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AMINO-ACID CONTENT IN GRAIN PROTEIN OF TETRAPLOID *OPAQUE-2* MAIZE

Andrei PALII, *Grigorii BATÎRU

Department of Plant Biology, Faculty of Agronomy, State Agrarian University of Moldova, Chishinau, 2049, Republic of Moldova,
grigore.batiru@mail.ru
*Corresponding author
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Abstract: *Amino acid composition in grain protein of a diploid (2x=20) single-cross maize hybrid containing opaque-2 (o2) gene and tetraploid (4x=40) derivatives of this hybrid was assayed. This mutant gene is widely used in maize breeding to improve quality of grain protein. Protein content was determined by the Kjeldahl method and analysis of protein amino acids - by ion exchange chromatography. Tetraploids, depending on genotype, showed higher protein content in grains than diploid forms and high variability in amino acid composition. Some derivatives contained higher levels of such amino acids like, lysine, arginine, aspartic acid and lower levels of leucine in protein in comparison with the diploid grains indicating possibilities to select valuable tetraploid forms. Grains with o2 endosperm from duplex-heterozygote self-pollinated ears showed higher content of lysine, arginine, valine, aspartic acid and glycine and reduced content of leucine, glutamic acid, proline and tyrosine than vitreous grains from the same ears, which highlights the specificity of gene effect. The analysis of dosage effect of the o_2 gene revealed that lysine content increases proportionally to the allele number of o_2 in the endosperm of diploid and, partly, in tetraploid grains. A possible maternal influence is indicated to explain the result.*

Keywords: *maize – diploid – tetraploid – gene – opaque-2 – amino acid – lysine.*

1. Introduction

Maize has spread in culture as a diploid species (2x=20). The first tetraploids (4x = 40) were obtained in 1932 by L. F Randolph using high temperature [1]. Since the first research on tetraploid forms a number of valuable morphological, physiological and biochemical features were highlighted. The grains of tetraploid maize are characterized by high content of protein, carotenoids and low fat [2-7]. Increasing the amount of grain protein is an important objective in maize breeding, as compared to other cereals, maize is low in protein content with an average of 10%. Studies have shown, however, that with increasing protein in grain, respectively, increases the rate of alcohol - soluble fraction - zein, very low in some essential amino acids such as lysine and tryptophan [8]. The research on improving the quality of maize protein opened up new perspectives after the discovery of the biochemical effect of the recessive mutation *opaque-2* (o2), which determines a floury texture of the endosperm and a dramatic shift in protein metabolism resulting in considerable increase of lysine and tryptophan [9]. Even in the early years of investigations of new forms created on the basis of the o2 gene in various institutions valuable practical results have been obtained. After using mutant maize grains in animal feed it was revealed a significant increase in the weight of different species of monogastric animals (mice, rabbits, pigs, poultry) and an

essential reduction of feed consumption [10-12].

Currently the biochemical effect of the o2 mutation is quite well studied in diploid maize, but, virtually, no information is known about the action of this gene in tetraploid maize. In just one paper of M. I. Hadjinov and V. S. Sherbak [7] is shortly stated that studying the biochemical effect of o2 gene in tetraploid maize, no significant changes in the content of lysine in grain protein was found. Given the hexaploid nature of the endosperm in tetraploid grains remains still unclear the dosage effect of the o2 recessive gene on the content of lysine and other amino acids from protein. In this paper we present the results of studying the action of o2 mutation on amino acid composition of proteins in tetraploid maize grain compared to the original diploid form. Also, the influence of the gene dosage in the endosperm of diploid and tetraploid grains on aminoacid composition is shown.

