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MORPHO-BIOLOGICAL DIVERSITY OF WINTER WHEAT VARIETIES ASSESSMENT

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A R T I C L E I N F O

A B S T R A C T

The work uses the statistical analysis methods for selecting the parent components for winter wheat hybridization. The research was performed in 2005-2016 in the conditions of the Central Chernozem Region. The research object included the varieties of soft winter wheat of various ecological and geographical origins; in different years, with 200-350 varieties. To assess the morpho-biological diversity of genotypes, the authors have tested various methods of statistical data analysis using Statistica®6.1, i.e. cluster analysis (sequential dichotomy, k-means clustering, and hierarchical classification) and neural network data processing (Cohen network). It is found that the k-means method (without limiting the number of groups) provides the most satisfactory results for forming the clusters of genotypes characterized by similar morphological and biological characteristics and used as parent material for selection of soft winter wheat. In this respect, the clustering of varieties is affected by three attributes, i.e., the length of seedling-earing period, plant height, and weight per 1,000 grains. For hybridization crosses, the parent components are selected with the account of their belonging to different clusters. The crossing is performed between the varieties with contrasting characteristics. The k-means method to plan crosses allows obtaining valuable winter wheat hybrids characterized by polymorphism, favorable transgressions, high productivity, winter hardiness, and lodging resistance.

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1. INTRODUCTION

At present, there are many methods for selecting the pairs for hybridization (Bondarenko and Artyukh, 1989; Borojevich, 1984; Briggs and Knowles, 1967; Vavilov, 1935, 1987; Girko and Sabadin, 2001; Egortsev, 2003; Zhegalov, 1930). Vyushkov, *et al.*, 2012 have developed the following structure of methods for selecting the parent components for hybridization in self-pollinating crops:

1) methods based on the gene-related principle (the methods of backcrosses, accumulation, and gene stacking);
2) methods based on the trait-related principle (the methods of maximum, minimum, complex stepwise crossing, selection by elements of a complex trait and by combining ability);
3) methods based on the principle of expected transgressions (the methods of ecological and geographical distance, genealogical distance, and genetic distance).

The method of genetic distance includes cluster analysis by the complex of morpho-physiological parameters, biochemical, and molecular markers. The use of the latter two methods of analysis to assess hundreds of breeding samples is currently impossible due to the complexity and high cost of analyses. Therefore, the authors propose to assess the breeding material by morphological and biological indicators.

The selection of parent components for crosses is a crucially important stage of breeding work, which largely determines its success (Kadyrov, 2001). Every year parent forms for crosses are selected in breeding nurseries out of hundreds (and sometimes thousands) of studied varieties. Moreover, only some of these varieties are unique, while most of them are characterized by similar traits and properties. Therefore, the authors consider it advisable to combine all the studied samples into groups on the basis of their comprehensive assessment. At the same time, the parent components for crosses should be selected with the account of their belonging to a certain group, which will significantly reduce the amount of work on hybridization, the costs, and terms of the selection process.

As a rule, previous studies on the assessment of parent material in field crop selection were based on the fact that a subset of samples was artificially divided into separate classes according to one trait, and samples were then assessed within these classes. However, the authors believe that this approach does not allow taking into account several traits simultaneously. Therefore, there is a need to find a new way to create groups (clusters) of breeding samples that are homogeneous by several traits in order to simplify the selection of parent components for hybridization.

The assessment of varieties by several traits can be performed using the methods of multivariate statistics, which have recently become increasingly popular in scientific research, particularly inbreeding. In order to use these methods two main tasks must be solved: 1) a method for grouping the varieties should be chosen; 2) the traits for this grouping should be identified.

2. MATERIALS AND METHODS

The work was performed at Voronezh State Agrarian University in 2005-2016. The crop production practices used in field experiments were conventional for the Central Chernozem Region. The materials included the varieties of soft winter wheat of various ecological and geographical origins; in different years the number of these varieties ranged from 200 to 350. Statistical data analysis was performed using Statistica 6.1 package (Borojevich, 1984; Khalafyan, 2010).

3. RESULTS AND DISCUSSION

Several scientists (Nikolaeva and Evstratova, 2005; Smiryaev and Kil'chevsky, 2007; Tishchenko and Chekalin, 2005) believe that the degree of genetic relationship can be expressed through a quantitative parameter. Back in the 1930s, it was proposed to use the Mahalanobis distance as a measure of distance (Rao, 1973). Its special case is the Euclidean distance.

