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GENETIC DIVERSITY ANALYSIS OF WINTER WHEAT ACCESSIONS OF DIFFERENT GEOGRAPHICAL ORIGINS BY PCA

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Agronomic valuable and morphological traits in 177 winter wheat varieties and breeding lines of different geographical origins from 20 countries were investigated. Significant variations for all studied traits were found and the accessions were differentiated by principal component analysis (PCA). The most complete geographical differentiation of the accessions was seen in winter hardiness scores, field resistance to Septoria spp., earing dates, ear morphology, wax bloom intensity.

Keywords*: winter wheat, PCA, economically valuable traits, morphology, starting material*

Introduction. Modern adaptive plant breeding is based on the extensive use of global genetic resources, their systematization and creation of genetic collections with identified valuable agronomic and adaptive traits. Genetic resources are fundamental to support the global wheat production today and in the future [1, 2]. Different parts of the world have different morphological types of wheat with a wide range of adaptive features [3]. Varieties of different geographical origins differ in expression of morphological traits, physiological responses to adverse environmental factors and have a clear morpho-physiological specifics related to environmental conditions of their origins and, accordingly, differ in their adaptability or resistance to limiting environmental factors [4].

Adaptability and resistance to adverse environmental conditions are the most important features for winter wheat varieties under stressful environmental conditions [5, 6]. Recently in Ukraine there has been a trend of extensive variety trials and subsequent registration of foreign varieties (mainly from European countries). Analysis of data from the State Register of Plant Varieties of Ukraine until 2000 showed that about 90% of varieties were Ukrainian. During the period of 2001-2010, the rate of Ukrainian varieties was 75%. During the period of 2011-2020, the percentage of Ukrainian varieties further decreased by 10% − to 65%. As for 2021, the State Register of Varieties of Ukraine contains 630 winter wheat varieties from 14 European countries. This indicates a strong integration of the seed market of European countries into the agriculture in Ukraine.

The purpose was to study the genetic diversity of collection accessions of different geographical origins; to analyze expression of morphological and agronomic valuable traits in the Left-Bank Forest-Steppe of Ukraine; to select valuable accessions; and to evaluate the winter wheat collection depending on the region of origin by PCA.

Literature review. The wheat remains the most cultivated crop in the world [7] and covers the nutritional needs of 60% of the world's population [8]. There is a serious challenge for breeding due to climatic changes to maintain food balance [9].

A number of researchers confirm that, at the present stage of breeding, most of commercial varieties of many crops are based on a narrow genetic diversity [10], leading to severe genetic erosion [11, 12].

Foreign varieties play an important role in expanding the varietal diversity and in the breeding in a country [13, 14]. Analysis of accessions of different origins includes field assessments of economically valuable traits and evaluation of responses of plants to changes in climatic conditions different from those in the place of their origin [15]. There are a lot of statistical approaches and methods to analyze data. Principal component analysis (PCA) is one of them. It helps to identify unknown trends in multidimensional data sets [16]. This statistical approach simplifies relationships between elements of large sets of variables without losing original data. PCA reduces a large sample of data to fewer components, searching for groups that have very strong correlations in the set of variables, and each component explains the percentage (%) of variation related to overall variability.

Materials and methods. Winter wheat varieties and breeding lines of different geographical origins were taken as the study material. A total of 177 varieties and breeding lines from 20 countries were studied. They were divided into groups according to their geographical origin: Eastern Europe − Russia (14 accessions), Belarus (1 accession); Northern Europe − Estonia (7 accessions), Latvia (1 accession), Sweden (1 accession); Central and Western Europe: Austria (2 accessions), Bulgaria (3 accessions), Czech Republic (2 accessions), Germany (24 accessions), France (4 accessions), United Kingdom (2 accessions), Hungary (2 accessions), Hungary (5 accessions), Netherlands accession), South-Eastern Europe: Serbia (1 accession), Croatia (1 accession), Romania (4 accessions), Southern Europe: Turkey (27 accessions); North American countries: USA (7 accessions), Canada (1 accession). To comprehensively represent the Ukrainian genetic diversity, we included 69 varieties bred at all Ukrainian research and breeding institutions in different years to our collection of varieties.

The study was conducted in the experimental field of Poltava State Agrarian University (soil-climatic zone of Ukraine: eastern steppe part of the Left-Bank Forest-Steppe in the Vorskla river valley) in the 2020−2021 vegetation year.

