0.75	0.74	1					
‘Limousine'	0.67	0.74	0.68	0.74	0.66	0.67	0.71	0.72	0.75	0.78	0.74	0.69	0.78	1				
'Eska 46'	0.71	0.71	0.68	0.69	0.71	0.70	0.68	0.70	0.72	0.71	0.78	0.72	0.74	0.72	1			
'Skiz'	0.72	0.71	0.71	0.71	0.72	0.65	0.71	0.70	0.74	0.75	0.77	0.70	0.74	0.73	0.76	1		
'Chałupy'	0.63	0.63	0.67	0.67	0.63	0.64	0.64	0.66	0.67	0.71	0.67	0.68	0.71	0.65	0.67	0.69	1	
'Dresa'	0.64	0.73	0.67	0.66	0.63	0.63	0.68	0.67	0.69	0.71	0.70	0.69	0.71	0.67	0.69	0.73	0.71	1

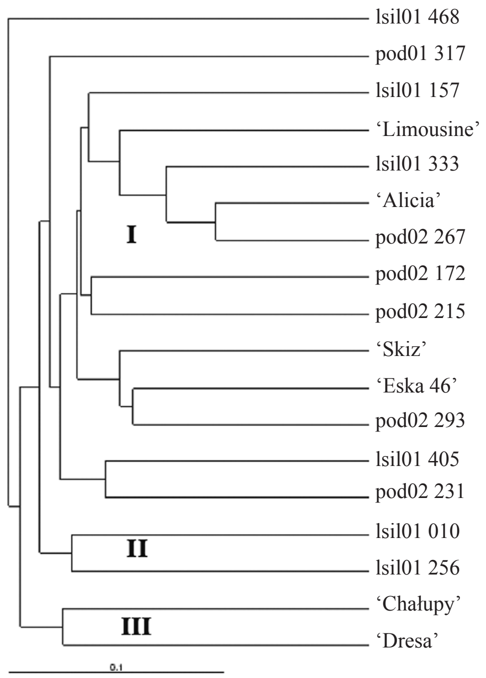

Fig. 3. UPGMA dendrogram depicting the genetic similarity of *Poa pratensis* forms based on the Dice's coefficient (TreeView 1.6.6.). Three main clusters were distinguished, of which the first included over 70% of the forms studied. The forms similar in terms of their phenotypes were also genetically similar

B10 primer (8 products), C06 (7) and P08 (6). 55 unique products were obtained from the ecotypes, in particular from dos01 468 (11) and pod02 317 (9). These forms can therefore be considered to be the most genetically different among the forms of *Poa pratensis* tested. Dice's genetic similarity matrix was also determined for the tested *Poa pratensis* forms, and the average value shown was about 0.7. This indicated a significant genetic similarity among most of the forms evaluated (Table 4). The range of genetic similarity index values was small, ranging from 0.617 for Lower Silesian ecotypes dos01 405 and dos01 468 to 0.855 for the lawn cultivar 'Alicia', and ecotype pod02 267. Dice's genetic similarity matrix was used to draw up a dendrogram using the UPGMA method and TreeView 1.6.6 software (Fig. 3). A total of 13 of the forms were assigned to this first cluster, 12 of which were divided into 4 smaller subclusters, and the remaining ecotype pod02 317 was not classified as belonging to any of these subgroups. The first subcluster comprised 5 of the forms – two lawn cultivars, two ecotypes from Lower Silesia (dos01 157 and dos01 333) and one ecotype from Podlaskie (pod02 267). The greatest genetic similarity was found between the 'Alicia' cultivar and ecotype pod02 267. It is worth noting that these two forms were also similar in terms of the analysed morphological traits. The second, smaller subgroup contained two forms from Podlaskie (pod02 172 and pod02 215) and the third subgroup both of the forage cultivars and the ecotype pod02 293. Forms dos01 pod02 405 and 231 showed significant genetic relatedness and were assigned to the last, fourth subcluster. Both, the second and the third main clusters contained only two forms of *Poa pratensis*: dos01 010 and dos01 256 as well as breeding lines 'Dresa' and 'Chałupy'. They both were genetically similar, whereas in the UPGMA tree diagram plotted using STATISTICA 10.0 and based on the mean values of morphological traits, the software assigned these objects to separate subclusters of cluster II. Ecotype do02 468 turned out to be the most genetically distinct and, thus, was not assigned to any of the main clusters.

