https://doi.org/10.61308/KRQB2968

Evaluation of genetic purity of parental lines and hybrids of sunflower

Igor Aksyonov
ORCHID 0000-0002-8293-4848
Education-Scientific Institute of Natural and Agrarian Sciences of Lugansk National University named Taras Shevchenko, Mirgorod, Ukraine
E-mail: *iva281256@gmail.com*

Citation: Aksyonov, I. (2024). Evaluation of genetic purity of parental lines and hybrids of sunflower. *Bulgarian Journal of Crop Science, 61*(5) 44-53.

Abstract: A high level of genetic purity, preservation and maintenance of genetic uniformity of parental lines and hybrids are necessary conditions in heterotic breeding of a sunflower. The main method for determining the genetic purity of sunflower genotypes is the method of field soil control with the assessment of plants based on morphological traits. The purpose of the experiment was to study the possibility of effectively using the method of electrophoresis of storage proteins of seeds in determining the typicalness of parental lines and the hybridity of sunflower hybrids. 13 samples of maternal sterile lines, 26 samples of hybrids were analyzed using the methods of soil control and the method of analyzing the spectra of seed storage proteins. Research results have proven the highly effective method of electrophoresis of storage proteins in determining the genetic purity of seed of lines and hybrids. The concurrence between the results of the two methods in determining genetic purity was 84.4% for maternal sterile lines, 69.2% for hybrids. The method of analysis allelic variants of helianthinin increased the reliability of the results obtained in determining the level of genetic purity of lines and hybrids by 7.7%. The presence of atypical plants in sunflower crops on the trait of "high" and "low" leads to unreliable, increased indicators of the level of genetic purity by 23.1% with the method of analysis of the spectra of storage proteins of sunflower seeds. The electrophoresis method, based on the analysis of the spectra of storage proteins, can be used as a rapid method in determining the genetic purity of sunflower genotypes.

Keywords: sunflower; line; hybrid; genetic purity; morphological trait; protein spectrum

INTRODUCTION

The heterotic breeding of sunflower is based on obtaining self-pollinated homozygous parental lines and maintenance of a high level of genetic purity of the parental lines in the plots of propagation and hybridization in seed production.

In the creation of sunflower hybrids is used the homozygous linear material: sterile analogues of the maternal lines with cytoplasmic male sterility (CYT^Srfrf), fertile analogues of the maternal line - fixer of sterility (CYT^Nrfrf), paternal lines-restorers of fertility, carrying nuclear genes for restoring the fertility of pollen R*f.* Fertile analogues - lines fixer of sterility of pollen from the maternal line are necessary for the propagation of a sterile analogue of maternal forms with CYT^S cytoplasm. The parental lines of sunflower, that the breeder uses to create hybrids must have a high level of genetic purity and have as much genetic uniformity as possible (Chelyustnikova et al., 2017).

Therefore, sunflower breeding using heterosis requires a high culture of seed production of the parent lines. Genetic purity of parental lines is a necessary condition for using the potential of sunflower hybrids (Hafiz Ghulam Muhu-Din Ahmed at. el., 2022).

The practice of production of the sunflower seed indicates that the typicalness of lines and the hybridity of hybrids does not fully meet the requirements of seed production: the actual level of genetic purity is lower than the level that is provided for by state standards for the sowing qualities of seed material (Malakhova & Pershin, 1999; Poperelya, 2000).

The results of research by many scientists indicate that the level of seed hybridity has a significant impact on the yield and technological properties of sunflower hybrids (Libenko, 1988; 2007; Piskov & Petrov, 1986; Nikitchin, 1999).

A decrease in the level of genetic purity of hybrids has a significant impact on the variation in plant height and head diameter of sunflower plants, leads to a decrease in the technological properties. When sowing of sunflower seeds F_1 with different levels of genetic purity, the cost of gross production per 1.0 ha decreases in proportion to the decrease in the level of genetic purity of the hybrid (Gridnev, 2008). The presence of an admixture of maternal form seeds from 30 to 50% in sunflower hybrids significantly reduces the yield of hybrids (Ryabota, 1997).

One of the pressing problems when growing seeds of hybrid sunflower is contamination of crops with the fertile maternal form in the plots of hybridization. Loss of genetic purity or varietal changes can occur for various reasons: when storing seeds in warehouses, natural crossings, non-compliance of the spatial isolation by seeds production on the plots of propagating and hybridization, genetic mutations, genetic drift and other selection factors (Bochkovoy, 2011; Nikolić et al., 2007).