At the initial stage of research, the authors grouped the varieties using the sequential dichotomy method with cluster analysis (the k-means method) (Bukhovets, *et al.*, 2006), which was based on sequential dividing of samples into two classes by a set of traits in a multidimensional space with a dichotomous tree being formed.

This results in the formation of classes, in which the variability of traits decreases and the homogeneity of genotypes increases. The distinguished classes are characterized by unique combinations of trait values, taking into account the effect of the external environment. As a result of studying of 246 varieties, 32 groups were identified. Some of them contained only one genotype, which indicated their uniqueness.

The main disadvantage of this method is the artificial limitation of the number of groups. Therefore, in further studies, the authors used a neural network data processing, i.e. the Cohen network (Excel Neural Package). The input nodes of the neural network are supplied with the data containing the values of the studied traits. The algorithm finds the data and builds two-dimensional matrix maps of multivariate data in geometric hyperspace with minimum possible distortions for each trait. The main disadvantage of neural networks is that they work as a "black box" (it is not always clear why they come to a certain decision). Therefore, in order to determine the genetic divergence of varieties, the authors used cluster analysis, where the Euclidean distance was used as a measure of similarity. The main disadvantage of determining the genetic diversity of varieties using the Euclidean distance is the inability to verify the quality of clustering and the trait value for the quality of clustering.

In this regard, the authors propose using the k-means method for clustering of varieties, as it enables an iterative choice of a given number of centers, for which the distance is minimal within the clusters and maximal between the clusters. The adequacy of dividing the entire mass of observations into clusters can be assessed using the analysis of variance. The efficiency of classification is verified using the discriminant analysis (Katsko and Paklin, 2009).

The next stage of studies was to search for the traits for "grouping".

Initially, the samples were grouped on the basis of the assessment of 16 traits:

1) *plant height (PH);*
2) *spike length (SL);*
3) *total number of spikelets per spike (TSS);*
4) *number of productive spikelets per spike (PSS);*
5) *number of unproductive spikelets per spike (USS);*
6) *number of grains per spike (GS);*
7) *plant mass (PM);*
8) *spike mass (SM);*
9) *mass of grain per spike (MGS);*
10) *mass of 10 grains (MG 10);*
11) *coefficient of economic use (Keu);*
12) *length of the seedling-earing period (Lse);*

13) length of the earing-ripening period (Ler);
14) length of vegetation period (Lv);
15) productive forage density (PFD);
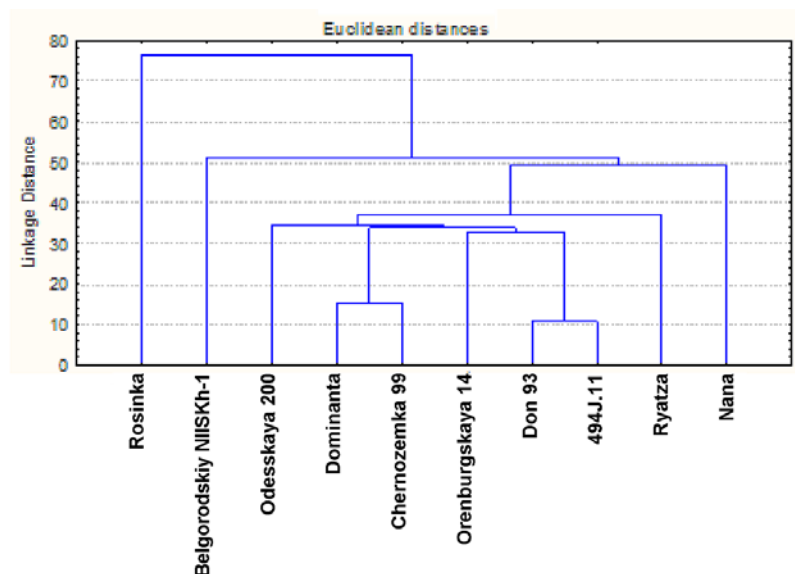*16) yield (Y) (see Figure 1).*

Figure 1: Tree diagram of Euclidean distances between winter wheat varieties (the calculation includes 16 traits).

The calculations have shown that Dominant – Chernozemka 99 and Don 93 – 494J.11 are the most genetically close varieties in terms of all traits, while Nana – Rosinka and Belgorodskiy NIISKh-1 – Ryatza and the most genetically distant varieties.

