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The Possibility of Using Allelic Variants of Electrophoretic Spectra of Storage Proteins in the Breeding of Sunflower

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Abstract:

Relevance of the provided research work lies in the need to create parental lines of sunflower with high genetic purity, high combinatorial ability to obtain modern hybrids capable of forming a high level of yield in the arid conditions of the Steppe of Ukraine. Analysis and study of allelic variants of electrophoretic spectra makes it possible to expand the genetic diversity of the source material identified by the spectra of seed storage proteins. The main goal was to establish and study allelic variants of sunflower seed storage proteins, their use in individual plant selection in the process of creating self-pollinated lines, with the subsequent inclusion of lines in the breeding process. The source material for creating self-pollinated lines were sunflower varieties and interspecific hybrids. Plant selection was carried out according to morphological characteristics and allelic variants of the electrophoretic spectra of sunflower seed storage proteins according to the method of F. O. Poperel. The research was carried out in accordance with generally accepted methods in plant breeding. As a result of the conducted studies, allelic variants of electrophoretic spectra of sunflower seed storage proteins Hel 1, Hel 2, Hel 3, Hel 4, Hel 5, Hel 6, Hel 7, Hel 9, Hel NK were established. New self-pollinated sunflower lines with a high level of genetic purity with identified loci Hel 7, Hel 9, Hel NK were created. The creation of starting material from interspecific hybrids ensured the production of genotypes with new helianthine spectra and contributed to the expansion of the genetic diversity of sunflower. Based on allelic variants of protein spectra, protein spectra were developed and recorded for each self-pollinated sample and line created. Self-pollinated lines were created using protein spectra and clustering of the resulting genotypes was performed. Sunflower hybrids with the participation of lines from the most genetically distant clusters form a yield higher by 0.5-0.8 t ha^{-1}. The established allelic variants of the electrophoretic spectra of seed storage proteins are recommended to be used in individual plant selection in the process of obtaining self-pollinated sunflower lines with a high level of genetic purity. The obtained sunflower lines are recommended to be used in the creation of hybrids intended for cultivation in arid steppe conditions of Ukraine.

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1. INTRODUCTION

The feasibility of applying sunflower growing technologies (crop productivity, energy conservation and environmental safety) is determined by the choice of hybrids and varieties as factors in the production basis. The production of highly productive hybrids and varieties of sunflower with valuable breeding signs is based on the expansion of genetic diversity, the formation of characteristic collections of sunflower and the creation of unitial material [1]. In the process of creating genetic collections, source material, the selection, classification and identification of genotypes is carried out on the basis of morphological characteristics of plants [2]. Evaluation and selection of plants according to morphological characters, identification of genes that control the appearance of morphological characteristics of sunflower plants, allows you to create characteristic collections and source material that will be further used in the breeding of sunflower [3-6].

The use of monogenic morphological signs in seed production is one the best option for increasing efficiency in determining varietal purity of crops that does not require lengthy measurements and mathematical processing [7, 8]. At the same time, the efficiency of the traditional method of selection of plant according to morphological signs in the early stages of creating the unitial material is still not at a high level. When conducting selections of plants only on morphological signs, phenotypic characteristics are observed, the complexity of genetic control of signs is noted due to the presence of various forms of latent genetic variability. The phenotypic demonstration of signs depends on the presence of certain genes or their alleles in the homo- or heterozygous state in the genotype, which significantly reduces the efficiency of plant selection during the creation of source material and self-pollinated sunflower lines and does not provide selection material with a high level of genetic purity [9]. Selections based on only one morphological signs of plants lead to the obtaining of a genetically heterogeneous source material also because different morphotypes can be the result of the same mutation, the same morphotypes are the result of changes in different genes in the selected genotypes [10]. Field conditions, especially in the arid conditions of the growing season, do not always create favorable conditions for the growth and development of plants.

Unvaforable conditions of environmental factors can have a strong effect on the expression of a sign, and when combined with exposure to plants with high air temperature during the growing season, they increase, in particular, gene expression after flowering and during the formation and filling of seeds. With such an assessment and selection of plants, due to the numerical limitations of morphological signs, it is not always possible to reveal latent genetic variation and to control the genetic homogeneity of the created material [11].

