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Variability of morphological characters of collection accessions of perennial ryegrass (*Lolium perenne* L.)

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Abstract

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The aim of the study is to determine the variability of morphological characters for the vegetative development of perennial ryegrass collection and to make a selection of genotypes for different directions of use – for fodder and ornamental purposes. During the period 2015-2018 in the Institute of Forage crops – Pleven a collection nursery was studied in field non-irrigated conditions on leached black soil, in block method, in 2 replications of a total of 21 accessions of perennial ryegrass, with 50 individual plants each (15 varieties and 6 ecotypes, 11 tetraploid and 10 diploid, from Bulgaria, Belgium, Romania and China). Mean, minimum, maximum values, standard deviations and variation coefficients for plant height, tuft width at base and top, habitus, number of leaves, leaf length and width, stem thickness, stem number were present. A great genetic diversity was found in the morphological features of the perennial ryegrass collection accessions, both between them and within population. Highest values of variation coefficients (CV) were obtained for plant stems – 43.05%, habitus – 33.85%, leaf length and width – 25.43% and 26.16%, respectively. In the collection the tetraploid varieties have higher plants, longer and broader leaves and more upright habitus, which ensures higher productivity of fodder and for ornamental and amenity purposes are suitable diploid varieties and ecotypes, with lower plants, short and narrow leaves.

Keywords: perennial ryegrass; collection; morphological characters; variability; selection
Abbreviations: H – height, cm; TWA – tuft width above, cm; TWB – tuft width on the base, cm; HS – habit, score; LN – leaf number; LL – leaf length, cm; LW – leaf width, mm; SW – stem width, mm; SN – stem number/plant (for all tables)

Introduction

Perennial or English ryegrass is one of the most studied and valuable grazing grasses in the temperate climate in the world (Cunningham et al., 1994; Peeters, 2004; Humphreys et al., 2010; Katova, 2005, 2016). It is considered to be the first grass introduced into crop growing. Perennial ryegrass is one of the most important forage (high yielding, highly digestible and tolerant of intensive grazing) and ornamental perennial grass species (Sampoux, et al., 2013; Birer & Gokkus, 2017). New varieties should not be only stable and uniform but also distinguishable from others (Katova, 2011, 2017 a, b). Therefore phenotypic and genotypic variability of morphological features is important for breeding (Kemesyte et al, 2014).

Morphological features are counted and measured in the Diversity, Uniformity and Stability Tests (DUS Tests) set out in the Protocols of the Common Plant Variety Office (CPVO-TP / 004/1: 23/06/2011) and in methodologies of the Union for plant variety protection (UPOV TG / 4/8: 2006-04-05). Morphological traits and their variability are also a valuable source in defining the directions of breeding. Perennial ryegrass plants vary strongly in habitus and height (Peeters, 2004; Katova, 2005).

The height of the stems varies in the range of 10-60 cm, and there are forms up to 90 cm (Peeters, 2004). The plant habitus can be from fully upright to prostrate. It is determined by measuring the angle that the stem forms with respect to the soil surface. It is an important morphological feature that determines the way grass is used. The upright is desirable in the hay and the prostrate – in the pastoral use (Katova, 2005). This feature is valuable from a breeding point of view because high-standing upright forms are competitive in control of the weed plants for light and mixture with legume components (Katova, 2016). A positive correlation has been established between late maturity and prostrate habit and early maturity and upright habit (Arents & Witze, 1978). Other important morphological features are the length and width of the leaves, which determine the size of the leaf surface and the location of the foliage in the space. According to Loomis and Williams (1963), they are limiting factors in the yield of perennial ryegrass. Spatial arrangement of leaf surface determines the angle of incidence of direct sunlight and access to scattered such that determines the activity and efficiency of photosynthesis. According to Frame (1994), the activity of photosynthesis is greatest at the top of the sward, and its efficiency is strongest in young leaves, gradually decreasing with aging.

The aim is to determine the variability of morphological characters for the vegetative development of perennial ryegrass collection and to make a selection of genotypes for different directions of use – for fodder and ornamental purposes.

Material and Methods

During the period 2015-2018 in the Institute of Forage crops (IFC) – Pleven a collection nursery was studied in field non-irrigated conditions on leached black soil, in block duplicate method, in 2 replications of a total of 21 accessions of perennial ryegrass, with 50 individual plants each (15 varieties and 6 ecotypes, 11 tetraploid and 10 diploid, from Bulgaria, Belgium, Romania and China).

