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BIOCHEMICAL FEATURES OF *OPAQUE-2* GENE EXPRESSION IN THE TETRAPLOID MAIZE GRAINS

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Abstract

The purpose of the research was to study the expression of the opaque-2 (o2) gene in the tetraploid genome of maize by the biochemical characteristics determining the protein quality and the nutritional value of grains. Diploid and tetraploid populations of maize containing the o2 gene, created at the State Agrarian University of Moldova, were used as research objects. Tetraploids showed an increase in the content of "lysine in the protein" of the endosperm by 7-12%. Several populations were identified, which combine a high level of accumulation of protein and lysine in the dry matter of grain and the content of "lysine in protein". The biochemical parameters determining the nutritional and energy value of corn grain did not change significantly. The obtained tetraploid forms of maize with the o2 gene can be recommended as models for a more in-depth study of the expression of monogenic mutations in the polyploid genome.

Key words: *maize, diploid, tetraploid, opaque-2 gene, biochemical indices.*

INTRODUCTION

It is generally recognized that polyploidy is one of the most promising synthetic breeding methods (Lutkov, 1966).

According to Udall and Wendel (2006), polyploidy provides genome buffering, increases allelic diversity and heterozygosity and allows the generation of new phenotypic variations.

However, for the purposeful use of polyploidy in combination with mutagenesis, it is important to focus on the features of the expression of a particular gene (or complex of genes) in the polyploid genome.

The analysis of literature data shows that the maize crop (*Zea mays* L.) with the gene *opaque-2* (*o2*) is one of the unique model objects for discussing the formulated problem (Palii and Batiru, 2011).

More than half a century of experience in studies of o2 gene expression in diploid maize genomes testifies to the effectiveness of the biochemical approach to the study of the specificity of o2 gene expression, which causes significant changes in the synthesis of protein - the first product of the gene action (Plotnikov, 2005; Palii, 2008), characterized by a significant improvement in the balance of some essential amino acids (primarily lysine). It has been experimentally demonstrated that the nutritional value of diploid high-lysine forms, determined by significant changes in the biochemical composition of corn grain, is substantially improved (Rotari, 2013).

The logical connection between the comparative analysis of diploid and tetraploid forms of maize (Ellis et al., 1946) allows us to continue the study of the *o2* gene expression in the tetraploid genome and, primarily, by the biochemical characteristics determining the protein quality and the nutritional value of corn grain, which was the main task of the presented work.

MATERIALS AND METHODS

The populations of tetraploid maize with the *o2* gene, which were created at the Department of Plant Biology of the State Agrarian University of Moldova, were used as research objects.

The basis for the creation of these populations was the regionalized hybrids of the Moldavian breeding, improved in biochemical quality: 1) Chișiniovschi 307 PL hybrid - its genome is the carrier of the highly-lysine *o2* gene combined with the specificity of polygenic control of the increase in synthesis of the maize grain storage protein; 2) hybrid Chişiniovschi 401 L - for its creation, the *o2* gene was also used.

It was these hybrids that were transferred to the tetraploid level by the method of colchicination (Palii and Batiru, 2011).

The method of Yudin was used (1964) to accelerate the process of creating tetraploid populations with the *o2* gene.

This method allowed already in the first year of reproduction to obtain tetraploid hybrids with the *o2* gene as a result of artificial intrapopulation pollination.

Place of reproduction of the experimental material: central zone of the Republic of Moldova (Cosernita village, Criuleni district). According to general meteorological conditions, the vegetation period of the experiment was characterized by the optimum for *Zea mays* L. crop. However, there was noted a water deficit in the critical phase for the reproduction of the maize plant (flowering and grain filling). This stress factor (due to lack of moisture) in the process of reproduction of diploid and tetraploid forms, caused a wide range of variation in the kernel set.

