

THE INFLUENCE OF GENETIC DIVERSIFICATION ON ASPECTS OF THE BIOCHEMICAL COMPOSITION OF SOME MAIZE ISONUCLEAR INBRED LINES

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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 extraction
- dextrin extraction
- glucose extraction
- maize oil.

Maize oil can also be used to produce:

- biodiesel
- paints
- pharmaceutical products, etc.

Of the total dry matter, the predominant biochemical elements of maize are starch (57-73%), protein (8-15 %), fat (2-9%), sugar (2-3%), fiber (2-7%) and ash (1-2%).

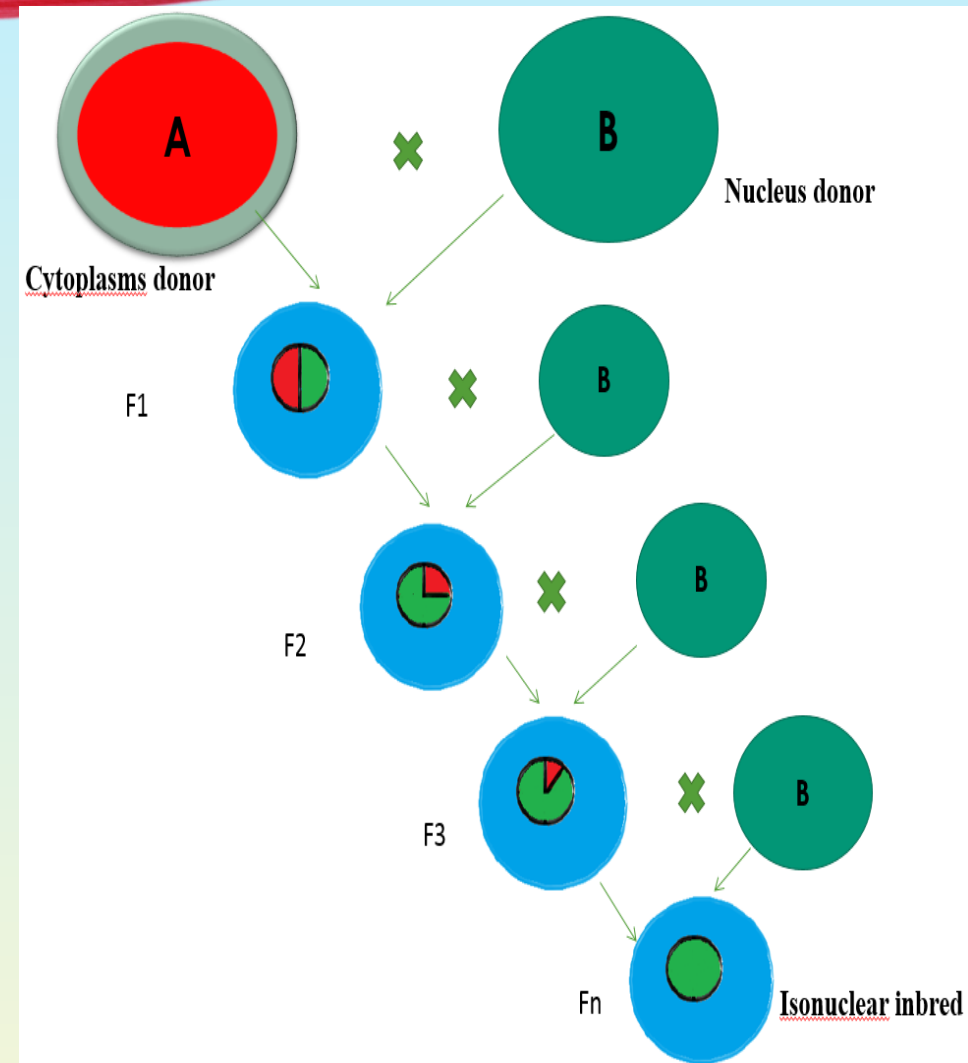
Introduction



- The discovery of cytoplasmic male sterility has led many researchers to move their studies towards cytoplasmic diversification, extrachromosomal heredity and the influence the cytoplasm can have on the transmission of some agronomic characters in hybrids.
- Helminthosporium maydis race T epidemic (1969-1970) is an example of the danger that is the use of a single source of cytoplasm. The epidemic triggered concerns regarding cytoplasm diversification.

