Use of Protein Spectra in Increasing the Genetic Homogenous Traits of the Parental Lines of Sunflower

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Citation Information: Helia. Volume 37, Issue 60, Pages 87–98, ISSN (Online) 2197-0483, ISSN (Print) 1018-1806,DOI: 10.1515/helia-2014-0007, July 2014

Publication History

Received:
2013-09-02
Accepted:
2014-05-20
Published Online:
2014-07-03

Abstract

This paper shows the efficiency of the individual selection of plants of the maternal line of sunflower belonging to the type ZL95 on electrophoretic spectra of storage proteins of seeds, with a simultaneous assessment of morphological traits for the increase and maintenance of genetic uniformity. On the localization of components of polypeptides, their mobility and intensity were installed and identified the allelic options of the electrophoretic spectra: *Hel-1*, *Hel-2*, *Hel-3*,*Hel-4* and *Hel-6*. By the method of individual selection of plants on the set protein spectra with simultaneous control on morphological traits increased the genetic purity of male analogue line ZL95 from 44.0 to 98.4%, fixer of sterility from 47.0 to 98.5% and the assessment on protein spectra 100.0% at both analogues of the line.

Keywords: sunflower; line; genetic homogenous; protein spectra; morphological traits

Introduction

Creation of the inbred lines of sunflower used for breeding hybrids is based on receiving genetically homogenous plants with important agronomical and certain morphological traits. In the future process

of seed-growing, the importance will be given to maintain the genetically homogenous parental lines, without reducing the level of their varietal purity.

The analysis and maintenance of the genetic purity of self-pollinated sunflower lines is carried out on the basis of the assessment of plants according to their morphological traits.

At the same time, the authentic definition of typical plants and rejection of untypical ones, based on the reproduction of parental lines of sunflower and their morphological traits, is not always effective for a number of reasons.

This results from the fact that in case of many parental lines their morphological traits are weakly expressed (Kirichenko, 2005). The assessment of plants based only on their morphological traits does not always reveal the hidden genetic variability and as a result does not carry out the analysis of genetic purity of the self-pollinated lines of parental forms (Konarev *et al*., 2000). The mistakes and incorrectness in determination of typical parental lines according to their morphological traits can be explainable in such a way that different morphotypes of sunflower, in certain cases, grow out of the same mutation and, on the contrary, identical morphotypes grow out of the change of different genes (Popov *et al*., 2002).

Besides that, developing adverse field conditions for growth and development of plants can cause the factors of environment to have strong impact on the expression of traits and considerably reduce efficiency of the assessment and selection of parental forms on traditional morphological traits (Krupnov *et al*., 2010).

The selection of parental lines during the reproduction of plants based only on morphological traits leads to decrease in genetic purity of both lines, and typical features of hybrid combinations of sunflower with participation of these parental components.

Noted shortcomings of the selection of plants based on morphological traits in the course of seed-growing lead to the emergence of atypical plants, reduction in typical features of parental components and hybrids, and finally cause the application of new methods of an assessment, selection of self-pollinated lines and the maintenance of the high level of their genetic homogenous features.

After the cultivation of parental lines and selection of sunflower plants on reproduction plots based only on morphological traits and without monitoring the procedure on the molecular level, the method

of electrophoresis of storage proteins, by means of comparison of protein spectra of each separate seed, shows the low level of typical features of lines on the molecular level.

Therefore, molecular markers are used for the identification of genotypes, determination of their genetic purity in the practice of breeding and seed-growing of crops (Knapp, 1998; Moose *et al*., 2008).

Application of fenotypical markers of protein molecules and products of individual genes significantly expands the possibilities of selection of plants and control of genetic purity of parental lines on the molecular level (Sobko *et al*., 1999).

Molecular-genetic markers have numerous advantages, including bigger informational content, in comparison to morphological traits.

Proteins are used as storage marker traits. They are characterized by requirements which, in the process of breeding, are shown as a marker for independence of conditions of cultivation and fixing genetic distinctions among generations (Konarev, 1982).

Despite a rather low degree of polymorphism of storage proteins (helianthin) in sunflower seeds, scientific research has saved up the material about the presence of polymorphism alleles helianthin in self-pollinated samples and the possibility of its use in breeding (Gavrilova *et al*., 2003).

The established spectra of possible positions of storage protein in sunflower seeds expand the possibilities of identification of a genetic diversity, including lines, hybrids and varieties.

However, the analysis of scientific sources of the obtained research results shows the insufficient exploration of efficiency of carrying out intra linear selection of parental sunflower lines during seed-production breeding in reproduction and hybridization plots.

