SECTION: BIOLOGY SCIENCE

УДК 577.21: 575.224.234.2

Batiru Grigorii, Comarova Galina, Rotari Eugen*, Cojocari Dumitru,
Bounegru Serghei, Adamciuc Arcadi
State Agrarian University of Moldova
(Chisinau, Moldova),
***Institute of Crop Science „Porumbeni"**
(v. Pascani, Moldova)

IMPACT OF COLCHIPLOIDIZATION ON POLYMORPHISM OF STORAGE PROTEIN IN ENDOSPERM OF MAIZE LINES

Abstract. *The paper aims at exploring the experimental possibilities of colchiploidization to obtain tetraploid forms from maize inbred lines in order to study the polymorphism of the zein – the prolamine fraction of the maize endosperm protein. It was revealed specificity of the zein fraction polymorphism of tetraploid lines endosperm. The existence of possible relationships between the degree of zein peptide subunits polymorphism in the zones of middle and fast migration in the EF spectra of the genotypes selected for polyploidization and their sensitivity to colchiploidization, are discussed.*

Keywords: *maize, colchiploidy, diploid, tetraploid, zein polymorphism.*

INTRODUCTION

The success of the selection process is in many cases due to hybridization, mutagenesis, and the creation of polyploid forms [1]. The argumentation of the legitimacy of the formulated postulate can be quite clearly demonstrated on *Zea mays* L. - one of the leading agricultural crops of the Republic of Moldova. The studies of the last five decades, vividly illustrate the effectiveness of using endosperm genes (*o2, fl2, su2, wx1*, etc) in the heterosis breeding of maize, which improve the quality of maize grain. The following hybrids of improved quality maize were created and released from 1980 to 2012: Moldovan 423 VL, Chisinau 307 VL, Chisinau 401 VL, Chisinau 297 wx1, Chisinau 333 wx1, Chisinau 403 wx1 [2].

These high-lysine hybrids were obtained at the Department of Breeding, Genetics and Biotechnology of Agricultural Crops of the State Agrarian University of Moldova. These hybrids were the optimal model for the development of a new research direction related to the production of maize tetraploid forms containing the opaque2 (*o2*) gene in their genotype. The selected genomes of maize hybrids with the *o2* gene (in particular, maize hybrids Chisinau 307 PL and Chisinau 401 L) were a good genetic substrate for colchicine treatment and, accordingly, successful production of the first forms of tetraploid maize with the *o2* gene in the Republic of Moldova. It was on these polyploidized heterozygous forms that evidence was obtained for the possibility of increasing the content of protein and lysine in maize grain by combining genomic (polyploidy) and genic (opaque-2 gene) variability [3, 4]. Thus, a new reserve for expanding the spectrum of genetic variability in terms of protein metabolites of maize grain quality was experimentally demonstrated. On a representative sample of maize hybrids of Moldovan

selection, as well as a number of synthetic populations with the *o2* gene, the possibility of targeted and successful induction of genome mutations in tetraploid maize was experimentally proven [4]. Various tetraploid populations with the *o2* gene demonstrated an increase in the content of crude protein by 1–3% (in absolute values), and an increase in the content of lysine in maize protein by 7–12% (in relative values) [5]. Undoubtedly, the results of the studies carried out under the project during 2015-2018, made it possible to deepen knowledge about the effect of polyploidy on the phenotype and expression of specific genes in grain for the end products of protein metabolism at the heterozygous level [3].

However, it is necessary to develop methodological principles for the purposeful creation of new tetraploid homozygous forms of maize as a model for studying the effect and interaction of genes and genomic mutations. Thus, it is possible to continue intensifying the process of breeding for heterosis by using the original maize lines that combine gene and genome mutations. Such a broad program provides a step-by-step algorithm of actions that reveal the versatility and logical sequence of experiments.

The purpose of this work was to explore the experimental possibilities of colchiploidization to obtain tetraploid forms from maize inbred lines in order to study the polymorphism of the prolamine fraction of the maize endosperm protein (zein).

MATERIALS AND METHODS

The work was carried out in the Department of Agronomy and Environment of the Faculty of Agronomy of the State Agrarian University in cooperation with the Institute of Crop Science "Porumbeni" and the Central Phytosanitary Laboratory of the Republic of Moldova. 17 homozygous maize genotypes were used for *colchiploidization* (polyploidization by colchicine) starting from 2017. Each genotype was represented by a monogenic endosperm mutation and its normal isogenic line (+).