Introduction

- Maize breeders from ARDS Turda have created a set of isonuclear inbred lines using the backcross method for 10 years.
- After backcrossing, it was estimated that the paternal nucleus was transferred 99.9 % in the cytoplasm of the donor line.
- Cytoplasm donor inbred lines were used as the maternal form and the recurrent parent gave the nucleus (Has et al., 2011).
- The transfer was initiated in order to diversify the genetic basis of the cytoplasm and for a possible improvement of the characters of these lines (characters of the cobs, plants, grains, production elements or biochemical composition).



Aims

- Biochemical analysis regarding the percentage of protein, fat (% of total dry matter) and NCGD (Neutral Cellulase Gammanase Digestibility) (% of total insoluble fiber) for 100 hibrids using isonuclear inbreds or the original inbred lines as maternal parent.
- Finding if the cytoplasms have any influence on the three biochemical constituents.
- Identify some hybrids using isonuclear inbreds as maternal parent with higher values for protein, fats and NCGD.

Material and methods

Biological material



✓ Nucleus

- ❖ TC 209
- ❖ TC 316
- ❖ TC 243
- ❖ TB 367
- ❖ D 105

✓ Cytoplasm

- ❖ Original
- ❖ T 248
- ❖ TB 329
- ❖ TC 177
- ❖ TC 221

✓ Testers

- ❖ TA 367
- ❖ TC 344
- ❖ TC 385 A
- ❖ TE 356

Material and methods

- Each hybrid was self pollinated
- For each genotype, 10 cobs were milled in order to realize the biochemical analysis.
- Biochemical analyzes were carried out using Tango FT-NIR spectrophotometer from the Bruker company.
- 3 repeats for each analysis
- The experimental data were statistically analyzed using analysis of variance by ANOVA, for polifactorial experiences.
- There were calculated indices for correlation and regression and also the general combining ability for grain composition and using the model proposed by Lein (1960) and Ceapoiu (1968), quoted by Haş et al. (2011).