2. Material and Methods

As biological material were used: diploid single-cross maize hybrids Chisiniovschi 307PL with o2 endosperm and Porumbeni 331Mrf with regular texture of the endosperm, tetraploid synthetic population B (USA) with normal consistency (regular) of endosperm (kindly offered by dr. E. Hatefov from Kabardino Balkarian Institute of Agriculture, Rusia) and tetraploid o2 maize forms obtained by colchicine treatment from hybrid Chisiniovschi 307 PL at the State Agrarian University of Moldova in 2010 [13]. As these o2 tetraploids derived from a hybrid that segregated in the following generations of reproduction, they were grouped in several segregating families (P38-3, P38-5, P39-3, U3-2, U4-12 and U7-11) in order to assess the possibility to select lines for protein quality.

Field Experiments were performed at the Moldavian Institute of Plant breeding "Porumbeni" in 2010-2012. Study of gene dosage effect was accomplished on material obtained from reciprocal crosses between genotypes with vitreous and o2 endosperms at both ploidy levels. Grains for biochemical analysis were obtained by controlled pollination. Protein content was determined by the Kjeldahl method for the quantitative determination of nitrogen and subsequent conversion to protein by the coefficient 6.25, specific for maize. Analysis of protein amino acid content was performed by ion exchange chromatography on an automatic amino acid analyser T339M.

3. Results and Discussion

The product of the o2 gene is a transcriptional activator of zein gene expression. This gene is active in normal grains and inactive in mutant grains, which causes a sudden reduction of zein synthesis and increase content of albumins, globulins and glutelins. As zeins lack lysine, and other fractions are rich in this amino acid, redistribution of protein fractions conditions increased lysine content in the mutant endosperm [14, 15]. At the amino acid level of the protein it was found that o2 mutation also causes a higher content of histidine, arginine, aspartic acid, glycine, cysteine, tryptophan, reduces the content of glutamic acid, alanine, methionine, leucine, tyrosine, phenylalanine, as well as, increases the content of free amino acids [9, 14, 16-18]. In our research, in the study of protein amino acid composition of diploid and tetraploid grains containing o2 gene, it was found that with increasing ploidy level, with some exceptions, there is a tendency to increase the content of lysine, threonine, leucine, glutamic acid, aspartic acid and serine (Table 1).

Table 1

Content of amino acids in protein of diploid and tetraploid grains of maize, %

Amino acids	Genotype/family/ploidy									Mean for o2 tetraploids
	Porumbeni 331 Mrf 2x (+)	Chisinio vschi 307 PL 2x (o2)	Sin. B 4x (+)	P38-3 4x (o2)	P38-5 4x (o2)	P39-3 4x (o2)	U3-2 4x (o2)	U4-12 4x (o2)	U7-11 4x (o2)	
Essential amino acids										
Lys	3.02	4.78	3.17	6.08	4.30	5.08	5.18	3.90	4.36	4.82
His	2.71	3.53	3.37	3.82	2.97	3.28	3.63	3.22	3.09	3.34
Arg	4.39	6.32	4.85	3.69	3.78	6.73	7.31	4.15	3.33	4.84
Thr	2.66	2.76	2.23	3.16	2.85	3.21	3.06	3.09	2.95	3.05
Phe	4.22	3.60	3.98	3.39	3.76	3.41	3.44	4.05	2.94	3.50
Ile	3.03	2.87	2.51	2.36	2.10	2.16	2.43	2.22	2.09	2.23
Leu	12.78	9.66	13.54	10.45	11.91	7.24	10.18	10.10	10.77	10.11
Met	0.53	0.47	0.63	0.43	0.56	0.46	0.63	0.23	0.56	0.48
Val	4.42	5.03	4.14	4.29	3.54	4.24	4.32	3.59	3.91	3.98
Σ	37.75	39.04	38.41	37.67	35.77	35.83	40.19	34.55	34.01	36.34
Non-essential amino acids										
Glu	26.92	20.55	22.86	23.56	22.23	24.59	22.71	25.03	24.16	23.72
Pro	9.12	8.56	10.95	6.98	7.15	5.83	6.82	9.19	7.81	7.30
Ala	6.84	5.32	6.14	5.63	6.02	5.21	5.31	5.73	4.90	5.47
Asp	5.58	7.51	5.01	9.17	8.71	10.51	10.36	8.62	9.58	9.49
Tyr	2.69	2.69	3.35	3.15	3.19	2.94	3.10	1.94	2.75	2.85
Cys	0.89	1.18	1.02	0.84	0.84	1.03	1.10	1.08	1.14	1.01
Ser	4.60	4.34	4.54	5.97	5.30	5.16	5.11	5.37	4.73	5.27
Gly	3.30	4.69	3.60	4.34	3.45	3.58	3.60	4.08	3.10	3.69
Σ	59.95	54.82	57.47	59.65	56.88	58.85	58.11	61.06	58.17	58.79
Σ total	97.69	93.86	95.88	97.32	92.65	94.68	98.29	95.60	92.19	95.12
Protein, % dry matter	12.90	11.91	13.60	13.81	14.06	13.50	13.74	14.30	14.13	13.92

