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THE NEW WAY OF EVALUATING THE PROTEIN POLYMORPHISM FOR MAIZE BREEDING AND SEED PRODUCTION

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Abstract

The paper proposes to discuss the possibility of a logical connection between the existing methods of digital and visual documentation of protein electrophoretic spectra (PES). Therefore, the aim of this work was to develop a methodological approach to the study of zein polymorphism (ZP) as a basis for assessing the hybridity degree of corn seeds and to determine the effect of heterosis level using protein molecules. The method of electrophoresis in gel polyacrylamide was used to study ZP of 49 maize genotypes (hybrids and their parent forms) with the following calculation of their PES formulas. It's known that during the identifying of hybridity using the marker zones in protein profiles arises the personalization item of visual estimation of the maize heterosis effect. Therefore, it is necessary to improve the accuracy of the PE documentation. So, it is suggested to characterize every component of the genotype protein track by using the number of zein molecules subunits (ZMS), each of which is equal to 0,1 rf. This technique has been used as a basis for the development of software "FOREZ" for computer modeling, storage, and for the synthesis of hybrid PES from parental forms PES on the basis of codominance principle. As a result of its use it is possible to create the corresponding matrix of the analyzed electrophoregram. It was found that the quantitative analysis of ZMS using PE matrices greatly increases the methodological possibility of identifying the hibridity with PE markers: the number of homogeneous peptide subunits is increased by 55% for lines; 63% - for the simple parental hybrids and 68% - for zoned hybrids. Thus the proposed methodological modification extends the study of heterosis effect using protein profiles.

Key words*: maize, protein, zein, hybridity, electrophoregram.*

INTRODUCTION

Traditional methods to help the high quality seeds production of maize hybrids for the purpose of their further commercialization is the method of soil-control. Soil-control is carried out in the field conditions on the 2nd vegetation season of plants grown from hybrid maize seeds of the first generation. Exactly these hybrid seeds of the first generation is estimated in accordance with the requirements of the International Union of the Protection of New Varieties of Plants - UPOV (www.upov.int) using the large complex of morphological traits (from 40 to 60 or more). However, the morphological traits are the manifestation result of the interaction between genotype and the environment.

Thus became necessary to overcome these difficulties. Therefore, in the 80th years of the last century an alternative method has been proposed: the method of the maize genome marking at the level of protein molecules by electrophoresis - along with other cultures (Comarova, 1998; Rotari, 2011).

The protein electrophoresis method, compared to the traditional field methods of soil-control is more effective to assess the hybridity level of corn seeds (Rotari et al., 2008): by saving time (one growing season) and due to the higher accuracy (proteins are the primary product of the gene activity).

Zein electrophoresis method used to determine the hybridity level of hybrid seed maize from the first generation is based on the following two principles:

1) the use of specific properties of the protein prolamin fraction in maize endosperm – the polymorphism of zein;

2) the use of codominance effect, i.e. the phenomenon of the simultaneous presence in the zein (storage protein) electrophoretic spectrum (ZES) of hybrid F_1 seeds the amount of peptide subunits that characterize the maternal and paternal parent forms of the estimated hybrid ("hybrid" spectrum).

Accordingly, at the level of protein molecules, the hybridity degree determination of comercial F_1 seeds is doing by using protein subunits

markers namely paternal forms of the estimated hybrid combinations. At the same time, evaluation of the heterosis effect degree in the first generation (F_1) should be carried out using the zein molecular forms (ZMF) from both parental forms (Rotari et al., 2004).

Consequently, at a higher zein polymorphism value of studied genotype the possibilities of selection and genetic analysis at the protein level are increasing for such important indicators as maize hybridity degree and value of the heterosis effect (Palii et al., 2005; Comarova et al., 2011).

There was typical to carry out the visual discussion of the ZES in the period 60 -80-ies of the last century. However, this method was limited and uninformative. At present, the interpretation of zein polymorphism is based on digital ZES documentation and analysis with using a variety of techniques and methods (Comarova et al., 2012).

In the present article, we propose to discuss possibilities of logical connection between the existing methods of digital and visual documentation of zein electrophoretic spectrum (ZES) in order to develop a new way for evaluating zein polymorphism as a basis of more precise definition of the seeds hybridity degree and heterosis effect by using protein markers.

MATERIALS AND METHODS

As biological material was used maize endosperm of 49 genotypes, namely: 12 commercialized hybrids and 37 parent forms (11 simple hybrids and 26 lines). ZMF study was conducted by electrophoresis on polyacrylamide gel in the acidic buffer in the presence of urea (Rotari et al., 2003). ZES formulas calculation of maize hybrids and their parental forms were performed according the method of Konarev et al.(1987). The scheme design of initial ZES was performed using standard computer programs EXCEL and PAINT. Drawing up of zein profile matrices of studied genotypes was performed using the software «FOREZ», developed by A. Adamchuk through the technical idea proposed by G.Comarova (Comarova et al., 2003).

RESULTS AND DISCUSSIONS

By zein electrophoretic spectrum (ZES) of the studied genotypes can be stated that the number of zein electrophoretic zones (bands) vary from 6 to 11. However, the electrophoretic zones have different sizes, i.e. peptide subunit of zein are heterogeneous that is experimentally provable on ranges of variation of the relative electrophoretic mobility (rf) each of these zones. For example, one of the most stable zein electrophoretic zones (ZEZ) is a zone with rf (55-60) almost at every investigated genotype and to a lesser degree - the zone with rf (38-40) and others.