It can be assumed that some of the considered traits duplicate the other ones and give no additional information. Therefore, there is a problem of screening out the excess traits. The authors used cluster analysis with the correlation coefficient as a measure of similarity of traits to build a tree diagram of clusters of traits and identify the groups of closely related traits. At the level of r = 0.9, only two pairs of all considered traits formed clusters, and hence duplicated each other. These were the spike mass/mass of grain per spike and the number of productive spikelets/the total number of spikelets (see Figure 2).
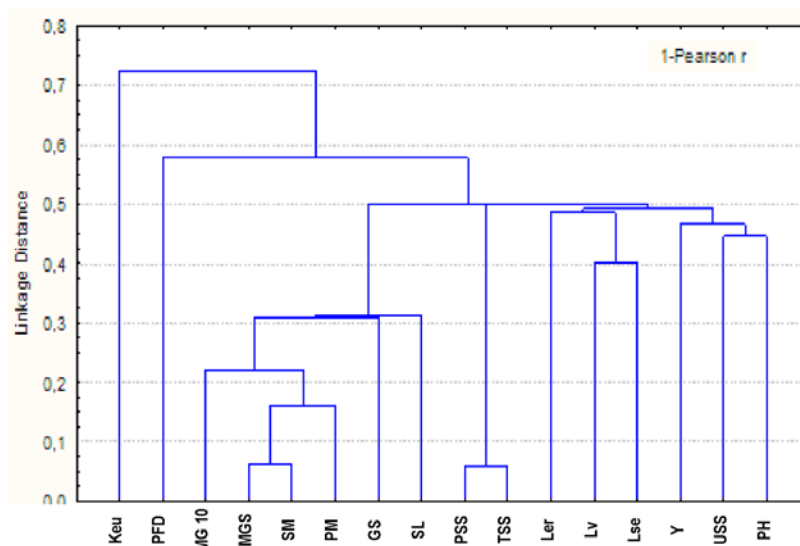

Figure 2: Tree diagram of relationships between the traits of winter wheat varieties

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Therefore, the authors have excluded such traits as the mass of grain per spike (as a more labor-intensive trait compared to the spike mass) and the number of spikelets from further calculations.

The calculation of Euclidean distances by 14 traits showed that the distance between the varieties remained unchanged. Hence, the excluded traits, provided no additional information (see Figure 3).
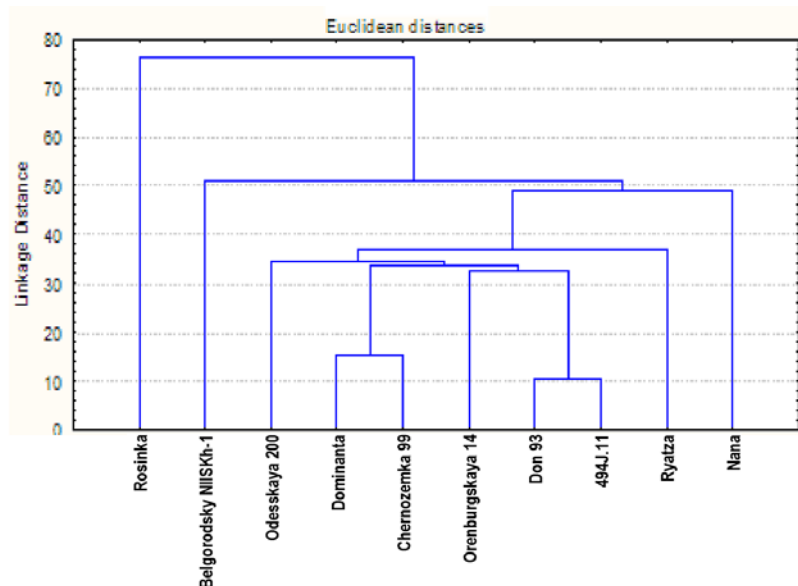

Figure 3: Tree diagram of Euclidean distances between winter wheat varieties (the calculation includes 14 traits)

Similar calculations were performed to exclude the duplicating traits until the results of the assessment of morpho-biological diversity of varieties matched the original calculations, where 16 traits were used. As a result, only eight traits instead of sixteen were sufficient to assess the morpho-biological similarity of winter wheat varieties. These traits included the winter hardiness, length of vegetation period, yield, plant height, the mass of grain per spike, gluten content in the grain, the mass of 1,000 grains, and the number of grains per spike (Rusanov, *et al.*, 2010). However, the authors took into account the fact that most quantitative traits (such as the yield and number of grains per spike) were strongly affected by the environmental conditions and therefore it would be difficult to use them for assessing the morpho-biological characteristics of varieties, and it would be possible to assess the winter hardiness of varieties only in certain years (Baldzhi, 1976; Batygin, 1993; Vyrikova, 1990; Zadontsev, *et al.*, 1971).