The morphological traits: plant height (cm), tuft width at base and top (cm), habit, leaf numbers, length (cm) and leaf width (mm), thickness of stems (CPVO-TP / 004/1: 23/06/2011) as well as the number of stems per plant according to the Diversity, Uniformity and Stability Tests (DUS) laid down in the Protocols of the Common Plant Variety Office in the methodologies of the Union for the Protection of Plant Varieties (UPOV TG/4/8: 2006-04-05) were measured early in spring before first cut for forage.

Experimental data on morphological features are characterized by: marginal values (min and max), arithmetic mean (x), standard deviation (SD) and coefficient of variation (CV, %) for 2016, 2017 and 2018. for each accession by individual phenotypic assessment, average for a group (ecotypes, varieties, diploid and tetraploid) and for the collection.

Variation is considered weak, moderate or strong at CV values, respectively: up to 10%, > 10-20%, and > 20% (Dimova & Marinkov, 1999). The samples are grouped and differentiated for different directions of use: for fodder and ornamental purposes.

Results and Discussion

In Table 1 and Table 2, the representations of mean values, according to 7 and 9 morphological characters are recognized as a common number of accessions in the collection of perennial ryegrass per year, somehow and coefficients of variation. The report for the year 2016 recognizes all characters had lower values in comparison with 2017, and less for comparison with 2018. There is strong variation for most characters during three years and medium for character leaves number.

From 2016, the highest coefficient of variation (CV) were obtained for: height per plant – 40.14%, stem number – 36.90%, and habit – 35.98%; in 2017, stem number – 43.05%, habit – 33.85%; leaves length and width – 26.16% and 25. 43%; for 2018, habit – 143.15%, leaf width – 119.97% and tuft width at base – 99.36%.

In Table 3, Table 4 and Table 5, the representations of the specificity of an all-around character for an all-accession – ecotype are shown by years. For 2016, for the ecotypes the highest CV was found with respect to the habit of 39.69%, following by the stem number of 29.66% and the height of

Table 1. Variability by 7 morphological characteristics of collection assesions of perennial ryegrass in 2016

Traits accessions n = 21	H, cm	TWA, cm	HS, score	LN	LL, cm	LW, mm	SN/ plant
average	38.32	38.95	4.85	3.92	14.06	5.37	24.76
Min	16.16	22.52	2.12	3.00	6.44	2.16	8.52
Max	81.80	55.88	9.00	5.04	19.07	9.24	44.08
STDEV	15.38	8.39	1.75	0.53	3.02	1.39	9.14
CV, %	40.14	21.55	35.98	13.56	21.46	25.88	36.90
confidence 0.01	8.65	6.98	3.31	2.37	4.72	0.98	0.30

29.64%; for 2017, stem number – 31.37%, in 2018, stem number – 38.24%, leaves length and width, 32.76% and 26.39%.

Observing a weak variation in 2017 for the most characters, medium strength, for habit and tuft width, and strong only for stem number. Locale ecotypes are distinguished with wide tufts above, prostrate habit, from short to medium length of the leaves, and medium to big stem number.

According to the ploidy level the ecotypes are diploid, you can't cope with tetra-ploidy or other ploidy levels when perennial ryegrass is in nature.

Table 2. Variability by 9 morphological characteristics of collection assesions of perennial ryegrass in 2017 and 2018

Traits accessions n = 21	H, cm	TWA, cm	TWB, cm	HS, score	LN	LL, cm	LW, mm	SW, mm	SN/plant
	2017								
average	53.54	63.35	16.07	5.33	4.63	17.14	5.83	2.14	131.64
min	24.94	26.60	6.18	1.08	3.60	9.43	3.24	1.46	10.28
max	74.55	83.33	22.68	8.26	5.52	25.46	10.88	2.69	221.89
STDEV	11.06	13.28	3.99	1.80	0.50	4.36	1.53	0.27	56.67
CV, %	20.67	20.96	24.83	33.85	10.77	25.43	26.16	12.41	43.05
confidence 0.01	6.22	7.46	2.24	1.01	0.28	2.45	0.86	0.15	31.85
	2018								
average	37.21	48.70	18.43	5.18	4.84	15.22	5.04	2.74	85.40
min	16.78	23.32	7.12	1.32	4.00	8.50	2.56	1.67	11.60
max	59.42	78.50	25.64	8.19	5.75	26.50	8.76	4.65	172.44
STDEV	9.96	13.22	18.31	7.42	1.39	6.06	6.05	1.61	51.17
CV, %	26.78	27.14	99.36	143.15	28.77	39.81	119.97	58.83	59.92
confidence 0.01	5.60	7.43	10.29	4.17	0.78	3.41	3.40	0.91	28.76

