Bulgarian Journal of Agricultural Science, 29 (No 2) 2023, 338–350

Morpho-agronomic assessment of genetic diversity among rye accessions using multivariate analyses

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Abstract

Desheva, G. & Valchinova, E. (2023). Morpho-agronomic assessment of genetic diversity among rye accessions using multivariate analyses. *Bulg. J. Agric. Sci., 29 (2)*, 338–350

The aim of the study was to assess the genetic diversity of 27 rye (*Secale cereale* L.) accessions by using multivariate statistical methods. The experiment was carried in the block method with 3 replications on plots of 2.5 m^2 during 2017/2018, 2018/2019 and 2019/2020 growing seasons. Nine qualitative and fifteen quantitative morpho-agronomical characters were evaluated. General Shannon-Weaver diversity index for all included in the study traits was moderate (H' = 0.631). The analysis of variance exhibited the presence of significant differences among the tested genotypes for all quantitative characters indicating the existence of variability. The high heritability with high genetic advance was recorded for leaf area of a flag leaf, leaf area of a sub-flag leaf, weight of the main spike, weight of the grain in the main spike, number of spikelets in the main spike and grain yield combined indicating that they are important characters to be considered for selection and improvement of *Secale cereale* L. Cluster analysis based on the Between groups linkage and Squared Euclidean Distance grouped the genotypes into six clusters. The highest distance values were measured for the following pair wise genotype combinations: B4000206-B7000123, B4000205-B7000123 and B4000210-B7000123. The first 8 principal components explained a very large proportion of the total variation (81.80%). The results of the study will be useful for the breeding improvement programs of rye.

Keywords: rye, morpho-agronomical traits, Shannon-Weaver diversity index, cluster analysis, PC analysis

Introduction

Rye (*Secale cereale* L.) is a diploid allogamous plant species with 2n = 14, a relatively high degree of gametophytic self-incompatibility and orthodox seeds (Lu, 2017). It is one of the most important cereal crops cultivated in Eastern and Northern Europe due to its tolerance to abiotic and biotic stress, high yield potential under marginal conditions and a unique nutritional value (Targońska et al., 2013, 2016; Vendelbo et al., 2020; Sluková et al., 2021). Globally for 2020 year *ex situ* rye collections include 20018 accessions maintained in 76 holding institutes. From them 18298 accessions are under *medium-term* and *long-term* conditions in 66 genebanks located in 50 countries and 16931 accessions belong to *Secale cereale* L. (http://www.fao.org/wiews/data/ex-situ-sdg-251/search/en/?no_cache = 1#results).

Assessing the genetic diversity is an essential step for breeding programs and the optimal use of genetic resources (Gholizadeh et al., 2018). Kuneva et al. (2018) noted that the contemporary selection is directed to creating economically advantageous varieties. The initial material is necessary to be of a wide genetic base. The constant expansion of the existent collections, as well as the accumulation, addition and systematizing of the received data help for higher effectiveness of the selective-improving activity.

Recently, nevertheless, a distinct reduction of cultivation area and yield of rye has been observed compared to other cereals. This is mainly caused by a relatively slow breeding progress connected, predominantly, with a high self-incompatibility and inbreeding depression (Targonska et al., 2013). Progress in rye breeding has been significantly reduced due to involving a small number of cultivars and landraces in crosses (Daskalova et al., 2020).

Improvement depends on a continuous supply of new germplasm as donors of various genes of agronomic importance. Future gains in yield potential and quality standard of products are desirable and can be achieved by exploiting the genetic diversity of related cereal species (Mengistu et al., 2016).

Effective tools for evaluation of diversity in the collected gene resources of a given species are multivariate statistical methods. They provide information on phenotypic and genetic variability of collected material. Multivariate cluster analysis and PCA are the methods highly useful for evaluation of genetic diversity of collection forms. They permit effective management of collection and facilitate the choice of parental forms that can be used for plant breeding (Pyza et al., 2015).

The aim of the study was to assess the genetic diversity of 27 rye (*Secale cereale* L.) accessions maintained in the National collection of Bulgaria by using multivariate statistical methods.

Material and Methods

The study was performed on 27 winter rye accessions (*Secale cereale* L.). The accessions differed in the origin and biological status (Table 1).

Table 1. List of 27 rye accessions (*Secale cereale* L.) included in the study

№	Accession number	Biological status	Accession name	Origin
1	A2BM0326	Cultivars	Milenium	BGR
2	76569	Cultivars	Danaya	DEU
3	A9000461	Cultivars	AC Rifle	CAN
4	B4000202	Cultivars	Brasov	ROM
5	B4000203	Cultivars	Svaloff	SWE
6	B4000204	Cultivars	Tetra	DEU
7	B4000205	Cultivars	Perennial	AUS
8	B4000206	Cultivars	Tetraploide Vilmorin	FRA
9	B4000207	Cultivars	Yigasske	HUN
10	B4000208	Cultivars	Zidlochovicke Panis	CZE
11	B4000209	Cultivars	Detenicke	CZE
12	B4000210	Cultivars	Irlanda	IRL
13	B4000211	Cultivars	CL 108	JPN
14	B4000212	Cultivars	Montalegre	PRT
15	B4000213	Cultivars	Valzanja	RUS
16	B4000214	Cultivars	Mikklikie Wezesne	POL
17	B4000215	Cultivars	TU85-012-01	TUR
18	B4000216	Cultivars	Oklon	USA
19	B4000217	Cultivars	FL-NSC	USA
20	B4000218	Cultivars	UCRR1-2001	USA
21	B4000219	Breeding lines	TJK 0383	TJK
22	B4000220	Breeding lines	TJK 03-367	TJK
23	B6E0208	Landrace		NOR
24	61148	Landrace	Petkusova	BGR
25	B7E0055	Landrace		BGR
26	B7E0131	Landrace		BGR
27	B7000123	Cultivars	Yaselda	BLR

The experiment was set by the block method of 2.5 m^2 reporting area in 3 repetitions in the experimental field of IPGR "K. Malkov" Sadovo in the area "Dolusene"during 2017/2018, 2018/2019 and 2019/2020 growing seasons. The soil type was meadow-cinnamon resinous soil. Sowing was carried out in October. Sowing rate was calculated on the basis of 500 germination seeds/m^2. During the vegetation the plants were grown according to the generally accepted technology for the country. Biometric analysis was performed on 10 randomized plants from each replication.