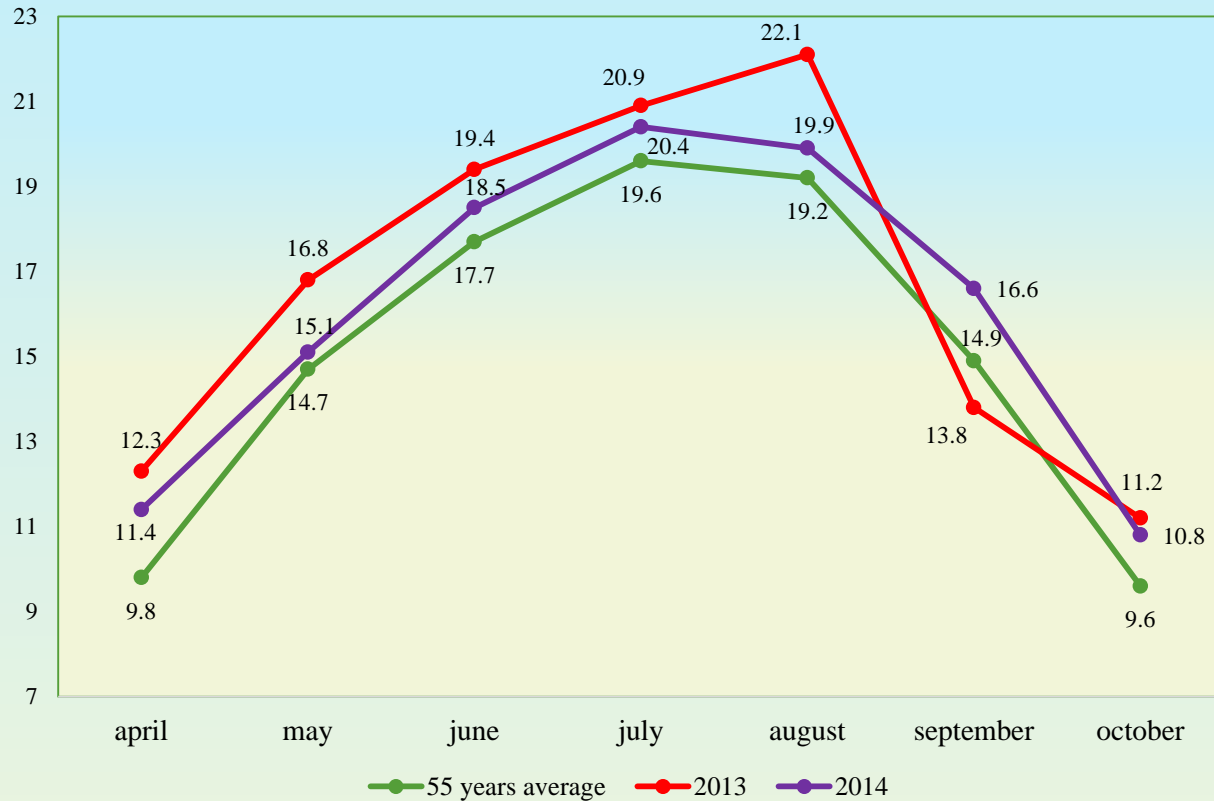


Near Infrared Spectroscopy

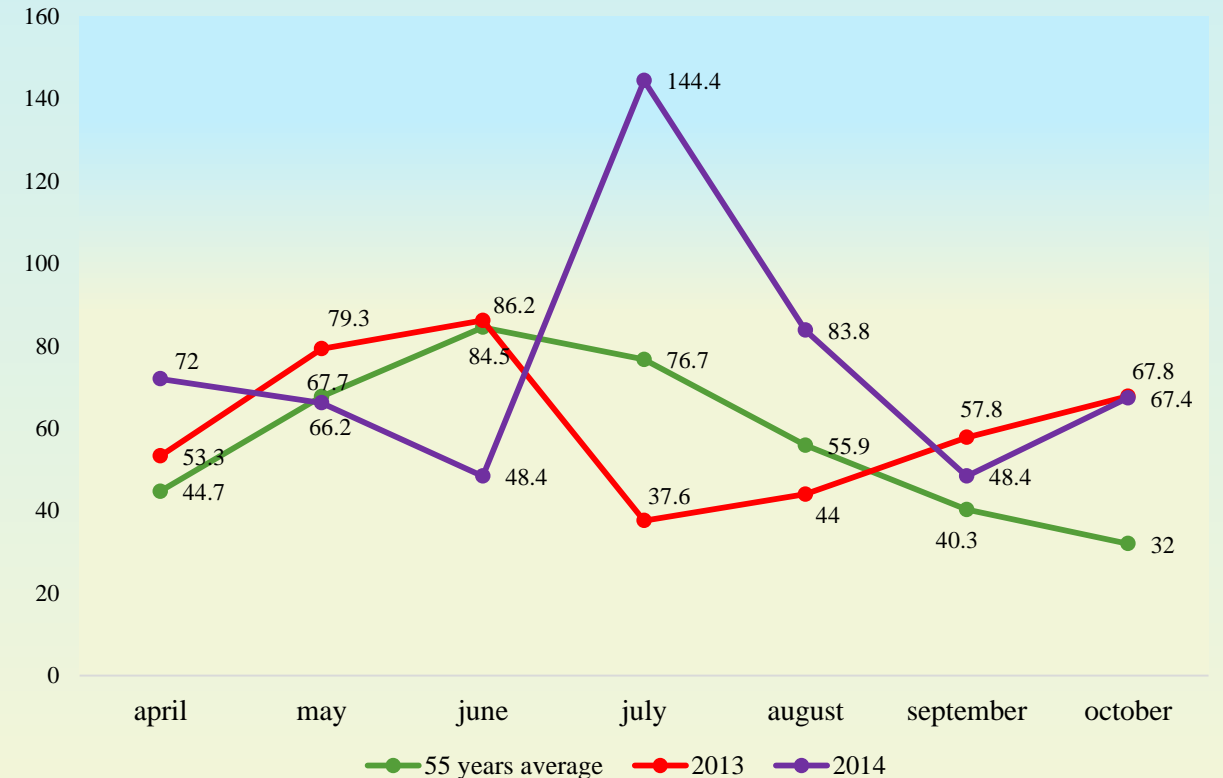
- Near infrared spectroscopy (NIR) offers several advantages over traditional analytical methods:
 - ✓ it is fast and non –destructive
 - ✓ no sample preparation is required or the preparation is very fast and simple
 - ✓ can provide simultaneous determination of multiple components
- In agriculture, NIR methods are widely used for the quantitative determination of components such as:
 - ✓ moisture
 - ✓ protein
 - ✓ fat
 - ✓ starch
 - ✓ fiber
 - ✓ sugar
 - ✓ grain hardness
 - ✓ wet gluten
 - ✓ ADF
 - ✓ NDF
 - ✓ NCGD etc.