Table 3. Morphological characteristics of perennial ryegrass ecotypes, 2016

Ecotype	H, cm	TWA, cm	HS, score	LN	LL, cm	LW, mm	SN/ plant
Sokolare	42.2	55.88	5.32	5.04	14.3	5.04	25.12
Ravnogor 1	19.6	30.28	2.44	4.08	10.03	3.92	10.8
Topolovgrad	50.44	51.92	6.28	3.84	14.94	5.36	28.12
Bekovi skali	31.24	38.96	3	4.32	12.34	4.72	17.68
Ravnogor 2	32.8	39.64	2.64	3.28	10.62	4.24	25.56
average	35.26	43.34	3.94	4.11	12.45	4.66	21.46
Min	19.60	30.28	2.44	3.28	10.03	3.92	10.80
Max	50.44	55.88	6.28	5.04	14.94	5.36	28.12
STDEV	10.45	9.32	1.56	0.58	1.94	0.52	6.36
CV, %	29.64	21.51	39.69	14.06	15.60	11.21	29.66
confidence 0.01	12.04	10.74	1.80	0.67	2.24	0.60	7.33

Table 4. Morphological characteristics of perennial ryegrass ecotypes, 2017

Ecotype	H, cm	TWA, cm	TWB, cm	HS, score	LN	LL,cm	LW, mm	SW, mm	SN/ plant
Sokolare	45.76	65.44	12.68	6.2	4.56	12.76	4.88	1.99	101.96
Ravnogor 1	44.84	72.21	17.32	8.26	5.05	15.09	4.74	1.87	151.74
Topolovgrad	49.56	61.92	16.88	5.8	4.64	14.44	5.32	1.9	127.64
Bekovi skali	43.8	67.88	19.4	7.8	4.88	14.28	4.36	2.04	152.12
Ravnogor 2	53.31	60.44	13.69	5.5	4.56	15.92	5.5	2.33	53.69
average	47.45	65.58	15.99	6.71	4.74	14.50	4.96	2.03	117.43
Min	43.80	60.44	12.68	5.50	4.56	12.76	4.36	1.87	53.69
Max	53.31	72.21	19.40	8.26	5.05	15.92	5.50	2.33	152.12
STDEV	3.52	4.22	2.47	1.11	0.20	1.04	0.41	0.16	36.84
CV, %	7.41	6.44	15.43	16.51	4.12	7.20	8.24	8.08	31.37
confidence 0.01	8.53	7.41	17.77	19.02	4.75	8.29	9.50	9.31	36.14

Table 5. Morphological characteristics of perennial ryegrass ecotypes, 2018

Ecotype	H, cm	TWA, cm	TWB, cm	HS, score	LN	LL, cm	LW, mm	SW, mm	SN/ plant
Sokolare	37.45	48.65	18.6	4.2	4.1	10.55	3.65	2.3	104
Ravnogor 1	26.33	45.67	21.17	6.33	5	14.46	3.58	2.25	58.33
Topolovgrad	35.53	52.67	18.78	6	4.72	15.44	4.39	2.39	94.22
Bekovi skali	33.04	57	21.6	6.36	4.28	10.14	4.48	2.64	145.4
Ravnogor 2	43.33	56.33	17	5.67	5.75	23.63	6.92	3.5	49.18
average	35.14	52.06	19.43	5.71	4.77	14.84	4.60	2.62	90.23
Min	26.33	45.67	17.00	4.20	4.10	10.14	3.58	2.25	49.18
Max	43.33	57.00	21.60	6.36	5.75	23.63	6.92	3.50	145.40
STDEV	5.56	4.37	1.72	0.80	0.58	4.86	1.22	0.46	34.50
CV, %	15.83	8.39	8.84	13.95	12.24	32.76	26.39	17.66	38.24
confidence 0,01	6.41	5.03	1.98	0.92	0.67	5.60	1.40	0.53	39.74

In Table 6, Table 7 and Table 8 the biometrics data are presented for morphological characters for diploid varieties by year. In 2016 the highest variability strong in degrees was observed for the characters: stem number per plant, height, the width and length of the leaves and habit, for 2017 and 2018: stem number per plant, the habit, height and the tuft width.

Diploid varieties are average when comparing with ecotypes on a low level, up to the middle by height, small tuft, and the stem number increase from 2016 to 2018 is higher from this of the ecotype. The Iljo variety is typically ornamental, low, with narrow and short leaves, and the Harmoniya variety is suitable for fodder and ornamental purpose.

