Genetic Diversity of Genetic Resources of Winter Barley Maintained in the Genebank in Slovakia

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Abstract: A set of 140 winter barley genetic resources of foreign and domestic origins was tested on experimental basis of RIPP in 1997–1999 to characterise the variability of the accessions based on agronomic data using multivariate methods. In the set tested, variability was studied of selected traits and characteristics such as: plant height (PH), weight of 1000 grains (W), grain number per a spike (SNG), grain uniformity – ratio of front seeds over 2.5 m sieve (GU), vegetation period – sowing/full maturity (VM) and seed yield (Y). Agronomic characters show great variability between cultivars. The study of matrix interrelationships between different variables showed that the yield is greatly correlated with traits: vegetation period – sowing/full maturity, grain uniformity and grain number per a spike. High positive correlation was obtained between the grain uniformity and the weight of 1000 grains. Negative correlation was found between the grain number per a spike and weight of 1000 grains in six-row barley. Correlations between agronomic traits differed between two- and six-row barley sets. The study revealed the existence of genetic differences among accessions as well as differences between two- and six-row winter barley and between the genotypes of domestic and foreign country origin, respectively. Results of this study provided information about diversity which should be of particular interest for the further collecting of genetic resources.

Keywords: barley; *Hordeum vulgare* L.; diversity; morphology; variation; agronomic characters; cluster analyses; principal component; cultivars

Barley is one of the most important crops in Slovakia. Winter barley is characterised by high stability of yield and a lesser demand on soil conditions than spring barley. Lower resistance to freeze is its liability. The breeding of winter barley is orientated mainly on fodder quality and at two-row barley there is perspective to achieve malt production. Actual studies on barley genetic resources collections show that the right utilisation of appropriate varieties can substantially put forward and make effective the breeding work. Selection of suitable genetic resources is the first step to breeding barley varieties of high quality (Sleziak 2000). There are 11 winter barley varieties registered in Slovakia (Anonymous 2003). The present varieties registered have biologic-agronomic characters suitable for intensive growing and have appreciable resistance to leaf diseases and resistance to freeze.

The study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analysed by a specific method or a combination of methods. The use of established multivariate statistical algorithms is an important strategy for classifying germplasm, ordering variability for a large number of accessions, or analysing genetic relationships among materials. Multivariate algorithms appear particularly useful. This particular statistics has several advantages (Peters & Martinelli 1989). First, it allows mixing of both qualitative and quantitative data and therefore all the available information on the sample can be utilised. The statistics has

been used in widely different fields (Murphy *et al*. 1986).

This work describes phenotypic diversity observed in winter barley cultivars. A similar work has been carried out with spring barley (Žáková & Žák 1999). The multivariate analysis, and in particular, the principal component and cluster analyses have been utilised for the evaluation of a collection of 140 spring barley genotypes from the Gene Bank of RIPP Piešťany. The main objectives of this study were: (a) to assess the phenotypic diversity for agronomic descriptors of winter barley, (b) to determine the associations between these descriptors, (c) to estimate and compare the phenotypic diversity in two-row and six-row winter barley accessions which are maintained by the genebank in Slovakia, (d) to determine the within-accessions and between-accessions agronomic variations of domestic and non domestic accessions.

MATERIAL AND METHODS

Plant material. A total of 140 accessions (47 two-row and 93 six-row) of winter barley maintained at the Gene Bank of RIPP Piešťany were studied. They were grown during two years 1997/1998 and 1998/1999 in the experimental fields of the institute. The experiment was set up as a randomised block design.

The accessions were evaluated for six agronomic descriptors (Table 1) according to the Descriptors list for barley (Anonymous 1994; Lekeš *et al*. 1986). The traits not scored in the field (SNG, W, GU) were measured in the laboratory on thirty plants sampled from the middle of the plot. The materials studied included 53 domestic accessions (Slovakia and previous Czechoslovakia) and 87 accessions from other countries, especially from Germany

(DEU – 71), France (FRA – 9), Belgium (BEL – 2), United States of America (USA – 2), Great Britain (GBR – 2) and Romania (ROM – 1).