These factors show, that a control of seed quality becomes important meaning in increasing genetic purity of lines and hybrids, requires improved methods for assessing plants, identification parental lines and hybrids in the available germplasm and among elite breeding material (Rasoulzadeh Aghdam et al., 2020).

In the breeding and seed production of lines and hybrids of a sunflower, the main method for evalution of the genetic purity of seed material is soil control (field control). By soil control, a control batches of the seeds of lines and hybrids are sown in the soil in field conditions for further observation of plants on morphological traits, that are typical for the genotype. However, soil control is possible only in field conditions next year, after receiving seeds, or in winter - in greenhouses, phytotrons. Carrying out soil control in the field can be complicated by climatic conditions. Carrying out soil control in greenhouses and phytotrons in winter requires a lot of financial costs, heat, and electricity. In addition, not all seeds germinate in field conditions. If a batch of hybrid seeds contains seeds of parental lines (smaller seeds, seeds have less viability), such seeds germinate in smaller quantities in the field conditions. This leads to the fact that the level of hybridity of a hybrid can be incredible and overestimated (Bochkovoy et al., 2014; Tikhonov et al., 1991). Description and identification of plants only by phenotype may be less reliable and incorrect (Zeinalzadeh-Tabrizi et al., 2018).

In this regard, determining the genetic purity of lines and hybrids based on the typicality or difference in the allelic state of protein loci on electrophoretogram is of practical interest in breeding and seed production of sunflower (Ahmed et al., 2012; Zia et al., 2014).

Advances in studying the nature of protein function have made it possible to solve the problem of determining the typicality of lines and hybrids with using a quick and reliable laboratory method - electrophoresis of storage proteins (helianthins) of sunflower seeds (Konarev at.el., 2000; Pochinok, 1976; Poperelya, 1996). Protein spectra of electropherograms make it possible to identify the typicality lines and hybrids of a sunflower and establish their genetic purity, since they have greater information compared to the morphological traits of plants (Aksyonov, 2005a; Konarev, 1986; Poperelya & Netsvetaev, 1994).

Protein markers that are observed on electrophoregrams and that have a monogenic type of inheritance can be used to identify sunflower genotypes that solves the problem of determining their genetic purity (Darvishzadeh et al., 2010; 2020; Hafiz Ghulam Muhu-Din Ahmed et al., 2022; Rauf et al., 2020; Yadav et al., 2018).

According to Pallavi et al. (2014) biochemical markers such as the spectrum of storage proteins of seeds, are least affected by environmental conditions and can be used in the identification of breeding and seed material. Protein spectra of storage proteins of seed are highly effective in solving of the problems of seed production: unintentional mixing of seeds of lines and hybrids,

uncontrolled pollination of plants, errors during reproduction of seeds, etc (Gerić et al., 1989; Zlokolica et al., 1996).

In breeding and seed production of a sunflower, the method of electrophoresis of storage proteins of the sunflower seeds (helianthinin) shows good results in determining the genetic purity of lines and hybrids (Aksyonov, 2005 b; Anisimova, 1989; Zheng et al., 2016). Established as a result of research, allelic variants of the electrophoretic spectra of storage proteins of seeds and their use in identifying genotypes increase the effectiveness of this method in determining the genetic purity of parental lines and hybrids of sunflower (Aksyonov, 2016).

In Ukraine, the producers of the sunflower seed have insufficient information regarding the correspondence of data on the genetic purity of lines and hybrids obtained by methods of soil control and electrophoresis of storage proteins of seed (helianthinins).

The aim of research was to compare the results of evaluation of the genetic purity of lines and hybrids of a sunflower, obtained by methods of the soil control and the electrophoresis of the storage proteins of seeds; to study the possibility of effectively using the method of electrophoresis of storage proteins of seeds in determining the level of typicality of source lines, hybrids of sunflower, on establishing ways to more broadly determine the genetic purity of genotypes at the molecular level in industrial seed production acquires the relevance.

MATERIAL AND METHODS

The experiment was carried out in the period 2018 –2021.

The object of the research was the maternal line and hybrids of sunflower.

An experiment has been conducted at Education-Scientific Institute of Natural and Agrarian Sciences of Lugansk National University named Taras Shevchenko (Mirgorod, Ukraine).

Determination of the genetic purity (typicalness) of sunflower hybrid lines was done by two methods – field soil control and electrophoresis of storage proteins of seeds.

The soil of the experiment plots for the field soil control was the black steppe soil, with the humus content in the soil layer 0-30 cm of 3.4-.6 and the pH of the soil solution from 6.8 to 7.0.