The weather in the 2020−2021 vegetation period was not favorable for assessing drought resistance during the generative phase. The winter was generally favorable for winter dormancy of winter wheat plants, but there were frosts down to -15^{o}C combined with thin snow cover, which allowed us to assess the genotypes for resistance to low temperatures. The weather in May and June was atypical for this area. It rained throughout the period. Such a great precipitation amount in May–June (135 mm) was not seen in all years of scientific observations, making the weather in June 2021 unique.

The following parameters were assessed in the field and laboratory: winter hardiness (with a 9-point scale, where 1 point corresponds complete or partial death of plants; 9 points – 100% survival of plants in the plot); earing date (the number of days after January 1); Septoria-induced damage (with a 9-point scale, as a percentage of affected vegetative organs of plants); of morpho-biological traits such as the flag leaf width, awn length, wax bloom intensity (1 point − light green vegetative organs, 9 points − dark bluish-gray), and plant height (in cm). The above parameters were visually assessed in accordance with the recommendations of UPOV and the state-approved methods for examination of wheat bread varieties (*Triticum aestivum* L.) for distinctness, uniformity and stability[17] as well as in accordance with the CIMMYT methods of physiological studies in breeding [18]. In addition, the following performance constituents were measured: ear length, spikelet number, grain number per ear, and 1000-grain weight; the linear density index of the ear was calculated. Visualization and statistical processing of data were performed in R-Studio.

Results and discussion. Under the soil and climatic conditions of the Left Bank of Ukraine, there were significant variations in all the studied traits, especially agronomic traits that characterize the resistance of plants to biotic and abiotic environmental factors (winter hardiness and resistance to leaf blotch). Table 1 presents the descriptive statistics of each studied trait for all the varieties under investigation.

It was found that such traits as winter hardiness and Septoria resistance had high coefficients of variation (27.3−36.8%). Analysis showed that the studied accessions could be differentiated by the above indicators. In general, almost all of the studied traits varied moderately or highly, which can be attributed to wide diversity of the studied accessions.

Table 1

Descriptive statistics of the wheat genotypes

Trait	Mean ± standard error	Standard deviation	Min value	Max value	Coefficient of variation, %
Winter hardiness (WH), score	6.11 ± 0.12	1.67	1	9	27.3
Days to earing date (after January 1) (EAR)	157.3 ± 0.32	4.5	149	171	2.9
Plant height (HP), cm	69.3 ± 0.78	10.7	35	95	15.4
Field resistance to *Septoria spp.* (Sept), score	5.7 ± 0.15	2.1	1	9	36.8
Flag leaf wax bloom (Wax), score	5.4 ± 0.09	1.3	3	9	24.1
Flag leaf width (FL), cm	1.4 ± 0.02	0.2	1.0	2.0	14.3
Ear length (EL), cm	9.2 ± 0.09	1.27	6.0	12.6	13.8
Spikelet number (Qsp)	17.8 ± 0.16	2.1	11	24	11.8
Grain number (Qgrain)	40.3 ± 0.6	7.9	22	73	19.3
Grain weight (Wgrain), g	2.0 ± 0.03	0.4	1	3.4	20
1000-grain weight (W1000), g	48.9 ± 0.41	5.35	31.3	56.7	10.9

Analysis of the sample (Figure 1) demonstrated that the differentiation of winter wheat varieties of different origins (country or region of origin) was based on winter hardiness and resistance to Septoria. The earing date, which is an important adaptive indicator of varieties of different origins in new conditions, was also a distinguishing character.

We investigated if the characteristics could be divided into groups depending on the origin. To analyze the genetic diversity of the varieties and possibility of their differentiation by country of origin, we chose accessions from four countries supplied the largest numbers of varieties, namely Ukraine − 69 accessions, Turkey − 27 accessions, Germany − 24 accessions, Russia – 14 accessions. All these countries differ in their soil and climatic conditions.