The content of arginine, isoleucine, valine, proline, cysteine, glycine decreased, at the same time no clear trend was found in the contents of histidine, phenylalanine, methionine, alanine and tyrosine.

A relatively high quantity of lysine was revealed in family P38-3 (6.08%) and U3-2 (5.18%). The sum of the essential amino acids of o2 tetraploids tends to be lower than that of diploids and the amount of non-essential higher. However, the results can be influenced both by ploidy level and gene action. In order to broader assess the biochemical effect of the o2 mutation on grain quality in our experiments we analyzed the amino acid composition of the protein from vitreous and o2 grains developed on the same tetraploid ears after self-pollination of heterozygous plants for o2 gene (O2O2/o2o2) which segregated in a phenotypic ratio of 35:1, grains with vitreous and floury endosperm, respectively (Table 2).

From the data obtained it can be observed that on the average, in tetraploid genome of maize the o2 gene increases the content of lysine, arginine, valine, aspartic acid and glycine in grain protein, while the content of leucine, glutamic acid, proline and tyrosine tends to lower.

Table 2

Content of amino acids in protein of tetraploid grains with vitreous and o2 endosperm selected from self-pollinated duplex heterozygotes ($O_2O_2o_2o_2$), %

Amino acid	Number of segregating ear										Mean	
	1		2		3		4		5			
	+	o2	+	o2	+	o2	+	o2	+	o2	+	o2
Essential amino acids												
Lys	3.24	4.63	3.11	5.06	3.98	5.30	3.10	4.45	3.02	5.78	3.29	5.05
His	3.23	3.14	2.91	3.94	3.80	3.65	3.76	3.46	3.35	2.85	3.41	3.41
Arg	4.98	5.21	2.98	3.20	3.89	6.85	3.83	5.22	1.45	5.36	3.43	5.17
Thr	2.94	3.42	2.90	3.16	3.40	3.55	3.05	3.11	2.97	2.89	3.05	3.22
Phe	3.71	3.46	3.50	3.95	3.83	3.20	3.44	3.89	3.78	2.96	3.65	3.49
Ile	2.17	2.41	2.19	2.31	2.44	2.21	2.41	2.20	2.12	2.56	2.27	2.34
Leu	12.38	9.31	12.68	10.75	12.94	9.11	11.17	9.91	13.26	7.99	12.49	9.42
Met	0.30	0.38	0.14	0.47	0.45	0.66	0.44	0.47	0.22	0.45	0.31	0.49
Val	3.62	4.70	3.48	3.82	3.58	4.12	3.44	4.20	3.47	4.79	3.52	4.33
Σ	36.57	36.66	33.89	36.66	38.31	38.66	34.64	36.90	33.65	35.63	35.41	36.90
Non-essential amino acids												
Glu	25.06	26.14	26.11	24.96	26.95	23.82	25.99	24.72	27.75	23.63	26.37	24.66
Pro	7.50	4.97	8.30	5.82	3.76	5.45	10.59	6.73	7.63	7.32	7.56	6.06
Ala	5.58	5.91	5.42	5.71	4.29	4.92	6.59	5.73	6.11	5.10	5.60	5.48
Asp	5.71	10.88	6.17	9.79	7.10	9.98	5.91	8.71	6.40	9.55	6.26	9.78
Tyr	3.20	2.61	3.32	3.34	3.52	3.07	2.79	2.84	3.03	3.10	3.17	2.99
Cys	0.85	1.24	0.95	0.78	1.31	1.03	1.20	1.27	0.97	1.40	1.06	1.14
Ser	5.13	5.65	5.20	5.48	5.51	5.45	5.42	5.58	5.43	4.77	5.34	5.39
Gly	2.84	3.52	2.99	4.09	2.33	3.90	3.26	3.91	2.76	3.41	2.83	3.77
Σ	55.87	60.93	58.46	59.97	54.78	57.63	61.74	59.48	60.07	58.29	58.18	59.26
Σ total	92.44	97.59	92.34	96.63	93.09	96.29	96.38	96.38	93.72	93.92	93.59	96.16
Protein, % dry matter	13.85	12.97	15.16	14.44	13.25	13.19	12.97	13.75	14.19	13.93	13.88	13.66