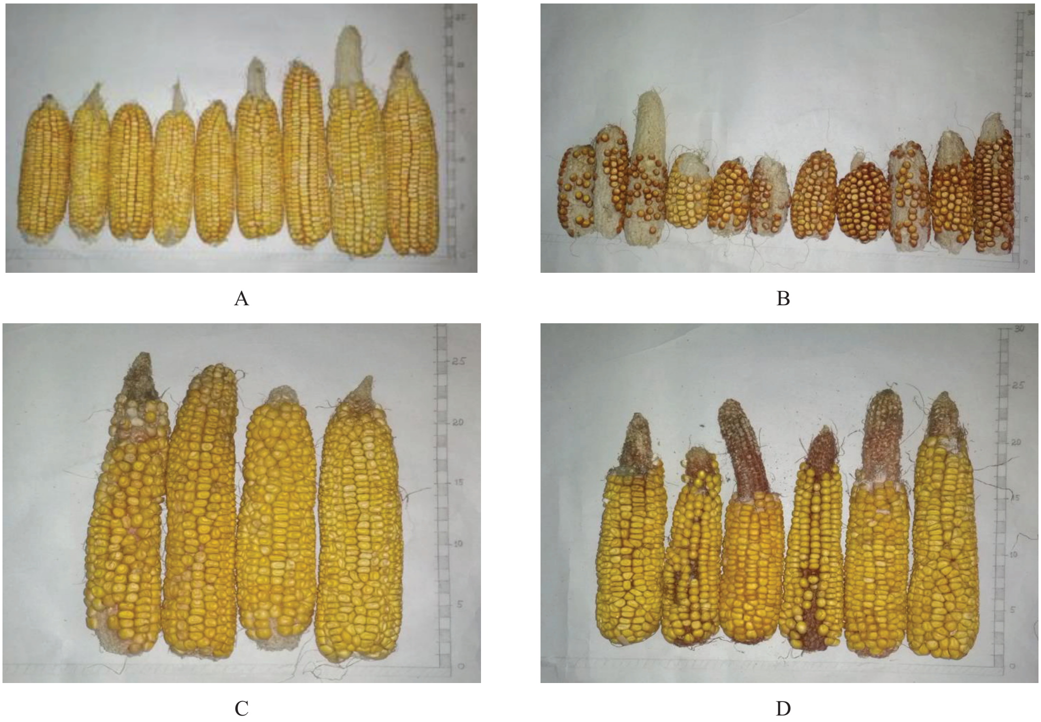
A B
C D

Figure 1. Visual variation of the kernel set of different genotypes of maize: A- diploid hybrid Chişiniovschi 307 PL (2x); B- tetraploid hybrid Chişiniovschi 307 PL (4x); C- tetraploid population SP 383 (4x); D- tetraploid population SP 461 (4x)

Figure 1 shows quite clearly the significant phenotypic differences in the kernel set between the diploid (2x) and tetraploid (4x) form of the hybrid Chişiniovschi 307 PL.

The revealed phenotypic specificity justified the need to select more homogeneous corn cobs for carrying out biochemical analysis (Figure 1, A, B).

At the same time, a visual analysis of the 12 tetraploid created populations (Figure 1, C, D) indicated the possibility of selecting the samples with similar elements of yield structure (cob length, rows and number of grains in the cob) for a more objective biochemical parameters comparison between tetraploid and diploid corn forms.

Such a thorough approach to sampling for studying the biochemical characteristics of the grain with *o2* gene expression in the genome of tetraploid maize is due to the previously established regularity (Ellis et al., 1946; Batiru, 2014) of increasing the protein content in the grain of defective ears.

This specificity may be due to:

- the effect of moisture deficiency in the critical phases of generative and reproductive development of maize;
- abnormalities that occur in tetraploid forms at the cytogenetic level.

The analysis of diploid and tetraploid forms of maize was carried out according to the key metabolites of grain, which determine:

a) the expression of the *o2* gene on the main biochemical marker of this gene - the content of "lysine in protein" of the corresponding genotype under study, which provided for a biochemical assessment of the content of "crude protein" and "lysine" in absolutely dry matter of the grain;

b) expression of the *o2* gene by the main biochemical indicators of the nutritional value of corn grain: "crude protein", "crude fat", "crude fiber", "ash" and "nitrogen-free extractives (NFE)", and also the leading component of nitrogen-free extractives, which determines the energy value of grain, - the content of "starch".

The content of all listed metabolites was evaluated by Near Infrared Spectroscopy on the IR 4500 "Interagroteh" (Krishchenko, 1997), which was calibrated using standard classical methods for analyzing of the biochemical indicators studied (Rotari, 2013).

RESULTS AND DISCUSSIONS

In accordance with the task at the first stage of the experiments, a comparative analysis was made of the *o2* gene expression on the main biochemical marker of this mutation - the content of lysine - the essential amino acid in the prolamin fraction of the grain protein of diploid and tetraploid heterozygous genotypes of maize.

As the data in Table 1 show, the transfer of the Moldovan breeding hybrids: Chişiniovschi 307 PL and Chişiniovschi 401 L, to the tetraploid level, induces an increase in the dry matter of the grain the protein content by 4-6%, the lysine content by 12-18% and enhances biochemical marking of the *o2* gene expression by content "lysine in protein" in the tetraploid genome.