The characterizations of the morphological and agronomical characters were performed according to the International Classification of Species *Secale cereale* L. of VIR (1984).

The following qualitative and quantitative morpho-agronomical characters were recorded:

1. Indicators characterizing the sample at a young age in a score:
   - ✓ color of the coleoptile: 1 – light green, 2 – dark green, 3 – purple;
   - ✓ type of the bush: 5 – semi-erect (46-55º), 7 – strongly declinated (56-70º), 9 – prostrate (> 70);
   - ✓ leaf color: 1 – light green, 3 – green, 7 – dark green;
   - ✓ presence of wax: 3 – weak, 5 – medium, 7 – strong.
2. Indicators characterizing the samples in the heading phase:
   - ✓ plant height, cm;
   - ✓ length of the upper internode, cm;
   - ✓ distance from the flag leaf to the spike, cm;
   - ✓ length of the 2nd from bottom internode, cm;
   - ✓ leaf area of a flag and sub-flag leaves, cm^2.

The leaf area of a flag and sub-flag leaves were determined by the following formula:

$$A = k.l.b,$$

where: k – coefficient, different for individual crops (for rye 0.65); l – length of the leaf along the central vein; b- maximum leaf width;

3. Indicators characterizing the spike:
   - ✓ shape of the spike (score) –1 – prismatic, 2 – fusiform, 3 – oval;

- ✓ length of spike, cm;
- ✓ length of awn, cm;
- ✓ spike thrashing (score): 1- thrashed, 2- non thrashed;
- ✓ density of the spike (score):1-very lax (<12), 3-lax (15-17), 5-intermediate (21-23), 7-dense (27-30), 9-very dense (>34);
- ✓ weight of the main spike, g;
- ✓ weight of the grain in the main spike, g;
- ✓ number of spikelets in the main spike;
- ✓ number of the grain in the main spike;
- ✓ weight of the grain in the main spike, g.

4. Indicators characterizing the grain:

- ✓ shape of the grain (score) – 1. ovate-oblong; 2. ovate; 3. barrel-shaped;
- ✓ grain color (score) – 1. white; 2. yellow; 3. green; 4. gray-green.

5. Economic characters:

- ✓ grain yield, t/ha;
- ✓ mass of 1000 grains, (g) (according to BDS 601-85);
- ✓ hectolitre mass of the grain, kg /hl (according to BDS 13381-76).

Data obtained from the different characters were analyzed using analysis of variance (ANOVA) with SPSS software version 19. The mean and standard deviation (SD) calculated for 9 qualitative and fifteen quantitative traits were used to group the accessions into five classes according to Iannucci & Codianni (2019): ≤ mean – 2SD; ≤ mean – SD; > mean +/– SD; ≥mean + SD; ≥ mean + 2SD. The phenotypic frequency data of each trait were analyzed as the Shannon-Weaver diversity index, (H') using the formula of Jain et al. (1975). Each value of H' was divided by its maximum value, log n, and normalised in order to keep the values between zero and one. The arbitrary rating scale adapted from Jamago (2000) was used to categorize the indices as following: maximum diversity (H' = 1.00), high diversity (H' = 0.76 – 0.99), moderate diversity (H' = 0.46 – 0.75), poor or low diversity (H' = 0.01 – 0.45), and no diversity or non-variable (H' = 0.00).

The environmental, genotypic and phenotypic variances (σ_e^2, σ_g^2, σ_p^2), phenotypic and genotypic coefficient of variations (PCV, GCV) for the quantitative morpho-agronomical traits were calculated according to Burton & Devane (1953). The PCV and GCV values are ranked as low (0-10%), medium (10-20%) and high (>20%) according to Shivasubramanian & Menon (1973). Broad sense heritability was calculated according to Falconer & Mackay (1996). Heritability values are categorized as low (0- 30%), moderate (30-60%) and high (>60%) according to Robinson et al. (1949). Genetic advance (GA) and genetic advance in terms of percentage of means (GAM) were calculated by using the formula given by Robinson et al. (1949). The GAM was categorized as low (0-10%), medium (10-20%) and high (>20%) according to Johanson et al. (1955).

Correlation coefficient analysis was done by using formulae developed by Johnson et al. (1955). The strength of the correlation as the absolute value was given below: very weak (0.00 – 0.19), weak (0.20 – 0.39), moderate (0.40 – 0.59), strong (0.60 – 0.79) and very strong (0.80 – 1.00) (Evans, 1996).)

Cluster analysis was used for classification of the examined genotypes. The variables were standardised because of various units that were measured. Squared Euclidean distance was used as a measure of distance between genotypes. Between – Group's linkage method was used for agglomeration of the clusters.

Principal component analysis (PCA) was performed using SPSS 19 software and the related cluster analysis was plotted based on the first eight principal components.

Results and Discussion

Phenotyping is an important activity to evaluate the utilization of the germplasm collection in a genebank. In this study, 27 rye accessions conserved at the National genebank of Bulgaria were scored and measured using 9 qualitative and 15 quantitative morpho-agronomical characters.

Qualitative morphological characters

In Table 2 are presented the score descriptions of 9 morphological characteristics.

The studied accessions did not differ in terms of morphological parameters related to the color of the coleoptiles and spike- thrashing. All genotypes presented a violet color of coleoptiles and non-thrashing spike.

Leaf color was a characteristic that, in studies of characterization and diversity tend to be very variable. In this study, the difference in this characteristic formed three groups: 55.55% of the genotypes presented a green color, 25.92% dark green and 18.52% light green. Shape of the spike and shape of the grain also showed high variable (respectively CV = 39.92% and CV = 38.95%) (Table 2).

According to shape of the spike genotypes were divided to genotypes with prismatic shape (48.1% of genotypes), fusiform (44.44% of genotypes) and ovate shape (7.41%). According to shape of the grain genotypes were grouped as followed – with ovate shape (44.44% of genotypes), with ovate-oblong shape (37.04% of genotypes) and with barrel-shaped form (18.52% of genotypes) (Table 2).