In this regard, the use of protein markers is more effective and justified in conducting selections and identification of plant [12]. This is because proteins are a complex system associated with all metabolic, morphogenetic and genetic processes. The structure of the obtained electrophoretic spectra of the storage proteins does not have modification variability and reflects the specific demonstration of a gene or gene block, and therefore can serve as a model sign [13]. Their peculiarity lies in a precise genetic determination and independence of the phenotypic demonstration from growing conditions. It is fundamentally important that storage proteins are contained in seeds, a fixed phase of ontogenesis, and are characterized by the absence of catalytic functions [14].

Storage proteins of seeds often remain unchanged for many years and this provides the opportunity for good reproduction of the results of electrophoresis of seeds proteins. The use of storage proteins of seeds as markers is associated with real, practical advances in the identification and registration of hybrids and varieties, namely, proteins polymorphism is the scientific basis for assessments, plant selection at the molecular level, and molecular marking of newly created breeding material [15]. The use of molecular markers provides particular advantages in assessing genetic diversity for identifying and determining the genetic purity of varieties [16]. Of fundamental importance is availability allelic genes, which make it possible for each created genotype to have a component composition of storage proteins and a characteristic combination of alleles of the genes encoding the protein [17, 18]. It should be noted that in sunflower, unlike many other agricultural plants, the number of identified components of the electrophoretic spectra is relatively small, therefore it is relevant to conduct studies to establish new allelic variants of protein spectra while expanding genetic diversity. Evaluation of new unitial material by morphological and electrophoretic determinate signs in sunflower breeding helps to solve the problem of obtaining parental lines with high combining ability, highly productive varieties and hybrids of sunflower [19, 20].

Thus, the creation of a new unitial material of sunflower necessitates studies to establish the characteristics, variability, inheritance of morphological signs of plants and components of the electrophoretic spectra of storage proteins, which would make it possible to use allelic variants of the electrophoretic spectra of proteins in breeding and to create varieties and hybrids with a high level of genetic typicalness adapted to growing conditions in the Steppe of Ukraine.

2. MATHERIAL AND METHOD

2.1. Electrophoresis Technique of Sunflower Seed Storage Proteins

The object of the research was the allelic variants of electrophoretic spectra of storage proteins, self-pollinated samples of sunflower.

Before sowing, after selfing and harvest of plant, the seeds of options with the repollinated plants were exposed to a method of an electrophoresis of storage proteins for determination of allelic variants of electro-phoretic spectra of storage proteins at molecular level.

Electrophoresis of storage proteins of sunflower seeds was performed by the method of A.Ph. Poperelya. For preparation of helianthin solution, kernel of each seed was crushed and defatted. The kernel of each seed was conditioned separately and placed in a centrifuge test-tube. The fat removal was done with a mixture of glacial acetic acid and acetone. One ml of glacial acetic acid and acetone (30 ml of glacial acetic acid in 1 1 of acetone) was added to each test-tube. Test-tube content was stirred with a mechanical mixer for 30-40 s. After that, the working solution of helianthin was added to a mixture of glacial acetic acid and urea (1.0 l of solution contained 30 ml of glacial acetic acid and 120 g of urea). Pyronine Y was used as the quality marker.

Electrophoresis was performed in vertical polyacrylamide gel slabs, at 500 V and initial current of 50 mA on the each plate, during 2.5 h. Fixation and staining of proteins was done in the solution. The staining solution composition was ethyl alcohol, glacial acetic acid, trichloroacetic acid extra pure and Coomassi Brilliant Blue R-250. The gel slabs were washed with water. The obtained electrophoretograms were analyzed. Identified and selected according to the components of the electrophoretic spectra of proteins and morphological signs of sunflower plants were sown in the field for further self-pollination, to obtain homozygous breeding material.

2.2. Soil Characteristics and Seed Sowing

The soil of the experimental plot is dark-gray forest. The mechanical composition of the soil is medium loam. Soil acidity pH 5.7. The humus content is 3.8 %. The content in the arable layer of mobile phosphorus is 12.9 mg/100 g of soil, exchange potassium – 15.9 mg/100 g of soil. In the main soil preparation system, plowing was carried out to a depth of 27.0-30.0 cm.