Questions of studying the efficiency of selection of plants according to their morphological traits, protein spectra and the maintenance of a high degree of genetic homogenous features demand carrying out further researches.

In accordance to that, the objectives of this research were as follows:

- –

to study and establish the efficiency of selection of self-pollinated lines of sunflower on their morphological traits and protein spectra;

- –

using the assessment of plants on their morphological traits and electrophoretic spectra of storage proteins to compare the efficiency of the selection of parental lines of sunflower.

Materials and methods

The experiment was conducted in the laboratory of genetics at The Institute of Oilseed Crops (Zaporozhie, Ukraine).

The subject of the research was the maternal line of sunflower ZL95. This line was chosen for carrying out the research since it showed a noted high level of typical features of plants on morphological traits, while the method of electrophoresis of storage proteins in seeds established a considerably low level of typical plant features.

The research consisted of laboratory and field experiments.

For the analysis and definition of the level of typical features by the method of electrophoresis of seed proteins, a sterile analogue and a fixer of sterility of the line after its cultivation on a reproduction plot were selected separately.

After electrophoresis and the comparison of the received electrophoretic spectra, the level of typical features of sterile analogue was 44.0%, while for the sterility fixer it was 47.0%.

From these samples of separately selected seeds, they were individually numbered and cut in half.

Every selected half of the seeds without a germ was subjected to electrophoresis for the localization of strips of polypeptides (allelic options) of protein spectra.

On the received electrophoretograms, allelic options of electrophoretic spectra were individually defined and analyzed on each half of a seed.

Parts of seeds were identified for electrophoretic spectra of helianthin.

Parts of seeds with a germ, having electrophoretic spectra which are inherent in the ZL95 line, and according to the individual number of analyzed halves without a germ, were selected in one variant for sowing as typical.

Also, a half of seeds of the line with atypical electrophoretic spectra of protein, but with the identical morphological traits inheritable for plants of the line ZL95, were selected in separate options.

The selection was made on a sterile analogue and a fixer of sterility of the line.

Established and identified on electrophoretic spectra of protein, a half of seeds with a germ were sown by options in pots with a soil mix for receiving seedlings. In the phase of the emergence of 1–2 real leaves, the plants were sown in the open ground.

The sterile analogue and fixer of sterility of the line were sown separately for the assessment in field conditions using the method of field trial on morphological traits of plants and reproduction of plants by the following options:

- – plants with the actual level of typical features when the selection was made only on morphological traits;
- – line plants when selection was made by the method of dividing the seeds with identical typical electrophoretic spectra of protein;
- – plants of lines having identical morphological traits and differing by allelic options of the sixth electrophoretic spectrum.

For the assessment of plants in the method of field trial, 500 seeds of each option were sown in each plot, two seeds per hill. Thinning was not performed. The experiment was organized in two replications. The sunflower was sown on plots with a width of row spacing of 70 cm. Depth of seeds was 6–8 cm. For the establishment of genetic purity, plants were assessed for morphological characteristics during growing season. The level of genetic purity was defined on the basis of number of typical plants. The results were recalculated and expressed on 100 plants.

The soil of the experimental plots was the black steppe soil, with the humus content in the soil layer of 0–30 cm and the pH of the soil solution from 6.8 to 7.0.

Optionally, if plants were sown for reproduction, during the phase of flowering a sterile analogue was pollinated by the pollen of a fixer of sterility separately in each option.

After harvest, the seeds of options with the repollinated plants were exposed to the method of electrophoresis of storage proteins for determination of typical features at molecular level.

The repollinated and selected plants on morphological traits and proteins spectra were sown by options in the following vegetative period for defining the genetic purity and efficiency of the selection in the field conditions on electrophoretic spectra of storage proteins.

Electrophoresis of storage proteins of sunflower seeds was performed by the method of Poperelya (2000).

For the preparation of helianthin solution, kernel of each seed was crushed and defatted. It 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 millilitre of glacial acetic acid and acetone (30 ml of glacial acetic acid in 1 l of acetone) was added to each test-tube. Test-tube content was stirred with a mechanical mixer for 30–40 s. After that, the solution of helianthin was added to a mixture of glacial acetic acid and urea (1 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 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. The level of genetic purity was determined on the basis of typical and atypical spectra of proteins.

The obtained results were analyzed by MSTAT test and compared by Tukey’s multiple comparison tests at 5% level.