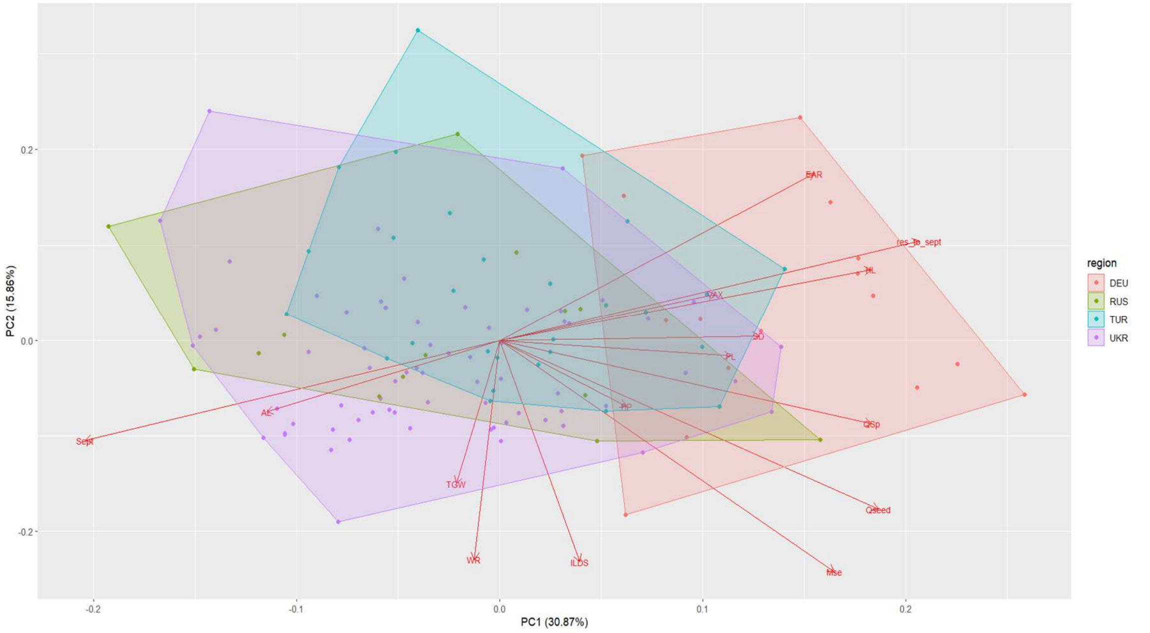

Fig. 1. Principal component scatter diagram for the traits in the wheat genotypes of different geographical origins

Using PCA, we analyzed the accessions locations on the diagram for the 1st and 2nd principal components. Together, PC1 and PC2 describe only 46.73% of the total variation, which indicates the diversity of the sample. Figure 1 shows the locations of the accessions from the four selected countries. The vector locations of the studied traits in multidimensional space and their standardization give an idea of distribution of the accessions according to the economically valuable and morphological features and relationships between them.

Analysis of the scatter diagram revealed that the German varieties differed significantly from the Ukrainian, Russian or Turkish varieties. The German varieties were different because of the following characteristics: earing date (ripeness group), Septoria resistance score, ear length, spikelet number per ear, and morphology (straw thickness, wax bloom, flag leaf width). As to the Ukrainian and Russian varieties, they had the following unifying traits: 1000-grain weight, awn length, and a higher percentage of Septoria-damaged leaves. Most of Ukrainian varieties were located along the awn length and winter hardiness vectors. The Turkish accessions were located on the diagram somewhat separately and did not have clearly distinctive characteristics.

PCA helps to analyze data on agronomic valuable and morphological traits and can serve both as the main and as an auxiliary tool to categorize accessions of different origins by a set of agronomic traits.

For a more in-depth analysis, we further analyzed on morphological and economic features.

Winter hardiness is a complex of resistances to adverse weather conditions in winter. Recent climatic changes are increasing the number of adverse weather events, including thin snow cover and thaws. High photoperiodic susceptibility and long vernalization play an important role in maintaining winter hardiness of plants.

There was a significant variation in the winter hardiness score, from one to nine. Figure 2 shows the accession distribution on the 9-point scale of winter hardiness (ordinate axis) against country of origin (abscissa axis). This figure shows the countries, which supplied the largest numbers of accessions: Germany, Estonia, France, Hungary, Romania, Russia, Turkey, Ukraine, and USA.

