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THE INFLUENCE OF PLOIDY LEVEL ON SOME BIOCHEMICAL CHARACTERISTICS OF MAIZE GRAINS WITH *OPAQUE-2* ENDOSPERM

Andrei PALII[1], Grigorii BATÎRU[1], Alexandru ROTARI[2], Eugen ROTARI[2], Galina COMAROV[1]

[1] State Agrarian University of Moldova, 44 Mirceşti str., 2049, Chisinau, Republic of Moldova
[2] Institute of Phytotechny "Porumbeni", 4834, Paşcani, District Criuleni, Republic of Moldova

Corresponding author email: g.batiru@uasm.md

Abstract

Maize is an important souse of vegetable protein. However, maize grains contain low protein and the protein is of poor quality, due to deficiencies in essential amino acids like lysine and tryptophan. Conventional breeding methods have been used to overcome this problem, yet, the only possible solution is the use of specific genes. Two of them are opaque2 and floury2, which have been extensively used in breeding maize for protein quality. Difficulties regarding pleiotropic effects of the genes on some important cultural characteristics, determine to find new ways of using these genes. This paper aims to present the results of biochemical analysis of diploid and tetraploid maize grains that contain the opaque-2 (o2) gene. Experiments were conducted at the Department of Plant Biology of the State Agrarian University of Moldova and at the Institute of Phytotechny "Porumbeni" in 2013. The research involved two special maize hybrids approved in Moldova and their tetraploid forms obtained by colchicine treatment. Biochemical analysis of diploid and tetraploid o2 grain was performed by infrared spectroscopy and traditional methods. As a result of the study it was revealed that tetraploid grains show higher protein content and a decrease in starch and fat. Lysine content was almost similar in the two types of grains. As hexaploid endosperm cells of the tetraploid grains are bigger in volume than triploid endosperm cells of the diploid grains, it was concluded that lysine content per cell is double in tetraploid grains.

Key words*: diploid, opaque-2(o2), protein, tetraploid, Zea mays L.*

INTRODUCTION

Maize plays an important role in agriculture and the global economy providing vegetable protein. However, the maize grains contain relatively low protein (10%), with low quality due to the limitation in such essential amino acids as lysine and tryptophan (Palii, 1989; Vasal, 2001; Shewry, 2007). The biological value of maize protein is approximately 32% of casein protein quality (FAO, 1992).

It is known that protein content of maize can be increased by applying traditional methods like selection (Dudley, Lambert, 2004), polyploidy (Ellis et al., 1946; Rotary et al, 1970; Hatefov and Novoselov, 2011), mutagenesis (Blyandur, 1974) and distant hybridization (Borovsky et al., 1973), however, this increase is usually not accompanied by improved quality. The issue of protein quality of maize grain took a great turn after the discovery of the biochemical effects of spontaneous recessive mutations *opaque2* (*o2*) and *floury2* (*fl2*) that determine a floury endosperm texture and cause an increase in the content of lysine and tryptophan (Mertz et al., 1964; Nelson et al., 1965). However, pleiotropic effects of genes on certain cultural traits of maize (floury endosperm that cause kernel damage by mechanical harvest, high moisture of grains at harvest that increased pathogen attack, reduced yield etc.) reduced the interest in these forms. In this context, finding new ways to exploit these mutations are an essential objective in genetics and improvement of maize.

At the State Agrarian University of Moldova, research are carried out that aim at studying the possibility of using genomic (polyploidy) and genetic (mutation recessive *o2*) variability to improve the quality of maize grain. In this paper we present the results on some biochemical characteristics of diploid and tetraploid grains of maize that contains the *o2* gene.

MATERIALS AND METHODS

The research was conducted at the Department of Plant Biology of the State Agrarian University of Moldova and at the Institute of Phytotechny "Porumbeni" in 2013. The biological stock used consisted of two simple hybrid maize Chişiniovschi 307 PL and Chişiniovschi 401 L (Figure 1) approved for cultivation in the Republic of Moldova. Both genotypes contain the *o2* gene that determines an essential increase in lysine and tryptophan in grain protein. The Chisiniovschi 401L also contains modifier genes that change the physical structure of the floury endosperm in mosaic, partly glassy.