4. Discussion

In recent years, pursuing narrow specializations of cultivars, besides improving crop productivity, contributed to the reduction of phenotypic and genetic diversity. This situation creates a demand for new sources of variability in the starting materials to be used for developing new cultivated plants or to improve the existing ones. Therefore, proper assessment of differences between the traits of starting materials, and, especially, distinguishing genetic variability from environment variability (Prosperi *et al*. 2006; Mądry 2007) seem important. When assessing phenotypic variability, it is morphological traits obtained through *in situ* (pot experiments) or field experiments and *ex situ* collections that should be paid special attention to. For knowing the values of morphological and biological traits of grasses allows, among other things, to assess their suitability for different uses. Evaluation of morphological traits supported by molecular analysis can be helpful in the classification system of grasses and selection of output forms to create new cultivars (Shortell *et al*. 2009; Tamkoca & Arslan 2010). In this study, the evaluation of morphological traits variability was based on a pot experiment. Within two years, a total of 630 *Poa pratensis* plants were tested (35 plants for each form). The lawn cultivars used in the study showed the greatest alignment of traits, which is particularly true for German, apomictic cultivar 'Limousine'. What is more, these lawn cultivars were characterized by a superior ability to propagate, more abundant foliage, and smaller assimilation surface of leaves. The differences observed in the study are consistent with the results from the previous studies (Szenejko 2010, 2013) and the results obtained by other authors (Rogalski *et al*. 2007; Prończuk & Prończuk 2008). Greater alignment between lawn cultivars when compared to that between forage cultivars can be explained by a more restrictive selection of breeding materials in the process of their creation, which is consistent with the previous observations by Goliński & Walerowska

(2007). Statistically significant differences in the mean values of the morphological traits indicate a different way of selecting the criteria depending on the purpose any given cultivar is to be used for. During two years in the course of the research (2007-2008), the only significant differences between the evaluated ecotypes, cultivars and breeding lines were found in the number of vegetative shoots per plant, the length of the vegetative shoot, and the width of leaves. This can be explained by the fact that some of the traits and differences between specific genotypes may show only after a few months from the beginning of the experiment, while others appear only in subsequent years. Similar observations were made by Prończuk & Prończuk (2008). These authors, when assessing the suitability of cultivars and ecotypes of *Poa pratensis* for extensive lawns, showed that, in the first year of research, the greatest differences could be observed, primarily, in the capacity of plants to propagate and in the width of leaves.

