

THE SECTION I

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ALGORITHM FOR CREATING ELECTROPHORETIC PASSPORTS OF MAIZE HYBRIDS

ADAMCIUC A., BATIRU G., COMAROVA Galina, BOUNEGRU S., ROTARI E.
State Agrarian University of Moldova, Chisinau, Republic of Moldova
e-mail: grigore.batiru@gmail.com

One of the topical issues of seed production in the Republic of Moldova is the timely export of hybrid maize seeds and ensuring their quality. In this regard, it is necessary to involve the latest methods of express diagnostics of hybrid purity of these seeds and their passportization in order to be able to carry out their objective and adequate certification. The project “*Creating the catalog of electrophoretic passports of parental forms and maize hybrids approved in the Republic of Moldova for export*” currently being developed under State Program 2020-2023, provides for the effective use of software for storing and synthesizing electrophoretic spectra based on the codominance principle.

Therefore, the purpose of this work was to create a new version of the FOREZ-2 Program adapted to the modern level of operating systems and new requirements for the creation of model electrophoretic passports of storage proteins of maize hybrids and their parental forms.

For the required modifications, the FOREZ program, developed in 2003 to work in the Windows XP environment, was used in the initial program database for the required modifications.

New generation maize hybrids and their parental forms, selected according to the principle of the greatest commercial demand in the Republic of Moldova and abroad, were used to test the newly created modified version of the FOREZ-2 Program. Sampling was carried out in accordance with the recommendations of ISO 9001 and a number of classical sampling methods that ensure reproducibility and reliability.

The method of electrophoresis in polyacrylamide gel (in accordance with the national standard SM-2003) was used to obtain electrophoretic spectra of the maize seeds storage protein (zein). Stained gels with electrophoretic (EP) tracks formed the basis for parameterization of the obtained peptide subunits spectra.

Formula calculation of the obtained electrophoretic spectra was carried out on the basis of determining the value of the relative electrophoretic mobility (r_f), which was calculated according to the “internal standard” unified by the EF component with $r_{f_{st}}=60$.

When compiling the formulas of the analyzed electrophoretic spectra, the following was performed:

- 1) binarization of intensity: the present band - designation (1) or absent band-designation (0);
- 2) determination of the band (peptide subunits) borders: rf_{in} & rf_{fin}
- 3) formation of a text file with a list of EF band boundaries [$rf_{in} - rf_{fin}$] for each genotype.

The new version of the FOREZ-2 Program is designed to work on the Windows 10 operating system in both 32-bit and 64-bit versions. To create the program, the Visual Studio 2019 software development environment was installed and configured. The developed version of the FOREZ-2 software and its verification testing on 27 maize genotypes demonstrated the ability to process binary electrophoretic spectra of low resolution (1 - 0.5 mm) and high resolution (band detection accuracy of 0.1 mm) according to the following algorithm:

Step 1. Sequential input of the names of maize initial parental lines, corresponding to the certified hybrid, in the category “*Parent forms*”, with the subsequent possibility of editing and deleting (if necessary).

Step 2. Introduction of the range of indicators “relative electrophoretic mobility - (rf)” of each of the peptide subunits that make up the electrophoretic spectrum of the maize parent line. It is provided for the possibility of editing or, if necessary, deleting erroneous information.

Step 3. Entering the name of the maize hybrid and its formula, using its parental forms entered earlier, in the category “*Hybrids*”.

Step 4. Synthesis of electrophoretic spectra of single cross, single modified cross, three-way cross, double cross hybrids. Automatic assembly of these hybrid spectra is carried out from the spectra available in the database, collected according to the «FOREZ-2» Program algorithm (steps 1-2).

Step 5. Creation of the final computer matrices, in graphical and tabular forms, as a basis for further development of maize hybrids and their parental forms passports with indicating hybridity protein markers

Thus, the step-by-step execution of the presented algorithm makes it possible to automatically identify on the obtained computer matrices:

- a) quantitative specifics of zein polymorphism for each studied genotype used in heterosis maize breeding;
- b) quantitative specifics of marking by binary intensity of molecular forms of zein of maize hybrids certified by protein electrophoresis.

In order to expand the effectiveness of using the «FOREZ-2» Software for creating electrophoretic passports of certified hybrids, there was also introduced a new processing element for the created database according to the «FOREZ-2» Program,

namely: a digital indicator of the «area/width» for every zone of peptide subunits obtained on electrophoregrams.

The specificity of the digital expression of this indicator is that each analyzed electrophoretic spectrum is binarized: along the common axis of the analyzed EF spectrum (conventionally denoted by the total rf length of 100 mm and the intensity of any zone equal to 1), there are zones of peptide subunits (ZPS), which are present a set of different number of bands (molecular forms of zein - MFZ). Therefore, the area index (S_{rf}) coincides with the specific rf (width) range for the respective area under assessment, denoted by the difference between rf_{in} (upper limit of the ZPS) and rf_{fin} (lower limit of the ZPS). Therefore, the area of each analyzed EF zone (S_{rf}) can be expressed in mm: $S_{rf} = [(rf_{in} - rf_{fin}) \text{ in mm}]$.

The new version of the «FOREZ-2» Program made possible to effectively use the discussed indicator to automatically determine:

- the «total area» of the entire set of electrophoretic zones characterizing the protein profile of the studied sample;

- the «area» of each discussed marker zone and «area» of their total set for the corresponding EF spectrum;

- the «area percentage » of marker molecular forms of zein (MFZ) of the total area of the MFZ of the electrophoretic hybrid protein profile, which expands the possibilities for express diagnostics of the hybridity level of certified hybrid maize seeds lots.

Thus, the developed algorithm for the creating of electrophoretic passports based on the modified version of the «FOREZ-2» Program should be considered as a successful experimental tool for modeling the electrophoretic passports of storage proteins in maize hybrids and their parental forms.

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