Statistical analyses. Two multivariate analyses, and in particular, the principal component and cluster analyses (Veasey *et al*. 2001) were utilised for the evaluation of our collection. Principal component analysis (PCA) was used to group the variables into subsets that are relatively independent from each other as well as for reducing the dimensionality of the structure. Thus it produced new accumulated variables (principal components, PCs) represent underlying processes responsible for inter-correlations of variables in the original dataset. The first two principal component scores were plotted in a two-dimensional plane to inspect the sample for interesting patterns of cultivars. PCA and character correlations were obtained by STATGRAPHIC.

The cluster analysis (CA) was used to see whether the cultivars fell into groups or clusters. The CA's were performed using the program SPSS that adopts squared Euclidian distance as a measure of dissimilarity and the Ward's method as the clustering algorithm (Ward 1963). Before computing the distance between genotypes, our data were standardised as recommended by Fox and Rosielle (1982). Correlations between all characteristics were calculated.

RESULTS AND DISCUSSION

Agronomic characters show great variations within cultivars of winter barley (Lasa *et al*. 2001; Atanassov *et al*. 2001) 47 two-row and 93 six-row selected accessions of winter barley were included. Table 2 shows the range of selected agronomic characters and significance between them. NS indicates non-significant trend. Two and six-rowed cultivars differed significantly ($P < 0.05$) for any characters investigated. The yield of six-row barley cultivars has a greater range than that of two-row barley cultivars. The results show significant phenotypic differences ($P < 0.01$) between two- and six-row winter barley, similar results were obtained by Ortiz (2002a,b) in his work.

Phenotypic correlations in two- and six-row barley were determined independently and the data are presented in Table 3. In the set tested during 1997–1999 positive straight strong correlation appeared between the yield and the traits: grain uniformity and grain number per a spike; negative strong correlation was observed between the yield

Table 1. Name, abbreviations of agronomic characteristics evaluated in winter barley collection

Characters	Codes
Plant height (mm)	PH
Vegetation period – sowing/full maturity (days)	VM
Weight of 1000 grains (g)	W
Grain uniformity (%) (ratio of front seeds over 2.5 mm sieve)	GU
Grain number per a spike	SNG
Yield (t/ha)	Y

Table 2. Range of selected characters of two-row and six-row winter barley cultivars (number of cultivars indicated in brackets)

Characters	2-row barley (47)		6-row barley (93)		Significant
	min–max	variance	min–max	variance	
PH	80.30–127.70	66.99	84.10–117.2	35.41	NS
VM	272.0–285.00	21.34	272.0–285.0	21.15	NS
W	49.0–60.10	7.34	36.80–52.30	7.33	**
GU	89.0–99.60	6.46	59.90–98.0	7.63	**
SNG	12.40–29.90	10.26	34.10–63.40	31.91	**
Y	3.28–7.29	5.64	3.80–8.98	11.76	*

[NS]non-significant; *$P < 0.05$; **$P < 0.01$

and the traits – the vegetation period and plant height in six-row barley, which is not in agreement with Costa and Bollero (2001). Another negative correlation is between the grain number per a spike and traits: vegetation period, weight of 1000 grains, which confirmed Ortiz *et al.* (2001) and Kebebew *et al.* (2001) data. In two-row barley positive strong dependence was found between the weight of 1000 grains and: the vegetation period, grain uniformity and negative correlations between the weight of 1000 grains, vegetation period and yield. In six-row barley, the correlation between the grain number per a spike, weight of 1000 grains, vegetation period and the yield were negative and significant, but in two-row barley no correlation existed. More details are given in Table 3.