In the spring, two cultivations were performed:

- early spring soil leveling by the KPS-4 cultivator to a depth of 8.0-10.0 cm with simultaneous harrowing of the;
- pre-sowing cultivation with the cultivator KPS-4 to a depth of 6.0-8.0 cm.

Sowing of sunflower was carried out at the end of April: April 25-30 with a row spacing of 70 cm. The density of plants standing before harvesting is 50 thousand/ha. The plots are four-row. The accounting area of the plot is 12.2 m^2. The experience had three repetitions. During the period of crop care, two inter-row cultivations were performed. In the period of research performed:

- the formation of seedlings in the phase of development of sunflower 2-3 true leaves;
- phenological observations;
- a description of the morphological signs of plants;
- self-pollination and hybridization of plants in the flowering phase;
- plant biometrics;
- determination of plant productivity (head diameter, number and weight of head seeds), oil content of seeds;
- electrophoresis of storage protein seeds to establish the manifestation, inheritance, variability of the components of the electrophoretic spectra of proteins, the selection of sunflower samples with specific protein spectra;
- establishment, selection of plants and samples with new electrophoretic spectra, protein markers, morphological signs;
- determination in laboratory conditions of resistance of sunflower samples to infection by broomrape, downy mildew.

Sunflower harvesting from experimental plots was carried out hand with the subsequent threshing of heads in laboratory conditions. The experiments were cleaned manually with individual threshing of each head separately. In the process of creating and expanding genetic diversity, creating new source material, in research were used the samples, lines of breeding of the Institute of oilseed crops NAAS and varieties of other scientific institutes. During the assessment and selection of plants at the molecular level, allelic variants of the components of the electrophoretic spectra of proteins, where 11S globulins are the main components of the protein fraction, were used as a systematic sign.

2.3. Statistical Analysis

Statistical data processing was performed according to Lakin [1990], Myatlev [2009]. For statistical processing, the programs by MSTAT test, STATISTICA, EXCEL, ADIS, DAD were used.

3. RESULTS AND DISCUSSION

During the creation of the source material and lines of sunflower, the assessment and selection of plants at the molecular level as a systematic sign used allelic variants of the components of the electrophoretic spectra of storage proteins, where the main components of the protein fraction are 11S globulins. Alleles of genes that control the synthesis of a particular polypeptide are represented in separate strips on electrophoretograms. An analysis of electrophoretic spectra found that the genetic control of heliantinins is performed by at least 5-6 locuses : Hel 1, Hel 2, Hel 3, Hel 4, Hel 5, Hel 6 (Figure **1**).

One or more linked inherited polypeptides (blocks of electrophoretic components of heliantinin) controls a separate locus. Heliantin-coding locuses Hel 1, Hel 2, Hel 4, Hel 6 are polymorphic. Each of these locuses encodes several polypeptides that are represented by a series of allelic variants. Locus Hel 2 is always represented on electrophoregrams by two components of the polypeptides.

Locus Hel 4 controls the synthesis of a series of components with different mobility and different molecular masses, which leads to different allelic variants of this locus. In all samples, the locus Hel is characterized by the presence or absence of polypeptides and in its composition has from 7 to 14 components of different intensities.

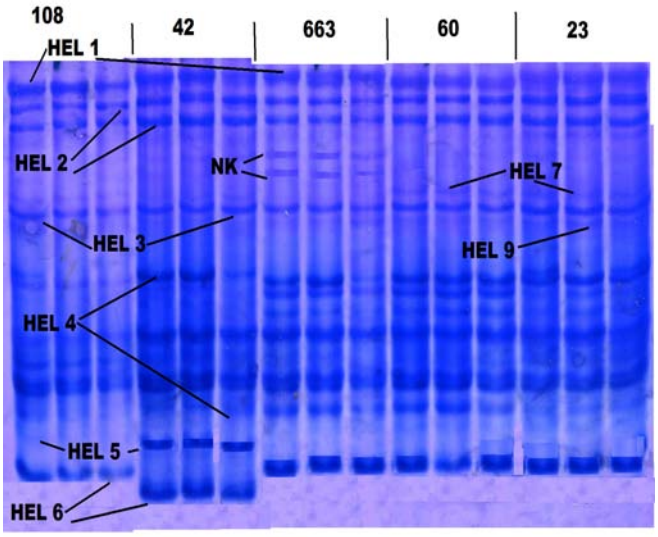

Figure 1: Allelic variants of the protein spectra of self-pollinated sunflower samples created by the method of selection by electrophoretic spectra, 2015-2021.