Field soil control was carried out using the plot method. The experiment had the two replications. One genotype (sample) under study was sown in each plot. The sample of plants to determine genetic purity using the method of field soil control consisted of 500 plants. The sowing was done in optimal time. Depth of seeds seal was 6-8 cm. The seeds of sunflower were sowed on plots with a width of row spacing of 70 cm. 500 seeds of each sample were sown in a separate experimental plot. For establishment of genetic purity (typicalness), plants of the sterile maternal lines and hybrids were assessed for morphological traits, on sterility, fertility during growing season. Typical and atypical plants for each sample were established during field soil control. The results of plants evaluation were recalculated and expressed on 100-plants.

An electrophoresis of storage proteins of seed of sunflower was performed by the A.Ph. Poperelya method. For analysis, 102 seeds were selected from each line and hybrid sample.

For preparation of storage proteins (helianthin) solution, kernel of each seed of sample of line and hybrid was crushed and defatted. Each seed kernel was conditioned separately and placed in a centrifuge test-tube. The fat removal was done with a mixture of glacial acetic acid and acetone. 1.0 ml solution of glacial acetic acid and acetone (30 ml of glacial acetic acid in 1,0 1 of acetone) was added to each test-tube. Test-tube content was stirred with a mechanical mixer during 30-40 s. After that, the working solution of storage proteins (helianthin) was added to a mixture of glacial acetic acid and urea (1.0 1 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 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. Coomassie Brilliant Blue R-25 was used as a dye of protein in an electrophoretogram. The gel slabs were washed with water.

The obtained electrophoretograms were analyzed. Level of genetic purity was determined on the basis of typical and atypical spectra of proteins.

The genetic purity (typicalness) of lines and hybrids of sunflower with soil control was determined according to the typical morphological traits of plants. The genetic purity (typicalness) of lines and hybrids using the method of electrophoresis of storage proteins was established according to the allelic variants of the electrophoretic spectra of storage proteins typical for each line, each hybrid.

In our work, lines and hybrids are represented by a serial number.

The obtained results were analyzed by MSTAT test, and means were compared by Tukey's multiple comparison test at 5% level.

RESULTS AND DISCUSSION

The established allelic variants of the electrophoretic spectra of storage proteins of seeds proteins made possible in our experience to determine the genetic purity of lines and hybrids of sunflower and compare these data with the results of assessing plants using the method of soil control.

There are studies that show that the level of polymorphism of storage proteins in sunflower seeds is not distinct enough for the polymorphism of storage proteins to be used in the identification of the sunflower genotypes (Nikolić et al., 2008).

At the same time, our studies show the promise of using the method of electrophoresis of storage proteins in the identification sunflower genotypes and correspond with the results of studies by other authors. Khlyostkina (2013) argues that molecular markers with unknown localization can be successfully used in identification of the plant genotypes, since the protein marker corresponds to a gene whose alleles have differences (different molecular weight) at the level of the protein product.

The experiment showed in 74.4% concurrence between the data obtained by methods of soil control and electrophoresis of storage proteins of seeds. The concurrence in the level of typicalness of sterile parental lines between the two methods was 84.6%. The concurrence between the level of hybridity of hybrids was less and equaled 69.2%.

The concurrence of the level of typicalness of self-pollinated sunflower lines was obtained at samples of lines of 16, 18, 22, 24, 25 30, 32 34, 40, 42, 44 (Table 1).

The difference in determining the typicalness of these lines by two methods ranged from 0.1 to 5.1% (LSD_{095}% 6.1). Since the P-value of the F-test was lower than 0.05, it had a statistically

Table 1. The level of typicalness of inbred lines of sunflower determined by methods of soil control for morphological traits and electrophoresis of storage proteins of seeds using electrophoretic spectra

Sample number of line	Level of typicalness		Difference in level of typicalness, %
	Evalution by morphological traits of plants, %	Evalution by electrophoretic spectra of storage proteins, %	
16	67.7	68.7	1.0
18	93.8	93.9	0.1
22	87.9	86.6	1.3
24	76.4	76.5	0.1
25	80.0	74.9	5.1
27	71.8	87.5	15.7
30	74.4	75.0	0.6
32	43,9	43.8	0.1
34	90.3	90.6	0.3
35	92.5	84.0	8.5
40	85.2	87.5	2.3
42	88.4	87.5	0.5
44	97.9	98.9	1.0
LSD_{095} %	*6,2*		

significant difference of data from one method to another method at the 95.0% confidence interval.

A discrepancy between the data for the two methods of determining typicalness was noted for sunflower lines 27, 35.

The level of typicalness in the sample of line 27, which was determined by electrophoretic spectra of proteins, was 87.5% and was higher on 16.0% than when assessing plants by morphological traits.