Colchicine treatment was carried out according to the method of V. S. Shcherbakov and M. I. Khadzhinov [6] modified by G. Batiru [4, 7]. To implement the task of studying protein polymorphism, the induced tetraploid forms were analyzed by comparing with the original diploid forms using the method of polyacrylamide gel electrophoresis in an acidic environment [8].

The calculation of the formulas of the obtained electrophoretic (EF) spectra, the compilation of EF matrixes of protein profiles for the studied genotypes and their digital processing were carried out using the FOREZ-2 software [9]. The technology for automatic synthesis of hybrid EF spectra of two compared protein profiles of zein was also used [4]. This procedure was carried out in order to identify the enriching and eliminating effect of colchiploidization in maize at the level of protein molecules.

RESULTS AND DISCUSSION

Despite careful manipulations during the experimental polyploidization of maize lines during several years of experiments (2017-2020), a low efficiency of colchiploidization of this homozygous material was observed. Thus, out of 17 homozygous maize genotypes, each of which represented by a monogenic endosperm mutation and its normal isoline (i.e., colchicine was carried out annually on 32-34 homozygous forms), only 5 genotypes (15% of the total number of colchicine genotypes) produced fertile offspring: lines C05*o2*, MK 131+, SL 343+, C81+ and its mutant high-lysine analogue - C81*o2*.

It is these forms that were used for a comparative analysis of the polymorphism of the *molecular forms of zein* (MFZ) of the diploid and tetraploid endosperm of each line.

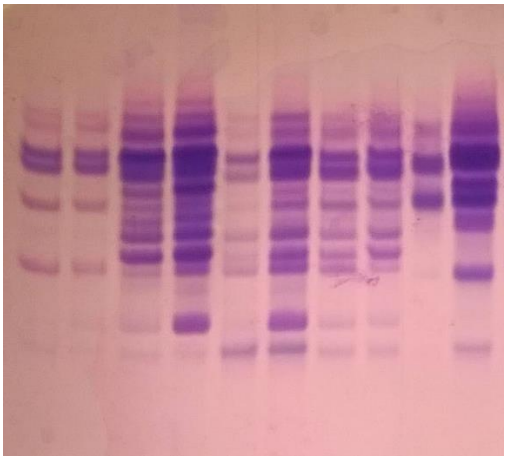


Fig.1. Electrophoretic spectra of endosperm zein of diploid and tetraploid maize lines: C05 *o2* (1 – 2x; 2 – 4x); C81+ (3 – 2x; 4 – 4x); C81*o2* (5 – 2x; 6– 4x); MK131 + (7 – 2x; 8 – 4x); SL343 + (9 – 2x; 10 – 4x).

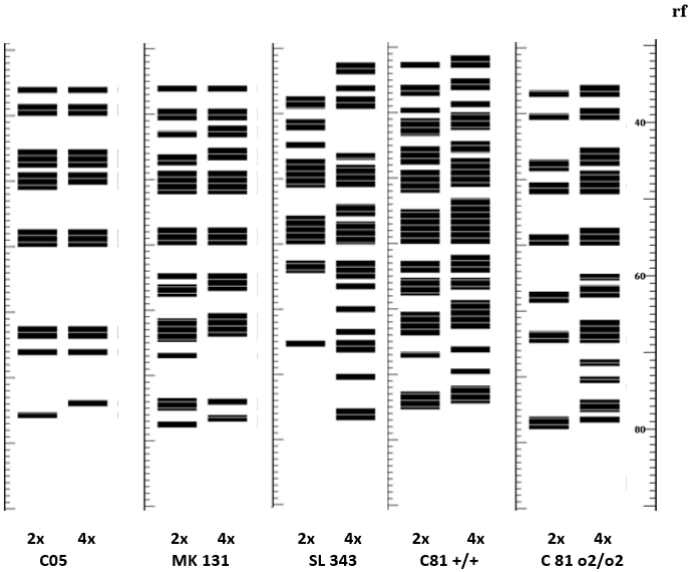


Fig.2. Matrix of electrophoretic profiles of endosperm zein of diploid (2x) and tetraploid (4x) maize lines.