Average temperature and rainfall Turda 2013-2014

Average temperature 2013-2014



Average rainfall 2013-2014



Primary data: **Turda weather station**
(23° 4' E longitude; 46°35' N latitude; 427 m altitude)

Results and discussions

Analysis of variance of some maize isonuclear inbred lines - Turda (2013-2014)

The cause of variability	DOF	Protein (% dry matter)		Fats (% dry matter)		NCGD (%/ % fiber)	
		S ²	F test	S ²	F test	S ²	F test
Total	599						
Years	1	44.06	806**	1.96	156**	16.84	34.53-
Testers	3	8.78	986**	7.03	1188**	39.88	265**
Years x Testers	3	0.65	73.48**	0.29	48.95**	2.11	13.98**
Nuclei	4	7.43	663**	15.14	2667**	50.20	163**
Years x Nuclei	4	3.85	344**	17.96	3162**	143	467**
Testers x Nuclei	12	1.74	115**	1.09	192**	4.03	13.10**
Years x Testers x Nuclei	12	2.25	201**	0.28	48.95**	3.18	10.31**
Cytoplasms	4	0.10	11.77**	0.18	28.67**	6.44	25.32**
Years x Cytoplasms	4	0.69	80.75**	0.49	76.95**	17.15	67.43**
Testers x Cytoplasms	12	0.60	71.08**	0.14	21.80**	2.71	10.67**
Years x Testers x Cytoplasms	12	0.19	22.29**	0.51	79.16**	3.33	13.09**
Nuclei x Cytoplasms	16	0.50	58.50**	0.21	32.68**	9.02	35.46**
Years x Nuclei x Cytoplasms	16	0.70	82.13**	0.79	122.68**	8.55	33.61**
Testers x Nuclei x Cytoplasms	48	0.49	58.16**	0.23	36.06**	3.83	15.05**
Years x Testers x Nuclei x Cytoplasms	48	0.47	55.51**	0.22	34.69**	2.70	10.62**
Repetitions	2	0.04		0.03		19.16	
Error Years	2	0.05		0.01		0.49	
Error Testers	12	0.01		0.006		0.15	
Error Nuclei	64	0.01		0.006		0.31	
Error Cytoplasms	320	0.01		0.006		0.25	

Protein content

Protein content (%) of maize grain for five groups of isonuclear inbred lines
Turda 2013-2014

Cytoplasm	Nucleus					Cytoplasm average	± original cytoplasm	GCA	GCA Testers	
	TC 209	TC 316	TC 243	TB 367	D 105					
Original	8.99	9.58	9.63	9.51	9.38	9.42	-	0.03		
cit T 248	8.85	9.75	9.72	9.16	9.31	9.36	-0.06	-0.02		
cit TB 329	8.96	9.59	9.60	9.09	9.52	9.35	-0.07	-0.04		
cit TC 177	9.06	9.62	9.38	9.24	9.68	9.40	-0.02	0.01		
cit TC 221	9.18	9.54	9.51	9.37	9.40	9.40	-0.02	0.01		
Average	9.01	9.62	9.57	9.27	9.46	9.39				
GCA	-0.38	0.23	0.18	-0.11	0.07					
									TA 367	-0.29
									TC 344	0.16
									TC 385 A	-0.11
									TE 356	0.24

LSD cytoplasm (p 5%) 0.02 (p 1%) 0.03 (p 0.1%) 0.04
 LSD nuclei (p 5%) 0.03 (p 1%) 0.04 (p 0.1%) 0.05
 LSD testers (p 5%) 0.02 (p 1%) 0.03 (p 0.1%) 0.05
 LSD cytoplasm x nuclei (p 5%) 0.05 (p 1%) 0.07 (p 0.1%) 0.09

Protein content for maize hybrids using isonuclear inbreds as maternal parent (2013-2014 – Turda)