There was a color variation in grain (CV = 21.95%), resulting in the formation of three groups. Group 1, with the green grain (51.85% of genotypes), the second with gray-

Table 2. The score descriptions of 27 rye accessions (*Secale cereale* L.) by 9 morphological characteristics

Accession number	Color of the coleoptile	Shape of the rosette	Presence of wax	Leaf color	Shape of the spike	Density of the spike	Spike thrashing	Shape of the grain	Grain color
A2BM0326	3	7	7	3	1	7	2	2	3
76569	3	7	3	3	1	7	2	2	3
A9000461	3	9	5	1	1	7	2	2	4
B4000202	3	9	5	3	2	8	2	1	3
B4000203	3	9	5	3	2	7	2	1	3
B4000204	3	7	5	1	1	9	2	2	4
B4000205	3	9	3	1	1	7	2	2	3
B4000206	3	9	3	1	3	8	2	1	3
B4000207	3	7	7	3	3	7	2	1	3
B4000208	3	9	7	3	2	7	2	2	4
B4000209	3	7	5	3	2	7	2	2	4
B4000210	3	9	3	1	1	7	2	2	3
B4000211	3	9	3	3	2	8	2	1	3
B4000212	3	9	5	3	1	8	2	1	4
B4000213	3	9	5	3	1	8	2	1	3
B4000214	3	7	3	3	2	8	2	3	2
B4000215	3	7	7	3	2	8	2	3	2
B4000216	3	7	5	7	1	7	2	1	3
B4000217	3	9	7	7	1	7	2	1	3
B4000218	3	9	3	3	2	7	2	2	4
B4000220	3	9	7	7	2	7	2	2	3
B4000219	3	7	5	7	1	7	2	2	4
B6E0208	3	9	7	3	2	8	2	2	3
61148	3	7	3	3	1	8	2	3	2
B7E0055	3	9	5	7	2	8	2	3	2
B7E0131	3	7	5	7	2	8	2	3	2
B7000123	3	9	5	7	1	8	2	1	2
CV, %	0.00	12.31	29.49	59.77	39.92	8.23	0.00	38.95	21.95

Note: color of the coleoptile: 1 – light green, 2 – dark green, 3 – purple; type of the bush: 5 – semi-erect (46–55º), 7 – strongly declinated (56–70º), 9 – prostrate (>70); leaf color: 1 – light green, 3 – green, 7 – dark green; presence of wax: 3 – weak, 5 – medium, 7 – strong; shape of the spike-1 – prismatic, 2 – fusiform, 3 –oval; density of the spike – 7 (39-40), 8 (40-41), 9 (> 41); spike thrashing: 1 – thrashed, 2 – non thrashed; shape of the grain: 1 – ovate-oblong, 2 – ovate, 3 – barrel-shaped; grain color: 1 –white, 2 –yellow, 3 –green, 4 – gray-green

green grain (25.93% of genotypes) and the third with yellow grain (22.22% of genotypes).

Regarding the type of bush genotypes were grouped in two groups. Group 1, with strongly declinated bush (40.74% of genotypes) and group 2 with prostrate bush (59.26% of genotypes) (Table 2).

In the presence of leaf wax descriptor, it was observed that 44.44% of the genotypes were characterized with medium presence of the wax, 29.63% of genotypes with weak and 25.93% with strong presence of wax (Table 2).

Quantitative morpho-agronomical characters

In Table 3 are presented the results of the analysis of variance for fifteen quantitative morpho-agronomical traits. The analysis of variance exhibited the presence of significant differences among the tested genotypes for all characters indicating the existence of variability. The genotypes differed significantly ($p<0.01$) for length of the 2nd from bottom internode and distance from the flag leaf to the spike, and were highly significant ($p<0.1$) for plant height, length of the upper internode, leaf area of a flag leaf, leaf area of a sub-flag leaf, length of spike, length of awn, weight of the main spike, weight of the grain in the main spike, number of spikelets in the main spike, number of grain in the main spike, mass of 1000 grains, hectolitre mass of the grain, and grain yield. Relatively the most variable for the studied period were the characters: weight of the grain in the main spike (CV = 37.14%), number of spikelets in the main spike (CV = 31.04%), leaf area of a flag leaf (CV = 30.88%), and grain yield (CV = 28.05%). The traits with the lowest de-

Table 3. Analysis of variance for fifteen morpho-agronomical traits in 27 rye accessions

Characters	Mean	Mean Square	CV, %
Plant height, cm	167.54	168.52***	5.68
Length of the upper internode, cm	45.90	35.34***	9.30
Length of the 2nd from bottom internode, cm	5.74	1.41**	15.37
Distance from the flag leaf to the spike, cm	24.60	25.51**	15.31
Leaf area of a flag leaf, cm^2	5.73	9.15***	30.88
Leaf area of a sub-flag leaf, cm^2	17.70	36.60***	20.98
Length of spike, cm	13.98	7.74***	13.46
Length of awn, cm	3.46	2.21***	27.04
Weight of the main spike, g	2.71	0.63***	15.98
Weight of the grain in the main spike, g	2.37	2.02***	37.14
Number of spikelets in the main spike	47.46	802.07***	31.04
Number of grain in the main spike	60.32	172.72***	11.89
Mass of 1000 grains, g	30.36	18.45***	7.83
Hectolitre mass of the grain, kh/hl	70.16	29.47***	5.16
Grain yield, t/ha	4.13	4.12***	28.05

*$p < 0.05$, ** $p < 0.1$, *** $p < 0.01$

Table 4. Estimates of genotypic variance, environmental variance, phenotypic variance, genotypic and phenotypic coefficients of variation, broad sense heritability and genetic advance for some quantitative morpho-agronomical traits in rye

Characters	σ_e^2	σ_g^2	σ_p^2	GCV, %	PCV, %	h^2, %	GA	GAM, %
Plant height, cm	65.64	25.72	91.36	3.03	5.71	28.15	554.29	3.31
Length of the upper internode, cm	12.70	5.66	18.36	5.18	9.34	30.82	272.07	5.93
Length of the 2nd from bottom internode, cm	0.58	0.21	0.78	7.92	15.43	26.31	48.02	8.37
Distance from the flag leaf to the spike, cm	10.55	3.74	14.29	7.86	15.37	26.17	203.82	8.29
Leaf area of a flag leaf , cm^2	1.20	1.99	3.19	24.60	31.17	62.31	229.23	40.01
Leaf area of a sub-flag leaf, cm^2	6.46	7.54	13.99	15.51	21.14	53.84	414.94	23.44
Length of spike, cm	2.19	1.39	3.58	8.42	13.53	38.75	151.01	10.80
Length of awn, cm	0.44	0.45	0.89	19.28	27.19	50.30	97.48	28.17
Weight of the main spike, g	0.05	0.14	0.19	14.02	16.19	74.97	67.76	25.00
Weight of the grain in the main spike, g	0.37	0.41	0.78	27.14	37.28	53.01	96.49	40.71
Number of spikelets in the main spike	23.84	194.56	218.40	29.39	31.14	89.08	2712.00	57.14
Number of grain in the main spike	12.53	40.05	52.57	10.49	12.02	76.18	1137.81	18.86
Mass of 1000 grains, g	1.54	4.23	5.77	6.77	7.91	73.31	362.64	11.94
Hectolitre mass of the grain, kh/hl	7.83	5.41	13.24	3.32	5.19	40.87	306.31	4.37
Grain yield, t/ha	0.45	0.92	1.37	23.18	28.32	67.00	161.41	39.08