Figure 1 shows the initial polyacrylamide electrophoregram of the entire set of zein spectra of the studied homozygous forms of maize.

Visual analysis of the obtained protein profiles indicates a wide range of polymorphism of MFZ in the studied diploid and tetraploid maize lines.

Subsequent computer processing of the calculation formulas of the discussed EF spectra using the modified FOREZ-2 program made it possible to obtain the corresponding electrophoretic matrices (Fig. 2). On its base, it was possible to directly compare the quantitative characteristics of the zein spectra according to the MFZ.

As the diagrams in figure 3 show, colchicine treatment of homozygous forms is not an absolute determinant of the quantitative increase in peptide subunits of zein and the obtained tetraploid maize lines. For each of the studied homozygous lines, a specific reaction of the genotype to colchiploidization is followed.

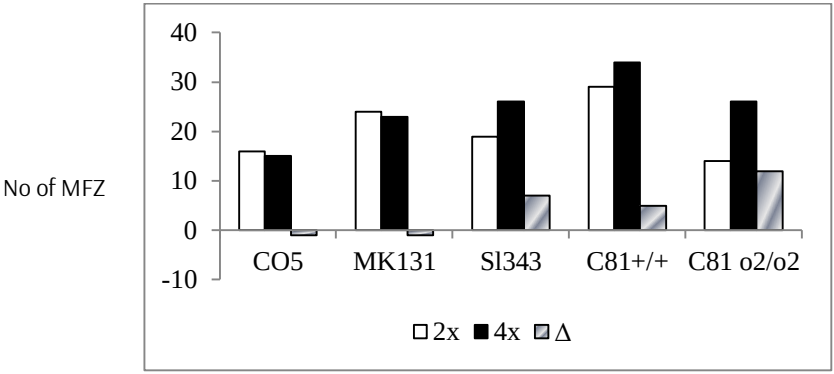


Fig.3. Zein polymorphism specificity (according to MFZ) in diploid and tetraploid maize lines

The conditional division of the analyzed EF tracks into four zones of migration of zein molecular forms according to their relative electrophoretic mobility (rf) reveals a tendency for the manifestation of maximum zein polymorphism in the zone of average migration of MFZ (0.4<rf<0.6) with a subsequent decrease in the amount of MFZ in the zone of fast migration (0.6<rf<08). However, a direct comparison of the quantitative values of peptide subunits does not clearly identify *marker molecular forms of zein* (mMFZ) in tetraploid maize lines (Table 1).

Table 1

Quantitative characterization of the zones of migration of molecular forms of zein (MFZ) in the protein EF profiles of the prolamine fraction of the endosperm of diploid and tetraploid maize lines.

Genotype	Number of molecular forms of zein (MFZ)									
	By general EF track		Slow Migration Zone (SMZ) rf<0,4		Middle Migration Zone (MMZ) 0,4<rf<0,6		Fast Migration Zone (FMZ) 0,6<rf<08		Ultrafast Migration Zone (UfMZ) rf>0,8	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
C05 *o2*	16	15	3	3	9	8	3	3	1	1

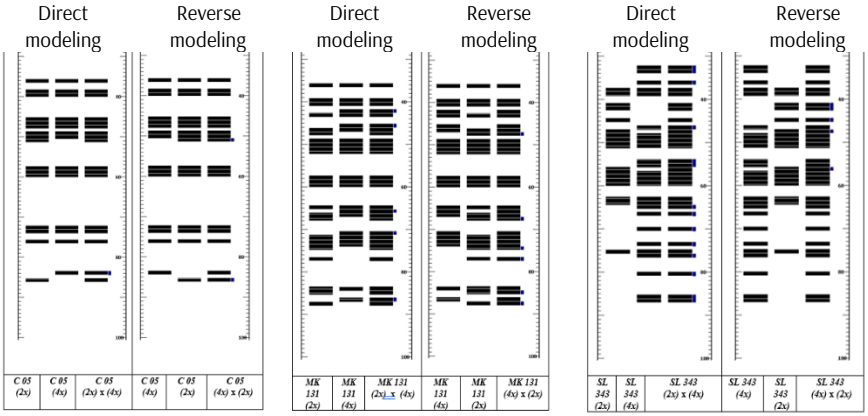
Issue 8(88) ISSN 2524-0986

MK 131 +	24	23	2	2	11	12	8	7	3	2
SL 343 +	19	26	2	5	13	10	4	8	0	3
C81 +	29	34	4	5	14	16	9	11	2	3
C81 *o2*	14	26	1	2	7	10	4	9	2	3
$\bar{X}$	20	25	2	3	11	11	6	8	2	2