Results and discussion

Identification of the electrophoretograms of lines and the sunflower hybrids, performed at The Institute of Oilseed Crops, shows that alleles whose code synthesis of storage proteins of seeds were collected on loci, on electrophoretograms are presented by the block (spectrum) of electrophoretic components of polypeptides. As a result of the conducted researches between 5 and 9 protein spectra were established and identified, depending on a genotype: *Hel-1, Hel-2*, *Hel-3*, *Hel-4*, *Hel-5*, *Hel-6*, *Hel-7*, *Hel-8* and *Hel-9*. Each locus is identified by allelic options which differ quantitatively in electrophoretic components of polypeptides, their mobility and intensity (Figure 1).

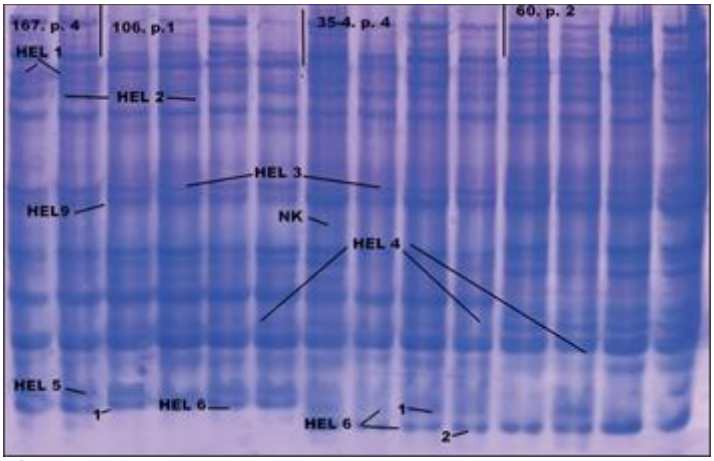

Figure 1:

The identified by allelic options of electrophoretic spectra of genotypes of sunflower

At the maternal line of sunflower of ZL95, five electrophoretic loci were established: *Hel-1*, *Hel-2*, *Hel-3*, *Hel-4* and *Hel-6*.

The analysis of component structure of seeds helianthin of the maternal sterile line of sunflower of ZL95A after its reproduction and selection of plants on morphological traits on a plot of reproduction showed distinctions on proteins spectra *Hel-1*, *Hel-4* and *Hel-6*. Distinctions on proteins spectra consisted of the absence or localization of strips of polypeptides in electrophoretic spectra and degree of intensity of their expressiveness. The established distinctions at molecular level showed genetic heterogeneity and a low level of typical features of the lines of sunflower. Typical level, which was determined by a molecular method – by comparison of protein spectra, showed typical features of the male analogue line – 44.0%; fixer of sterility of 47.0%. The decrease in genetic purity of the line caused the emergence of atypical features for the line of allelic options on loci *Hel-1*, *Hel-4*, and substantially on *Hel-6*. The lack of control of typical features of the line at molecular level, maintenance of purity of the line only on morphological traits led to considerable decrease in its genetic purity. The

line use with such level of typical features in seed-production for receiving hybrids was already inadmissible.

For the increase of genetic purity of the line, the selection of individual plants separately both on morphological traits and on electrophoretic spectra of proteins with simultaneous control on morphological traits was made. After a four-year cycle of selection, the assessment of reproduction of plants of lines, the final assessment of efficiency of methods of selection by the assessment of plants on morphological traits by the method of field soil control in field conditions and at molecular level on typical features of proteins spectra was carried out. The selection of plants only on their morphological traits provided the increase of typical features of sterile analogue of the line to the level of 90.1%, while for the sterility fixer it was up to 89.8% (Table 1).

Tab.
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Table 1:

Level of typical features of the maternal line of sunflower of ZL95 on morphologicaltraits in different methods of selection of plants (%)

In the selection on morphological traits at sterile analogue of the line, the quantity of fertile plants ranging from 38.7% to 8.5% and atypical plants from 3.4% to 1.4% decreased. At fertile analogue of the line in such a way of selection, the absence of atypical plants on morphological traits was reached, while the decrease in the level of atypical plants on height was not reached. At fertile analogue the percent of atypical plants in height made 10.2%.

The assessment of plants in the method of field trial showed that in the selection of plants only on their morphological traits atypical plants on height and on morphological traits were observed. This method failed to fully reach a higher level of genetic purity of the ZL95 line (Figure 2).