In this regard the authors propose to choose the traits of grouping according to the following requirements:

1) the traits must be stable, i.e. it should be possible to assess them annually (Korobeynikov, 1981);
2) the traits should characterize the belonging of genotypes to a certain variety type and ecotype since one of the basic principles for selecting the pairs for crosses is still the eco-geographical principle developed by Vavilov (the founder of scientific selection);
3) the assessment of traits should be as less labor-intensive as possible.

The work with spring wheat can achieve good results if the breeding material is aligned according to the following traits: botanical variety (or at least beardedness), plant height, vegetation period, and drought resistance (Kumakov, 1984). Since there are no reliable objective parameters for defining the drought resistance, this trait was excluded by the authors.

In order to group the breeding samples, the authors used the traits that characterize the belonging to a particular variety, ripeness group (length of the seedling-earing period), variety type (plant height) and productivity level (spike length, number of spikelets per spike (productive and unproductive), and mass per 1,000 grains). The authors have established the following: despite the fact that the absolute values of these traits in varieties may vary over the years, their belonging to a certain group is constant.

The analysis of variance found that all the traits had a significant influence on the results of classification (see Table 1).

Table 1: Assessment of the significance of traits for grouping of the varieties

Traits	Between SS	df	Within SS	df	F	p-value
Variety	3.6	3	24.9	116	5.7	<0.01
Length of seedling-earing period	5335.6	3	2268.2	116	91.0	<0.01
Plant height	15004.3	3	4792.8	116	121.1	<0.01
Spike length	6.8	3	89.7	116	2.9	0.04
Number of unproductive spikelets	11.1	3	33.4	116	12.9	<0.01
Number of productive spikelets	20.1	3	261.4	116	3.0	0.03
Mass per 1,000 grains	2096.5	3	5812.3	116	13.9	<0.01

The next stage of grouping was to determine the number of clusters. The authors performed clustering by a various number of groups, determined the classification function using the discriminant analysis, and achieved the 100% distribution of objects into groups.

The quality of discrimination was assessed using the Wilks' Lambda indicator. Its value was 0.023, which indicated that the discrimination was successful.

The participation of each variable in discrimination was estimated using the Wilks' Lambda indicator, which is the result of the exclusion of the corresponding variable from the model. When this variable is excluded from the model, a part of the information about the object is lost. Therefore, the worse the consequences of such an exception, the more important the variable in the model is, i.e. the greater the value of the Wilks' Lambda, the more desirable is the presence of this variable in the discrimination procedure. The value of the partial Wilks' Lambda characterizes the individual contribution of the corresponding variable to the dichotomous power of the model. The smaller these statistical values, the greater the contribution to overall discrimination is. The larger the F-to-remove value and the smaller the p-level, the greater the contribution of the variable to the discrimination procedure is. The variables with the significance level of greater than 0.05 can be excluded from the model (Khalafyan, 2010).

The calculation of these criteria shows that such traits as the spike length and the number of productive spikelets, for which the p-level is greater than 0.05, can be excluded from the model (see Table 2). In Tables 2 and 3, we highlighted the coefficients at the 5% level of significance.

Table 2: Results of analysis of discriminant functions

Traits	Wilks' Lambda	Partial Lambda	F-remove (4.109)	p-level	Toler.	1-Toler. (R-Sqr.)
Variety	**0.031**	**0.901**	**2.984**	**0.022**	**0.941**	**0.059**
Length of seedling-earing period	**0.060**	**0.458**	**32.234**	**<0.001**	**0.921**	**0.079**
Plant height	**0.128**	**0.216**	**98.909**	**<0.001**	**0.878**	**0.122**
Spike length	0.028	0.973	0.759	0.554	0.684	0.316
Number of unproductive spikelets	**0.032**	**0.855**	**4.624**	**0.002**	**0.855**	**0.145**
Number of productive spikelets	0.030	0.936	1.868	0.121	0.708	0.292
Mass per 1,000 grains	**0.040**	**0.695**	**11.940**	**<0.001**	**0.917**	**0.083**

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As a result of clustering, the authors have obtained 19 clusters with 100% distribution of objects. In this respect, the clustering of varieties was affected by only three traits, i.e. the length of seedling-earing period, plant height, and weight per 1,000 grains. The Wilks' Lambda value of 0.0074 indicates good discrimination (see Table 3). In Table 3 we highlighted the coefficients at the 5% level of significance.