The method of soil control of line 27 showed the presence in this sample of a fairly large number of atypical plants in height, which are assessed as tall and short plants. Analysis of allelic variants in the electrophoretic spectra of seed storage proteins does not make it possible to identify these atypical plants on their protein spectra, since the genes that control this trait are not represented in the electropherograms.

Analysis of the typicalness of line 27 showed a concurrence of data on the presence of heterozygous fertile branching plants, which were described by the soil control method and ascertained by electrophoresis of storage proteins. The inability to identify atypical plants using the electrophoresis method on the trait "plant height" unreasonably increased the typicalness of the line compared to the soil control method. The level of typicalness of line 27 is reliable using the soil control method (plants are evaluated by morphological traits).

The level of typicalness of line 35, established by electrophoresis of seed storage proteins, was lower on 8.1% compared to the soil control method and amounted to 84.4%.

The line typicality level using the soil control method was 92.5%. Analysis of the protein spectra of electropherograms established that the seeds of the line 35 had an atypical spectrum of proteins for this line within 15.6% of the total number of analyzed seeds. Atypical spectra of storage protein reduced the typicalness level to 84.4%. During the field soil control, these plants (15.6%) did not differ in morphological traits from the typical plants of the lines. Consequently, the level of typicality of line 35, determined from protein spectra, is equal to 84.4% and is a reliable level of typicalness of the line.

The obtained experimental data correspond to the scientific postulate that the same morphotypes of plants can be the result of changes in various genes that control such morphological traits of plants which are not externally manifested when describing plants (Konarev, 1998; Konarev et al., 2000).

At evaluation plants using the method of soil control based on the morphological traits of plants, these "genetically dirty" plants were classified as typical, which led to an artificial increase in the level of typicalness and to an overestimated indicator of genetic purity. The level of typicalness of line 35 at this method was incorrectly and erroneously inflated and amounted to 92.5%. According to Chesnokov et al. (2020) the main disadvantages of morphological genetic markers are related to the fact that they are few in number and are susceptible to the influence of the environmental factors or depend on the developmental stage of plants. In our opinion, evaluation plants only by morphological using the method of soil control can lead to erroneous and unreliable conclusions in determining the genetic purity of sunflower genotypes.

26 samples of sunflower hybrids were analyzed in an experiment on ascertaining the level of hybridity.

Almost, the concurrence of level of hybridity was ascertained by two methods in 18 samples of hybrids (1, 2, 4, 5, 6, 8, 9, 10, 11, 15, 16, 17, 19, 20, 22, 23, 25, 26). For these samples of hybrids, the difference in hybridity between the two methods (soil control and electrophoresis of seeds storage protein) ranged from 0.2% to 5.5% (LSD_{095}% 6.4) (Table 2). The intervals shown of the indicators of the level of hybridity in the table are based on least significant difference of Fisher (LSD). They are designed so that if two middle indicators are the same, then their intervals will be covered in 95.0% of cases.

The difference in determining the genetic purity of hybrids by two methods wasascertained in several hybrids. The difference in hybridity in-

dicators between the two determination methods ranged from 9.0 to 29.8%.

Using the method of electrophoresis of storage proteins, samples of hybrids 3 and 12 (7.7% of the total number of hybrids) had lower indicators of hybridity by 9.0% and 20.2% compared to the method of soil control.

This can be explained by the following factors. Selection and varietal weeding (removal and culling of atypical plants) of parental components were carried out only according to the morphological traits of plants without analysis and control of allelic variants of electrophoretic spectra of storage proteins. This approach led to the appearance in the hybrids crops of atypical plants, which did not have the differences in the morphological traits of the plants, but had differences in the protein spectra of storage proteins of seeds.

With using the method of proteins electrophoresis, all differences in the alleles of the helianthinin spectra of the seeds of these hybrids, were identified as qualitative, which made it possible to accurately register and more reliably determine the genetic purity. In this case, the soil control method unreasonably overestimated the level of hybridity of hybrids 3 and 12 and led to

Table 2. Comparative data on determining the level of hybridity of sunflower hybrids using the methods of soil control and electrophoresis of storage proteins of seeds