In Table 9, Table 10 and Table 11, the biometrics data are presented by morphological characters for tetraploid varieties, by years. Tetraploid varieties on average during three years period are higher than diploid varieties and ecotypes, with wider tuft on base after cutting. According to habit prevailing semi upright to upright, have the longest and widest leaves and widest stems. There is significant variation by most characters.

Table 6. Morphological characteristics of perennial ryegrass diploid varieties, 2016

Variety – 2n	H, cm	TWA, cm	HS, score	LN	LL, cm	LW, mm	SN/ plant
Harmoniya	30.88	37.04	5.32	4.09	11.67	4.5	11.59
Strandja	58.56	51.92	6.68	3	12.28	4.24	44.08
Iljo	16.16	22.52	2.12	3.52	6.44	2.16	8.52
Mara	38	41.36	3.88	4.56	15.13	6	26.44
average	35.20	37.16	4.71	3.54	10.13	3.63	21.40
Min	16.16	22.52	2.12	3.00	6.44	2.16	8.52
Max	58.56	51.92	6.68	4.09	12.28	4.50	44.08
STDEV	17.63	12.19	1.96	0.68	3.62	1.58	16.28
CV, %	50.09	32.79	41.55	19.18	35.76	43.49	76.11
confidence 0.01	26.22	18.12	2.91	1.01	5.39	2.35	24.22

Table 7. Morphological characteristics of perennial ryegrass diploid varieties, 2017

Variety -2n	H, cm	TWA,cm	TWB, cm	HS,score	LN	LL,cm	LW, mm	SW, mm	SN/ plant
Harmoniya	51.42	69.58	12.47	6.47	4.58	15.19	5.32	2.42	144.16
Strandja	50.28	51.6	14.2	3	3.92	13.17	4.72	2.14	91.76
Iljo	24.94	44.35		8.18	4.35	13.09	3.24	1.46	69.47
Mara	56.83	69.52	17.87	5.96	5.09	17.93	5.09	1.94	174.39
average	42.21	55.18	13.34	5.88	4.28	13.82	4.43	2.01	101.80
Min	24.94	44.35	12.47	3.00	3.92	13.09	3.24	1.46	69.47
Max	51.42	69.58	14.20	8.18	4.58	15.19	5.32	2.42	144.16
STDEV	14.24	12.80	2.76	2.16	0.49	2.27	0.93	0.40	47.93
CV, %	33.74	23.20	20.68	36.64	11.38	16.46	21.12	20.16	47.09
confidence 0.01	21.18	19.04	4.10	3.21	0.72	3.38	1.39	0.60	71.28

Table 8. Morphological characteristics of perennial ryegrass diploid varieties, 2018

Variety -2n	H, cm	TWA, cm	TWB, cm	HS, score	LN	LL, cm	LW, mm	SW, mm	SN/ plant
Harmoniya	36.93	48.87	18.73	5	5.07	15.31	5.13	2.73	131.33
Strandja	46.03	35.44	15.31	3	4	10.96	4.19	2.44	94.88
Iljo	16.78	23.67	16.78	3.22	5.11	8.5	2.56	1.67	172.44
Mara	35.24	54.71	18.41	6.18	5.18	11.38	4.41	2.41	56.88
average	33.25	35.99	16.94	3.74	4.73	11.59	3.96	2.28	132.88
Min	16.78	23.67	15.31	3.00	4.00	8.50	2.56	1.67	94.88
Max	46.03	48.87	18.73	5.00	5.11	15.31	5.13	2.73	172.44
STDEV	12.26	13.91	1.58	1.51	0.56	2.82	1.09	0.45	49.48
CV, %	36.88	38.65	9.34	40.46	11.89	24.31	27.41	19.82	37.23
confidence 0.01	18.24	20.69	2.35	2.25	0.84	4.19	1.61	0.67	73.58

In 2016 the strongest variation was found for height, stem number and habit: CV – 29.23%, 23.85% and 23.33%, respectively, for 2017 – stem number, habit and length of the leaves: CV – 26.23%, 25.05% and 21.33%, respectively, for 2018 – the stem number, habit and height: CV – 43.43%, 35.26% and 26.02%.

In Table 12 collection accessions of perennial ryegrass are grouped according to morphological characters, based on marginal values and classes for 2017, when the full potential for plant development was revealed.

Habitus and height of perennial ryegrass plants

The habitus is an important morphological character determining the way of sward use. In the ecotype's group the habitus is from erect to prostrate for most accessions (average score above 6 -7). The habitus of the diploid varieties is from semi-erect to prostrate. As a hole the habitus of tetraploid varieties is more erect than that of the diploids (average score 4.8) to fully erect for the varieties Tetramis, Meltador and Merkem.