Six-row barley cultivars showed a more extensive phenotypic variation in the traits studied than two-row barley cultivars, according to Aikasalo (1988).

Most of the variations observed in the agronomic characters in six-row barley were significantly affected by the country of the cultivar origin, except for grain of uniformity. One-way analysis of variance was carried out to confirm the geographical partitioning of diversity (Tables 4 and 5).

The results show that there was significant phenotypic difference between the accessions of SVK, CZE origin and the rest of others in six-row winter barley in these traits: the plant height, the vegetation period, the weight of 1000 grains and grain number per a spike. Genotypes LU-74 (CSK), KM-660 (CSK), KM-987 (CSK), KM-1065 (CSK) were found optimal from the point of view of these traits. Significant differences in the yield exist in both types of two- and six-row winter barley. The best uniformity of grain was found domestic genotypes of two-row winter barley. Considering the history of the breeding, the yield of domestic genotypes is higher comparing to yield of other genotypes.

Table 3. Correlation coefficients among selected agronomic characters (two-row above diagonal, *n* = 47 and six-row below diagonal, *n* = 93 winter barley cultivars)

Characters	PH	VM	W	GU	SNG	Y
PH		–0.099 [NS]	0.043 [NS]	–0.086 [NS]	0.014 [NS]	–0.169[NS]
VM	0.063 [NS]		0.191**	–0.324*	–0.041	–0.699**
W	–0.034 [NS]	0.293*		0.456 **	–0.029 [NS]	–0.275*
GU	–0.215*	–0.294*	0.548**		–0.218*	0.281*
SNG	–0.034 [NS]	–0.349**	–0.206*	0.030 [NS]		–0.076 [NS]
Y	–0.186*	–0.804**	–0.155 [NS]	0.430**	0.425**	

[NS]non-significant; *$P < 0.05$; **$P < 0.01$

Table 4. Range of selected agronomic characters of domestic and foreign two-row winter barley cultivars (number of cultivars indicated in brackets)

Characters	SVK, CZE (12)		Others (35)		Significant
	min–max	variance	min–max	variance	
PH	80.30–106.3	40.73	81.0–127.7	76.9	NS
VM	272.0–278.0	6.81	272.0–285.0	19.98	**
W	49.0–60.10	10.50	49.40–59.10	6.53	NS
GU	93.1–99.60	4.03	89.0–99.5	7.11	NS
SNG	23.4–29.0	2.62	12.4–29.9	11.93	NS
Y	3.87–5.93	1.62	3.28–7.29	6.43	**

[NS]non-significant; *P < 0.05; **P < 0.01

Table 5. Range of selected agronomic characters of domestic and foreign six-row winter barley cultivars (number of cultivars indicated in brackets)

Characteristics	SVK, CZE (41)		Others (52)		Significant
	min–max	variance	min–max	variance	
PH	84.10–101.50	15.66	84.70–117.20	47.14	*
VM	272.0–283.0	5.50	272.0–285.0	17.87	**
W	36.80–47.10	5.29	40.40–52.30	7.55	**
GU	59.90–98.0	63.39	63.20–96.0	82.07	NS
SNG	45.7–63.4	22.33	34.10–59.7	33.36	**
Y	3.62–8.98	4.97	3.80–8.48	9.98	**

[NS]non-significant; *P < 0.05; **P < 0.01

Our domestic genotypes are adapted to our natural environment, they are genetically very similar and qualitatively homogeneous. Genotypes PY-2-O, PY-5-O and PY-14-O were good in the traits: yield, SNG, W and GU.

More detailed relationships were revealed by PCA. The first step in PCA was to calculate eigenvalues, which define the amount of total variation that is displayed on the PC axes. The PCs with eigenvalue > 1.0 were used as a criteria to determine the number of PCs, 3 in our case. The variance accumulated by the first three components was 74.7% and 81.4% for two- and six-row barley accessions, a relatively high percentage of the total variation which, according to VEASEY *et al.* (2001), explains satisfactorily the variability manifested between individuals.