σ_e^2 – environmental variance, σ_g^2 – genotypic variance, σ_p^2 –phenotypic variance, GCV – genotypic coefficients of variation, PCV – phenotypic coefficients of variation, h^2 – broad sense heritability, GA – genetic advance, GAM – genetic advance expressed as a percentage of the mean

grees of variation included plant height (5.68%), hectolitre mass of the grain (5.16%), mass of 1000 grains (7.83%) and length of the upper internode (9.30%). Therefore, the analysed samples were homogeneous by these indicators.

Variance of components estimates (σ^2) revealed that environmental variance strongly influenced on the traits: plant height and number of spikelets in the main spike (Table 4).

The genotypic variance ranged from 0.14 (weight of the main spike) to 194.56 (number of spikelets in the main spike). The magnitude of genotypic variances was higher than their corresponding environmental variances for the following traits: leaf area of a flag leaf, leaf area of a sub-flag leaf, weight of the main spike, weight of the grain in the main spike, number of spikelets in the main spike, number of grain in the main spike, mass of 1000 grains, hectolitre mass of the grain, grain yield. This indicates that the genotypic component of variation was the major contributor to total variation in these studied traits (Table 4).

PCV ranged from 5.19% for hectolitre mass of the grain to 37.28% for weight of the grain in the main spike, while GCV range from 3.03% for plant height to number of spikelets in the main spike (29.39%). The high values above 20% for the both coefficients were recorded for leaf area of a flag leaf (PVC = 31.17%, GVC = 24.60%), weight of the grain in the main spike (PCV = 37.28%, GVC = 27.14%), number of spikelets in the main spike (PCV = 31.14%, GVC = 29.39%) and grain yield (PCV = 28.32%, GVC = 23.18%). PCV was also high for the following characters-leaf area of a sub-flag leaf (PVC = 21.14%) and length of awn (PVC = 27.19%). This indicated that the genotype could be reflected by the phenotype and the effectiveness of selection based on the phenotypic performance for these characters (Meles et al., 2017). As expected, phenotypic coefficient of variation was greater than genotypic coefficient of variation for all the characters (Table 4).

Broad sense heritability (h^2) was calculated in order to estimate the proportion of total variance attributed to genotypic differences. The values obtained were low in three characters, moderate in six characters and high in six of the all analysed 15 characters. High heritability indicated that the characters were less influenced by the environment (Dyulgerova & Valcheva, 2014). Our results showed that leaf area of a flag leaf, weight of the main spike, number of spikelets in the main spike, number of grain in the main spike and grain yield possessed a wide range of genetic variability and improvement could be achieved with mass selection alone (Table 4).

The expected genetic advance expressed as a percentage of the mean varied between 3.31% and 57.14%. The high GAM was calculated for the following characters: leaf area of a flag leaf, leaf area of a sub-flag leaf, length of awn, weight of the main spike, weight of the grain in the main spike, number of spikelets in the main spike and grain yield. These traits, with the exception of the length of the awn, combined high heritability with high genetic progress, indicating that they are important characters to be considered for selection and improvement of *Secale cereal*e L. (Table 4).

Using Pearson's correlation, an analysis was done to assess the relationship among the quantitative morpho-agronomical traits (Table 5). It is useful to determine the relationship among the morphological traits since this information will be useful in the utilization of the germplasm as well in the collection of the germplasm based on the target traits (Rabara et al., 2014).

Table 5. Pearson correlation coefficients between 15 morpho-agronomical traits in 27 rye accessions (*Secale cereal*e L.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1														
2	0.522**	1													
3	-0.458*	-0.429*	1												
4	0.599**	0.845**	-0.401*	1											
5	0.279	0.234	-0.162	0.143	1										
6	0.321	0.388*	-0.365	0.356	0.745**	1									
7	-0.26	-0.026	0.127	-0.123	0.22	0.021	1								
8	-0.036	0.202	0.277	0.217	0.151	0.27	0.387*	1							
9	-0.068	0.053	-0.107	-0.118	0.061	-0.001	0.478*	0.291	1						
10	-0.085	0.011	0.029	-0.216	-0.084	-0.004	0.193	0.175	0.509**	1					
11	0.438*	0.378*	-0.161	0.473*	0.503**	0.421*	0.498**	0.414*	0.236	-0.022	1				
12	0.134	0.089	-0.148	-0.076	0.051	-0.007	0.597**	0.16	0.727**	0.436*	0.368	1			
13	-0.068	0.225	-0.044	0.238	-0.015	0.167	-0.318	-0.022	0.032	0.096	-0.268	-0.135	1		
14	-0.247	-0.255	0.085	-0.36	-0.375*	-0.28	-0.499**	-0.465*	-0.096	0.088	-0.875**	-0.275	0.438*	1	
15	0.387*	0.22	-0.064	0.277	0.407*	0.306	0.338	0.073	0.348	0.166	0.701**	0.296	-0.059	-0.363	1

Note:**Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

1 – plant height, 2 – length of the upper internode, 3 – length of the 2nd from bottom internode, 4 – distance from the flag leaf to the spike, 5 – leaf area of a flag leaf, 6 – leaf area of a sub-flag leaf, 7 – length of spike, 8 – length of awn, 9 – weight of the main spike, 10 – weight of the grain in the main spike, 11 – number of spikelets in the main spike, 12 – number of grain in the main spike, 13 – mass of 1000 grains, 14 – hectolitre mass of the grain, 15 – grain yield