The plant height at the full heading stage varied by accessions and by years. In the ecotype's group the tallest were plants of Ravnogor 2 – 53.31 cm and Topolovgrad – 49.56 cm, and the smallest were these of Bekovi skali – 43.8 cm. Plants of the diploid varieties were very different as the smallest were plants of variety Iljo 24.94 cm, and the tallest 56.83 cm for variety Mara. Tetraploids at full development were taller compared to diploid as the tallest were these from varieties Meltador, Meracoli and Melverde. Plants of all tetraploid varieties were taller than those of diploid, and during the second growing year height was in the average 60.04 cm, and in third year they were smaller – 38.04 cm.

Table 9. Morphological characteristics of perennial ryegrass tetraploid varieties, 2016

Variety – 4n	H, cm	TWA, cm	HS, score	LN	LL, cm	LW, mm	SN/ plant
Tetramis	57.48	50.32	7.28	3.16	13.44	5.96	25.28
Tetrany	42.76	45.88	4.56	4.24	16.4	5.84	28.08
Merlinda	35.13	37.24	7.08	4	15.51	5.84	20.64
Roy	33.08	35.96	3.76	3.6	16.81	6.4	24.32
Meltador	34.68	39.04	4.68	4.24	17.83	7.04	27.4
Meracoli	28.56	34.32	4.52	4.76	19.07	6.64	38.4
Melpetra	23.88	31	3.84	3.92	16.07	5.16	24.12
Floris	22.96	30.92	3.76	3.68	12.8	4.58	16.04
Magura	49.56	36.28	5.6	3.56	13.8	5.24	34.92
Melverde	47.16	36.36	4.72	3.88	12.64	5.32	25.8
Merkem	27.8	30.28	5.44	4.32	14.69	5.24	19.36
average	36.64	37.05	5.02	3.94	15.37	5.75	25.85
Min	22.96	30.28	3.76	3.16	12.64	4.58	16.04
Max	57.48	50.32	7.28	4.76	19.07	7.04	38.40
STDEV	10.71	5.94	1.17	0.42	2.00	0.70	6.17
CV, %	29.23	16.02	23.33	10.65	13.03	12.14	23.85
confidence 0,01	8.32	4.61	0.91	0.33	1.55	0.54	4.79

Table 10. Morphological characteristics of perennial ryegrass tetraploid varieties, 2017

Variety -4n	H, cm	TWA, cm	TWB, cm	HS, score	LN	LL, cm	LW, mm	SW, mm	SN/ plant
Tetramis	52.52	53.43	17.3	3	4.04	17.87	6.13	2.4	103.7
Tetrany	48.96	66.7	17.7	5.35	4.78	14.69	5.39	2.17	127.52
Merlinda	59.05	68.9	16.48	5.1	4.05	20.8	7.24	2.36	123.95
Roy	61.78	76.35	19.13	6.14	5.04	18.59	5.91	2.21	196.43
Meltador	74.55	68.55	18.64	4	4.91	24.49	7.36	2.22	210.05
Meracoli	68.74	79.39	19.91	5	5.52	25.46	6.74	2.13	
Melpetra	63.53	71	21.68	5.44	5.47	24.29	6.95	2.3	221.89
Floris	61.74	78.57	22.68	5.7	5.17	20.53	6.22	2.28	177.13
Magura	60.5	56.5	13.5	6.4	3.6	11.16	4.2	2.04	
Melverde	65.22	83.33	16.44	4.56	4.67	19.61	5.67	2.35	
Merkem	43.83	48.17	9	2.33	4.33	17.67	6	2.18	
average	60.04	68.26	17.50	4.82	4.69	19.56	6.16	2.24	165.81
Min	43.83	48.17	9.00	2.33	3.60	11.16	4.20	2.04	103.70
Max	74.55	83.33	22.68	6.40	5.52	25.46	7.36	2.40	221.89
STDEV	8.39	10.85	3.63	1.21	0.59	4.11	0.87	0.10	43.50
CV, %	13.98	15.89	20.72	25.05	12.63	21.00	14.12	4.65	26.23
confidence 0.01	6.52	8.43	2.82	0.94	0.46	3.19	0.68	0.08	33.78

Table 11. Morphological characteristics of perennial ryegrass tetraploid varieties, 2018