Hybrid	Original cytoplasm	T 248 cytoplasm	
	%	%	± citoplasma originală
TC 209 x TA 367	9.09	8.24	-0.86
TC 243 x TC 344	9.64	10.27	0.62
TB 367 x TA 367	9.82	9.17	-0.66

Hybrid	Original cytoplasm	TC 177 cytoplasm	
	%	%	± citoplasma originală
TC 243 x TA 367	9.33	8.71	-0.62
TC 243 x TE 356	10.57	9.73	-0.85
TB 367 x TA 367	9.82	9.14	-0.68
TB 367 x TC 344	9.53	8.80	-0.73
D 105 x TC 344	9.42	10.20	0.78

Hybrid	Original cytoplasm	TB 329 cytoplasm	
	%	%	± citoplasma originală
TB 367 x TC 344	9.53	8.80	-0.72

Hybrid	Original cytoplasm	TC 221 cytoplasm	
	%	%	± citoplasma originală
TB 367 x TA 367	9.85	9.06	-0.76
TC 209 x TE 356	8.90	9.56	0.67

LSD cytoplasms x nuclei x testers
 (p 5%) 0.10
 (p 1%) 0.14
 (p 0.1%) 0.18

Fat content

Fat content (%) of maize grain for five groups of isonuclear inbred lines
Turda 2013-2014

Cytoplasms	Nucleus					Cytoplasm average	± original cytoplasm	GCA
	TC 209	TC 316	TC 243	TB 367	D 105			
Original	4.45	4.21	4.91	5.14	4.45	4.63	-	-0.0
cit T 248	4.58	4.33	5.03	5.14	4.39	4.69	0.06	0.05
cit TB 329	4.50	4.21	4.76	5.17	4.62	4.65	0.02	0.01
cit TC 177	4.52	4.20	5.03	5.02	4.44	4.64	0.01	0
cit TC 221	4.40	4.21	5.02	4.90	4.40	4.59	-0.05	-0.06
Average	4.49	4.23	4.95	5.07	4.46	4.64		
GCA	-0.15	-0.41	0.31	0.43	-0.18			

GCA Testers	
TA 367	0.10
TC 344	0.25
TC 385 A	-0.12
TE 356	-0.23

LSD cytoplasms (p 5%) 0.02 (p 1%) 0.03 (p 0.1%) 0.035
 LSD nuclei (p 5%) 0.02 (p 1%) 0.03 (p 0.1%) 0.035
 LSD testers (p 5%) 0.02 (p 1%) 0.03 (p 0.1%) 0.04
 LSD cytoplasms x nuclei (p 5%) 0.05 (p 1%) 0.06 (p 0.1%) 0.08

*Fat content for maize hybrids using isonuclear inbreds as maternal parent
(2013-2014 – Turda)*

Hybrid	Original cytoplasm	TB 329 cytoplasm	
	%	%	± citoplasma originală
TC 209 x TC 385 A	4.50	4.95	0.45
TC 243 x TC 344	5.36	4.76	-0.60
TC 243 x TC 385 A	4.89	5.40	0.51

Hybrid	Original cytoplasm	TC 177 cytoplasm	
	%	%	± citoplasma originală
TB 367 x TC 385 A	4.89	4.37	-0.52

Hybrid	Original cytoplasm	TC 221 cytoplasm	
	%	%	± citoplasma originală
TB 367 x TC 344	5.33	4.59	-0.74

LSD cytoplasms x nuclei x testers
 (p5%) 0.09
 (p1%) 0.12
 (p 0.1%) 0.15

NCGD percent

NCGD percent (%/% fiber) of maize grain for five groups of isonuclear inbred lines
Turda 2013-2014

Cytoplasms	Nucleus					Cytoplasm average	± original cytoplasm	GCA
	TC 209	TC 316	TC 243	TB 367	D 105			
Original	89.53	91.81	91.92	91.37	93.09	91.55	-	-0.25
cit T 248	90.76	91.30	91.63	91.77	92.94	91.68	0.14	-0.12
cit TB 329	91.10	91.21	92.51	92.19	92.06	91.81	0.27	0.01
cit TC 177	91.87	91.57	90.95	91.57	92.97	91.78	0.24	-0.01
cit TC 221	91.87	91.99	91.49	92.52	92.96	92.17	0.62	0.37
Average	91.03	91.58	91.70	91.88	92.80	91.80		
GCA	-0.77	-0.22	-0.10	0.09	1.01			

