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The Influence of Cytoplasmic Diversification on the Biochemical Composition of some Maize (*Zea mays*) Isolines

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Abstract

Cytoplasmic male sterility discovery has led many researchers to move their studies towards cytoplasmic diversification and their influence on the heredity of some agronomic traits in maize (*Zea mays*). This study reflects the results regarding the influence of cytoplasmic diversification on some biochemical components. The isolines were created using the backcross method with the nucleus donor inbred line. Four cytoplasm donor inbred lines were used as maternal genotypes and each of them was crossed with one of the five nucleus donor lines. All these isolines were crossed with four testers, elite inbred lines, thus resulting 100 hybrids. The hybrids were self-pollinated in order to conduct biochemical analysis to achieve a thorough description for the influence of cytoplasm on the content of proteins, starch, fats, fiber and NCGD. Using isolines as maternal parent for some hybrids has led to a slight decrease of the protein content, three cytoplasm led to a slight increase of fats and all four-cytoplasm influenced in a positive way the NCGD.

Keywords: cytoplasm, fat, nucleus, protein, starch, NCGD.

1. Introduction

The maize (*Zea mays* spp. *Mays*) is one of the most important crops worldwide, ranking first in terms of production due to the multiple uses: food, animal feed, producing alcohol, starch, dextrin, glucose or maize oil. It is therefore one of the most studied plants, with a very high intraspecific diversity. Increasing the genetic diversity of maize is one of the main objectives of breeding programs, so that this plant can be as well adapted to a multitude of stress factors. The southern corn leaf blight epidemic (1969-1970) caused great yield losses due to the use of a single type of cytoplasm (Texas male sterile cytoplasm) and triggered concerns regarding cytoplasm diversification. Genetic vulnerability is still a current issue due to the low number of cytoplasm that are used for breeding new inbred lines and hybrids [26,13]. From the early use of cytoplasmic male sterility, it was noted the role of maize cytoplasm in the heredity of some traits [10]. A number of important agronomical traits are influenced by cytoplasmic effects, including yield, ear and plant height, erect plants, date of tasselling and silking, grain quality, the tassel size, stalk breaking resistance, dry matter, cob weight and length, biochemical components, germination, number of dead seedlings due to *Fusarium* sp., diseased plants, plant vigour, vegetative period etc. [1-11;13-24, 27, 29]. In 1992, at the Agricultural Research and Development Station Turda (A.R.D.S.

Turda), Romania, a maize breeding program was initiated: the transfer of some elite inbred lines nucleus on several types of conventional cytoplasm, in order to diversify the genetic basis, to avoid the cytoplasm uniformity and also for a possible improvement of the inbred lines [6].

Two inbred lines are required in order to create an isoline: one nucleus donor and one cytoplasm donor. After crossing the two inbred lines, backcrossing is performed for several generations (until the nucleus is transferred 99.9% into the new cytoplasm) using the nucleus donor line as recurrent parent [9, 15, 28, 29]. By creating isolines, the maize genetic basis can be widened and studies regarding the cytoplasmic influence on some agronomic traits can be conducted. These isolines were also crossed with testers in order to study the cytoplasmic influence on hybrids and the inheritance of several agronomic traits.

2. Material and Method

The biological material used in this study is represented by 100 maize hybrids, and the data presented below refer to their content in proteins, starch, fats, fibers and NCGD. The 100 hybrids were created after crossing 5 groups of isogenic lines (each group comprising 5 lines) with 4 testers. The five groups were obtained by transferring the nuclei of elite inbred lines TC209, TC316, TC243, TB367 and D105 on four cytoplasm sources: T248, TB329, TC177 and TC221. In order to test the value of the isolines, they were used as maternal parent in crosses with four inbred lines testers: TA367, TC344, TC385A and TE356. The resulting hybrids were self-pollinated in two experimental years and for each genotype, 10 self-pollinated cobs were milled in order to realize the biochemical analysis. Biochemical analyzes were carried out using Tango FT-NIR spectrophotometer from Bruker company. Each analysis was repeated three times. This study presents results on the percentage of protein, fat, fiber (% of total dry matter) and NCGD (Neutral Cellulase Gammanase Digestibility) (% of total fiber). The experimental data was statistically analyzed using analysis of variance by ANOVA, for polyfactorial experiences (1st factor: experimental year, 2nd factor: testers, 3rd factor: nucleus of the maternal parent and 4th factor: cytoplasm of the maternal parent).

3. Results and Discussions

Testers had a major influence on biochemical composition of maize grain. The nucleus of the inbred line generating a group had also influence on the four traits, but after statistical analysis it can be said that the cytoplasm had also influenced the studied traits, even if its contribution is lower compared to the other factors. The interaction between cytoplasm, nucleus and testers had significantly influenced all four traits. Like most traits, the year has an overwhelming influence on the quality of the grains (Table 1).