Variety -4n	H, cm	TWA,cm	TWB, cm	HS, score	LN	LL, cm	LW, mm	SW, mm	SN/ plant
Tetramis	45.35	47.78	17.33	5.22	4.17	16.12	5.44	2.89	81.5
Tetrany	27.1	36.85	20.25	3.1	5.35	13.94	4.25	2.8	98.45
Merlinda	47.47	71.29	21.12	7.59	4.82	20.41	5.88	2.94	120.65
Roy	44.6	56.6	24.3	3.7	4.55	16.86	5.1	2.45	146.1
Meltador	34	44.24	20.95	3.2	4.81	13.17	5	2.57	76.05
Meracoli	36.94	47.48	19.53	8.19	5.24	16.42	6.42	4.65	92.14
Melpetra	31.5	53.56	21.81	7.75	5.44	14.53	4.69	2.31	57.13
Floris	38.55	64.86	25.64	8	5.27	14.27	5	2.64	94.82
Magura	59.42	78.5	16.5	6.67	4.83	26.5	7.67	3.5	43.67
Melverde	27.2	36.6	13.8	3.8	4.4	16.12	4	2.56	33
Merkem	26.33	38.67	12.33	4.33	4.67	13.9	4.33	1.93	31.67
average	38.04	52.40	19.41	5.60	4.87	16.57	5.25	2.84	79.56
Min	26.33	36.60	12.33	3.10	4.17	13.17	4.00	1.93	31.67
Max	59.42	78.50	25.64	8.19	5.44	26.50	7.67	4.65	146.10
STDEV	9.90	13.50	3.92	1.97	0.39	3.68	1.02	0.69	34.55
CV, %	26.02	25.76	20.18	35.26	8.11	22.21	19.49	24.16	43.43
confidence 0.01	7.69	10.48	3.04	1.53	0.31	2.86	0.80	0.53	26.83

Tuft width

The tuft width above (measured at full heading) is related to the plant habitus and height, whereas tuft width in the base (measured after first harvest – cut) depends on tillering and tuft density. Tetraploid varieties Melverde – 83.33 cm and Meracoli – 79.39 cm were distinguished with the widest tuft above average for the period 2016-2018. With age advance the tuft increased its diameter. Sometimes the intensive tillering and the great density of shoots of plant resulted in physiological dying of its oldest shoots (in the middle of the tuft), whereas those in its periphery continued to develop normally. For diploid varieties significant variation for the character was observed. The values of the character tuft width above were higher during the second year and they decreased in third year. From diploid ecotypes Sokolare and Ravnogor 2 had the smallest tuft width (12.68 and 13.69 cm – in the base). Tetraploid varieties had higher average values for tuft width in the base and the tuft density

Table 12. Grouping of perennial ryegrass accessions by morphological characteristics

Character	Class	Interval of class	Accessions number			
Habit			ecotypes E	varieties		total
				D	T	
	Erect	score 1-3,5	1	1	2	4
	Semierect	score 3,6-6	1	1	7	9
	Prostrate	score > 6	4	2	2	8
Height at full heading	Short	< 53 cm	4	3	3	10
	Medium	> 53-57 cm	1	1		2
	Tall	> 57 cm	1		8	9
Tuft width above	Narrow	< 44 cm	1			1
	Medium	> 44-47 cm		1		1
	Wide	> 47 cm	5	3	11	19
Tuft width on the base	Narrow	< 19 cm	5	4	4	13
	Medium	> 19-20 cm	1		1	2
	Wide	> 20 cm			6	6
Leaf length	Short	< 15 cm	3	2	2	7
	Medium	15-19 cm	2	2	2	6
	Long	> 19 cm	1		7	8
Leaf width	Narrow	< 4 mm		1		1
	Medium	4-5 mm	3	1	1	5
	Wide	>5 mm	3	2	10	15
Leaf number	Small	< 4		1	1	2
	Medium	4 – 5	5	2	6	13
	Big	≥ 5	1	1	4	6
Stem width	Thin	≤ 1.8 mm	1	1		2
	Medium	1.9-2 mm	2	1		3
	Thick	> 2 mm	3	2	11	16
Stem number per plant	Small	≤ 60	2			2
	Medium	60-110	1	2	2	5
	Big	> 110	3	2	9	14

Legend: Ecotypes – E; Diploid varieties – D and Tetraploid varieties – T

was smaller. The tuft width in the base increased during every next year. The characteristics of tuft width above and in the base, together with the height allowed to record the shape and volume of the plant in space.