Sample number	Sample type	Level of typicalness, %		Sample number	Sample type	Level of typicalness, %	
		GC* (standard)	EF			GC (standard)	EF
1	Hybrid	79.2	80.1	14	Hybrid	67.2	82.9
2	Hybrid	82.3	84.5	15	Hybrid	86.9	87.7
3	Hybrid	92.9	83.9	16	Hybrid	90.6	93.1
4	Hybrid	67.2	65.3	17	Hybrid	93.0	92.4
5	Hybrid	90.9	87.5	18	Hybrid	65.3	95.1
6	Hybrid	73.2	73.4	19	Hybrid	88.5	93.8
7	Hybrid	75.7	85.6	20	Hybrid	89.9	87.6
8	Hybrid	71.0	75.0	21	Hybrid	75.0	91.8
9	Hybrid	53.8	51.7	22	Hybrid	91.8	87.7
10	Hybrid	62.0	63.5	23	Hybrid	81.1	83.3
11	Hybrid	65.0	62.7	24	Hybrid	82.9	95.1
12	Hybrid	58,9	38.7	25	Hybrid	90.0	95.5
13	Hybrid	63.7	79.7	26	Hybrid	76.2	76.6

LSD_{095}% 6.3
*Note: *GC – soil control; EF – electrophoresis*

Table 3. Results of the analysis of the genetic purity of sunflower samples using methods of soil control and electrophoresis of helianthins of seeds

Sample number	Soil control (standard): morphological of traits plants					Electrophoresis of storage protein: protein spectrum				
	level of hybridity, %	typical plants, %	atypical plants, %	maternal form, %	fertility restorer line, %	level of hybridity, %	typical plants, %	atypical plants, %	maternal form, %	fertility restorer line, %
3	92.9	92.9	4.0	2.0	2.0	83.9	83.9	9.4	4.8	1.9
12	58.9	58.9	23.5	7.1	10.5	38.7	38.7	39.7	11.4	10.2

an unreliable result. Consequently, in this case, the ascertaining of the hybridity of hybrids was more reliable with using the analysis of protein spectra of seeds.

The data in Table 3 shows and confirms that, on the allelic state of loci of protein spectra, the electrophoresis method makes possible to identify a larger number of atypical plants (38.7% of plants by the soil control.

Allelic variants of the electrophoretic spectra of storage proteins made it possible to identify a larger number of plants of maternal lines in samples of hybrids 3 and 12, respectively of 4.8 and 11.4%. The method of soil control was identified plants of the maternal form in crops of hybrid of 3 less on 2.8% and in crops of hybrid of 12 less on 7.3%. Such data indicate about the high proportion of plants of the fertile analogue (a fixer of sterility) of sterile maternal lines in the crops of hybrids.

These plants, were not promptly removed from the hybridization plot during varietal weeding. In hybrid crops, such plants did not differ on phenotype from plants of F_1 hybrids. Evaluation of hybrid plants on morphological traits did not ascertain the plants of the fertile analogue (a fixer of sterility) sterile maternal lines. Plants of the fertile analogue of the sterile maternal lines, were identified only by the difference between the allelic variants of the protein spectra of the storage proteins of the hybrids and the fertile analogues of the maternal lines.

In the experiment the method of electrophoresis of storage proteins of the seeds increased the level of hybridity by 9.9-29.8% in 23.8% of the analyzed hybrids (7, 13, 14, 18, 21, 24) compared with evaluation plants by morphological traits in the field conditions.

In this case, the method of protein electrophoresis increased the level of hybridity and was less reliable compared to the soil control. This difference in the level of hybridity was typical for sunflower samples with a low level of genetic purity, which was determined by the morphological traits of the plants: 63.7-82.9%. These samples of sunflower hybrids were characterized by a large number of plants, which by soil control were noted as atypical plants on the traits of "tall" and "low" plants. The level of hybridity, determined by soil control was significantly reduced reliably by the factor of the presence in the crops of hybrids of such plants.

By analysis electropherograms, the electrophoretic spectra of storage proteins of the seeds of such plants did not differ from the spectra of plant hybrids. This factor contributed to an unreasonable and unreliable increase the level of hybridity of hybrids (samples 7, 13, 14, 18, 21, 24) by evaluation plants by electrophoretic spectra of proteins. Determinations of the level of hybridity of these hybrids by electrophoresis of storage proteins were less reliable compared with data of the soil control method.

CONCLUSIONS

The results of studies on ascertaince the level of genetic purity of sterile maternal lines and hybrids of sunflower with using soil control and electrophoresis of storage proteins coincide in most cases. The concurrence between the research results was 84.6% by determining the typicalness of maternal lines and 69.2% by determining the hybridity of hybrids. The differences between the indicators of the genetic purity of the lines ranged from 0.1 to 5.1%, the differences between the indicators of the genetic purity of the hybrids ranged from 0.2% to 5.5%.