Table 1. Comparative analysis of the *opaque-2* gene expression in the grain of diploid and tetraploid forms of maize by the protein content and the essential amino acid - lysine

Nr.	Genotype, population	Ploidy	% dry matter		% lysine in protein (g/100 g)
			protein	lysine	
1	Ch.307 PL	2x	12.77	0.49	3.84
2	Ch.307 PL	4x	13.32	0.55	4.13
		Δ	+0.55	+0.06	+0.29
		Δ %	+4.32%	+12.24%	+7.55%
3	Ch.401 L	2x	12.22	0.33	2.70
4	Ch.401 L	4x	12.91	0.39	3.02
		Δ	+0.69	+0.06	+0.32
		Δ %	+5.64%	+18.18%	+11.85%

Moreover, the heterozygous genome of the hybrid Chişiniovschi 401 L transferred to the tetraploid level showed more significant changes in the protein metabolism of corn grain by the content of "lysine in protein" - by almost 12%, while in the genome of the hybrid Chişiniovschi 307 PL, as a result of colchicination, the *o2* gene expression by the content of "lysine in protein" only increased by 7.6%.

Probably, the revealed biochemical specificity for each of the analyzed hybrids is caused by genetic differences of the initial maize hybrids genomes of Moldavian breeding. The hybrid Chişiniovschi 401 L was created by traditional heterotic selection using only one monogeneity characteristic controlled by the *o2* gene, while the Chişiniovschi 307 PL hybrid was created on the basis of the introduction of the *o2* gene in combination with the germplasm of the high-protein forms of maize, i.e. its genome is characterized by a more significant genetic modification of the original complex of protein metabolites of corn grain.

A study of the variation in the biochemical parameters of *o2* gene expression among tetraploid populations obtained as a result of colchicination (Table 2) allows us to conclude that the coefficient of variation is characterized by a weak significance (V% = 4.32) by the content of "crude protein", but for the content of “lysine” in the dry matter of the grains of studied populations, the coefficient of variation is determined by the average significance (V%=11.61).

Table 2. Variation of the protein content and the essential amino acid - lysine - in the grain of tetraploid forms of maize with the *opaque-2* gene

Nr.	Genotype, population	Ploidy	% dry matter		%/100 g protein
			protein	lysine	lysine/protein
1	Ch.307 PL	2x	12.77	0.49	3.84
2	Ch.401 L	2x	12.22	0.33	2.70
	Average	*2x*	***12.50***	***0.41***	***3.27***
3	Ch.307 PL	4x	**13.32**	**0.55**	**4.13** *(Δ %+26%)*
4	Ch.401 L	4x	**12.91**	0.39	3.02
5	C34	4x	13.33	0.49	***3.68***
6	SP 270	4x	12.67	0.41	3.24
7	SP 383	4x	**13.25**	**0.50**	3.77
8	SP 369	4x	12.77	0.48	3.76
9	SP 458	4x	**13.35**	**0.53**	**3.97** *(Δ %+21%)*
10	SP 459	4x	**13.41**	**0.53**	**3.95** *(Δ %+21%)*
11	SP 461	4x	12.41	0.39	3.14
12	SLG 2306	4x	**13.33**	0.49	***3.68***
13	SLG 3232	4x	**12.92**	0.47	***3.64***
14	SLG 3330	4x	**13.16**	**0.51**	**3.88** *(Δ %+19%)*
15	SFA	4x	**13.77**	0.49	***3.56***
16	C34-181	4x	**12.91**	0.46	***3.56***
	min	4x	*12.41*	*0.39*	*3.02*
	max	4x	*14.77*	*0.55*	*4.13*
	V%		**4.32**	**11.61**	**9.97**

Table 3. Comparative analysis of the expression of the *opaque-2* gene in the grains of diploid and tetraploid forms of maize according to the content of biochemical parameters determining the nutritional and energy value

Nr.	Genotype, population	Ploidy	% dry matter					
			protein	lipids	cellulose	ash	NFE	starch
1	Ch.307 PL	2x	12.77	4.69	3.94	1.32	77.28	71.23
2	Ch.307 PL	4x	13.32	4.33	3.92	1.33	77.10	69.93
		Δ	+0.55	-0.36	- 0.02	+0.1	-0.18	-1.3
		Δ %	**+4.3%**	-7.7%	-0.5%	**+7.6%**	-0.2	**-1.8%**
3	Ch.401 L	2x	12.22	4.76	3.93	1.32	77.77	71.00
4	Ch.401 L	4x	12.91	4.79	3.91	1.33	77.06	69.33
		Δ	+0.69	+0.03	-0.02	+0.1	-0.71	-1.67
		Δ %	**+5.6%**	**+0.6%**	-0.5%	**+7.6%**	-0.9%	**-2.34%**

The variation coefficient of marker *o2* gene expression by content of "lysine in the protein" is on the border of weak and medium significance (V% = almost 10%).