Therefore, for the subsequent comparative interpretation of the specificity of zein polymorphism in diploid (2x) and tetraploid (4x) homozygous maize forms, the principle of direct and inverse modeling of the calculation formulas of two compared EF spectra was used: 2x and 4x for each of the analyzed lines. A detailed description of the methodology for automatic synthesis of two compared zein EF profiles is presented in the article by Komarova et al. [10] and extrapolated for the design of the experiment on the direct and reverse modeling of mMFZ, which allow discussing the enriching and eliminating effect of colchiploidization.

In *direct modeling*, as a result of an automatic combination of formulas of the diploid and tetraploid lines of the corresponding genotype, according to the principle of codominance, an EF matrix is obtained, on which markers of the enriching effect of colchiploidization on the prolamine fraction of the protein (zein) of the maize endosperm are indicated. Those MFZ that appear in the protein molecule under the influence of colchiploidy are automatically indicated.

In *reverse modeling*, as a result of an automatic combination of formulas of 4x and 2x genotypes, an EF matrix is synthesized according to the principle of codominance, on which markers of the elimination effect on the prolamine fraction of the protein (zein) of the maize endosperm are indicated. Those molecular forms of zein that disappear are automatically indicated, probably as a result of structural modifications of the eliminated molecular forms of zein, as a result of colchiploidy.



C05 *o2* MK 131 + SL 343 +

Issue 8(88) ISSN 2524-0986

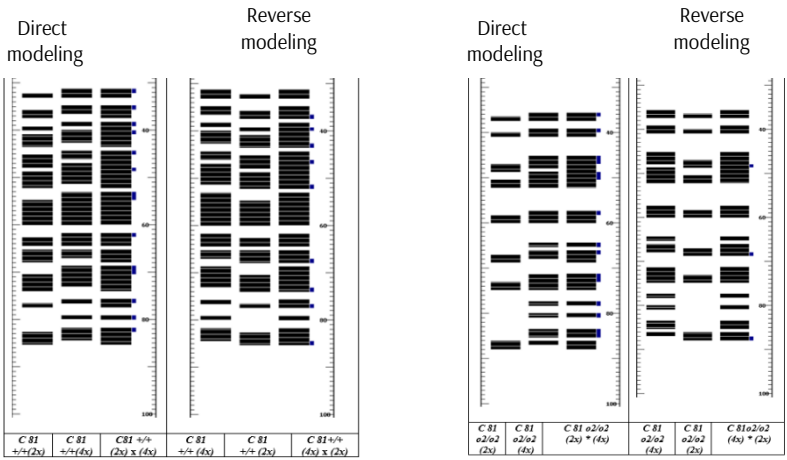


Fig.4. Genotypic diversity of diploid and tetraploid maize lines by marker molecular forms of zein (mMFZ) based on reciprocal combinations of zein EF spectrum matrices using the codominance principle.

In accordance with the above methodological technique, express marking of all five tetraploid lines by MFZ was carried out.

Figure 4 presents all the information about the genotypic diversity of protein markers in the endosperm of diploid (2x) and tetraploid (4x) maize lines based on the reciprocal combination of matrices of zein electrophoretic spectra.

Thus, it was possible to conduct a quantitative analysis of the enriching and eliminating effect of colchiploidization on the zein prolamine fraction.

For a more in-depth interpretation of the results obtained, new processing elements in the FOREZ 2 software were used, in addition to the quantitative determination of marker zones of molecular forms of zein. The new FOREZ-2 software introduces automatic data processing to determine the area of each marker zone [(rf_{in} – rf_{fin}) in mm]. This area is characterized along the single and common axis of the analyzed EF spectrum (conventionally denoted by the total *rf* length of 100 mm and the intensity of any band is equal to unity), a specific range of *rf* for the corresponding marker zone, denoted by the difference between rf_{in} (upper limit of the marker zone) and rf_{fin} (lower limit of the marker zone). Therefore, the area of each marker zone (S*rf*) is expressed in millimeters.