Table 3: Results of analysis of discriminant functions

Traits	Discriminant Function Analysis Summary (Spreadsheet126) No. of vars in the model: 3; Grouping: CLUSTER (19 groups) Wilks' Lambda: .00074 approx. F (54.289) = 54.881 p < 0.0000					
	Wilks' Lambda	Partial Lambda	F-remove (4.109)	p-level	Toler.	1-Toler. (R-Sqr.)
Length of seedling-earing period	0.007	0.110	43.7	<0.001	0.999	0.001
Plant height	0.017	0.044	115.7	<0.001	0.992	0.008
Mass per 1,000 grains	0.004	0.179	24.6	<0.001	0.992	0.008

According to the importance of classification, these traits can be ranged in the following sequence: plant height, length of seedling-earing period, and mass per 1,000 grains. These traits fully meet the requirements that the authors presented to them. For instance, the plant height is a characteristic of the variety type, the length of the seedling-earing period is a characteristic of the ecotype, and the mass per 1,000 grains is a productivity indicator, the formation of which is less affected by vegetation conditions than the number of grains per spike.

Having performed the clustering of varieties by the proposed method, the authors have combined similar varieties into groups. As a result, 17 clusters were obtained. Their characteristics are presented in Table 4.

Table 4: Characteristics of clusters of winter wheat varieties

Cluster number	Length of seedling-earing period (days)	Plant height (cm)	Mass per 1,000 grains (g)
1	262.8	82.6	43.8
2	262.1	95.4	43.9
3	264.2	87.7	40.1
4	258.6	84.1	38.5
5	262.9	77.4	40.6
6	257.9	91.0	41.9
7	256.4	79.4	45.6
8	255.8	93.7	40.5
9	262.0	90.2	31.5
10	262.1	72.8	45.0
11	256.8	75.1	40.7
12	260.1	66.8	40.9
13	255.9	79.6	34.7
14	265.0	99.9	33.5
15	256.6	100.2	42.2
16	262.8	105.7	43.2
17	264.0	115.1	38.0

It should be noted that the number of clusters and their representation varies annually since the assortment understudy is changing.

The authors select the parent components for hybridization, taking into account their belonging to clusters and trying to make crosses between contrasting varieties. This approach allowed obtaining the hybrids characterized by high productivity and favorable transgressions (see Table 5).

Table 5: Characteristics of winter wheat hybrids

Combination of hybrids	Number of grains per spike (pcs)	Mass of grains (g)	
		per spike	per 10 pcs
Rosinka × Nana	34.5	1.54	0.46
Don 93 × 494J6.11	38.0	1.73	0.45
Odesskaya 200 × Chernozemka 99	37.4	1.87	0.53
Zhatva Altaya × Zihtus	45.5	1.97	0.42
Bezenchukskaya 380 × CADET	45.8	1.91	0.42
Moskovskaya 56 × Nana	49.0	2.26	0.46

The authors have tested the method of k-means as a way of assessing the morpho-biological diversity of winter wheat varieties for the selection of parent components for hybridization. It is recommended to use this method for grouping the varieties according to such traits as plant height, length of seedling-earing period, and mass per 1,000 grains. This simplifies the selection of parent components for crosses from the point of view of their distance and allows creating the hybrids, which are characterized by high productivity, winter hardiness, and resistance to adverse environmental factors in the conditions of the Central Chernozem Region.

4. CONCLUSION

The authors used the methods of the cluster and discriminant analysis and determined the minimum number of traits for grouping the varieties of winter wheat, i.e. plant height, length of seedling-earing period, and mass per 1,000 grains. These are the criteria for assessing the variety type, ecotype, and productivity of winter wheat genotypes. The tested method of k-means (without limiting the number of groups) can be used as a way of assessing the morpho-biological distance of varieties and allows simplifying the process of planning the crossbreeding schemes for more efficient hybridization work.

5. AVAILABILITY OF DATA AND MATERIAL

Information can be made available by contacting the corresponding author.

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