The greatest numbers of accessions with high winter hardiness scores (7-9 points) came from Ukraine, USA, Estonia, and Russia, which corresponds to the climatic conditions of the country of origin, where winter-hardy genotypes had been selected. The accessions from France,

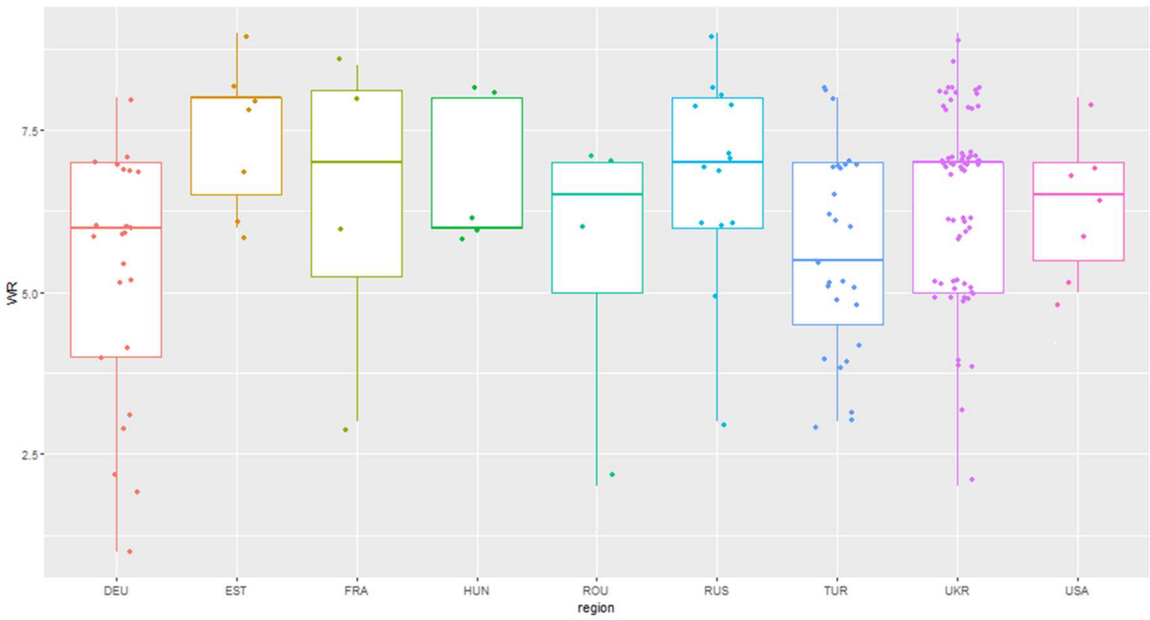

Fig. 2. Distribution of the genotypes by field winter hardiness (WH) depending on country of origin

Hungary, and Romania had slightly lower winter hardiness scores. Most of accessions with low frost resistance were German. This group also included accessions from Bulgaria, the CzechRepublic and the United Kingdom, but they are not shown in the diagram. These countries are characterized by milder winters and favorable conditions for overwintering. In Europe, varieties with low photoperiodic susceptibility and short vernalization dominate. However, the selected European accessions were quite winter-hardy, namely Bodycek (FRA), Cubus, Arctis, Skagen (DEU). Accessions from Turkey were characterized by moderate winter hardiness, despite the fact that the Turkish climate is not good for selecting genotypes for winter hardiness. There were three Turkish lines (T67 / X84W063-9-45 // Karl92 / 3, CUPRA-1/3, Pehlivan) with good winter hardiness combined with a high 1000-grain weight, which indicated their drought resistance. In general, analysis of the accessions confirmed the geographical orientation of conditions for selecting winter-hardy genotypes.

Analysis of the earing dates also confirmed the geographical orientation of the collection accessions. The earing dates varied from May 29 to June 20 in 2021. The earliest dates of earing were intrinsic to accessions from Ukraine, Turkey, USA, Russia, Romania, and Croatia. The climates in these countries require the cultivation of short-season genotypes for grain setting and filling before the dry weather onset. European varieties, with few exceptions, were late-ripening, which directly affected caryopsis setting and subsequently the 1000-grain weight. There was a negative correlation (r = -0.261) between the earing date and the 1000-grain weight. European varieties with earlier earing dates were distinguished: Bodycek (FRA) and Skagen (DEU).