The statistical analysis of the biochemical parameters variation allowed selecting three tetraploid forms from the analyzed samples: SP 458, SP 459 and SLG 3330. As follows from Table 2, these forms combine the high values of all the studied parameters of the protein complex: the content of "crude protein" and "lysine" in dry matter of tetraploid populations grain, as well as the parameter directly determining the biochemical effect of the *o2* gene - the content of "lysine in protein". The relative increase in the "lysine content in the protein" (*Δ% - Table 2) in the selected tetraploid populations in terms of the average value of this indicator in diploid high-lysine maize hybrids is 19-21%. However, the maximum increase in the content of "lysine in protein" (by 26%) is established in the tetraploid form obtained from the highly lysine high-protein hybrid Chişiniovschi 307 PL.

Table 4. Variation of the content of biochemical indicators determining the nutritional and energy value of grain of tetraploid forms of maize with the *opaque-2* gene

Nr.	Genotype, population	Ploidy	% dry matter					
			protein	lipids	cellulose	ash	NFE	starch
1	Ch.307 PL	2x	12.77	4.69	3.94	1.32	77.28	71.23
2	Ch.401 L	2x	12.22	4.76	3.93	1.32	77.77	71.00
	Average	***2x***	***12.49***	***4.72***	***3.94***	***1.32***	***77.52***	***71.12***
3	Ch.307 PL	4x	**13.32**	4.33	3.92	1.33	77.10	69.93
4	Ch.401 L	4x	**12.91**	**4.79**	3.91	1.33	77.06	69.33
5	C34	4x	**13.33**	3.75	**3.95**	1.31	77.66	70.28
6	SP 270	4x	12.67	4.51	3.94	1.32	77.56	70.94
7	SP 383	4x	**13.25**	4.57	3.93	1.33	77.00	70.34
8	SP 369	4x	12.77	4.45	**3.95**	1.31	77.52	71.03
9	SP 458	4x	**13.35**	4.58	3.93	1.33	76.81	70.45
10	SP 459	4x	**13.41**	4.40	3.93	1.33	76.93	70.09
11	SP 461	4x	12.41	4.19	**3.95**	1.32	78.13	70.51
12	SLG 2306	4x	**13.33**	4.38	3.93	1.32	77.04	70.55
13	SLG 3232	4x	**12.92**	4.27	3.94	1.32	77.55	70.43
14	SLG 3330	4x	**13.16**	4.66	3.92	1.33	76.93	70.44
15	SFA	4x	**13.77**	4.37	3.91	1.33	76.62	69.05
16	C34-181	4x	**12.91**	4.57	3.91	1.32	77.29	70.51
	min	4x	12.41	3.75	3.91	1.31	76.62	69.05
	max	4x	13.77	4.79	3.95	1.33	78.13	71.03
	V%		**4.32**	**5.66**	**0.37**	**0.56**	**0.53**	**0.77**

Discussion of the biochemical parameters characterizing the nutritional and energy value of diploid and tetraploid forms of maize allowed us to concentrate our attention on the analysis of the pleiotropic effect of the *o2* gene.

As the data of Table 3 show, in addition to the indicators of the content of "crude protein" and the content of "ash", it is impossible to establish the presence of significant differences between diploid and tetraploid forms of maize for one of the studied metabolites.

The pleiotropic effect of the *o2* gene on the "crude fat" content is largely dependent on the genotypic environment. There is also a certain tendency to decrease the content of biochemical indicators of the energy value of grain of tetraploid forms - both in the content of "nitrogen-free extractives" and in the content of "starch". However, the observed differences are no more than 2% (in relative values).

The statistical calculation of the coefficients of variation of the biochemical indicators of nutritional and energy values under discussion (Table 4) allows us to establish a variation with weak significance for each of the studied parameters.

The tetraploid population SP 461 is of some interest for a more in-depth study of nutritional and energy value, which requires the use of methods that go beyond biochemical studies.

CONCLUSIONS

The transfer of the heterozygous maize genome with the *o2* gene to the tetraploid level induces an increase in the content of "lysine in the protein" of the endosperm by 7-12%, depending on the level of genetic modification of the initial complex of protein metabolites.

Among the tetraploid forms of maize created by colchicination, three populations have been identified: SP 458, SP 459 and SLG 3330, that combine a high level of accumulation of the studied indices of the protein complex: “protein” and “lysine” content in the dry matter of grain and the content of "lysine in protein".

The pleiotropic effect of the *o2* gene on the tetraploid level did not significantly change (with the exception of the "crude protein") the quantitative characteristics of biochemical parameters determining the nutritional and energy value of corn grain.

The obtained tetraploid forms of maize with the *o2* gene can be recommended as models for a more in-depth study of the expression of monogenic mutations in the polyploid genome.

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