The FOREZ-2 program also makes it possible to automatically determine, from the relative EF mobility (rf), the total area of the entire set of MFZ that characterizes the corresponding EF spectrum of the studied sample [9].

Table 2

Influence of colchiploidy on the composition of peptide electrophoretic subunits of zein in maize lines according to the number of marker molecular forms of zein (mMFZ)

Line	By general automatically synthesized EF track (2x vs. 4x) 0,0<rf<1,0		By electrophoretic migration zones (rf range)							
			Slow Migration Zone (SMZ) rf<0,4		Middle migration zone (MMZ) 0,4<rf<0,6		Fast Migration Zone (FMZ) 0,6<rf<0,8		Ultrafast Migration Zone (UfMZ) rf>0,8	
	*Enr effect	**Elim effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect
C05 *o2*	1	2	-	-	-	1	-	-	1	1
MK 131 +	5	6	-	-	2	1	2	3	1	2
SL 343 +	11	4	2	-	2	4	5	-	2	-
C81 +	12	9	3	2	4	3	4	3	1	1
C81 *o2*	11	3	2	-	3	1	4	1	2	1

* Enriching effect; ** Elimination effect.

Table 3

Effect of colchiploidy on the composition of peptide electrophoretic subunits of zein in maize lines by total area of mMFZ (in mm)

Line	By general automatically synthesized EF track (2x vs. 4x) 0,0<rf<1,0		By electrophoretic migration zones (rf range)							
			Slow Migration Zone (SMZ) rf<0,4		Middle migration zone (MMZ) 0,4<rf<0,6		Fast Migration Zone (FMZ) 0,6<rf<0,8		Ultrafast Migration Zone (UfMZ) rf>0,8	
	Enr. effect	Elim. effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect
C05 *o2*	1,0	1,7	-	-	-	0,8	-	-	1,0	0,9
MK 131 +	4,1	5,2	-	-	1,7	0,8	1,5	2,6	0,9	1,8
SL 343 +	13,0	4,3	2,7	-	2,7	4,3	4,8	-	2,8	-
C81 +	12,0	7,7	2,7	1,6	4,0	2,6	4,4	2,6	0,9	0,9
C81 *o2*	13,6	2,4	1,6	-	4,4	0,7	4,7	0,8	2,9	0,9

Tables 2-4 summarize the results of processing the obtained electrophoregrams using the FOREZ-2 program. Moreover, the analysis of the results obtained was carried out not only for the general automatically synthesized electrophoretic tracks, but also for the migration zones of the MFZ.

Table 4

Influence of colchiploidy on the composition of peptide electrophoretic subunits of zein of maize lines: according to the mMFZ area from the total area of subpeptide band of the automatically synthesized EP track (in %).

Line	By general automatically synthesized EF track (2x vs. 4x) 0,0<rf<1,0		By electrophoretic migration zones (rf range)							
			Slow Migration Zone (SMZ) rf<0,4		Middle migration zone (MMZ) 0,4<rf<0,6		Fast Migration Zone (FMZ) 0,6<rf<0,8		Ultrafast Migration Zone (UfMZ) rf>0,8	
	Enr. effect	Elim. effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect	Enr. effect	Elim. effect
C05 *o2*	6,4	10,8	-	-	-	5,1	-	-	6,4	5,7
MK 131 +	15,6	19,8	-	-	6,5	3,0	5,7	9,9	3,4	6,9
SL 343 +	44,4	14,7	9,2	-	9,2	14,7	16,4	-	9,6	-
C81 +	28,6	18,4	6,4	3,8	9,5	6,2	10,5	6,2	2,2	2,2
C81 *o2*	51,3	9,1	6,0	-	16,6	2,6	17,7	3,0	11,0	3,4
$\bar{X}$	29,3	14,6	4,4	0,8	8,4	6,3	10,1	3,8	6,5	3,6

Discussion of the results of the simulation for all three parameters used (Tables 2-4), made it possible to make the following observations:

- both the enriching and eliminating effect of colchicine on the general automatically synthesized EF tracks of reciprocal combinations of matrices of zein electrophoretic spectra was established;

- it was found that the levels of the enriching and eliminating effect of the colchiploidization are genotype specific;

- the predominant elimination effect of colchiploidization on the zein polymorphism of most of the studied lines (with the exception of the MK 131 line) was noted, namely: the enriching effect can exceed the elimination effect from 1.5 to 2 times depending on the genotype.