The first and second principal components reduced the 6 original descriptors to two major components that accounted together for 60% of the variation among the accessions of barley. PC1, which is the first and most important component, accounted for over 31% of the total variation in two-row and 37% in six-row barley, respectively. Figure 1 shows a part of the results of PCA applied to the data set. The principal components generated are characterised by the loadings of the input variables. A higher absolute loading value means a more important contribution to the new PC. The first principal component is mainly influenced by the yield, grain number per a spike and the vegetation period, whereas grain uniformity and the weight of 1000 grains were the most important loadings for 2nd component. The third

1st component

2nd component
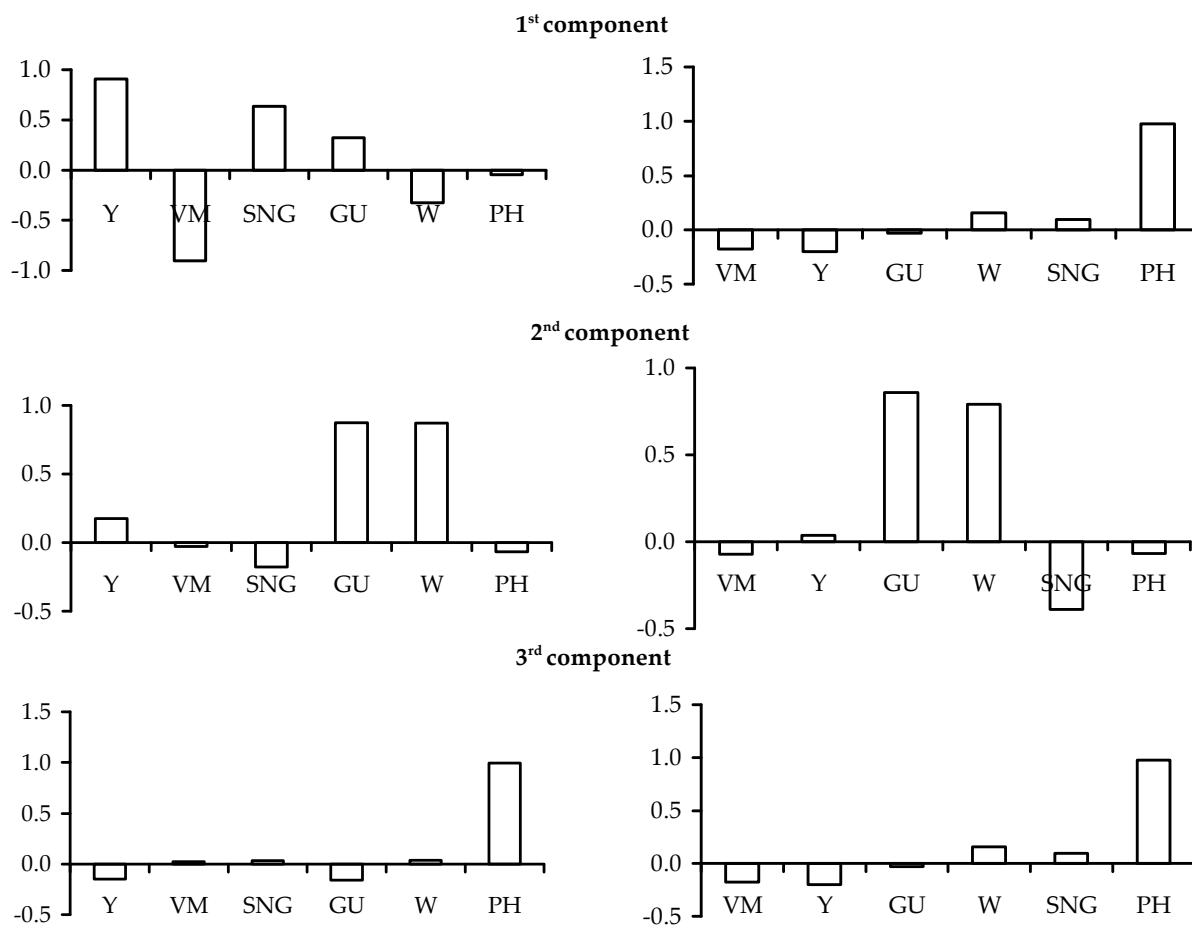
3rd component

PH – plant height, VM – vegetation period, W – weight of 1000 grains, GU – grain uniformity, SNG – number of grains/spike, Y – yield

Figure 1. Vectors loadings of agronomic input variables on three principal components of barley data

principal component was influenced mainly by the height of plant, according to the paper by Ruitz *et al*. (1997), which explained 17% of total variation. These components are different for two and six-row barley data sets (Figure 1), which is caused by the different variations in these sets.