A moderate and significant relation was found between plant height and length of the upper internode (r = 0.522**), while length of the 2nd from bottom internode correlated moderately negatively with plant height (r = -0.458*) and length of the upper internode (r = -0.429*). Distance from the flag leaf to the spike correlated strongly to very strongly positively with plant height and length of the upper internode (r = 0.599**, r = 0.845**) and moderately negatively with length of the 2nd from bottom internode (r = -0.401*). Leaf area of a sub-flag leaf correlated positively and weakly with length of the upper internode (r = 0.388*) and strongly with leaf area of a flag leaf (r = 0,745**). Relation between length of awn and length of spike was weak and statistically proven at $p \leq 0.5$ (r = 0.387*). Weight of the main spike correlated moderately with length of spike (r = 0.478*). Correlation between weight of the grain in the main spike and weight of the main spike was positively moderately and significant at $p \leq 0.01$ (r = 0.508**) (Table 5). A moderate and significant relation was found between number of spikelets in the main spike and the following characters: plant height (r = 0.438*), length of the upper internode (r = 0.378*),distance from the flag leaf to the spike (r = 0.473*), leaf area of a flag leaf (r = 0.503**), leaf area of a sub-flag leaf (r = 0.421*), length of spike (r = 0.498**) and length of awn (r = 0.414*). Number of grain in the main spike strongly correlated with length of the spike (r = 0.597**) and weight of the main spike (r = 0.727**), while moderate weight of the grain in the main spike (r = 0.436*). A significantly negative relation was found between hectolitre mass of the grain and leaf area of a flag leaf (r = -0.375*), length of spike (r = -0.499**), length of awn (r = -0.465*), number of spikelets in the main spike (r = -0.875**), while positive with mass of 1000 grains (r = 0.438*). Grain yield correlated weakly with plant height (r = 0.387*), moderately with leaf area of a flag leaf (r = 0.407*) and strongly with number of spikelets in the main spike (r = 0.701**). Correlations were statistically proven at $p \leq 0.5$ and $p \leq 0.01$, respectively (Table 5).

In accordance with our study Kuneva et al. (2018) also found significant correlations between: length of awn and length of spike, weight of the main spike and length of spike, weight of the grain in the main spike and weight of the main spike. Pyza et al. (2015) established positive and significant correlations between number of kernels per spike, weight of kernels per spike and length of spike. Laidig et al. (2017) also noted positive but not significant correlation between number of kernels per spike and grain yield.

Diversity analysis

The Shannon–Weaver diversity (H′) index was used to estimate phenotypic diversity. In the Table 6 are presented the computed indices for qualitative and qualitative traits. Variability and phenotypic diversity among rye accessions were observed in the most of the investigated traits in this study. For qualitative traits the highest diversity was observed in type of the bush with H' = 0.983 diversity index, followed by the traits connected with the presence of wax on the leaf and shape and color of the grain (H' = 0.944 and H' = 0.929 respectively). Leaf color, shape of the spike and density of the spike also showed high diversity index between 0.759 and 0.896. The color of the coleoptiles and spike- thrashing

Table 6. Shannon-Weaver diversity index for different morphological traits

Quantitative Traits	H'	Qualitative Traits	H'
Color of the coleoptile	0.000	Plant height	0.575
Type of the brush	0.983	Length of the upper internode	0.526
Presence of wax	0.954	Length of the 2nd from bottom internode	0.685
Leaf color	0.896	Distance from the flag leaf to the spike	0.575
Shape of the spike	0.824	Leaf area of a flag leaf	0.568
Density of the spike	0.759	Leaf area of a sub-flag leaf	0.646
Spike thrashing	0.000	Length of spike	0.491
Shape of the grain	0.944	Length of awn	0.542
Grain color	0.929	Weight of the main spike	0.494
		Weight of the grain in the main spike	0.457
		Number of spikelets in the main spike	0.515
		Number of grain in the main spike	0.613
		Mass of 1000 grains	0.660
		Hectolitre mass of the grain	0.633
		Grain yield	0.481
Mean	0.699		0.564
General diversity index – H' = 0.631			

were monomorphic traits- 100% of the genotypes presented a violet color of coleoptiles and non-thrashing spike (H' = 0.00). The average diversity index of all qualitative traits was moderate (H' = 0.699) (Table 6).

For quantitative traits, the moderate diversity indices were recorded for all of the study characters. The value of the diversity index was the highest for length of the 2nd from bottom internode (H' = 0.685), following from the mass of 1000 grains (H' = 0.660) and leaf area of a sub-flag leaf (H' = 0.660), while it was the lowest for weight of the grain in the main spike and grain yield (H' = 0.457 and H' = 0.481, respectively). As a whole, the Shannon–Wiener diversity index of qualitative traits was higher than that of the quantitative traits, which was indicative of more extensive genetic diversity (Table 6).

General Shannon-Weaver diversity index for all included in the study quantitative and qualitative traits was moderate (H' = 0.631) (Table 6).

Hierarchical cluster analysis

Cluster analysis based on the 22 morpho-agronomical traits from the total of 24 investigated traits grouped the genotypes into six clusters at 15 Squared Euclidean Distance. Cluster 1 consisted of 18 genotypes (B4000205, B4000210, A9000461, B4000204, B4000209, B4000218, B4000212, B4000213, B4000202, B4000203, B4000211, A2BM0326, B4000208, B4000214, B4000215, 76569 and 61148), which represents 66% of total genotypes. Genotypes were characterized with medium plant height, medium productive spikes and respectively medium grain yield per ha, but with relatively high hectoliters mass of grain. Cluster 2 consisted of only one genotype (B4000206 from France). This genotype had the lowest values of the flag leaf area and the subflag leaf area, as well as the lowest thousand grains mass. Cluster 3 consisted of 2 genotypes (B7E0131 and B7E0131). These two Bulgarian landraces were characterized with the highest plant height, length of the upper internode and distance from the flag leaf to the spike, and with the high value of the number of spikelets in the main spike but with the lowest value of the hectolitre mass of the grain. Cluster 4 included 4 genotypes (B4000217, B4000220, B4000219, B4000216), which represents 14.81% of total genotypes. These genotypes had the lowest plant height, number of spikelets in the main spike, number of grain in the main spike, mass of 1000 grains and grain yield but the relative high hectoliter mass of the grain. The Hungarian genotype -B4000207 was separated in cluster 5. It had the lowest length of spike but the highest weight of the grain in the main spike and hectolitre mass of the grain. Cluster 6 consisted of only one accession (B7000123). This genotype was with the highest areas of flag and sub-flag leaves, with the highest productive spike and respectively with the highest grain yield (Figure 1, Table 7).