Analysis of the helianthinn spectra of seeds increased the reliability of genetic purity indicators of lines on 7.7% and reduced the reliability of genetic purity indicators on 7.7% by the presence of atypical plants on trait of "plant height"compared to the soil control method.

Analysis of the helianthinn spectra of seeds increased the reliability of the level of hybrids hybridity on 7.7% and reduced the reliability of the level of hybrids hybridity on 23.1% by the presence of atypical plants on trait of "plant height"compared to the soil control method.

The soil control method in determining genetic purity is more reliable by evaluation genotypes with the presence of atypical plants in crops on the trait of "tall" and "low" plants.

The high efficiency of the method for determining the genetic purity of lines and hybrids of sunflower based on analysis epy allelic variants of the electrophoretic spectra of seeds storage proteins makes it possible to increase the reliability of the obtained data and, in some cases, to avoid labor-intensive field soil control.

The method of electrophoresis, based on the analysis of electrophoretic spectra of storage proteins of seeds, can be used as an express method in determining the level of typicalness of maternal forms and hybridity hybrids of sunflower, and can be used for control for the genetic purity of lines and hybrids.

REFERENCES

Ahmed, HGM-D., Zeng, Y., Iqbal, M, Rashid MAR. & Raza, H. (2022) Genome-wide association mapping of bread wheat genotypes for sustainable food security and yield potential under limited water conditions. *PloS One,* 17.

Aksyonov, I. V. (2005a). Use of albumin markers for defining genetic purity of sunflower parent lines and hybrids. *Helia*, 28 (43), 43-48.

Aksyonov, I. V. (2005b). Protein markers specificity of sunflower inbred lines. *Helia*, 28 (43), 49-54.

Anisimova, I. N. & Gavrilyuk, I. P., (1989). Heterogenity and polymorphism of the 11S globulin of sunflower seed. *Genetics*, 25 (7), 1248-1255 (Ru).

Bochkovoy, A. D., Antonova, T. S., Kamardin. V. A., Araslanova, N. M., Veter, I. I., Guchetl, S. Z. & Chelyustnikova, T. A. (2014). Efficiency of various methods for determining the genetic purity of batches of seeds of self-pollinated lines and hybrids of the first generation of sunflower. *Oilseed crops. Scientific and technical Bulletin VNIIMK*, 1 (157-158), 3-7 (Ru).

Bochkovoy, A. D. (2011). State and problems the seed production of sunflower hybrid at VNIIMK. *Oilseed crops. Scientific and technical Bulletin VNIIMK*, 2 (148-149), 23-27 (Ru).

Chelyustnikova, T. A., Guchetl, S. Z. & Antonova, T. S. (2017). Application of molecular markers to identify the CMS-Rf system in parental lines of sunflower hybrids. *Scientific and technical bulletin of the All-Russian Research Institute of Oilseeds*, 4 (172), 3-13 (Ru).

Chesnokov, Yu., Kosolapov, V. M. & Savchenko, I. V. (2020). Morphological genetic markers in plants. *Генетика*, 56 (12), 1366-1377 (Ru).

Chikkadevaiah & Nandini, R. 2003. Isozymes as markers for differentiating sunflower genotypes. *Helia*, 36(29): 51-58.

Dalgalarrondo, M., Raymond, J. & Azanza J. (1984). Sunflower seed proteins: Characterization and subunit composition of the globulin fraction. *Journal of Experimental Botany,* 35, 1618–1628.

Darvishzadeh, R., Azizi, M., Hatami-Maleki, H., Bernousi, I. & Mandoulakani, B. A. (2010). Molecular characterization and similarity relationships among sunflower (Helianthus annuus L.) inbred lines using some mapped simple sequence repeats. *African Journal of Biotechnology*, 9, 7280-7288.

Gerić, I., Zlokolica, M. & Gerić, C. (1989). Application of isoenzymes in control of genetic purity of seed of agricultural plants. *Agricultural News*, 3-4, 555-564.

Gonzalez-Perez, S., Vereijken, J.M., Merck, K.B., Van Koningsveld, G.A., Gruppen, H. & Voragen, A. G. J. (2004). Conformational states of sunflower (*Helianthus annuus*) helianthinin: effect of heat and pH. *Journal Agricultural Food Chemistry*, 52, 6770-6778.

Gridnev, A. K. (2008). Influence of the level of genetic purity of seeds on the yield and technological properties of hybrids. *Oilseed crops. Scientific and technical Bulletin VNIIMK*, 2 (139), 7-10 (Ru).