The results of PCA were combined with the results of cluster analysis in a plot of three components (Figure 2). Cluster analysis using the Ward's method as the clustering algorithm (Ward 1963) was approved as a suitable method, also suggested by Mohammedi and Prasanna (2003).

The comparisons between the clusters and the groups developed from PCA allowed us to deduce that the characters contributing substantially to the barley clusters involved the yield, the vegetation period, the weight of 1000 grains, grain uniformity, and the grain number per a spike. The distribution of the accessions on the scanner

plot is shown in Figure 2. The analysis based on agronomical components distributed both sets into 3 (two-row), eventually 2 (six-row) big clusters with small subclusters. Tables 6 and 7 give the mean values of the characters with standard errors of characters selected.

Analysis of variance indicated a significant difference between clusters formed on agronomic characters except grain number per spike in two-row barley set. The largest cluster on the left site is formed by 27 genotypes. The group is represented mostly by genotypes of Slovak origin with a high yield, grain uniformity and the shortest vegetation period, by which it statistically significantly differs from other clusters. The next clusters (2 and 3) are smaller with 15 and 5 genotypes. The smallest group is formed by the highest genotypes with the lowest weight of 1000 grains, grain uniformity and the yield. On
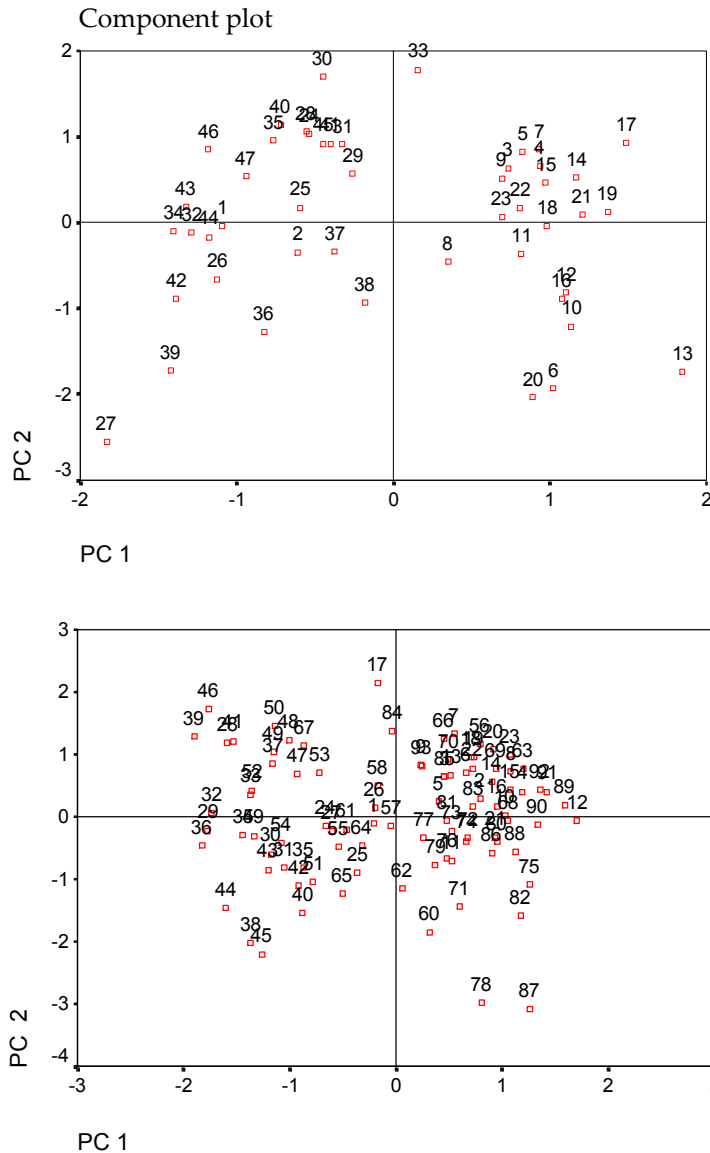
Component plot