Regarding the content of the amino groups (essential and nonessential) one can see that the essential group is higher in o2 grains. The results can be easily influenced by the fact that grains on the segregated ears contain different doses of the o2 gene.

It is known that maize endosperm is an important biological tissue resulting from double fertilization and is triploid by nature i.e. made up of two maternal and one paternal genomes. This allows, by directing pollination, to obtain and study grains with different endosperm genotypes and number of doses (1, 2 and 3) of endosperm genes (dosage effects). Dosage effect in maize endosperm was first reported by P. C. Mangelsdorf and G. S. Fraps in 1931 [19], who found a direct relationship between the number of alleles Y1 (yellow) and content of provitamin A in grains. In research conducted by L. S. Bates [17], related to dosage effect of the o2 gene in diploid maize it was established that lysine content in grain increases proportionally with allele number.

The endosperm of tetraploid grains of maize is hexaploid, contains 60 chromosomes (40 maternal and 20 paternal) that is four maternal and two paternal genomes. This allows by reciprocal crosses,

to obtain tetraploid grains with a different number (2, 4 and 6) of alleles of a gene (dominant and recessive). In special literature, at the moment, there is no information about obtaining and biochemical study of different doses of endosperm genes (gene dosage) in tetraploid maize grains. Based on the mentioned, one of the objectives of our research was to obtain o2 maize tetraploid forms containing high (almost double) lysine content in protein grain. It was also considered the possibility to obtain tetraploid forms of maize with 4 doses of the o2 gene in endosperm, which would have a normal endosperm (glassy), but with a higher content of lysine in protein than diploid grains homozygous for o2, which could facilitate the implementation of o2 maize in production.

In our experiments possible variants of endosperm genotype were obtained, which allowed us to study the influence of gene dose on protein quality at both diploid and tetraploid levels (Table 3).

Table 3

Dosage effect of o2 gene in endosperm on protein amino acids of diploid and tetraploid maize grains, %