Hafiz Ghulam Muhu-Din Ahmed, Muhammad Rizwan, Muhammad Naeem, Muhammad Ahsan Khan, Faheem Shehzad Baloch, Sangmi Sun & Gyuhwa Chung. (2022). Molecular characterization and validation of sunflower (*Helianthus annuus* L.) hybrids through SSR markers. *Plos One*, 1-12. https://doi.org/10.1371/journal.pone.0267383

Jasso de Rodríguez, D., Romero-García, J., Rodríguez-García, R. & Angulo Sánchez, J. L. (2002). Characterization of proteins from sunflower leaves and seeds: Relationship of biomass and seed yield. *In: J. Janick and A. Whipk*ey (eds.), *Trends in New Crops and New Uses*. *ASHS Press, Alexandria,* VA., 143–149.

Konarev, A. V., Konarev, V. G., Gubareva, N. K. & Peneva, T. I. (2000). Seed proteins as markers in solving problems of plant genetic resources, breeding and seed production. *Cytology and genetics*, 38 (2), 91-103 (Ru).

Konarev, V. G. (1983). *Seed proteins as genetic markers*. Kolos, Moscow, 320 (Ru).

Konarev, V. G. (1998). *Morphogenesis and molecular biological analysis of plants.*VIR, Sankt-Peterburg, 370 (Ru).

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the heads of bacteriophage. *Nature*, 4 (227), 680-685.

Lakemond, C. M. M., de Jongh, H. H. J., Hessing, M., Gruppen, H. & Voragen, A. G. J. (2000). Soy glycinin: influence of pH and ionic strength on solubility and molecular structure at ambient temperatures. *Journal of Agricultural Food Chemistry*, 48, 1985–1990.

Lakemond, C. M. M. (2001). *Heat denaturation of soy glycinin: structural characteristics in relation to aggregation and gel formation*. *PhD* Thesis, Wageningen University.

Khlyostkina, E. K. (2013). Molecular markers in genetic research and breeding. *Vavilov Journal of Genetics and Breedin*g, 17 (4/2), 1044-1053 (Ru).

Libenko, N. A. (1988). The influence of pollen impurities not provided for in the formula on the manifestation of valuable traits in F_1 sunflower hybrids. Scientific and technical Bulletin of VSGI, 2 (68), 22-26 (Ru).

Libenko, N. A., Babich, V. I. & Chervonis, M. V. (2007). Efficiency of methods for assessing the level of hybridity and typicality of sunflower hybrids. *Collection of scientific works of NAC NAI*C, 9 (49), 181-188 (Ukr).

Lombard, V., Baril, C. P., Dubreuil, P., Blouet, F. & Zhang, D. (2000). Genetic relationships and fingerprinting of rapeseed cultivars by AFLP: Consequences for varietal registration. *Crop Sciences*, 40, 1417–1425.

Malakhova, M. S. & Pershin, A. F. (1999). Correspondence of the results of assessing the genetic homogeneity of sunflower seeds by the method of soil control and electrophoresis of storage proteins. *Collection of scientific works of the Institute of Oilseed Crops of the UAAS*, 4, 7-13 (Ru).

Nikitchin, D. I. (1999). Features of the technology for growing parental forms of sunflower hybrids. *Collection of scientific works of the Institute of Oilseed Crops of the UAAS*, 4, 172-180 (Ru).

Nikolić, Z., Milošević, M., Taški-Ajduković, K. & Vujaković, M. (2007). Genetic purity as a component of seeds quality. *Proceedings 42nd Croatian and 2nd International Symposium on Agriculture*, Opatija, 245-248.

Nikolić, Z., Vujaković, M. & Jevtić, A. (2008). Genetic purity of sunflower hybrids Determined on the basis of isozymes and seed storage proteins. *Helia*, 31 (48), 47-54.

Pallavi, H.M., Gowda, R., Shadakshari, Y.G., Bhanuprakash, K. & Vishwanath, K. (2011). Identification of SSR markers for hybridity and seed genetic purity testing in sunflower (Helianthus annuus L.). *Helia*, 34 (54), 59-66.

Piskov, A. & Petrov, P. D. (1986). The influence of non-hybrid plants on the yield of seeds and oil of a sunflower hybrid. Start and possibilities of obtaining hybrid seeds with late sowing and irrigation. *Plant Sciences*, 23 (9), 28-32 (Bg).

Poperelya, F. O. & Netsvetaev, V. P. (1994). Genetic control of seed helianthin in sunflower (Helianthus annus L.). *Cytology and genetics*, 28 (4), 59-63 (Ru).

Poperelya, F. O. (2000). Genetic interpretation of electrophoregrams of helianthin in F1 sunflower seeds. *Cytology and genetics*, 34 (2), 59-63 (Ukr).