In 2020−2021, the accessions presented symptoms of the following diseases: Septoria blotch, brown rust, yellow rust, bacterial diseases, and Fusarium ear blight. However, the collection accessions could be only differentiated by Septoria-induced damage. The other diseases were insufficiently manifested to assess resistance scores. Significant variations in Septoria-induced damage were observed: from no signs of damage at all to 80% of affected plant organs. Figure 3 shows the accession distribution on the 9-point scale of field resistance to Septoria (ordinate axis) against country of origin (abscissa axis).

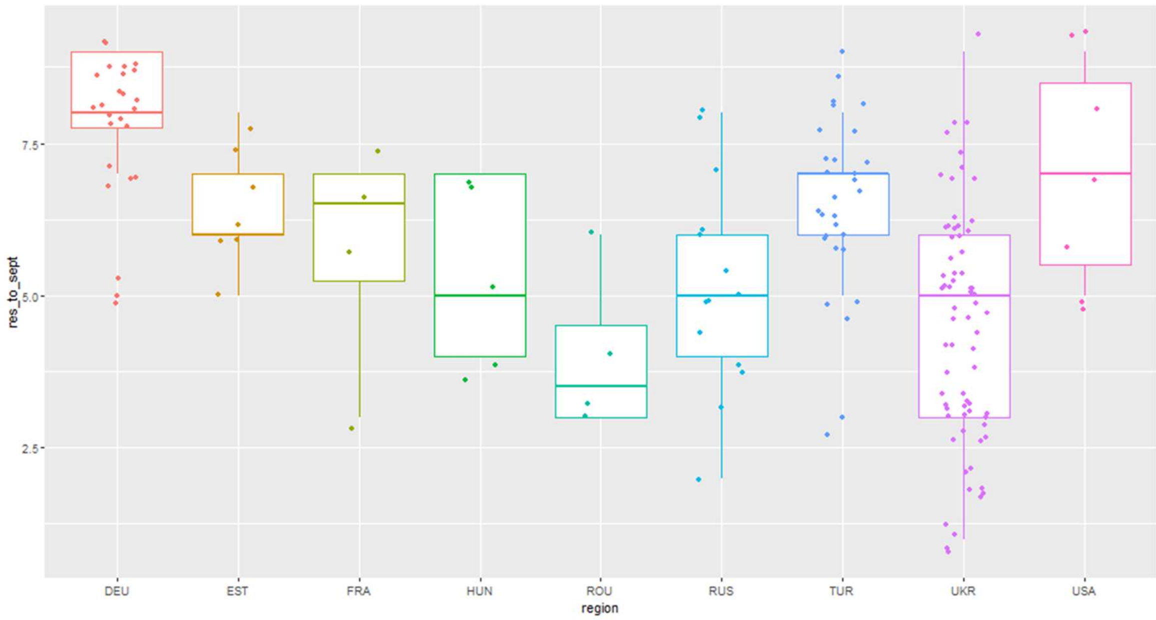

Fig. 3. Distribution of genotypes by resistance toSeptoria spp. (res_to_sept) depending on the country of origin

The resistance scores ranged from 1 to 9. The accessions from Germany, Estonia, and Turkey were the most resistant to Septoria spp.

Most of Ukrainian accessions were noticeable for low field resistance to Septoria spp. Four Ukrainian varieties (Raihorodka, Bohdana, Podilska Niva, and Krayevyd) were highly resistant to Septoria spp. in the field.

Septoria is one of the most common diseases of winter wheat, but its harmfulness for is much lower early-ripening genotypes than for late-ripening ones. Resistance to Septoria is more important for genotypes in Europe, as significant damage reduces photosynthetic surface and, consequently, yields. Analysis of the distribution of the genotypes by Septoria resistance scores also indicated a possibility of geographical differentiation. Septoria resistance assessment in the genotypes allowed us to select resistant accessions that can serve as sources of resistance to this disease.

The wax bloom intensity showed a certain geographical orientation in the collection accessions. The hue of green parts of plants indicates the ability of genotypes to absorb sunlight. Light green genotypes are common in regions with longer sunshine hours and higher intensity of irradiation. It was found that the European varieties were characterized by higher intensity of wax bloom (dark-green and bluish-gray genotypes). Genotypes with lower intensity of wax bloom are more common for countries with higher intensity of solar irradiation (Ukraine, Romania, USA, and Turkey).