Figure 2:

The crops of the maternal line of sunflower of ZL95 after selection of plants on morphological traits

In the initial assessment of plants of the line on electrophoretic spectra, a higher level of typical features of sterile analogue at molecular level was noted, while further at the line reproduction level it decreased. In the same way of selection of plants of the ZL95 line, the analysis electrophoretograms of sterile and fertile analogues of the ZL95 line showed differences by allelic options of electrophoretic spectra. Differences were noted on the fourth *Hel-4*, and sixth*Hel-6* electrophoretic spectra (Figure 3).

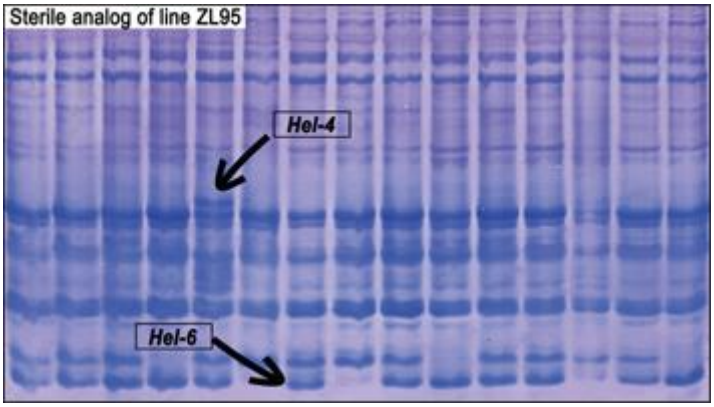

Figure 3:

Atypical allelic options of electrophoretic spectra of *Hel-4* and *Hel-6* of sterile analogue of the maternal line of sunflower of ZL95 at selection on morphological traits

Emergence of plants of sterile analogue with atypical allelic options on the fourth locus *Hel-4* is caused by the existence of such plants among plants of the most sterile analogue. Among plants of a fixer of sterility with atypical allelic options of the fourth locus *Hel-4* at molecular level, it was not discovered. Among plants of the fixer of sterility, atypical plants on the electrophoretic spectrum of *Hel-6* were noted at molecular level. In the selection on morphological traits among plants of a fixer of sterility, plants atypical on the sixth locus *Hel-6* were not excluded (Figure 4).

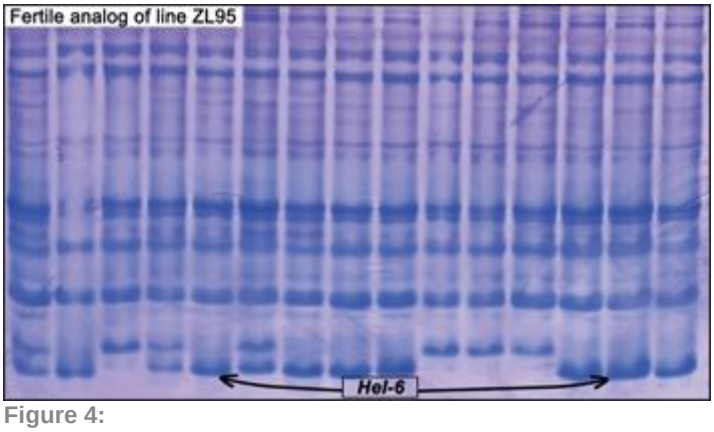

Figure 4:

Atypical allelic options of electrophoretic spectrum of *Hel-6* of fertile analogue of the maternal line of sunflower of ZL95 at selection on morphological traits

Individual comparison of plants for their morphological traits and protein ranges showed that the plants, available for differences in allelic options of electrophoretic spectra, were identical on a morphological sign.

These plants, during the reproduction of the line, led to the emergence of plants of sterile analogue which were fertile and atypical on the sixth locus *Hel-6*.

The selection of plants of the ZL95 line on electrophoretic spectra of proteins and with simultaneous control on morphological traits was more effective.

This method of selection allowed to allocate and exclude the plants which in their morphological traits did not differ from plants of the ZL95 line and at the same time had distinctions on the sixth electrophoretic spectrum of *Hel-6*. This method of selection will reach the highest level of genetic purity of the line. The analysis of electrophoretograms showed the identity of allelic options on all electrophoretic spectra of storage proteins of seeds. After carrying out the selection and reproduction of distinctions in allelic options of protein spectra of sterile analogue and a fixer of sterility of the line, it is not established (Figures 5 and 6).