Figure 2. Scatter diagram of two and six-row barley cultivars following principal components analysis

the other hand the component – grain number per a spike has substantially higher value in this cluster than in the groups 1 and 2.

A few accessions of German origin are distributed in these clusters. The first cluster contains apart genotype 8 (Filia), with high plants (PH – 90.5) and a higher yield (Y – 7.4 t/ha). The second cluster contains apart genotype 17 (Labea) with a higher value of the weight of 1000 grains (W – 59.1 g) and grain uniformity (GU – 95.5 g). In the third cluster is genotype 13 (Triesdorfer Stamm 441 Beschalt) with a lower yield (Y – 3.28 t/ha) and higher plants (PH – 115.7 mm) separately. On more detailed investigation of six-row barley, we can state that there are only 2 clusters with subgroups. Cluster analysis confirms the results obtained by analysis

of principal component analyses. As can be seen in Table 7, the other characters contributed also to the group difference, except the plant height. The lowest difference found (distance = 0.12) in the agronomic characters was between the domestic genotypes.

Into the first cluster fall separately domestic genotypes: 78 (PY-26-O) with a lower height of plant (PH – 90 mm) and grain number per spike (SNG – 47), genotype 87 (PY-27-O) with the weight of 1000 grains (W – 40.7 g), genotype 17 (LU-61) with high grain uniformity (GU – 91%) and grain number per spike (SNG – 55) in comparison to average. The highest differences found (distance = 132.91) were between the genotypes from Germany and those from France (distance = 104.51).

Table 6. Means and standard errors of characters in the clusters of two-row barley (number of cultivars in clusters indicated in brackets)

Characteristics	Clusters		
	1. (27)	2. (15)	3. (5)
PH	94.83 ± 0.95^{23}	89.17 ± 1.32^{13}	106.20 ± 6.9^{12}
VM	274.85 ± 0.53^{23}	283.071 ± 0.33^{1}	282.601 ± 0.51^{1}
W	53.57 ± 0.56^{2}	55.67 ± 0.42^{13}	52.24 ± 0.96^{2}
GU	96.97 ± 0.42^{3}	96.06 ± 0.38^{3}	91.46 ± 0.77^{12}
SNG	24.38 ± 0.74	25.01 ± 0.57	26.00 ± 0.61
Y	9.87 ± 0.35^{23}	6.73 ± 0.14^{13}	4.76 ± 0.49^{12}

[a]Superscripts 1–3 indicate: mean is significantly different from the means of clusters 1–3

Table 7. Means and standard errors of characters in the groups of six-row barley (number of cultivars in clusters indicated in brackets)