Due to different allelic variants, significant variability was established only at the locus Hel 4 in comparison with other loci. Locuses Hel 3, Hel 5 are monogenic, encode only one polypeptide. In different genotypes, the Hel 5 locus is characterized by the presence of a component with a different degree of intensity of its manifestation or its absence on electrophoregrams.

Involvement of wild annual forms in a combination of crosses made it possible to obtain samples with new loci established at the molecular level, providing a broader expansion of the genetic diversity of sunflower and a larger number of locus, allowing the genetic purity of lines and hybrids to be controlled. Self-pollination of plants of interspecific hybrids obtained by crossing cultivated sunflower with wild annual forms of H. Debilis, H. Praecox, contributed to the production of homozygous self-pollinated forms with previously unobserved in the lines, hybrids and varieties of sunflower locuses Hel 7, Hel 9, Hel NK. Lines 60 and 23, obtained from the interspecific hybrid combination of H. Annuus × H. Debilis, are characterized by the manifestation of new loci of Hel 7, Hel 9 on electrophoregrams. Locuses Hel 7, Hel 9 are monogenic and are represented by only single component. The 663 line, obtained from the interspecific hybrid combination of H. Annuus × H. Praecox, is characterized by the manifestation of a new locus of Hel NK on electrophoregrams. The locus of Hel NK is represented by two alleles that control the synthesis of the polypeptide and is localized between the Hel 2 and Hel 7 loci on the electrophoregram.

In hybrid crossing combinations involving lines 60, 23, 663, dominant inheritance of allelic variants of the locuses Hel 7, Hel 9, and Hel NK is noted. Since the locuses Hel 7, Hel 9, Hel 8, NK were established during the expansion of genetic diversity and isolated from new interspecific hybrids, their numbering was carried out as they were identified and new electrophoretic spectra were established in self-pollinated sunflower samples. Locuses Hel 1, Hel 2, Hel 3, Hel 4, Hel 6 are observed in all created lines, hybrids and varieties of sunflower, regardless of their geographical places of creation and the occurring genetic changes. The display of the components of the locuses Hel 5, Hel 7, Hel 9, Hel NK on electrophoregrams is characteristic of individual genotypes for which they can serve as protein markers. With self-pollination and hybridization, changes in allelic variants are always observed at the locuses Hel 1, Hel Hel 4, Hel 6. During self-pollination, hybridization at the locuses Hel 2, Hel 3, Hel 5, Hel 7, Hel 9, Hel NK, there are no changes in the allelic variants of the protein spectra, they are always constant, the locuses Hel 2, Hel NK on the electrophoregram have two polypeptides, the locuses Hel 3, Hel 5, Hel 7, Hel 9 - one. Locuses Hel 5, Hel 7, Hel 9, Hel NK are characterized by the presence or absence of components on the electrophoregrams of the spectra of heliantinins.

Determination allelic variants of the components of the electrophoretic spectra of storage seed proteins and the varying degree of display of their intensity in the spectra on electrophoregrams made it possible to create and describe the obtained sunflower genotypes in protein formulas (Table **1**).

According to the protein formula written by us, in each individual locus, the components responsible for the synthesis of the polypeptide are assigned a number as their molecular weight decreases. In the absence of a component at the locus, zero (0) is written in the protein formula. With a higher degree of manifestation of the component, the figure is emphasized. For example, in sample 108, a weakly intense manifestation of the Hel 5 gene allele is observed, and vice versa, in the created self-pollinated sample 42, an intense manifestation of the allele of the same gene is clearly visible. Only one difference in the degree of manifestation of the intensity of the component of the Hel 5 locus makes it possible to distinguish one sample from another.