Figure 1. Diploid and tetraploid maize ears of Chisiniovschi 307 PL (A) and Chisiniovschi 401 L (B) hybrids

The material was provided by the laboratory of genetic and improvement of maize from the State Agrarian University of Moldova. Tetraploid forms of these hybrids were obtained earlier by the conventional colchicine methods as described (Palii and Batîru, 2011).
Biochemical analysis of diploid and tetraploid *o2* grain was performed by infrared spectroscopy method (Krishchenko, 1997). Also it was used classic methods (Rotari, 1993). These analyzes were performed in the laboratory of biochemistry of the Institute of Phytotechny "Porumbeni".

RESULTS AND DISCUSSIONS

The results of biochemical analysis have shown that tetraploid o2 grains are characterized by higher protein content as compared with diploid grains (Table 1). These data confirm the information in the literature that supports higher protein content in grains of tetraploid maize (Ellis et al., 1946; Khadzhinov and Shcherbak, 1974; Hatefov and Novoselov, 2011).
It has to be mentioned that protein content in table 1 represents the average value of the tetraploid populations studied. An important fact is that the range of protein content in different ears was highly variable. The highest protein content obtained approached 15%. This increase shows possibilities to make selection for protein content at tetraploid level.

Table 1. Biochemical characteristics of diploid and tetraploid grains of maize with *o2* endosperm, 2013 (% dw)

Indici	Chisiniovschi 307 PL		Chisiniovschi 401 L	
	2x	4x	2x	4x
Protein	11.79	12.10	11.55	12.31
Starch	71.95	68.71	71.88	70.16
Lipids	4.98	3.88	4.70	3.51
Cellulose	4.02	4.03	4.13	4.18
Lysine	0.49	0.55	0.49	0.48
Lysine/protein	4.16	4.46	4.24	3.90

In the same time, it was noted by other researchers that protein content can be higher in grains if seed set is low (Rotary et al., 1970). In tetraploid maize, seed set is a very big problem especially for newly created tetraploids. In our research the ears were abundantly pollinated so that seed set could be as high as possible.
In this way well filled ears were used for biochemical analysis which assured a more relevant data for protein content.
Along with the increase in protein, starch content was reduced, compared to diploid grains. The same downward trend was found for lipid content.

From the literature it is known the ploidy level in maize is usually accompanied by a reduced fat content, which some research put on the cumulative action of the genes (Ellis et al., 1946).

The amount of cellulose in diploid and tetraploid grains was almost similar for both genotypes, a fact that has been mentioned in other sources (Ellis et al., 1946).

As it was mentioned above, the main biochemical effect of the *opaque2* gene is increasing lysine content in protein. Thus the most important was to determine whether doubling of chromosome number would at least increase the lysine if not double, because it is known that in diploid maize grain the *o2* gene showed dosage effect (Bates, 1966).

The obtained results for lysine content showed no significant differences among genotypes. If, however, taken into account the higher level of protein in tetraploids, then lysine gets higher for tetraploid Chisiniovschi 307 PL, and lower for Chisiniovschi 401L. Similar results were obtained in other crops, such as barley, where tetraploids showed similar lysine content as diploids (Tiwary et al., 1980).

Our results, in fact show, that the opaque2 gene lacks dosage effect as a result of genome doubling and thus, gene copies. However, certain factors need to be considered to better understand the mechanism of expression. First of all, it is necessary to mention that tetraploid cells are twice as big in volume as diploid ones (Kondorosi et al., 2000). Studies have shown, also that endosperm cells of tetraploid maize are even triple in volume compared with those of diploid grains (Randolph, Hand, 1938). The fact that polyploid cells are larger makes them fewer per unit mass. To confirm this hypothesis, Birchler and Newton (1981) determined the total hydrolysable DNA of diploid and tetraploid forms of maize and found that the amount of DNA in mg dry weight was almost similar in all forms. This means that if a gene has an additive effect, by comparison, biochemical analyzes of the two forms with different ploidy will show almost similar data. In this way, usually gene expression in polyploids is evaluated per cell.

In the present study, quite similar lysine content in *o2* maize grain at both diploid and tetraploid levels can be considered additive, i.e. double the level of lysine per cell of tetraploid maize forms.

CONCLUSIONS

The results of biochemical analysis of diploid and tetraploid maize grains that contain the *opaque2* gene revealed that tetraploid grains show higher protein content and a decrease in starch and fat. Lysine content was almost similar in the two types of grains. As hexaploid endosperm cells of the tetraploid grains are bigger in volume than triploid endosperm cells of the diploid grains, it was concluded that lysine content per cell is double in tetraploid grains.

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