Table 1. Analysis of variance for the biochemical content of 100 hybrids using isolines as maternal parent

Variability cause	DF	Protein	Starch	Fats	Fiber	NCGD
			F test			
Hybrids	99	7.3**	131**	177**	37.3**	9.2**
N	(4)	270**	663**	2666**	322**	163**
C	(4)	37.3**	11.8**	28.7**	3.9**	25.3**
T	(3)	370**	986**	1188**	942**	265**
N x C	(16)	65.0**	58.5**	32.6**	44.6**	35.5**
N x T	(12)	40.4**	155**	192**	16.6**	13.1**
C x T	(12)	18.1**	71.1**	21.8**	15.9**	10.7**
C x N x T	(48)	18.5**	58.2**	36.1**	18.2**	15.1**
Y	1	35.0*	806**	156**	2080**	34.5*
Error (Y)	2					
Error (H)	396					
Error (T)	(12)					
Error (N)	(64)					
Error (C)	(320)					

Y= year; T=testers; C= cytoplasm; N= nucleus; H= hybrids

Protein content. The change in the cytoplasm of the maternal line had a negative influence on the protein content (Table 2) of the hybrids obtained by crossing the line TB367 with TA367, respectively TC344, the differences recorded being up to 0.79 g/100g for TB367(cytTC221) xTA367, compared to TB367 x TA367. The biggest difference due to the change in the cytoplasm of the maternal line was registered in the case of the hybrid TC209(cyt T248)xTA367, 0.85 g/100g. By changing the cytoplasm of the maternal line, an improvement of the protein content was also achieved in the case of the hybrids D105(cytTC177) x TC344 (+0.78g/100g), TC209 (cyt221)xTE356 (+0.66g/100g), when compared to the hybrids using the original maternal genotype.

Starch content. The study of the triple interaction between the cytoplasm and the nucleus of the maternal line, respectively the tester used, highlighted the fact that by simply changing the cytoplasm of the maternal line, the starch content of some hybrids can be modified (Table 3).

Table 2. Influence of the cytoplasm x nucleus x tester interaction on the protein content (g/100g) of the studied maize hybrids

Hybrid	♀ Original inbred	♀ Isoline	± Original inbred
		T248 cytoplasm	
TC209 x TA367	9.09	8.24	-0.85^{000}
TC243 x TC344	9.64	10.27	0.63***
TB367 x TA367	9.82	9.17	-0.65^{000}
		TB329 cytoplasm	
TB367 x TC344	9.53	8.80	-0.73^{000}
		TC177 cytoplasm	
TC243 x TA367	9.33	8.71	-0.62^{000}
TC243 x TE356	10.57	9.73	-0.84^{000}
TB367 x TA367	9.82	9.14	-0.68^{000}
TB367 x TC344	9.53	8.80	-0.73^{000}
D105 x TC344	9.42	10.20	0.78***
		TC221 cytoplasm	
TC209 x TE356	8.90	9.56	0.66***
TB367 x TA367	9.85	9.06	-0.79^{000}
		LSD 5 %	0.10
		LSD 1 %	0.14
		LSD 0.1 %	0.18

Table 3. Influence of the cytoplasm x nucleus x tester interaction on the starch content (g/100g) of the studied maize hybrids

Hybrid	♀ Original inbred	♀ Isoline	± Original inbred
		T248 cytoplasm	
TC209 x TA367	69.47	70.29	0.82*
TC316 x TE356	71.02	70.07	-0.95^{00}
TC243 x TC385A	68.32	69.24	0.92**
TB367 x TA367	67.72	68.67	0.95**
		TB329 cytoplasm	
TC209 x TA367	69.47	70.65	1.18***
TC243 x TC385A	68.32	69.74	1.42***
D105 x TC385A	70.18	69.35	-0.83^{0}
		TC177 cytoplasm	
TC209 x TA367	69.47	70.59	1.12**
TC316 x TA367	70.03	70.91	0.88*
TC243 x TC344	68.20	68.93	0.73*
TC243 x TC385A	68.32	69.65	1.33***
TB367 x TA367	67.72	68.57	0.85*
		TC221 cytoplasm	
TC209 x TA367	69.47	70.55	1.08**
TC316 x TA367	70.03	70.9	0.87*
TC243 x TC385A	68.32	70.34	2.02***
		LSD 5 %	*0.68*
		LSD 1 %	*0.89*
		LSD 0.1 %	*1.14*

By changing the cytoplasm of the TC209 x TA367 hybrid, an improvement in starch content was achieved, with all four-cytoplasm leading to significant increases with values between 0.82 and 1.18 g/100g. The starch content of the TC243 x TC385 A hybrid was also influenced by all four cytoplasm, the values increasing between 0.92 and 2.02 g/100g. Using isolines as parental forms can be a good method for modifying starch content, but favorable interactions must be identified. The four-cytoplasm used proved to be favorable for increasing the starch content, but in the case of some interactions they had an unfavorable effect. **Fat content.** Transferring the maternal nucleus on different cytoplasm had a slight influence on the fat content of the studied hybrids (Table 4). Some hybrids were identified whose fat content was influenced, either by increasing or decreasing it. The use of t248 cytoplasm determined increases of 0.51 and 0.45 g/100g respectively for the TC243 x TC385A and TC209 x TC385A hybrids. However, the biggest recorded difference had negative values, of -0.74 g/100g and was found in the case of the hybrid combinationTB367 x TC344. **Fiber content.** The fiber content (Table 5) was also influenced by the change in the cytoplasm of the maternal line, the differences being both positive and negative.