The analysis of the obtained results was carried out not only on the general automatically synthesized electrophoretic tracks, but also on the migration zones of mMFZ.

The generalization of data on individual genotypes confirms the specificity of the reaction of the used homozygous form of maize to the process of colchiploidization for the zein prolamine fraction. So, for line C05 a single electrophoretic marker was revealed, moreover, in the zone of ultrafast migration (rf>0.8). As evidenced by the long-term experience of the laboratory of biochemistry and physiology of maize of the Institute of Crop Science „Porumbeni" [9], the indicated frontal region of the EF track in most cases does not include zein molecular forms, but only the accompanying components of the prolamine fraction - amino sugars.

For the rest of the studied 4 tetraploid lines, there is a tendency of the predominant positive effect of colchiploidization on the prolamine fraction of the grain endosperm in the zones of middle (0.4<rf<0.6) and, especially, fast migration (0.6<rf<08) mMFZ. The elimination effect of colchiploidy in the protein profiles of zein EF spectra manifests itself (albeit in a

weakened form) primarily in the zone of middle migration, and then in the zone of fast migration of mMFZ.

These dependences can be especially clearly traced by the average values ($\bar{X}$) of the percentage of the area of mMFZ from the total area of mMFZ of automatically synthesized EF tracks (Table 2 and Fig. 5).

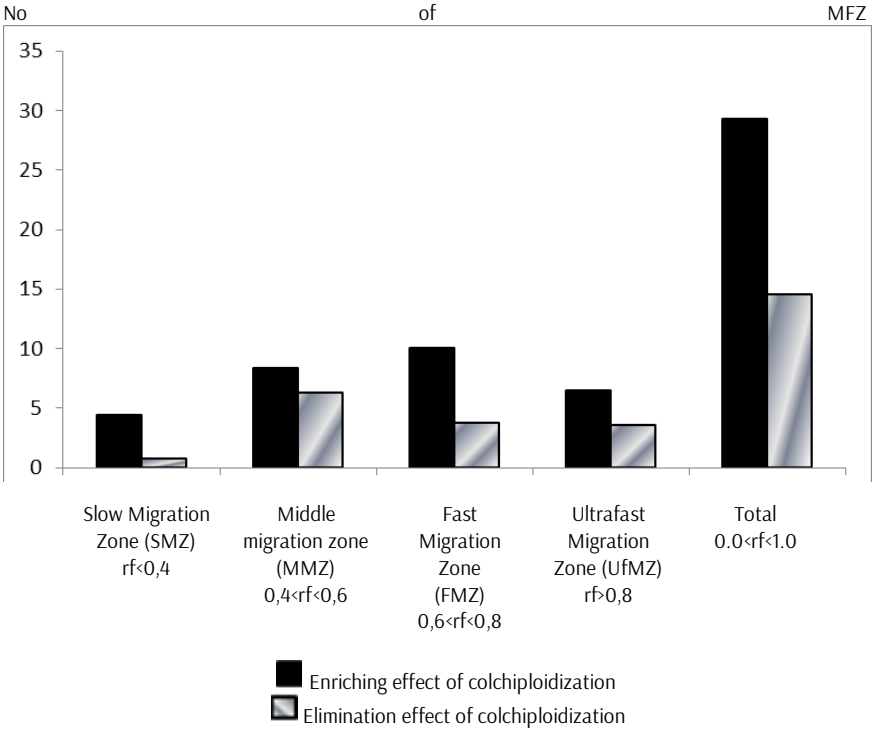


Fig.5. Enriching and eliminating effect of colchiploidization on marker MFZs (along the coordinate axis - averaged indicators - % of the area of MFZ from the total area of MFZ of automatically synthesized EF tracks).