Amino acid	Number of recessive alleles in endosperm							
	2x				4x			
	++/+	++/o2	o2o2/+	o2o2/o2	++++/ ++	++++/ o2o2	o2o2o2o2/ ++	o2o2o2o2/ o2o2
Essential amino acids								
Lys	3.02	3.63	3.76	4.78	3.17	4.07	3.03	5.18
His	2.71	3.49	3.60	3.53	3.37	3.28	3.39	3.63
Arg	4.39	5.68	5.53	6.32	4.85	4.22	5.53	7.31
Thr	2.66	2.60	2.54	2.76	2.23	3.14	2.99	3.06
Phe	4.22	3.58	3.46	3.60	3.98	4.12	3.90	3.44
Ile	3.03	2.79	2.77	2.87	2.51	2.25	2.42	2.43
Leu	12.78	12.91	11.81	9.66	13.54	10.27	14.45	10.18
Met	0.53	0.44	0.47	0.47	0.63	0.23	0.28	0.63
Val	4.42	3.66	4.77	5.03	4.14	3.65	3.70	4.32
Σ	37.75	38.78	39.17	39.04	38.41	35.24	39.69	40.19
Non-essential amino acids								
Glu	26.92	22.31	23.56	20.55	22.86	25.46	25.80	22.71
Pro	9.12	11.83	8.92	8.56	10.95	9.35	7.58	6.82
Ala	6.84	6.18	6.28	5.32	6.14	5.83	6.13	5.31
Asp	5.58	5.66	5.31	7.51	5.01	8.77	5.76	10.36
Tyr	2.69	3.05	3.13	2.69	3.35	1.98	3.50	3.10
Cys	0.89	1.24	1.53	1.18	1.02	1.10	0.93	1.10
Ser	4.60	4.74	4.93	4.34	4.54	5.46	5.38	5.11
Gly	3.30	3.94	4.19	4.69	3.60	4.15	2.69	3.60
Σ	59.95	58.96	57.85	54.82	57.47	62.10	57.76	58.11
Σ total	97.69	97.73	97.02	93.86	95.88	97.34	97.46	98.29
Protein. % dry matter	12.90	12.25	13.19	11.91	13.60	14.06	12.75	13.74

As a result of the study we have found that the content of lysine increased proportionately with dose number in endosperm of diploid grains, which confirmed the research carried out by L. S. Bates [17], but in the case of tetraploid grains data obtained are inconsistent. The presence of two recessive alleles of o2 gene in hexaploid endosperm determined an increase in the content of lysine in protein (4.07%) compared to the dominant homozygote genotype (3.17%). For reasons unclear it is difficult to explain lysine content reduction in protein in the presence of 4 doses in endosperm (3.03%). At the same time, in the case of maximum number of doses (six o2 recessive alleles), the content of lysine exceeded all other variants of gene dosage of diploid and tetraploid levels (5.18%). Therefore, with the exception of the variant with four doses, the phenomenon of gene dosage effect of the o2 gene has been found, at both diploid and tetraploid levels.

It is, also, to be mentioned the pleiotropic action of the mutation on the content of other amino acids. Thus, a similar pattern as lysine was found for arginine at both diploid and tetraploid levels, due, perhaps, to their biochemical interdependence, but leucine showed a negative trend. Other amino acids manifested an unclear tendency.

Uncertainties related to these data can easily be influenced by differences between genotypes involved in crosses, especially the effects of reciprocal crosses (maternal influence). In order to exclude this, similar genotypes and isogenic lines are needed.

Preventive information with respect to the positive influence of the number of doses of o2 gene on lysine content in tetraploid maize grains proves the need to extend the research in this field. The results allow us to admit the possibility to select tetraploid genotypes with a higher content of lysine in grain protein than diploid grains and obtaining of forms heterozygote for o2, with vitreous endosperm and high protein quality.

4. Conclusions

Tetraploid maize forms, that contain the o2 gene, exhibit considerable variability on proteinogenic amino acid content in grains. Lysine content in protein varied from 3.90 to 6.08 %. This allows performing selection for desirable quality characteristics of grain protein at tetraploid level.

The amino acid analysis of the protein composition of tetraploid grains with vitreous and o2 endosperm obtained from the same heterozygote self-pollinated ears showed that the o2 gene in tetraploid genome increases the content of lysine, arginine, valine, aspartic acid and glycine and reduces the content of leucine, glutamic acid, proline and tyrosine, discriminating gene effect from ploidy level. In a dosage effect study of the o2 gene it was found that the content of lysine increases proportionally with dose number in the diploid genome and, partly, in tetraploid. As we used different genotypes in the investigation, there could be differences in results due to maternal cytoplasm involved in crosses, so to further understand and confirm this expression pattern of o2 gene in tetraploid maize, a different series of genotypes and more sophisticated biochemical analyses are required.

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