Characteristics	Clusters		Significant
	1. (58)	2. (35)	
PH	95.55 ± 0.76	96.27 ± 10.6	NS
VM	273.66 ± 0.20	282.71 ± 0.14	**
W	43.89 ± 0.35	45.36 ± 0.44	**
GU	88.48 ± 10.7	82.85 ± 1.45	*
SNG	52.55 ± 0.64	48.73 ± 1.02	**
Y	11.64 ± 0.30	5.89 ± 0.21	**

NS = non-significant; *$P < 0.05$; **$P < 0.01$

CONCLUSIONS

The results of this study provide information about diversity of winter barley which should be of particular interest for the further collecting of genetic resources and show a wide spectrum of agronomic variability between the genotypes investigated. The results obtained have shown that sets of agronomic data are very useful for the initial description. The geographic origin was not the determining criterion for the genotype aggregation.

The cluster and principal component analyses together with the agronomic data of winter barley revealed the existence of genetic variations within accessions, as well as differences between two- and six-row winter barley and between genotypes of domestic and foreign origins. Ward's method produced 3 or 2 clusters, respectively. This study highlighted a strong 'resemblance' and, consequently, possible relationships between some clusters.

The study of matrix interrelationship between different variables showed that the yield is significant positive correlated to grain uniformity and the grain number per a spike. Significant negative correlation was found between the grain number per spike and the weight of 1000 grains. Correlations between agronomic traits differed between two- and six-row barley sets. The six agronomic characters separated the groups of two- and six-row barley. The most important characters contributing to the variability were the yield, vegetation period, grain uniformity and the weight of 1000 grains.

Principal component analysis provides interesting information about the associations of the characters

that are useful to formulate better hypotheses for the breeding work. The results achieved give a survey on the variability in genetic resources of winter barley of domestic and foreign origins. The comparison of foreign and domestic barley genetic resources in the set tested showed no differences in the grain uniformity. Significant differences were found in the vegetation period and in the yield. The highest seed yield, the highest weight of 1000 grains and good grain uniformity in Czech genetic resources, namely: LU-74, KM-660, KM-987 and KM-1065 (six-row), and in Slovak genetic resources: PY-2-O, PY-5-O and PY-14-O (two-row) were recorded.

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Abstrakt

Žáková M., Benková M. (2004): **Diverzita genetických zdrojov ozimného jačmeňa uchovávaného v slovenskej génovej banke**. Czech J. Genet. Plant Breed., **40**: 118–126.

V rokoch 1997–1999 sme zisťovali prostredníctvom mnohorozmerných štatistických metód variabilitu 140 genetických zdrojov ozimného jačmeňa domáceho a zahraničného pôvodu. Študovali sa niektoré agronomické znaky ako:

výška rastliny (PH), hmotnosť 1000 semien (W), počet zŕn na klase (SNG), rovnorodosť semena (GU), vegetačná doba – sejba – plná zrelosť (VM) a úroda (Y). Agronomické znaky poukázali na variabilitu medzi odrodami. Zistila sa kladná korelácia medzi úrodou a vegetačnou dobou, rovnorodosťou semena a počtom semien na klase, rovnorodosťou semena a hmotnosťou 1000 semien a záporná korelácia medzi počtom semien na klase a hmotnosťou 1000 semien. Korelácia medzi agronomickými znakmi sa líšila medzi dvoj- a šesťradovým jačmeňom. Vypočítaný rozptyl prvých troch komponentov analýzou hlavných komponentov bol 74,73 % (dvoj-) a 81,43 % (šesťradový) z celkového rozptylu. Potvrdila sa genetická variabilita medzi odrodami, ako aj rozdiely medzi dvoj- a šesťradovým a medzi genotypmi domáceho a zahraničného pôvodu. Výsledky tejto analýzy poskytli informácie o variabilite, čo sa môže ďalej využiť v zhromažďovaní genetických zdrojov.

Kľúčové slová: jačmeň; *Hordeum vulgare* L.; diverzita; morfológia; variácia; agronomická charakteristika; zhluková analýza; komponenty analýzy; odroda

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