Squared Euclidean Distance Matrix (Proximity Matrix) was produced by assuming 351 total possible pair wise combinations of the 27 rye genotypes (Table 8). Squared Euclidean Distance coefficients varied from 0.250 to 7.655. The lowest distance values were measured for the following pair wise genotype combinations: B4000210 – B4000205 (0.250, from IRL and AUS, respectively), B4000213-B4000212 (0.347, from RUS and PRT, respectively), B4000204 – A9000461 (0.354, from DEU and CAN, respectively) and B4000203 – B4000202 (0.380, from SWE and ROM, respectively), following from the next combinations with higher distance values: B4000205-A9000461 (0.471, from AUS and CAN, respectively), B7E0131-B7E0055 (0.574, from BGR and BGR, respectively), B4000213-B4000202 (0.579, from RUS and ROM, respectively), B4000213-B4000203 (0.641, from RUS and SWE, respectively), B4000212-B4000202 (0.662, from PRT and ROM, respectively) and B4000218-B4000209 (0.669, from USA and CZE, respectively). B7000123 (from

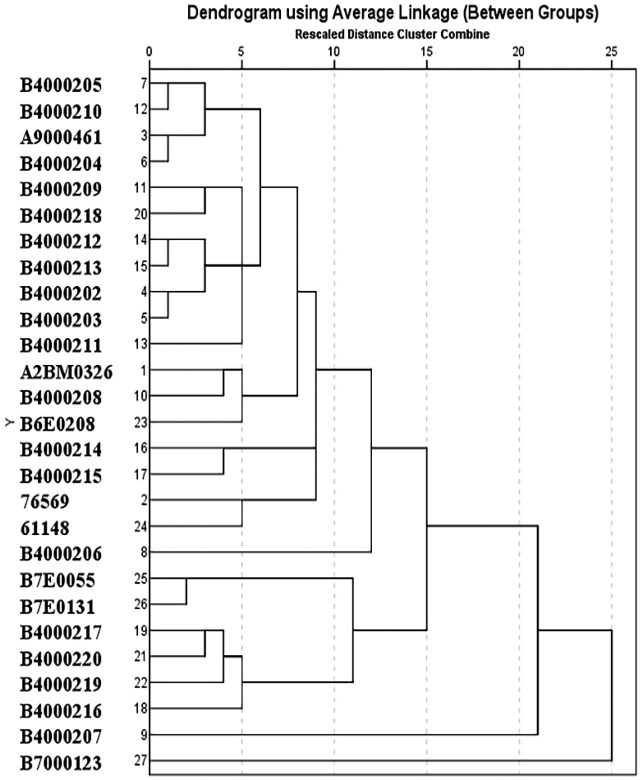

Fig. 1. Dendrogram of 27 rye genotypes generated by the Between – groups linkage method and Squared Euclidean distance

BLR) was generally the most diverged genotype from others. It had the highest distance value with B4000206 (7.655, from FRA, respectively), following from B4000205 (7.001, from SWE, respectively) and B4000210 (6.931, from IRL, respectively) (Table 8).

Principal component (PC) analysis

Principal component (PC) analysis for 7 qualitative and 15 quantitative morpho-agronomical data set revealed 8 components with Eigenvalues greater than 1 (Table 9). The first 8 principal components (PC) explained 81.80% of the total variation. In particular, the first principal component (PC1), which explained 14.56% of the total variation, was positively associated with plant height, length of the upper internode, distance from the flag leaf to the spike, number of spikelets in the main spike and grain yield, while it was negatively associated with grain color. The second PC (PC2), which explained 12.98% of the total variation, was strongly and positively associated with spike characters-number of grain in the main spike, weight of the grain in the main spike, weight of the main spike and length of spike (Table 8). The third PC (PC3) was associated with traits characterized the leaf- leaf color, leaf area of a flag leaf and leaf area of a sub-flag leaf. The forth PC (PC4) which explained 12.98% of the total variation, was strongly and positively associated with type of brush and length of the 2nd from bottom internode, while negatively with shape of the grain. The physical characters of grain – mass of 1000 grains and hectolitre mass of the grain were the main traits contributing to the fifth PC (PC5), while only the morphological trait- presence of wax was important in the sixth PC (PC6). The seventh PC (PC7) was strongly and positively associated with density of the spike and length of the upper internode, while shape of the spike was the main trait contributing with the eighth PC (PC8) (Table 9).

Cluster analysis based on the first eight Principal Components and the Between – groups linkage method grouped the genotypes into 4 clusters at 15 Squared Euclidean distance. Cluster 1 consisted of 17 genotypes (A2BM0326, 76569, 61148, B4000207, B4000220, B4000217, B4000208, B6E0208, A9000461, B4000203, B4000205, B4000213, B4000202, B4000218, B4000212, B4000215 and B7E0055), which represents 62.96% of total genotypes. Genotypes in this cluster were in the highest rate with respect to sixth PC. Second cluster comprised 4 genotypes (B4000214, B7E0131, B4000204, B4000219) including 14.81% of total genotypes and had the highest mean with respect to fifth and seventh PCs. Cluster 3 consisted of 5 genotypes (B4000209, B4000216, B4000210, B4000211, B4000206), which represents 18.52% of total genotypes. Genotypes in this cluster were in the lowest rate with respect to sixth PC and the highest with the eighth PC. B7000123 genotype was separated individually in the fourth cluster. It was highly associated with PC1, PC2, PC3 and PC4 and had the lowest rate with respect to PC8 (Figure 2, Table 10).