Table 4. Influence of the cytoplasm x nucleus x tester interaction on the fat content (g/100g) of the studied maize hybrids

Hybrid	♀ Original inbred	♀ Isoline	± Original inbred
		T248 cytoplasm	
TC209 x TC385A	4.50	4.95	0.45***
TC243 x TC344	5.36	4.76	-0.60^{000}
TC243 x TC385A	4.89	5.40	0.51***
		TC177 cytoplasm	
TB367 x TC385A	4.89	4.37	-0.52^{000}
		TC221 cytoplasm	
TB367 x TC344	5.33	4.59	-0.74^{000}
		LSD 5 %	0.09
		LSD 1 %	0.12
		LSD 0.1 %	0.15

Table 5. Influence of the cytoplasm x nucleus x tester interaction on the fiber content (g/100g) of the studied maize hybrids

Hybrid	♀ Original inbred	♀ Isoline	± Original inbred
		T248 cytoplasm	
TC209 x TA367	2.30	2.00	-0.30^{000}
TC243 x TC344	2.23	2.61	0.38***
TB367 x TC344	2.22	2.56	0.34***
D105 x TC385A	2.06	1.70	-0.36^{000}
		TB329 cytoplasm	
TC209 x TA367	2.30	1.89	-0.41^{000}
TC209 x TC385A	2.32	1.92	-0.40^{000}
TC209 x TE356	2.23	1.89	-0.34^{000}
		TC177 cytoplasm	
TC209 x TA367	2.30	1.77	-0.53^{000}
TC209 x TC385A	2.32	1.81	-0.51^{000}
TC316 x TC385A	1.58	2.10	0.52***
TB367 x TA367	2.13	2.43	0.30***
D105 x TC344	2.11	2.59	0.48***
		TC221 cytoplasm	
TC209 x TA367	2.30	1.91	-0.39^{000}
TC209 x TC344	2.37	2.06	-0.31^{000}
TC209 x TC385A	2.32	1.90	-0.42^{000}
TB367 x TC344	2.22	3.08	0.86***
		LSD 5 %	0.09
		LSD 1 %	0.12
		LSD 0.1 %	0.16

A slightly greater difference was recorded in the case of the hybrid TB367(cytTC221)xTC344, in which, by changing the cytoplasm, it was possible to increase the fiber content by 0.86 g/100g. It can be observed, like the other analyzed constituents, that some cytoplasm has a positive influence on some hybrids, and a negative one on others, which means that to improve the biochemical content by this method, it is necessary to test a large number of hybrid combinations, in order to be able to identify those with specific combining ability. **NCGD percent.** Neutral Cellulase Gammanase Digestibility (NCGD), an indicator of ruminant digestion, was significantly modified in the case of some maize hybrids, by changing the cytoplasm of the maternal line (Table 6). The change in the cytoplasm of the maternal line of the hybrid TC209xTA367 determined statistically significant increases in all 4 cases, the differences having high values, of 3.78% (cyt TB329), 4.70% (cyt T248), 5.80% (cyt TC221) and 5.84% (cyt TC177).

Table 6. Influence of the cytoplasm x nucleus x tester interaction on the NCGD (%) of the studied maize hybrids

Hybrid	♀ Original inbred	♀ Isoline	± Original inbred
		T 248 cytoplasm	
TC 209 x TA 367	87.51	92.21	4.70***
D 105 x TC 385 A	92.67	94.82	2.15***
		TB 329 cytoplasm	
TC 209 x TA 367	87.51	91.29	3.78***
		TC 177 cytoplasm	
TC 209 x TA 367	87.51	93.35	5.84***
TC 243 x TC 385 A	91.86	90.80	-1.06000
		TC 221 cytoplasm	
TC 209 x TA 367	87.51	93.31	5.80***
TB 367 x TA 367	91.71	93.92	-2.21000
TB 367 x TC 385 A	90.69	93.12	-2.43000
		LSD 5 %	0.57
		LSD 1 %	0.75
		LSD 0.1 %	0.97

4. Conclusions

The transfer of elite inbred lines nucleus on different cytoplasm influenced the biochemical content, by increasing or decreasing the protein, starch, fat, fibre or NCGD. Both cytoplasm, the interactions between the nucleus and cytoplasm, and the testers are involved in the determinism of the three analyzed constituents.

The hybrids using TC 209 isolines had significant differences compared to those using the original maternal inbred line for NCGD content. Cytoplasmic nuclear interactions have a very strong influence on NCGD, the differences between hybrids obtained by using the original maternal cytoplasm and those who use isolines being up to 5.84%.

Some cytoplasms have a positive influence on some hybrids, but a negative one on others, which means that in order to improve the biochemical content using this method, it is necessary to test a large number of genotypes, in order to identify those with specific combining ability.

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