Ukrainian, Turkish and American varieties have long awns. Long awns are an additional assimilation surface that allows plants to produce more dry matter. In most of Ukrainian varieties, the ear length was medium (7−10 cm) and ears comprised rather large numbers of spikelets per ear (17−20); the same features were intrinsic to genotypes from the USA. Turkish lines had the longest ear across the sample, but fewer spikelets and, consequently, less dense ears.

Varieties from Northern Europe (Germany, Estonia) were awnless, with long ears and increased numbers of spikelets per ear. The weather in these countries contributes to development of multiple diseases of the ear, and awns are an additional area for fungal and bacterial diseases. Countries with warmer climates (France, Romania) had awny genotypes, but with shorter awns compared to Ukrainian or Turkish genotypes.

As to the performance constituents (grain number and weight per ear), the highest values were recorded for European varieties (35−60 grains per ear). Genotypes from Ukraine, Russia, Turkey and the USA generally had similar values of the performance constituents (30−50 grains per ear and 1.5−2.3 g, respectively). However, varieties from Ukraine, Russia, Turkey, and the USA, despite fewer grains per ear, had greater grain weights per ear and larger and plumper grains. That is, European genotypes were able to fully realize the genetic potential of yield in arid conditions during the grain filling phase.

The traits that did not have a clear geographical differentiation included the following morphological features: plant height and flag leaf width. It was difficult to differentiate the accessions by origin for these traits.

In the accessions under investigation, the flag leaf widths varied from 1 to 2 cm. Varieties with wider flag leaves were typical for Europe, except for French varieties. Ukrainian varieties were characterized by strong variation of this trait, but a significant number of genotypes had a medium width of the flag leaf (1.2−1.8 cm). However, this feature also did not give a clear geographical differentiation.

The plant heights across the studied accessions varied from 35 cm in Trublion (France) and Sila (Russia) with pronounced signs of yellow dwarf barley virus to 95 cm in Turkish and Estonian breeding lines. Analysis of the distribution of the genotypes of different origins by plant height suggested that this trait is not differentiating and does not allow one to group genotypes by origin. The plant heights varied greatly across the genotypes of different origins, regardless of the region of their breeding. Breeding lines from countries with arid climates (Turkey, Iran, and Tajikistan) had the tallest plants in the sample under the environment influence (increased rainfall in spring and early summer).

Conclusions. It was established that the suitable for geographical differentiation traits were field winter hardiness, field resistance to Septoria, and earing date. All these traits are important adaptive traits to new conditions. The traits by which differentiation was possible, but with less accuracy, were the wax bloom intensity and ear morphological (awn length). It was impossible to differentiate the accessions by geographical origin for the 'plant height' and 'flag leaf width' traits.

In general, varieties from Ukraine and Eastern Europe (Belarus, Russia) were characterized by similar expression of agronomic characteristics. Characteristic features of the genetic pool included high winter hardiness, below-medium resistance to Septoria leaf blotch, medium values of the performance constituents with fairly large and plump grains. Varieties from Central and Western Europe were characterized by below-medium winter hardiness, high resistance to Septoria leaf blotch and above-medium performance constituents (grain number and ear length).

These two groups of varieties are the most distant in terms of morphological characteristics and expression of adaptive traits, which was confirmed by PCA of the data. The use of varieties of different origins with various expression of economically valuable traits for crossing allows for generation of valuable recombinations.

However, it should be noted that further research into this issue will be conducted on larger samples.

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АНАЛІЗ ГЕНЕТИЧНОГО РІЗНОМАНІТТЯ ЗРАЗКІВ ПШЕНИЦІ ОЗИМОЇ РІЗНОГО ГЕОГРАФІЧНОГО ПОХОДЖЕННЯ ЗА ДОПОМОГОЮ PCA-АНАЛІЗУ

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Мета дослідження: Метою дослідження був аналіз прояву цінних господарських та морфологічних ознак у колекційних зразків пшениці озимої в умовах Лівобережного Лісостепу України та встановлення відмінностей із сортами українського походження. Також метою дослідження було використання методу аналізу головних компонент при диференціації та доборі цінних для селекції для умов змін клімату зразків.

