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USE OF ALBUMIN MARKERS FOR DEFINING GENETIC PURITY OF SUNFLOWER PARENT LINES AND HYBRIDS

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SUMMARY

Experiments have been conducted at Institute of Oilseed Crops, UAAS, Ukraine, to study the usefulness of electrophoresis of seed storage proteins in defining genetic purity of sunflower seed material. They have shown it is possible to use albumin markers for genetic homogeneity definition of sunflower parent lines and hybrids.

Key words: sunflower, electrophoresis, albumin markers, genetic purity, line, hybrid

INTRODUCTION

Seed quality, i.e., genetic purity, is a prerequisite for high seed yield. Genetic purity of seed of sunflower lines and hybrids is usually defined by the traditional method - the field trial. Yield estimate is made on the basis of morphological parameters, i.e., on the basis of direct assessment of plants during flowering period.

This method has certain shortages. The morphological parameters are neither sufficiently conspicuous nor sufficiently stable. The environment affects them. Therefore, it is possible that the field trial method produces misleading results (Kalendar, 2002; Konarev, 1998). The new methods of molecular markers provide possibilities of defining genetic purity of sunflower seed (Konarev, 1983; Levontin, 1978; Sosinov, 1985).

One of the methods of molecular-genetic markers - electrophoresis of seed storage proteins (helianthins) - is most convenient for the analysis on the genetic homogeneity of sunflower lines and hybrids. Seeds are the final stage of ontogenesis. Seed storage proteins remain stable for many years. Electrophoretic protein patterns are easy to read and reproducible (Konarev, 1998; Poperelya, 2000). The analysis of markers allows to reliably identify homozygotes (lines), and heterozygotes (hybrids) in sunflower.

The objective of this investigation was to determine the efficiency of albumin markers in defining genetic purity of sunflower lines and hybrids.

MATERIAL AND METHOD

The genetic purity of sunflower samples was determined by the methods of field trial (standard) and electrophoresis of storage proteins (helianthins) of sunflower seeds.

In the field trial, 500 seeds were sown in each plot, two seeds per hill. Thinning was not performed. The experiment was organized in two replications. For establishment of genetic purity, plants were assessed for morphological characteristics during growing season. Level of genetic purity was defined on the basis of the number of typical plants. The results were recalculated and expressed on 100-plant basis.

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 working solution of helianthins was prepared in acid environment with glacial acetic acid and 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. Level of genetic purity was determined on the basis of typical and atypical spectra.

The objects of this study were 26 sunflower commercial hybrids and 4 parent lines.

Standard procedures of analysis of variance were performed and stability parameters calculated using the Lakin method (1990).

RESULTS AND DISCUSSION

The analysis of electrophoretograms indicated the presence of 6 albumin spectra (*Hel* 1, *Hel* 2, *Hel* 3, *Hel* 4, *Hel* 5, *Hel* 6). Variants of each albumin spectrum were established in the electrophoretograms (Figure 1).

The number of albumin spectra (polymorphism zones) corresponds to the number of subunits composed of helianthin molecule. The genes *Hel* 1, *Hel* 2, *Hel* 3, *Hel* 4, *Hel* 5, *Hel* 6 are polymorphic. Each allele controls the polypeptide synthesis. Each polypeptide on the electrophoretograms is presented as one segment of the band. We observed different numbers of segments in each albumin spectrum.

They allowed us to identify, precisely and reliably, homozygotes and heterozygotes of each allele.

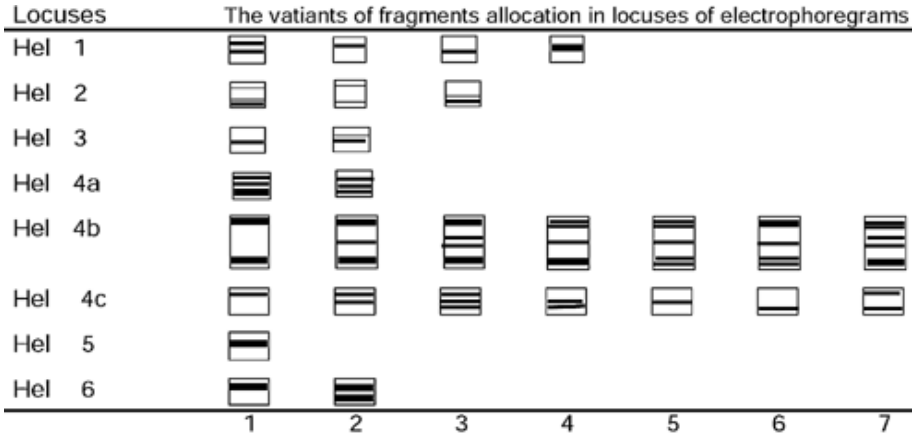

Figure 1: Standard helianthin electrophoretogram spectrums per locus of sunflower seeds. Albumin spectrums were determined and described in Laboratory for Sesame Breeding and Electrophoresis of Institute of Oilseed Crops, UAAS, 2001 - 2003.

The comparative analysis of genetic purity level of the hybrids and inbred lines showed that the methods of field trial and electrophoresis were in agreement in most cases. The coincidence of data was estimated at 73.3% (Table 1).