Table 7. Means for six clusters based on 15 quantitative traits

	Clusters					
Characters	I	II	III	IV	V	VI
Plant height. cm	167.0	168.2	175.5	163.7	168.9	173.1
Length of the upper internode. cm	45.7	45.0	49.5	44.5	45.6	46.7
Length of the 2nd from bottom internode. cm	6.0	6.5	5.0	5.3	5.8	5.5
Distance from the flag leaf to the spike. cm	24.5	24.8	29.3	23.2	22.7	25.7
Leaf area of a flag leaf. cm^2	5.5	3.2	6.5	5.8	5.2	9.5
Leaf area of a sub-flag leaf. cm^2	16.8	12.5	20.3	18.6	18.8	21.9
Length of spike. cm	13.9	14.8	13.8	14.1	13.7	16.4
Length of awn. cm	3.4	3.3	3.9	3.5	3.8	3.4
Weight of the main spike. g	2.6	2.7	2.5	2.8	2.9	3.7
Weight of the grain in the main spike. g	2.2	2.4	2.0	2.4	5.5	2.8
Number of spikelets in the main spike	45.6	42.8	69.1	38.0	41.6	87.1
Number of grain in the main spike	59.5	66.2	59.4	58.8	64.9	71.8
Mass of 1000 grains. g	30.4	32.3	30.7	30.3	31.7	30.0
Hectolitre mass of the grain. kg/hl	70.5	70.0	65.7	71.0	72.0	66.1
Grain yield. t/ha	4.0	3.9	4.6	3.3	4.8	8.2

Table 8. Squared Euclidean Distance coefficients between 27 rye genotypes

Proximity Matrix

	Squared Euclidean Distance																										
Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1:A2BM0326	0.000																										
2:76569	0.990	0.000																									
3:A9000461	1.172	1.610	0.000																								
4:B4000202	1.374	1.630	1.299	0.000																							
5:B4000203	1.554	2.244	1.346	**0.380**	0.000																						
6:B4000204	0.935	1.253	**0.354**	1.340	1.661	0.000																					
7:B4000205	1.775	1.909	**0.471**	1.621	1.347	0.776	0.000																				
8:B4000206	3.443	3.360	2.542	1.237	1.476	2.397	2.306	0.000																			
9:B4000207	4.498	5.306	4.393	2.359	3.050	4.596	4.971	3.046	0.000																		
10:B4000208	0.968	1.552	1.621	1.105	1.490	1.449	2.741	2.622	4.017	0.000																	
11:B4000209	1.517	2.419	1.267	1.134	1.068	1.207	1.407	1.531	3.636	1.451	0.000																
12:B4000210	1.843	2.047	0.564	1.578	1.538	0.833	**0.250**	2.182	5.223	2.638	1.246	0.000															
13:B4000211	2.270	1.910	2.044	0.851	1.350	1.981	2.213	1.364	4.619	1.702	1.282	1.793	0.000														
14:B4000212	1.166	1.355	0.965	**0.662**	0.947	0.816	1.321	2.513	3.886	1.507	1.432	1.474	1.606	0.000													
15:B4000213	0.985	1.428	0.831	**0.579**	**0.641**	0.969	0.980	2.332	3.787	1.643	1.241	1.123	1.171	**0.347**	0.000												
16:B4000214	2.051	1.484	1.944	1.752	2.129	1.786	1.699	2.408	4.143	2.127	1.608	1.829	2.217	2.374	2.181	0.000											
17:B4000215	1.632	2.736	1.889	1.884	1.933	1.988	2.040	3.225	3.190	2.100	1.670	2.345	3.516	2.538	2.229	0.915	0.000										
18:B4000216	2.389	2.830	3.612	2.187	2.211	3.592	3.921	4.884	6.023	2.793	2.377	3.708	2.175	1.952	1.635	3.547	3.975	0.000									
19:B4000217	2.240	2.942	3.620	2.008	2.484	3.627	4.270	5.425	4.402	2.819	3.180	4.422	3.379	1.651	1.810	3.729	3.249	1.005	0.000								
20:B4000218	1.670	1.494	0.974	0.834	0.734	1.208	1.072	1.486	4.043	1.208	**0.669**	1.094	1.071	1.193	1.143	1.112	1.856	2.439	3.117	0.000							
21:B4000220	2.078	2.868	3.563	1.784	2.129	3.586	4.208	4.375	3.536	1.800	2.271	4.297	3.080	2.289	2.323	2.420	1.931	1.483	0.790	2.223	0.000						
22:B4000219	2.051	2.311	2.931	2.282	2.612	2.869	3.468	5.216	5.358	2.522	2.174	3.489	2.930	1.832	1.973	2.682	2.887	0.908	0.839	2.074	0.895	0.000					
23:B6E0208	1.012	1.741	1.814	1.191	1.663	1.734	2.487	2.790	3.276	1.253	2.076	2.673	2.633	1.790	1.799	2.042	1.396	3.863	2.752	1.808	1.912	2.692	0.000				
24:61148	1.446	1.189	2.166	2.860	2.720	1.965	1.655	4.282	6.284	3.047	2.728	2.087	3.704	2.675	2.333	1.314	1.879	4.138	4.334	2.047	3.544	3.151	2.028	0.000			
25:B7E0055	2.465	2.564	4.555	3.246	3.355	4.420	4.595	5.512	6.027	2.821	3.419	4.967	4.099	3.846	3.588	2.046	2.218	2.920	2.612	2.747	1.162	1.960	2.174	2.010	0.000		
26:B7E0131	2.453	2.665	5.148	3.533	3.948	4.539	5.171	5.507	6.025	3.042	3.259	5.274	4.107	4.181	3.937	2.349	2.754	3.090	3.152	3.342	1.574	2.264	2.587	2.218	**0.574**	0.000	
27:B7000123	3.788	3.803	6.867	4.251	5.286	6.198	**7.001**	**7.655**	6.758	5.351	6.380	**6.931**	5.829	4.407	4.540	6.248	6.393	4.584	3.359	6.153	3.715	4.066	3.736	4.637	3.946	2.992	0.000
This is a dissimilarity matrix																											

Table 9. Eigenvector coefficient of 22 morpho-agronomical traits for the first 8 principal components with Eigenvalue, individual and cumulative percentages of the total variance