Poperelya, F. O. (1996). Three main genetic systems of winter wheat grain quality. *In: Realization of the potential capabilities of varieties and hybrids of the breeding and genetic institute in the conditions of Ukraine*. Breeding and Genetics Institute, Odessa, 117-132 (Ukr).

Pochinok, A. N. (1976). *Methods of biochemical analysis of plants*. Scientific thought, Kyiv, 334 (Ru)..

Ryabota, A. N. (1997). The influence of the quality of seed material of sunflower hybrids on their yield and other traits. *Collection of scientific works of the Institute of Oilseed Crops of the UAAS*, 2, 127-132 (Ru).

Prakash, V. & Narasinga Rao, M. S. (1988). Structural similarities among the high molecular weight protein fractions of oilseeds. Journal Bioscience, 13 (2), 171-180.

Rasoulzadeh Aghdam, M., Darvishzadeh, R., Sepehr E. & Alipour, H. (2020) Association analysis for agronomic traits with SSR markers in sunflower (Helianthus annuus L.) genotypes under optimum and phosphorus deficiency conditions. *Crop Biotechnology*, 10, 71-89.

Raymond, J., Mimouni, B., & Azzaza, J. L. (1994). Variability in the 11S globulin fraction of seed storage protein in *Helianthus* (*Asteraceae*). *Plant Systematics and Evolution*, 196, 69-79.

Raymond, J., Robin, J. M. & Azzaza, J. L. (1995). 11S seed storage proteins from *Helianthus* species (*Compositae*): biochemical, size and charge heterogeneity. *Plant Systematics and Evolution*, 198, 195-208.

Sabir, M. A., Sosulski, F. W. & MacKenzie, S.L. (1973). Gel chromatography of sunflower proteins. *Journal Agricultural Food Chemistry*, 21, 988-993.

Samarah, N. H., Mullen, R. E., Cianzio, S. R. & Scott, P. (2006). Dehydrin-Like Proteins in Soybean Seeds in Response to Drought Stress during Seed Filling. *Crop Sciences*, 46, 2141-2150.

Schwenke, K. D., Paehtz, W., Linow, K. J., Raab, B. & Schultz, M. (1979). On seed proteins. XI. Purification, chemical composition, and some physico-chemical properties of the 11S globulin (helianthinin) in sunflower seed. *Nahrung*, 23, 241–254.

Stuber, C. W., Wendel, J. F., Goodman, M. M. & Smith, J.S.S. (1988). *Technique and scoring procedures for starch gel electrophoresis of enzymes from maize (Zea mays L.)*. NC State University, Agricultural Research Service Tech.

Tikhonov, O. I., Bochkaryov, N. I. & Dyakov, A. B. (1991). *Biology, breeding and cultivation of sunflower*. Agropromizdat, Moscow, 160-164 (Ru).

Yadav, P., Tilak, I., Kisan, B., Goud, I. S. Naika, M. B., Ayyangouda Patil, Vikas Kulkarni, Kadirvel Palchamy &Praduman Yadav. (2018). Biochemical and Molecular Characterization of Parents and its Crosses for High Oleic Acid Content in Sunflower (Helianthus

annuus L.). *International Journal of Current Microbiology and Applied Sciences*, 7 (4), 200-2020. https://doi.org/10.20546/ijcmas.2018.704.231.

Zeinalzadeh-Tabrizi, H., Haliloglu, K., Ghaffari, M. & Hosseinpour, A. (2018) Assessment of genetic diversity among sunflower genotypes using microsatellite markers. *Molecular Biology Research Communications*, 7, 143.

Zheng, J., Wen, D., Zhao, H. & Zhang, C. (2016). Acetic acid urea-polyacrylamide gel electrophoresis: a rapid method for testing the genetic purity of sunflower seeds. *Quality Assurance and Safety of Crops & Foods*, 9 (1), 41-46.

Zia, Z. U., Sadaqat, H. A., Tahir, M. H. N., Sadia, B. Bushman B. S., Hole, D., Michaels, L. & Malik, W. (2014) Estimation of genetic diversity using SSR markers in sunflower. *Russian Journal of Genetics*, 50, 498-507. DOI: 10.7868/s0016675814050142

Zlokolica, M., Nikolić, Z., Turkav, S., Milošević, M., Graovac, M. & Škorić, D. (1996). Isozymic variability of self pollinated sunflower (*Helianthus annuus* L.) lines. *Heli*a 19, 113-120.

Received: August, 25, 2024; *Approved*: September, 22, 2024; *Published*: October, 2024