Table 1: Comparative data of genetic homogeneity level of sunflower hybrids and inbred lines (field trial and electrophoresis of storage proteins of seed)

Sample number	Sample type	Genetic homogeneity level (%)		Sample number	Sample type	Genetic homogeneity level (%)	
		SC	EF			SC	EF
1	hybrid	79.0	80.0	16	hybrid	90.5	93.7
2	hybrid	82.0	64.0	17	hybrid	93.0	92.1
3	hybrid	93.0	85.0	18	hybrid	65.9	94.8
4	hybrid	67.0	65.0	19	hybrid	88.4	93.7
5	hybrid	91.0	87.0	20	hybrid	89.8	87.5
6	hybrid	73.0	70.0	21	hybrid	75.3	91.6
7	hybrid	75.0	85.0	22	hybrid	91.7	87.5
8	hybrid	71.0	75.0	23	hybrid	81.1	83.3
9	hybrid	53.0	51.0	24	hybrid	83.0	94.8
10	hybrid	62.0	64.0	25	hybrid	90.1	95.5
11	hybrid	65.0	62.0	26	hybrid	76.2	76.6
12	hybrid	58.0	39.0	27	line	67.6	68.7
13	hybrid	63.7	79.7	28	line	93.8	93.9
14	hybrid	67.0	82.4	29	line	87.9	86.6
15	hybrid	86.9	87.5	30	line	76.4	76.5

LSD 0.05 % - 6.3; SC - soil-control (standard); EF - electrophoresis

Practically identical results of the two methods were obtained for 22 samples (1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 15, 16, 17, 19, 20, 22, 23, 25, 25, 26, 27, 28, 29, 30). In these samples, difference in genetic purity level was from 0.1 % to 5.4%.

Electrophoretic analysis of samples 3 and 12 (representing 6.7% of the total number of samples) showed lower genetic homogeneity, by 8.0% and 19%, respectively, compared with the field trial. The analysis of albumin spectra of electrophoretograms showed that prior to the introduction of this method it was easier to discard a valuable inbred line from selection program. Qualitative differences in helianthin alleles allow precise and reliable determination of genetic purity. Conversely, the morphological makeup of a plant is characterized by interaction between alleles which allows different combinations of genes to produce similar phenotypes. Therefore, the presently used field trial method increases the genetic homogeneity level.

Table 2 shows that the method of electrophoresis of storage proteins in sunflower seeds allowed us to define a larger number of atypical plants in sample 12 - 20 atypical plants were identified by the field trial method, 34 atypical plants by the electrophoretic method.

Table 2: Genetic purity of sunflower samples

Sample number	Soil-control					Electrophoresis proteins spectrum				
	Typical plants	Atypical plants	Female form of plants	Male forms of plants	Admix-tures	Typical plants	Atypical plants	Female form of plants	Male form of plants	Admix-tures
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
3	93	-	3	1	3	85	-	13	1	1
12	58	20	10	11	1	39	34	19	5	3

The electrophoresis of seed helianthin allowed us to identify a larger number of female lines in samples 3 and 12, 10% and 9%, respectively. The field trial method defined the number of female lines in samples 3 and 12 at 3% and 10%, respectively. It proves that the female plants in the field trial had a high percentage of fertile plants. These fertile plants were not removed by mechanical methods because they did not differ much from sterile plants. The results of helianthin electrophoresis were higher and more accurate.

Also, we found a different number of male plants in sample 12. The helianthin electrophoresis showed a lower number of male plants.

In 6% of the cases, the protein electrophoretograms of parent lines did not exceed the protein electrophoretograms of F_1 hybrids. It occurred when plants of female lines and F_1 hybrids were inadvertently harvested together. Only these plants could show the segregation of generations and have seeds with typical electrophoretograms of parent lines. In that case, the field trial showed more accurate data regarding seed maintenance of the male form.

The electrophoresis of storage proteins in seed showed that the genetic homogeneity of samples 7, 13, 14, 18, 21 and 24 (20% of the total number of samples) ranged from 10.0 to 28.9%. The values of genetic homogeneity were higher when analyzed by the method of electrophoresis. The electrophoretogram pattern for F_1 hybrids did not differ from the electrophoretogram pattern of female lines because in both, hybrids and female lines, the first albumin spectrum, *Hel* 1, has one homozygous locus.

Therefore, the electrophoresis method of storage proteins of sunflower seeds improved the genetic purity level of samples 7, 13, 14, 18, 21, 21 and 24 as compared with the field trial.

When the method of electrophoresis of seeds storage proteins is employed for determining the level of genetic homogeneity in sunflower, we are in fact simultaneously analyzing albumin markers (albumin spectrum) of the seeds of F_1 hybrids and their parent lines.

Consequently, use of albumin markers allows to define the genetic homogeneity level of hybrids at 80% level. The method of electrophoresis of storage proteins of sunflower seeds may be used as a rapid method for defining genetic purity of sunflower parent lines and hybrids.

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UTILIZACIÓN DE MARCADORES ALBUMÍNICOS EN DEFINICIÓN DE PUREZA GENÉTICA DE LAS LÍNEAS PROGENITORAS E HÍBRIDOS DE GIRASOL

RESUMEN

Los experimentos fueron realizados en el Instituto de Cultivos Oleaginosos UAAS, Ucrania, para determinar la eficacia de electroforesis de las proteínas de reserva de la semilla, en la definición de pureza genética del material de semilla del girasol. La utilización de los marcadores albumínicos para la determinación de homogeneidad genética de las líneas progenitores e híbridos de girasol, se mostró posible.

L'UTILISATION DE MARQUEURS ALBUMINEUX DANS LA DÉFINITION DE LA PURETÉ GÉNÉTIQUE DES SOUCHES PARENTALES ET DES HYBRIDES DE TOURNESOL

RÉSUMÉ

Des expériences ont été effectuées à l'institut des cultures oléagineuses UASS, Ukraine dans le but d'étudier l'efficacité de l'électrophorèse des protéines de réserve des graines dans la définition de la pureté génétique du matériel séminal du tournesol. L'utilisation de marqueurs albumineux pour établir l'homogénéité génétique des souches parentales et des hybrides de tournesol s'est avérée possible.