Characters	Principal Component							
	1	2	3	4	5	6	7	8
Type of the brush	-0.04	0.03	-0.01	**0.85**	-0.03	-0.05	-0.02	-0.03
Presence of wax	-0.28	0.31	0.39	-0.06	0.17	**0.43**	-0.26	0.26
Leaf color	0.25	0.14	**0.53**	-0.31	0.00	0.04	-0.54	-0.13
Shape of the spike	0.07	-0.05	0.01	0.01	-0.07	-0.07	0.07	**0.92**
Density of the spike	0.22	0.21	0.10	-0.11	-0.10	-0.04	**0.79**	0.01
Shape of the grain	0.22	-0.17	-0.18	**-0.54**	-0.42	0.44	0.11	-0.06
Grain color	**-0.80**	-0.15	0.03	0.14	0.16	-0.22	0.05	-0.14
Plant height	**0.78**	-0.10	0.20	-0.12	-0.03	-0.24	0.16	0.11
Length of the upper internode	**0.52**	0.02	0.24	-0.42	0.31	0.14	**0.51**	-0.01
Length of the 2nd from bottom internode	-0.27	-0.13	-0.29	**0.70**	0.00	0.52	-0.03	0.01
Distance from the flag leaf to the spike	**0.65**	-0.19	0.17	-0.42	0.22	0.20	0.31	-0.19
Leaf area of a flag leaf, cm^2	0.14	0.02	**0.89**	0.12	-0.09	0.00	0.10	-0.08
Leaf area of a sub-flag leaf	0.17	-0.08	**0.85**	-0.21	0.10	0.12	0.03	0.10
Length of spike	-0.19	**0.62**	0.21	0.08	-0.43	0.33	0.17	-0.24
Length of awn	0.14	0.17	0.13	-0.02	-0.03	0.86	-0.02	-0.04
Weight of the main spike	0.12	**0.90**	-0.01	0.01	0.08	0.08	-0.07	-0.05
Weight of the grain in the main spike	-0.02	**0.63**	-0.08	-0.03	0.20	0.12	-0.13	0.55
Number of spikelets in the main spike	**0.59**	0.26	0.42	-0.01	-0.42	0.32	0.15	-0.20
Number of grain in the main spike	0.07	**0.85**	0.00	0.01	-0.18	0.00	0.29	0.04
Mass of 1000 grains	-0.01	-0.02	0.07	-0.05	**0.90**	0.07	0.00	-0.08
Hectolitre mass of the grain	-0.39	-0.13	-0.32	0.12	**0.61**	-0.43	-0.07	0.17
Grain yield, t/ha	**0.56**	0.38	0.36	0.30	-0.08	-0.02	0.03	-0.08
Eigenvalues	**3.20**	2.86	2.69	2.17	2.03	2.01	1.56	1.47
Individual % of variance	14.56	12.98	12.24	9.88	9.24	9.12	7.09	6.70
Cumulative % of variance	14.56	27.54	39.78	49.66	58.89	68.01	75.10	81.80

Table 10. Mean values and standard deviations (SD) for each principal component (PC)

Clusters		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
I	mean	-0.037	-0.028	-0.070	0.267	**0.143**	**0.587**	-0.063	0.051
	SD	0.782	0.930	0.994	0.728	1.180	0.552	0.775	1.056
II	mean	-0.356	0.250	0.284	-1.498	-0.195	-0.213	**0.683**	-0.303
	SD	1.979	0.594	0.781	0.363	0.682	0.221	1.536	0.895
III	mean	0.018	-0.585	-0.384	-0.012	-0.261	**-1.676**	-0.255	**0.319**
	SD	0.149	0.920	0.912	1.077	0.583	0.279	1.315	0.904
IV	mean	**1.961**	**2.397**	**1.975**	**1.510**	-0.336	-0.756	-0.388	**-1.251**

Note: SD – standard deviation

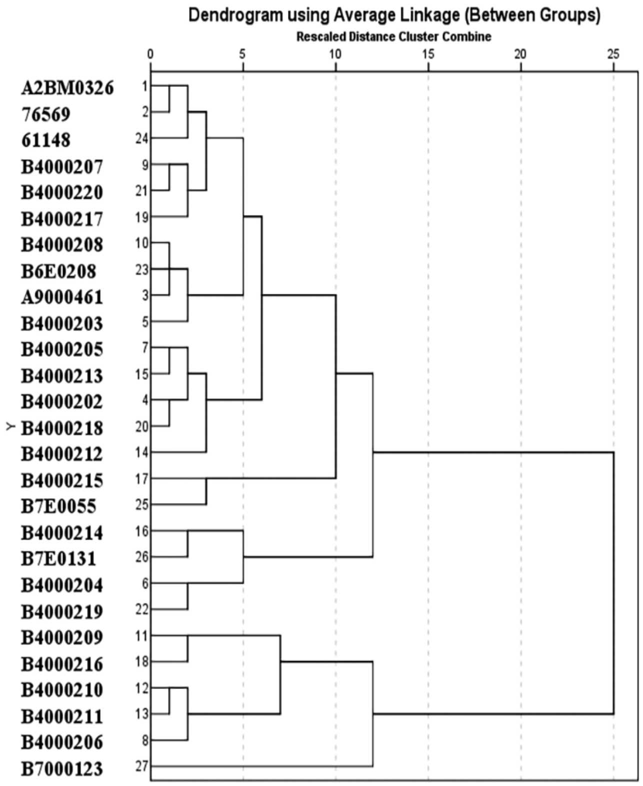

Fig. 2. Dendrogram of 27 rye genotypes generated by the Between – groups linkage method and Squared Euclidean distance on the base of the first eight principal components

Conclusions

The studied accessions did not differ in terms of morphological parameters related to the color of the coleoptiles and spike- thrashing. The analysis of variance exhibited the presence of significant differences among the tested rye genotypes for the all of the studied quantitative morpho-agronomical characters, indicating the existence of variability. Leaf area of a flag and sub-flag leaf, weight of the main spike, weight of the grain in the main spike, number of spikelets in the main spike and grain yield combined high heritability with high genetic progress, indicating that they are important characters to be considered for selection and improvement of *Secale cereale* L. Grain yield correlated positively and significantly with plant height, leaf area of a flag leaf, and number of spikelets in the main spike.

The level of phenotypic diversity (H' = 0.699) among 27 winter rye accessions based on the Shannon-Weaver Diversity Index indicated that these accessions could be very useful in utilizing these valuable accessions for varietal improvement under different rye breeding programs. The clustering of the studied rye genotypes was not related to their geographical origin but it referred to specific phenotypic characters. Genetically the most distant on the studied characters were measured for the following pair wise genotype combinations: B4000206-B7000123, B4000205-B7000123 and B4000210-B7000123. The first 8 principal components (PC) explained 81.80% of the total variation. B7000123 could be used as parents in hybridization programmes to develop high yielding rye varieties. Grouping of genotypes by multivariate methods in the study are of practical value for the rye breeders.

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Received: **August, 27, 2021**; *Approved:* **November, 24, 2021**; *Published:* **April, 2023**