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THE DOSAGE EFFECT OF THE OPAQUE-2 GENE ON THE AMINO ACID COMPOSITION OF PROTEIN FROM TETRAPLOID MAIZE GRAIN

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Abstract

In this paper we aimed at studying the influence of different doses of opaque-2 gene (number of recessive alleles) in endosperm on amino acid composition of grain protein from tetraploid maize in comparison to diploid forms. Biochemical analysis was performed on grains of a comercial hybrid Chişiniovschi 307 PL containing the mutant gene, tetraploid forms of this hybrid obtained by colchicine treatment, a commercial hybrid Porumbeni 331Mrf and tetraploid synthetic population B. Protein content was determined by the Kjeldahl method, amino acid composition was assayed by ion exchange chromatography on an automatic amino analyzer T339M. In diploid grains the o2 gene showed a clear dosage effect on the content of lysine. In tetraploid grains, two recessive alleles of o2 gene in the hexaploid endosperm determined an increase in the content of lysine in protein as compared to the dominant genotype, in the four dose endosperm, the lysine content was intermediate between the dominant and two dose genotypes and was significantly higher in six doses. The expression pattern of the o2 gene was similar in replicate experiments carried in different growing season. As a result, it was concluded that the o2 gene has a dosage effect in diploid maize and, partially, in tetraploid maize.

Key words: *diploid, lysine, maize, opaque-2, tetraploid.*

INTRODUCTION

Kernel mutants of maze have been extensively used as tools in maze breeding programs for quality traits. It is known that protein of maize grain has a low biological value due to deficiencies in such essential amino acids like lysine and tryptophan, so that, improving the amino acid composition of maize grain is an important objective in maize breeding (Palii, 1989; Vasal, 2001). The research on improving the quality of maize protein opened up new perspectives after the discovery of the biochemical effect of the recessive mutation gene *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 (Mertz et al., 1964).

Maize endosperm is an important biological tissue derived from double fertilization. In diploid maize the endosperm is made up of two maternal and one paternal genome (triploid) (Birchler, 1993; Pennington et al., 2008). By controlled pollination, different doses (1, 2 and 3) of a particular gene in endosperm can be obtained and their biochemical influence assessed. Many endosperm genes in maize show dosage effect, which means that each present allele determines a corresponding biochemical effect (Boyer, Hannah, 2001). The first notice of such an effect in maize was reported by Mangelsdorf and Fraps in 1931 (Egesel et al., 2003), who found a direct relationship between the number of alleles for Y_1 gene (yellow pigment) present in endosperm and the content of carotenoids in grains. A similar expression pattern was revealed for the *o2* gene by Bates (1966), who found that lysine content in grains increased proportionally with the number of recessive alleles in endosperm.

In tetraploid maize forms, grains have a hexaploid endosperm (40 maternal and 20 paternal), allowing greater variability of gene dosage (2, 4 and 6) (Pennington et al., 2008). Randolph and Hand (1938, 1940) noted that the Y_1 gene in tetraploid grains of maize doubles the content of carotenoids as compared to diploid grains. Nevertheless, no research has been made with other genes, including *o2*, so

far. At the State Agrarian University of Moldova (Chisinau) are carried out experiments with the aim of using polyploidy and *o2* gene in improving the quality of maize grain. In this paper we present the results on studying the dosage effect of the *opaque-2* gene on the amino-acid content in diploid and tetraploid maize grains.

MATERIALS AND METHODS

Experiments were performed at the State Agrarian University of Moldova and the Moldavian Institute of Plant breeding "Porumbeni" in 2012-2013. The biological material included a diploid (2x = 20) maize hybrid Chişiniovschi 307 PL, that incorporates the *o2* mutation, a tetraploid form (4x = 40) of this hybrid (U3-2) obtained by treatment with colchicine in 2010 (Palii, Batiru, 2011). Also, taken into study were a diploid hybrid Porumbeni 331Mrf and the tetraploid synthetic population B, both with normal texture of the endosperm. All genotypes were reproduced by controlled pollination. Different doses of o2 gene were obtained by reciprocal crosses between genotypes with vitreous and o2 endosperms at both ploidy levels. Protein content was determined by the Kjeldahl method for the quantitative determination of nitrogen (N × 6.25), and amino acid composition was assayed by ion exchange chromatography on an automatic amino acid analyzer T339M. The percent lysine content was obtained by dividing the lysine content per unit dry matter to protein content.

