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The dosage effect of the *opaque-2* gene on the amino acid composition of proteins from tetraploid maize grain

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Kernel mutants are widely used in maize breeding programs for quality traits. A great perspective in improving protein quality of maize was the discovery of the biochemical effect of the *opaque-2* (*o2*) gene, which determines a floury texture of the endosperm and a considerable increase of lysine and tryptophan. It was also established [Bates L. S. Amino acid analysis. Proc. of the high lysine corn conference. Washington, 1966, p.55-65] that the *o2* gene has dosage effect (lysine content in grain increases proportionally with allele number in endosperm). 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.

At the State Agrarian University of Moldova are carried out experiments with the aim of using polyploidy and *o2* gene in improving the quality of maize grain. For this purpose, tetraploid maize forms containing *o2* gene were obtained. Since tetraploid grains have hexaploid endosperm, in our research we considered the possibility to obtain grains with different doses of the *o2* gene in endosperm that would have a vitreous texture and a high level of lysine in protein. In this note we present the results of studying the action of the *o2* gene in different doses on amino acid content in tetraploid maize grain.

The biological material included a diploid ($2x = 20$) maize hybrid Chişiniovschi 307 PL, that incorporates the *o2* mutation, tetraploid forms ($4x = 40$) of this hybrid obtained by treatment with colchicine in 2010. Also, taken into study were a comercial hybrid Porumbeni 331Mrf and the tetraploid synthetic B both with vitreous texture of the endosperm. Protein content was determined by the Kjeldahl method for the quantitative determination of nitrogen ($N \times 6.25$), and amino acid composition was assayed by ion exchange chromatography on an automatic amino acid analyzer T339M.

Diploid maize grains have a triploid endosperm (2 maternal genomes and 1 paternal), and tetraploid grains have hexaploid endosperm (4 maternal : 2 paternal). By reciprocal crosses, it was possible to obtain different doses of the *o2* gene in endosperm and study the influence of gene dose on protein quality at both diploid and tetraploid levels (Table 1).

The data showed that the content of lysine increased with each dose number in endosperm of diploid grains, 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%) 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%). A similar pattern as lysine was found for arginine at both diploid and tetraploid levels, 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. The results show the possibility to create tetraploid forms with floury and vitreous endosperm with a high content of lysine in protein. However, these data can easily be influenced by genotypes involved in crosses, especially the effects of reciprocal crosses (maternal influence). In order to exclude this, isogenic lines are needed.

Table 1

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	++++/++	++++/ 2o2	o2o2o2o2/++	o2o2o2o2/o2o2
<i>Essential amino acids</i>								
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
<i>Non-essential amino acids</i>								
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