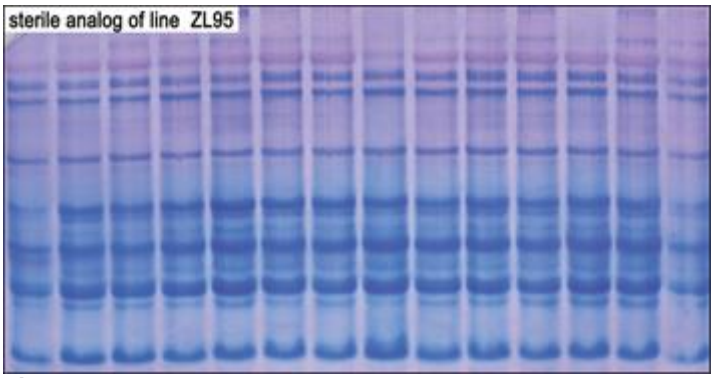

Figure 5:

Electrophoretic spectra of helianthin of seeds of the line of sunflower of ZL95 at selection of plants on protein spectra and morphological traits. All electrophoretic spectra of proteins are typical

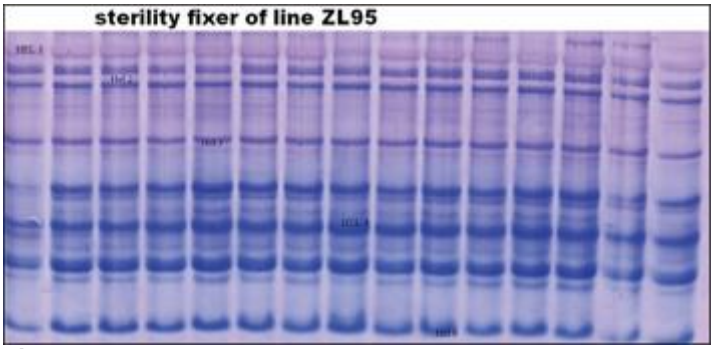

Figure 6:

Typical allelic options of electrophoretic spectra of fertile analogue of the maternal line of sunflower of ZL95 at selection on protein spectra and morphological traits

The assessment of plants on their morphological traits in the field trial was confirmed with a method of efficiency of selection on protein markers. The level of typical features of sterile analogue was 98.4%, while at a sterility fixer it was 98.5% (Figure 7).

At the same time, in such individual selection on protein markers during the field trial, the emergence of atypical plants and their morphological traits in sterile analogue 0.5%, on height 1.1%, was observed. At fertile analogue the emergence of atypical plants on morphological traits at the level of 1.5% was noted. It was explained by a small amount of electrophoretic spectra of storage proteins of seeds on which the selection of plants of sunflower and control at the molecular level is carried out in their reproduction.

Figure 7:

The crops of the maternal line of sunflower of ZL95 after selection of plants on protein spectra and morphological traits

In the option when the selection on electrophoretic spectra was carried out at an initial stage, and further control during the reproduction of seeds of the line was carried out only on morphological traits, a high level of genetic purity was reached also at the beginning. Originally the level of typical features of sterile analogue and a fixer of sterility of the line equalled 98.5%. However, further during the reproduction of the line and selection of plants only on their morphological traits the level of typical features of sterile analogue decreased to 92.9%, while a sterility fixer to 95.2%. During the assessment of plants the method of field trial noted the increase in atypical plants on height. The emergence of atypical plants on height led to decrease in typical features by 7.1% at sterile analogue and 4.8% at a fixer of sterility of the line. However, the level of typical features in this third way of selection showed, according to the electrophoresis method (by comparison of electrophoretic spectra), that the level of typical features of the line makes only 292%. Distinctions in allelic options of protein

spectra were recorded on the sixth locus of *Hel-6*. It was noted that plants were identical in morphological traits, but various in electrophoretic spectra.

It is possible to assume that when selection during reproduction of the line is made only on morphological traits without control at the molecular level, there is a genetic contamination the plants having differences in the sixth locus. In the assessment and selection of plants on morphological traits in the line reproduction, those plants joined in further reproduction. Finally, it led to decrease in genetic uniformity of the line at the molecular level, and simultaneously a real overestimation of typical features of plants in the assessment only on morphological traits.

The analysis of genetic uniformity of the ZL95 line in different ways of the assessment and selection of plants shows a possibility of increasing the genetic purity and its maintenance at high level by means of selection applied at the molecular level with a simultaneous control of morphological traits.

Conclusions

The most effective way of increasing and maintaining the genetic purity of parental lines of sunflower is the assessment and individual selection of plants on electrophoretic spectra of storage proteins of seeds with a simultaneous assessment on their morphological traits. This way of selection provided a level of typical features of the maternal line of sunflower of ZL95 during reproduction by applying a method field trial which was 98.4% (sterile analogue), 98.5% (a sterility fixer) and the assessment on protein spectra 100% at both analogues of the line.

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