GCA Testers	
TA 367	0.37
TC 344	-0.76
TC 385 A	0.28
TE 356	0.11

LSD cytoplasms	(p 5%) 0.13	(p 1%) 0.17	(p 0.1%) 0.22
LSD nuclei	(p 5%) 0.14	(p 1%) 0.19	(p 0.1%) 0.25
LSD testers	(p 5%) 0.10	(p 1%) 0.14	(p 0.1%) 0.19
LSD cytoplasms x nuclei	(p 5%) 0.29	(p 1%) 0.38	(p 0.1%) 0.48

NCGD percent for maize hybrids using isonuclear inbreds as maternal parent (2013-2014 – Turda)

Hybrid	Original cytoplasm	T 248 cytoplasm	
	%	%	± citoplasma originală
TC 209 x TA 367	87.51	92.21	4.69
D 105 x TC 385 A	92.67	94.82	2.15

Hybrid	Original cytoplasm	TC 177 cytoplasm	
	%	%	± citoplasma originală
TC 209 x TA 367	87.51	93.35	5.84
TC 243 x TC 385 A	91.86	90.80	-2.03

Hybrid	Original cytoplasm	TB 329 cytoplasm	
	%	%	± citoplasma originală
TC 209 x TA 367	87.51	91.29	3.77