Матеріали і методи: Матеріалом для дослідження були 177 сортів пшениці озимої різного походження з 20 країн світу, з них 69 сортів різних науково-дослідних установ України. Колекція включала в себе як сучасні та комерційні сорти, так і селекційні лінії. Диференціацію зразків проводи за наступними ознаками: польова зимостійкість, дата колосіння, висота рослин, польова стійкість до септоріозу, оцінка інтенсивності воскового нальоту, ширина прапорцевого листа, елементи продуктивності колоса (довжина, кількість колосків в колосі, кількість зерен та їх маса з колосу) та маса тисячі зерен. Дослідження проводилися шляхом візуальних обліків у полі відповідно до рекомендацій та вимірюванням частин рослин після доведення до повітряного-сухого стану.

Обговорення результатів. Установлено значне варіювання за всіма досліджуваними ознаками, коефіцієнт варіації становив більше 10%, що говорить про середній та високий рівень різноманітності досліджуваних зразків. Проведено аналіз оцінки генотипів за допомогою методу головних компонент та визначені ознаки, за якими можлива диференціація зразків за географічним походженням, а саме – за рівнем польової зимостійкості, польової стійкості до септоріозу та датою настання фази колосіння. Всі вищезазначені ознаки є адаптаційними ознаками та визначають перспективи використання сортів у селекційних програмах як джерел цінних ознак. Слід зазначити, що погодні умови 2020−2021 вегетаційного року були нетиповими (надмірне зволоження в весняний період – 135 мм).

Висновки: За результатами дослідження визначені зразки, що можуть використовуватися у комбінаціях схрещування як джерела цінних ознак. Встановлені 37 зразків різного походження з високою (вище 8 балів) польовою зимостійкістю та 40 зразків з високою стійкістю до септоріозу листя. Виділено сорти, що поєднували в собі високу зимостійкість і стійкість до септоріозу листя – Богдана та Подільська нива (Україна), селекційна лінії 653.1.5 (Естонія), Августина (Білорусь), Дуплет (Росія). Використання методу аналізу головних компонент та шляху візуалізації його результатів (PCA biplot) є зручним інструментом для відбору зразків різного походження та створення колекції вихідного матеріалу.

Ключові слова: *пшениця озима, PCA-аналіз, господарсько-цінні ознаки, морфологія, вихідний матеріал.*

GENETIC DIVERSITY ANALYSIS OF WINTER WHEAT ACCESSIONS OF DIFFERENT GEOGRAPHICAL ORIGINS BY PCA

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Purpose: The purpose of the study was to analyze expression of morphological and agronomic valuable traits in winter wheat in the Left-Bank Forest-Steppe of Ukraine; to select valuable accessions; and to evaluate the winter wheat collection depending on the region of origin by PCA.

Materials and methods. The material for the research were 177 varieties of winter wheat of varieties from 20 countries, including 69 varieties of different research institutions of Ukraine. The collection included modern commercial varieties and breeding lines. Differentiation of samples was carried out on the following traits: field winter hardiness, earing date, plant height, field resistance to Septoria spp., waxiness of flag leaf, width of flag leaf, yield components (ear length, spikelet number, number of grains and their weight from ear) and thousand grains weight (TGW). The research was carried out by visual surveys in the field conditions in accordance with the recommendations and measurements of plant parts after bringing to an air-dry state.

Results and discussion. Significant variation was found for all studied traits, the coefficient of variation was more than 10%, which indicates a medium and high level of diversity of the studied samples. The analysis of genotype assessment using the principal components analysis method was performed and the differential traits by geographical origin was established – field winter hardiness, field resistance to Septoria spp. and the earing date. All the above-mentioned traits are adaptive traits and determine the prospects for the using of varieties in breeding programs as sources of valuable traits. It should be noted that the weather conditions of 2020-2021 vegetation year were atypical (excessive moisture in the spring − 135 mm).

Conclusions: The results of the study identified samples that can be used in crossing combinations of as a source of valuable traits. The 37 samples of different origin with high (above 8 points) field winter hardiness and 40 samples with high resistance to leaf septoria were identified. Varieties that combined high winter hardiness and resistance to Septoria − Bogdana and Podilska Nyva (Ukraine), breeding line 653.1.5 (Estonia), Augustina (Belarus), Duplet (Russia). Using the principal component analysis and the approach of visualization of its results (PCA biplot) is a convenient tool for sampling of different origins and creating a collection of source material.

***Key words**: winter wheat, PCA, economically valuable traits, morphology, starting material.*