RESULTS AND DISCUSSIONS

The recessive o2 gene prevents zein synthesis in grain protein of maize which causes an increase content of albumins, globulins and glutelins.

This redistribution of protein fractions conditions a higher content of lysine, 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 (Mertz et al.1964; Bates, 1966; Vasal, 2001; Wang and Larkins, 2001; Plotnikov, 2005).

The results of our research show that protein content in diploid forms was at the same level with tetraploid grains, which can be explained by dry conditions of the 2012 season. The difference between the two diploid genotypes was 1% and negligible between the tetraploid genotypes. In the dosage series of diploid grains, protein content decreased with one and three recessive alleles and increased with two. In tetraploid grains, protein level was higher in two and six doses and lower in four as compared to the dominant genotype.

Lysine content in diploid grains increased with the number doses in endosperm, that confirm the research made by Bates (1966), but in the case of tetraploid grains, two recessive alleles of o2 gene in hexaploid endosperm determined an increase in the content of lysine in protein (4.07%) as compared to the dominant homozygote genotype (3.17%), while in grains with a four dose endosperm the content of lysine was lower (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%). A similar pattern as lysine was found for arginine at both diploid and tetraploid levels, due to their biochemical interdependence, but leucine showed a negative trend.

Therefore, with the exception of the variant with four doses, the phenomenon of gene dosage effect of the o2 gene was confirmed, at both diploid and tetraploid levels. However, these data can easily be influenced by genotypes involved in crosses, especially the effects of reciprocal crosses (maternal influence).

In order to minimize the effect of maternal influence and confirm the expression pattern of the dosage effect of the o2 gene in tetraploid maize grains, in 2013 we performed such a cross: o2 grains obtained in 2012 from segregating ears of the U3-2×Syn.B combination (that we named Seg. 1) were grown and during the silking period, some ears were isolated and their silks split in two halves. One half was self pollinated and the other half cross-pollinated with synthetic population B.

Table 1. Dosage effect of o2 gene in endosperm on protein amino acids of diploid and tetraploid maize grains (2012), %

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

In this way we obtained grains with four and six doses of o2 gene on the same ears and minimized the difference from the two progenitors involved in cross. To obtain zero and two doses of o2 gene in tetraploid grains, we used plants of tetraploid synthetic population B that developed two ears, one of which was self pollinated and the other cross-pollinated with Seg.1. The developed kernels were analyzed by protein and lysine content (Table 2).

Table 2. Dosage effect of o2 gene on protein and lysine content in tetraploid maize (2013)

Denumirea mostrei	Number of recessive alleles in endosperm	protein, % d.m.	Lys, %
Syn. B	++++/++	10.59	3.40
Syn.B x Seg.1	++++/o2o2	12.01	3.99
Seg. 1 x Syn.B	o2o2o2o2/++	11.07	3.61
Seg.1	o2o2o2o2/o2o2	10.83	4.61

The growing season of 2013 was favorable for maize, so that, tetraploid plants and ears developed normally, kernel set was complete and grains well filled.

In such conditions the protein content was lower than previous year by 2-3%. In two dose grains, the content of lysine was higher than wild type grains, slightly reduced with four doses (intermediate between wild type and two dose), and increased again in six, which confirm the expression profile of the o2 gene in tetraploid grains. The results show the possibility to create tetraploid forms with floury and vitreous endosperm with a high content of lysine in protein.

CONCLUSIONS

As a result of the study it was established that in tetraploid grains, two recessive alleles of o2 gene in hexaploid endosperm determine an increase in the content of lysine in protein as compared to the dominant homozygote genotype, in the four dose endosperm, the

lysine content is intermediate between the first two and is significantly higher in six doses.

The expression pattern of the o2 gene on lysine content in tetraploid grains was similar in replicate experiments carried out in different growing season. This fact allowed concluding that the o2 gene has a dosage effect in diploid maize and partially in tetraploid maize.

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