The greatest differences in the protein formulas of sunflower genotypes are observed in the fourth electrophoretic spectrum Hel 4. The developed protein

Table 1: Protein Formulas of Self-Pollinated Sunflower Samples, 2015-2021

Sample	Protein Spectra									Parentage
	HEL 1	HEL 2	HEL NK	HEL 7	HEL 3	HEL 9	HEL 4	HEL 5	HEL 6	
23	1	1, 2	0	1	1	1	2, 3, 4, 5, 7, 9, 10, 12, 13	1	1	H. Annuus × H. Debilis
25	2	1, 2	1, 2	1	1	0	2, 3, 4, 7, 9, 10, 11	0	1	H. Praecox × Variety Sur
42	1	1, 2	0	0	1	0	1, 3, 5, 7, 9, 10, 12	1	2	Variety Flagman
60	2	1, 2	0	1	1	0	2, 3, 4, 5, 9, 10, 12,	0	1	H. Annuus × H. Debilis
97-3	1	1,2	0	0	1	0	2, 3, 4, 7, 9, 10, 11,	0	1	H. Debilis × Variety Ermak
108	2	1, 2	0	1	1	0	2, 5, 7, 9, 10	1	1	Variety Sur
109	2	1, 2	0	1	1	0	1, 2, 5, 8, 9, 10, 11	1	1	Variety Flagman × Variety Faforit
663	1	1, 2	1, 2	0	1	1	2, 3, 4, 5, 7, 9, 10, 12	0	1	H. Annuus × H. Praecox

formulas made it possible to solve the problem of identifying the created self-pollinated sunflower samples at the molecular level and to conduct a cluster analysis with the preparation of a dendrogram of the created self-pollinated sunflower samples.

According to the protein formula written by us, in each individual locus, the components responsible for the synthesis of the polypeptide are assigned a number as their molecular weight decreases. In the absence of a component at the locus, zero (0) is written in the protein formula. With a higher degree of manifestation of the component, the figure is emphasized. For example, in sample 108, a weakly intense manifestation of the Hel 5 gene allele is observed, and vice versa, in the created self-pollinated sample 42, an intense manifestation of the allele of the same gene is clearly visible. Only one difference in the degree of manifestation of the intensity of the component of the Hel 5 locus makes it possible to distinguish one sample from another. The greatest differences in the protein formulas of sunflower genotypes are observed in the fourth electrophoretic spectrum Hel 4. The developed protein formulas made it possible to solve the problem of identifying the created self-pollinated sunflower samples at the molecular level and to conduct a cluster analysis with the preparation of a dendrogram of the created self-pollinated sunflower samples.

The results of cluster matrix analysis show that the expansion of genetic diversity during selection by protein spectra contributed to the isolation of 4 clusters (groups) of samples of the initial material (Figure **2**).

Clusters differ in genetic distance, which is mainly determined by differences in allelic variants of the locuses Hel 1, Hel 4 and Hel 6, and the presence or absence of components of the locuses Hel 7, Hel 9, Hel 5, NK. Samples 25, 97, 107 obtained by the method of selection from interspecific hybrids make up a separate 4 cluster. The selection of parental forms, based on the distribution of sunflower genotypes among clusters, made it possible to create the most highly productive sunflower hybrids. The maximum yield of all hybrid combinations was characteristic of hybrids obtained with the participation of parental forms from the most genetically distant clusters. The highest yields were observed in hybrid combinations with the participation of parental forms 50 × 97, 42 × 25, 104 × 31, 107 × 92.

The involvement of self-pollinated samples located in 1, 2 clusters with samples of 4 clusters ensured that hybrids capable of forming the maximum yield level – 3.39-3.51 t ha^{-1} (Table **2**).