To evaluate genetic polymorphism of *Poa pratensis* tested, RAPD molecular markers were utilized, using the polymerase chain reaction (PCR). Because of their universality, relative simplicity of application, they are often used for genetic analyses of different grass species, including *Lolium perenne* L*.*, *Cynodon dactylon* L. Pers., *Paspalum vagantum*, *Stenotaphrum secundatum* (Walt.) Kuntze, *Agrostis stolonifera* L., *Agrostis* spp., *Poa annua, Poa compressa* and *Poa pratensis* (Caetano-Anollés 1998; Al-Humaid & Motawei 2004; Ning *et al*. 2005; *Fard et al*. 2012). RAPD analyses of *Poa pratensis*, primarily, focused on: identification of sexual and apomictic genotypes (Huff & Bara 1993; Mazzucato *et al*. 1995; Barcaccia *et al*. 1997, 1998; Stephens *et al*. 2006), assessment of genetic diversity, identification of different genotypes and cultivars (Lickfeldt *et al*. 2002; Al-Humaid & Motawei 2004; Ning *et al*. 2005; Liang *et al*. 2009; Fard *et al*. 2012; Wang *et al*. 2012), attempts to determine the genetic relationships between different genotypes and species belonging to the genus *Poa* (Johnson *et al*. 2002; Curley & Jung 2004; Patterson *et al*. 2005), and identification of pathogens responsible for fungal diseases in grasses (Hsiang *et al*. 2000). As evidenced by the results of research presented in the literature (Johnson *et al*. 2002; Ning *et al*. 2005; Rajasekar *et al*. 2005; Szenejko *et al*. 2009; Szenejko 2013) various forms of *Poa pratensis* are characterized by a high level of band polymorphism, estimated to be over 80%. This was confirmed by the results of RAPD analyses presented in this study. Thus, in the presence of 18 primers, it was possible to obtain as many as 437 DNA fragments, ranging from 135 bp (for B10) to 2971 bp (for P08). High levels of polymorphism (84.2%) were observed among them. On average, in a reaction with one primer, approximately 26 bands were obtained. Usually, in RAPD analyses, a smaller number of products of amplification is obtained (Wang *et al*. 2012), ranging from several to no more than twenty (Fard *et al*. 2012). However, in some studies (Renganayaki *et al*. 2001; Broda *et al*. 2009), authors were able to obtain a larger number of bands in a single reaction with one primer: 34 and 51, respectively. This study demonstrated significant genetic similarity between forms of *Poa pratensis* evaluated, which is evidenced by the mean value of Dice's genetic similarity, which was about 0.7. Based on RAPD molecular characterization and yield component, Johnson *et al*. (2002) also showed significant genetic relatedness between the USDA collection consisting of 228 different forms of this species of grass. The mean value of Dice's coefficient obtained by these researchers was around 0.8. UPGMA dendrograms plotted on the basis of the genetic similarity coefficient and the mean values of 17 traits analyzed confirmed high genetic similarity and small differences in morphology and yield components of the objects studied. Most of the examined forms of *Poa pratensis* (62%) belonged to a common cluster of objects related genetically, suggesting that the unique genotypes did not play a significant role in the characterized collection. The high genetic similarity among the evaluated genotypes and cultivars of *Poa pratensis* was demonstrated by other authors (Ning *et al*. 2005; Tamkoca & Arslan 2010; Fard *et al*. 2012; Szenejko 2013). UPGMA tree dendrograms created in this study are based on mean values of morphological traits and Dice's similarity matrix. Similarly as in the work by Johnson *et al*. (2002), they showed little genetic diversity and substantial similarity of morphological traits of the forms of *Poa pratensis* investigated. For most of them (17), compatibility of clustering of both dendrograms was confirmed. Convergence was found to occur between phenotype and genetic similarity among the forms. Only ecotype dos01 468 showed discrepancies in the assessment of phenotypic and genomic variability. Compliance in clustering dendrograms, constructed on the basis of morphological and agronomic and molecular analyzes of grasses, was also shown by other researchers (Ning *et al*. 2005; Tamkoca & Arslan 2010). For comparison, Curley & Jung (2004), studying genetic relationships between different forms of *Poa pratensis,* including 85 commercial cultivars, discovered greater discrepancies in morphological and genetic assessment. They distinguished 12 morphological types among the cultivars they studied. Only two of them (i.e. 33%) showed genetic similarity. The other cultivars in their study, representing morphologically similar types, were genetically diverse, each with a unique genotype. These studies demonstrated significant intra-population genetic variability, which was also reported by Słominska-Walkowiak *et al*. (2008). The variability discussed here might be attributed to the fact that sexually reproducing forms comprised a significant portion of a population.

5. Conclusions

The studied forms of *Poa pratensis* were similar in terms of some of their phenotypic traits and showed significant genetic similarity. We believe that such a situation might be explained by the fact that apomixis is a dominant way in which they reproduce. Apomictic plants are usually characterized by lower genetic variability and significant phenotypic compensation. The offsprings generated in this way have the same genotype as the mother plant, since seed formation takes place without fertilization of the ovum. The greatest, statistically confirmed, differences among the studied forms of *Poa pratensis* related to the average number of vegetative shoots and the average width of leaves. This last trait proved to be the most stable, which was confirmed by the low value of the coefficient of variation (9.7%). On this basis, it can be concluded that the width of leaves is a trait that is largely heritable and independent of modifying influences of environmental factors. Some of the forms studied included ecotypes that were comparable to forage cultivars in terms of the mean values and the stability of morphological traits. Among them, dos01 468 and pod02 317 deserve special attention. They represented the most unique genotypes from among all the ecotypes of *Poa pratensis* studied. Although we did not assess the reproduction style of particular forms, we suspect that apomixis did not play such an important role in the process of their reproduction as it did for the remaining ecotypes. We suggest that they could reproduce mostly sexually.

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