Morphological characters of generative shoots, leaf number, length and width of the second leaf

The leaf number per generative stem and their size (length and width) are factors determining leafiness. The leaf number varied from 3 to 6 for the different varieties and populations. In the three groups (E, D and T) the accessions with 4-5 leaves prevailed and their number decreased with advance of sward age. Meracoli and Melpetra varieties had greatest leaf number.

The length of the second leaf of the generative shoots is closely related to vertical distribution of leafiness. The variation was significant. Longest leaves were observed for the tetraploid varieties. From diploids the shortest leaves had variety Iljo (6.44 and 8.50 cm) which is ornamental one. Hazard et al. (1995) and Katova (2008) also found that the ornamental varieties morphologically differ from the natural populations – they have small leaves with short and narrow blades. Among the tetraploids (4n), with an average value of 19.56 cm, the leaves of Meracoli и Meltador were longest (25.46 and 24.49 cm), and those of Magura were shortest (11.16 cm).

To calculate leaf area it is necessary to measure leaf width**.** The leaf width vary according to variety and ploidy level. Tetraploid Belgian varieties Meltador and Merlinda had the widest leaves 7.36 mm and 7.24 mm, respectively. Katova et al. (2016) also obtained that tetraploid varieties, as a whole, has longer and wider leaves. The narrowest leaves had variety Iljo – 2.16 mm and ecotype Ravnogor 1 – 3.9 mm. The varieties with narrow and short leaves are suitable

for pasture and ornamental use and those with longer and broad leaves for hay – pasture use.

Number of stems and stem thickness

Stem thickness is right proportional to the resistance to logging. From the other site, tender and thin stems had higher digestibility. For the tetraploid varieties the average stem thickness was greater (2.24 mm), than for the diploids (2.01 mm). From diploids variety Iljo had the thinnest stems – 1.67 mm.

Stem number on the average is the largest for tetraploid variety in 2017, followed by ecotypes and diploid varieties. Only for diploid varieties the stem number increase every next year.

The 9 morphological characters recorded (Table 12) are basic in the variety test required for variety registration according to DUS. Different sets of them were used by Bukhteeva et al. (1985) in Russia, Camlin, (1995) in Great Britain and Romani et al. (1999) in Italy, Kemesyte et al. (2014) in Lithuania.

The obtained data can be used for development of standard collections, for variety DUS testing procedure (UPOV) for registration of new varieties.

Analysis of variation on 9 morphological characters (habit, plant height at full heading, number, length and width of leaves, tuft width above and in the base, number of stems and stem thickness) for studied accessions showed great diversity, which is definitely effective for selection of genotypes and in two directions of use – for fodder and for amenity purpose.

For ornamental purpose are suitable diploid varieties and ecotypes with: prostrate to semi-erect habit, plant height at full heading up to 45 cm, length of leaves under 15 cm and leaves width under 4.5 mm, with thin stems. These criteria were met by: Iljo, Ravnogor 1, Sokolare, Bekovi skali, Harmoniya.

The tetraploid varieties Tetramis, Meltador, Meracoli, Melpetra, Merlindaq Melverde, Merkem were distinguished from the diploid ones for: semi-erect to erect habitus, greater plant height at full heading (60 cm as compared to 47 cm), longer (20 cm as compared to 15 cm) and broader (6.1 mm as compared to 4.4 mm) leaves, thicker stem (2.24 mm as compared to 2.01 mm) and as a result they had greater overground biomass.

Conclusions

- A great genetic diversity was found in the morphological features of the perennial ryegrass collection accessions, both between them and within population.
- Highest values of variation coefficients (CV) were obtained for plant stems – 43.05%, habit – 33.85% and leaf length and width – 25.43% and 26.16%, respectively.
- In the collection, the tetraploid varieties have higher plants, longer and broader leaves and a more upright habit, which ensures higher forage productivity.
- For ornamental and amenity purposes are suitable diploid varieties and ecotypes, with lower plants, short and narrow leaves.

References

Arents, R., & Witze, D. (1978). Eichhof Berichte, 1……

Birer, S., & Gokkus, A. (2017). The features of turf varieties commonly used in landscape areas, *Agriculture & Food*, *5*, 374-380.

Bukhteeva, A. V., Shutova, Z. P., Korneichuk, V. A., Vatsek, V., Krcmar, B., Bares, I., Horvat, L., Osinsky, B., Kelner, E., & Neaggu, M. (1985). The international comecon list of descriptors for the family *Poaceae*. Leningrad, USSR, 22-46.

Camlin, M. S. (1995). DUS testing procedures. Grasses & Forage Legumes, Crossnacreevy.