Thus, summing up the analysis of the obtained results, it should be concluded that the revealed specificity of the polymorphism of the zein fraction of the endosperm of tetraploid lines indicates the existence of possible relationships between the degree of polymorphism of the peptide subunits of zein in the zones of middle and fast migration in the EF spectra of the genotypes selected for polyploidization and their sensitivity to colchiploidization.

CONCLUSIONS

1. Empirical screening of the presented sample of maize lines for sensitivity to colchiploidization made it possible to transfer only 15% of the total experimental sample of homozygous genotypes to the tetraploid level.

2. Direct electrophoretic analysis of the prolamine fraction of the endosperm protein in tetraploid maize lines indicates that the proportion of the influence of the genotype on the specifics of zein polymorphism is more significant compared to the proportion of the influence of the polyploidization induction factor.

3. It has been established that the use of protein markers of maize zein peptide subunits based on computer automation of electrophoretic data processing (using the FOREZ 2 computer program) makes it possible to identify both the enriching and eliminating effect of colchicine homozygous maize forms.

4. The trend of the predominant effect of colchicine on the prolamine fraction of grain endosperm in the zones of fast and middle migration of mMFZ was revealed. The elimination effect of colchiploidization in the protein profiles of zein EF spectra manifests itself in a weakened form in the reverse order: in the zone of middle and rapid migration of mMFZ.

5. For subsequent experimental verification, a working hypothesis was formulated on the existence of a possible relationship between the degree of polymorphism of zein peptide subunits in the zones of middle and fast migration in the EF spectra of the genotypes selected and their sensitivity to colchiploidization.

REFERENCES

1. Palii A. Ameliorarea plantelor. Chişinău: S.n., 2014, 216 p.
2. Palii A. Cercetări privind ameliorarea şi genetica porumbului efectuate în cadrul Universității Agrare de Stat din Moldova. Materialele conf. internaţ. "Institutul de Fitotehnie Porumbeni - 40 ani de activitate ştiințifică". Paşcani, 2014, pp. 59-69.
3. Batiru G. Palii A., Comarova G., Cojocari D. Polyploidy in maize breeding for grain quality. In: XI-th International Congress of Geneticists and Breeders from the Republic of Moldova: Abstract Book, (CEP USM), Chisinau 2021, p. 71.
4. Batîru G. Expresia genei opaque-2 (o2) la porumbul tetraploid. Autoreferatul tezei de doctor în ştiințe biologice. Chişinău, 2014, 27 p.
5. Palii A., Batîru G., Rotari E., Comarova G., Cojocari D. Studiul unor particularități la forme diploide şi tetraploide de porumb de origine locală. Materialele Simpozionului Ştiințific Internațional „85 ani ai facultății de agronomie – realizări şi perspective" dedicat aniversării a 85 ani de la fondarea Universității Agrare de Stat din Moldova. 2018, pp. 269-273.
6. Щербак В. С., Хаджинов М. И. Получение тетраплоидных форм кукурузы. В: Генетика, 1969, т.5, № 3, с. 5-11.
7. Batîru G., Palii A., Comarov G., Cojocari D., Rotari E. Analiza unor indici biochimici la populații de porumb tetraploid ce conțin gena opeique2. В сборнике iScience «Актуальные научные исследования в современном мире». Вып. 9 (29), ч.2, Переяслав-Хмельницкий, 2017, с. 39-43.

8. SM 233:2003. Determinarea purității biologice a liniilor consangvinizate şi a gradului de hibridare la semințele hibrizilor de porumb de prima generație prin metoda de electroforeză a proteinelor. Departamentul „Moldova-Standard" Chişinău, 2003, 27 p.
9. Adamciuc A., Batiru G., Comarova G., Bounegru S., Rotari E.. Algorithm for creating electrophoretic passports of maize hybrids. Materials of Scientific International Symposium "Advanced Biotechnologies - Achievements and Prospects". Chisinau, Republic of Moldova, 2022 (in print).
10. Comarova G., Batîru Gr., Rotari E., Rotari A., Bounegru S., Cojocari D. Modelarea sistemului de markeri în ameliorarea porumbului. Partea 2. Posibilitățile de marcare prin compoziția biochimică a bobului şi prin formele moleculare de zeină. Culegerea de articole "Realizări ştiințifice in ameliorare şi tehnologii inovative la culturile cerealiere în contextul schimbărilor climaterice". Paşcani, 2020, pp. 43-55.