Lines selected for hybrid combinations from genetically close clusters or from one cluster formed a significantly

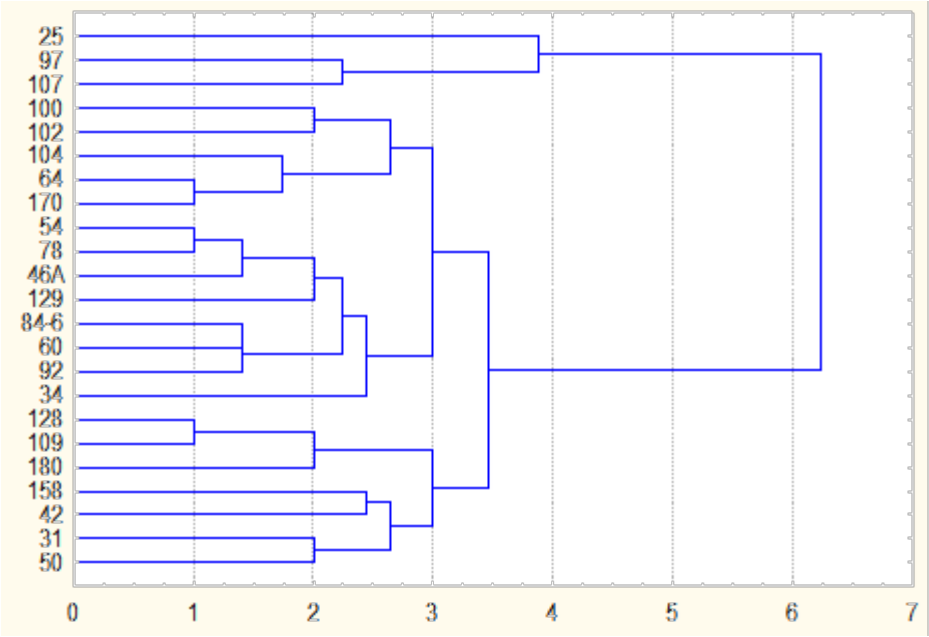

Figure 2: Dendrogram of samples and lines of sunflower, built on the basis of allelic variants of electrophoretic spectra of storage seed proteins, 2015-2021.

Table 2: The Yield Level of Hybrid Combinations of Sunflower Obtained with the Participation of Parental Forms of Different Clusters and Having Distinctive Protein Formulas, ton/ha, 2015-2021

Maternal Form	Cluster	Restorer of Pollen Fertility	Cluster	Yield of Hybrid Combination, t ha^{-1}	Heterosis Effect, %
42	1	25	4	3.51	140.1
50	1	97	4	3.47	138.1
104	3	31	1	3.48	139.2
107	4	92	2	3.39	130.0
46	2	97	4	3.44	130.0
102	3	129	2	2.90	62.1
60	2	84-6	2	2.92	65.2
46	2	92	2	3.00	65.5
104	3	100	3	2.50	49.6
Smallest significant difference, SSD $_{0.05}$				0.23	

lower yield level. Hybrids obtained by crossing lines 2 and 3 of the cluster 102 × 109, 60 × 84-6, 46 × 92, 104 × 100 formed a yield in the range of 2.5-3.0 t ha^{-1}. The lowest yield level of 2.5 t ha^{-1} was obtained by crossing the parental forms 104 and 100 belonging to cluster 3. At the same time, the maternal form 104 of 3 clusters, when crossed with the paternal form 31 of 1 cluster, more genetically removed, provided a hybrid with a higher yield of 3.48 t ha^{-1}.

4. CONCLUSION

The determination and description of allelic variants of the electrophoretic spectra of storage proteins of sunflower seeds provides for the replenishment of the genetic collection of plants, expands the possibilities for creating the source material, new varieties and hybrids. Creating source material from interspecific hybrids, select genotypes with new, previously unidentified and undescribed loci Hel 7, Hel 9, Hel NK. The expansion of genetic diversity, the analysis of allelic variants of the electrophoretic spectra of sunflower seed proteins, allows us to establish and isolate samples with new specific electrophoretic spectra of proteins at the early stages of plant selection. The establishment of allelic variants of electrophoretic spectra makes it possible to identify sunflower genotypes using recorded protein formulas. Identification of sunflower genotypes by allelic variants of the components of the electrophoretic spectra of proteins and protein formulas solves the problem of clustering parental forms at the molecular level and more reasonably carry out the selection of forms for cross combinations. In the conditions of the Steppe of Ukraine, the greatest heterosis effect and high yield of hybrid sunflower combinations of 3.3-3.4 t ha^{-1} are observed when parent forms are used in hybrid combinations, which, according to allelic variants of the components of the electrophoretic spectra, belong to different, most genetically distant clusters.

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