CPVO. (2011). Technical protocol for distinctness, uniformity and stability tests – Perennial ryegrass (*Lolium perenne* L.) – CPVO-TP/004/1.

Cunningham, P. J., Blumenthal, M. J., Anderson, M. W., Prakash, K. S., & Leonforte, A. (1994). Perennial ryegrass improvement in Australia. *New Zealand Journal of Agricultural Research, 37*(3), 295-310 DOI: 10.1080/00288233.1994.9513068.

Dimova, D & E. Marinkov, E. (1999). Experimental Case and Biometrics. Academic Publishing of Agricultural University, Plovdiv, pp 263, 137-138 (Bg).

Frame, J. (1994). Improved grassland management. Farming Press Books, Ipswich.

Hazard, L., Ghesquière, M., & Barraux, C. (1995) Genetic variability for leaf development in perennial ryegrass populations. *Canadian Journal of Plant Science*, *76*, 113-118.

Humphreys, M. W., Feurstein, U., Vandewalle, M., & Baert, J. (2010). Raygrasses. In: *B. Boller (ed.) Fodder Crops and Amenity Grasses. Handbook of Plant breeding 5. Springer Science Business Media, LLC*, 211 – 260.

Katova, A. (2005). Study of morphological traits, biological properties and agricultural value of plant germplasm of perennial ryegrass (*L. perenne* L.) with a view to breeding. PhD Thesis, Sofia.

Katova, A. (2008) Study of the perennial grass species and varieties for ornamental purposes. *Journal of Mountain Agriculture on the Balkans (JMAB)*, *11*(4), 744 – 757.

Katova, A. (2011). New perennial ryegrass variety (*Lolium perenne* L.) IFK Harmoniya. *Journal of Mountain Agriculture on the Balkans, 14*(4), 721-739.

Katova, A. (2016). Study of growth and development of perennial

ryegrass in pure stand and in mixtures with alfalfa. *Journal of Mountain Agriculture on the Balkans*, *19*(2), 111 – 135.

Katova, A. (2017a). Tetrany – The first Bulgarian tetraploid perennial ryegrass variety (*Lolium perenne* L.). *Journal of Mountain Agriculture on the Balkans*, *20*(1), 110-122.

Katova, A., (2017b). Tetramis – New tetraploid perennial ryegrass variety. *Journal of Mountain Agriculture on the Balkans, 20*(1), 123 – 134.

Katova, A., Baert, J., & Reheul, D. (2016). Comparative characteristics of newly developed perennial ryegrass varieties in Bulgaria. In: *Roldán-Ruiz et al. (eds.). Breeding in a World of Scarcity,* Proceedings of the meeting of the section "Fodder crops and amenity grasses" of Eucarpia, Ghent, 2015. Springer, Switzerland, 35-40.

Kemesyte, V., Statkeviciute, G., Lemeziene, N., Brazauskas, G., & Ivanciv, R. (2014). Morphological variation between perennial ryegrass (*Lolium perenne* L.) natural ecotypes. In: *Sokolovic et al. (eds). Quantitative Traits breeding for multifunctional grasslands and turf.* Springer Science & Business Media, Dordrecht, 81-84.

Loomis, **R. S., & Williams, W. M.** (1963). Maximum crop productivity: An extimate. *Crop Science*, *3*, 67-72

Peeters, A. (2004). Wild and sown grasses. Profiles of a temperate species selection: ecology, biodiversity and use. FAO and A. Peeters.

Romani, M., Piano, E., Falcinelli, M., & Torriccelli, R. (1999). Linee duida e protocolli technici per la valutazione dei requisiti di distinguibilita, omogeneita, stabilita e valore agronomico e di utilizzazione.: *Lolium perenne* L., in *E.Piano* (Cord.) Proprsta di revision del criteri e delle procedura di iscrizione delle varieta di specie foraggere al Registro Nazionale. II. Parte special. Instituto Sperimentale per le Colture Foraggere Lodi, 1.9, 1- 46.

Sampoux, J. P., Baudouin, P., Bayle, B., Beguier, V., Bourdon, P., Chosson, J. F., Brujin, K. D., Deneufbourg, F., Galbrun, C., Ghesquiere, M., Noel, D., Tharel, B., & Viguie, A. (2013), Breeding perennial ryegrass (*Lolium perenne* L.) for turf usage: an assessment of genetic improvements in cultivars released in Europe, 1974–2004. *Grass and Forage Science*, *68*(1), 33-48.

UPOV (2006). Guidelines for the conduct of tests for distinctness, uniformity and stability – TG/4/8.

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