The encountered difficulty in interpreting is especially clearly manifested in the process of identifying ZEZ marker of studied hybrid - by comparing ZEZ of its parental forms. During scrupulous comparison of protein profiles of maternal and paternal forms for the majority of hybrid combinations should be noted that in most cases of labeling hybridity it is necessary to carry out by the fused composite components of widely zein electrophoretic zones.

Thus, the detection of marker zones of hybridity in protein profiles has an personalization element of professional analyst who carries out a visual assessment of the analyzed hybridity degree for the corresponding commercial seed lots of hybrid maize.

Therefore, to improve the accuracy of documenting the results of protein electrophoresis in order to standardize their interpretations G. Comarova (2003) proposed to characterize each component of the protein electrophoretic spectrum of the corresponding genotype by the number of zein molecules subunits (hereinafter referred to as molecular forms of zein -) for each of them the relative electrophoretic mobility (rf) is equal to 0.1.

This approach is considered as a methodological principle, which is the basis of development of software "FOREZ", designed for computer modeling, storage, as well as for the synthesis of hybrid electrophoretic spectra from the electrophoretic spectra of parental forms by using the principle of codominance.

As a result of computer modeling of input formulas derived by calculating the rf ZEZ values, we obtain the matrix of analyzed electrophoregrams, consisting of corresponding zein molecular forms (ZMF), for each of them the relative electrophoretic mobility (rf) is equal to 0.1.

For example, the protein profile of the line F2mC (Figure 1, EF) consists of 9 zein electrophoretic zones (ZEZ), which differ not only by the electrophoretic mobility, but also on

size of the interval for each ZEZ (Figure 1, rf). If it is used the other methodological principle which underlies the program "Forez" to calculate the electrophoretic matrix, then the matrix of ZES for line F2mC is characterized by the 21st of ZMF (Figure 1, M).

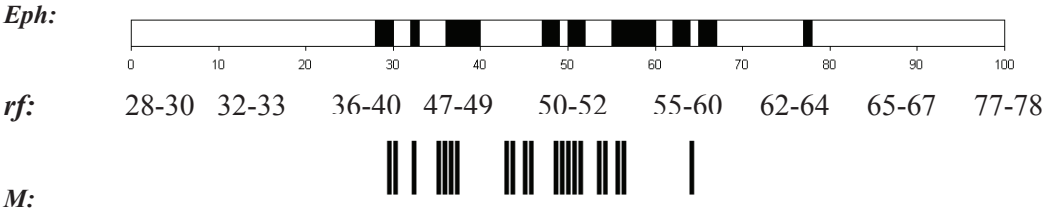

Figure 1. Comparison of the ways used to processing the results of electrophoretic zein analysis for the line F2 mC.
Symbols: ***Eph*** - scheme of the original zein electrophoregram of the studied line F2 mC ; ***rf*** - the calculation formula by rf, corresponding to the resulting electrophoretic spectrum; ***M*** - the matrix of protein profile for the line F2 mC (one unit of rf=0.1).

Analogous analysis of electrophoretic passports conducted for the all studied maize genotypes allowed to compare the level of zein polymorphism manifestation depending on the technique used for the documentation and analysis of the received electrophoregrams.

It was found that the range of zein electrophoretic zones variation for all studied initial zein electrophoregrams from the endosperm of the studied genotypes (lines, simple parent hybrids or regionalized hybrids) is quite small and limited: from 6 to 12 ZEZ.

Conversely, the zein polymorphism on the matrix of the zein electrophoregrams has a higher level and a wider variation range.

So for the lines - the total number of zein molecular forms (ZMF) varies from 14 to 24; for parental simple hybrids their level increases and varies from 21 to 27 ZMF and for regionalized hybrids the zein polymorphism is even higher: from 23 to 33 ZMF.

Therefore, quantitative analysis of ZMF by electrophoretic matrixes greatly increases the methodological ability to identify electrophoretic markers of hybridity: the number of homogeneous peptide subunits is increased by 55% for lines; 63% - for the simple parental hybrids and 68% - for regionalized hybrids.

CONCLUSIONS

As a result the study of zein polymorphism in 49 maize genotypes by various methods of a digital and visual documentation were formulated recommendations for the use of these methods and techniques of processing results of zein electrophoretic analysis in the following order:

1. production of the starting electrophoregrams with colored zein electrophoretic zones (ZEZ);
2. carrying out of one type of intermediate documents: densitometry, or photographing, or conservation the plates;
3. measuring the relative electrophoretic mobility *(rf)* each of the colored electrophoretic zones characterizing the electrophoretic protein profile of the corresponding studied genotypes;
4. the calculation formulas by *rf*, corresponding to the electrophoretic spectrum of zein endosperm hybrids and their parental forms;
5 - design the scheme of the original zein electrophoretogram using standard computer programs EXEL and PAINT, if it is necessary to carry out an official electrophoretic certification of studied genotype;
6. using the software "FOREZ", that allows to carry out:

- the compilation of a databank on the protein profiles of the parental forms;
- the computer modeling for the synthesis of hybrid electrophoretic spectra from the electrophoretic spectra of parental forms by using the principle of codominance;
- the comparison of the hybrid synthesized zein electrophoretic spectrum (ZES) with calculation formulas and visual documentation of hybrid ZMF by the original hybrid ZES;
- the identification of marker zones hybridity based on analysis the computer matrixes of zein electrophoregrams;

- the assessment of the heterosis effect manifestation by ZES of hybrids: a quantitative analysis the total and marker zones of ZMF.

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