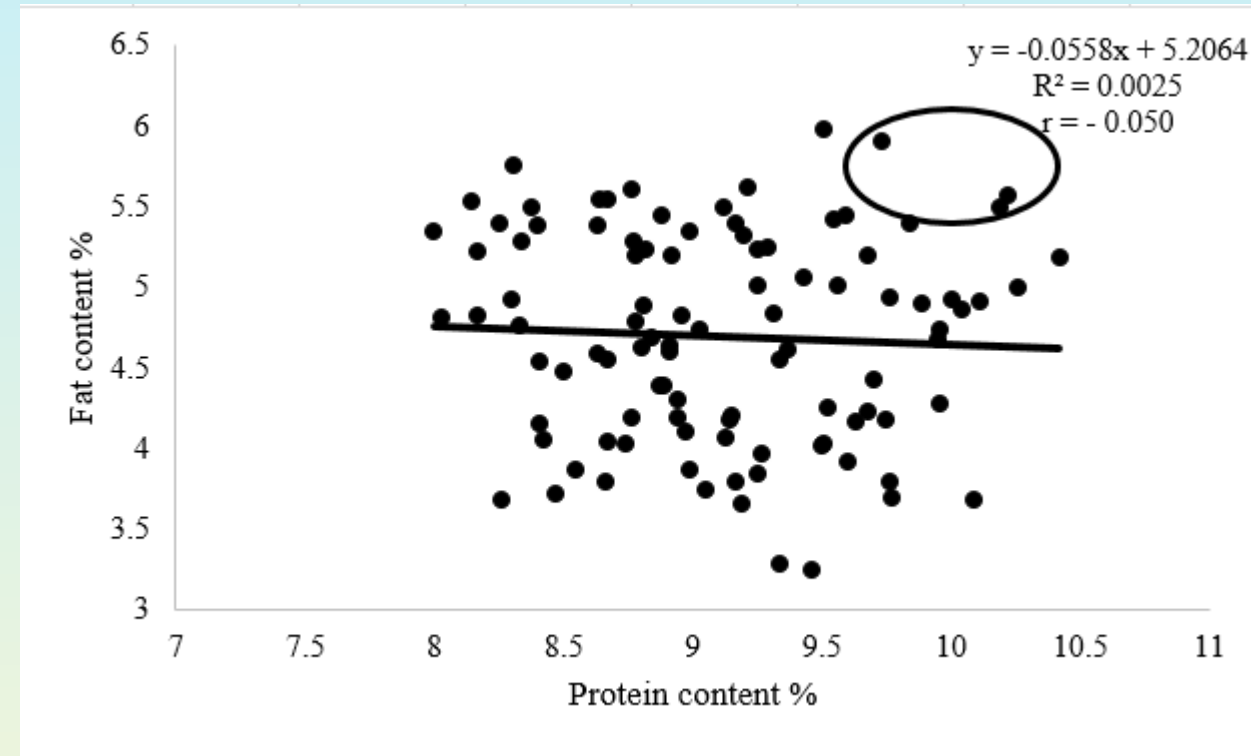
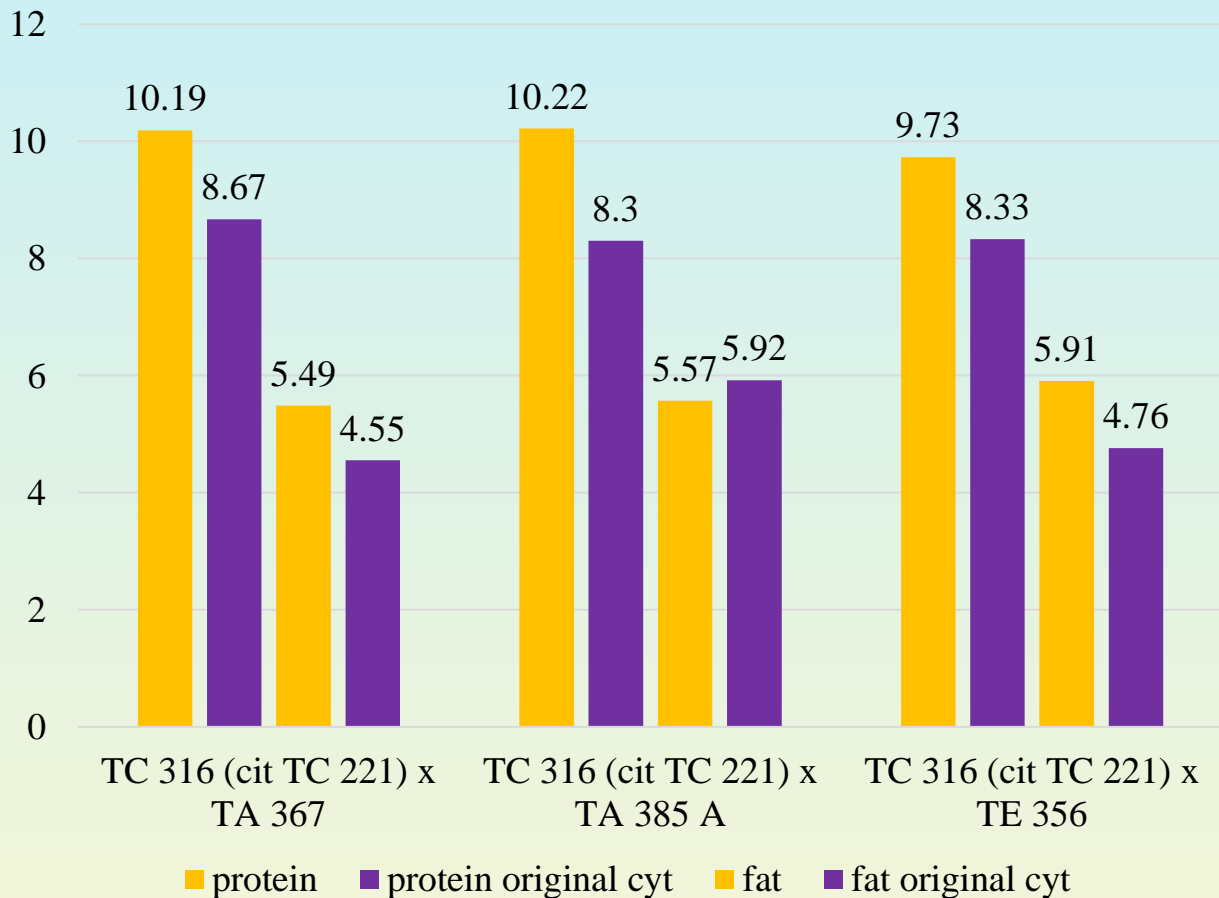
Hybrid	Original cytoplasm	TC 221 cytoplasm	
	%	%	± citoplasma originală
TC 209 x TA 367	87.51	93.31	5.79
TB 367 x TA 367	91.71	93.92	2.21
TB 367 x TC 385 A	90.69	93.12	2.44

LSD cytoplasms x nuclei x testers

(p 5%)	0.10
(p 1%)	0.14
(p 0.1%)	0.18

Regression analysis for protein and fat content for some maize hybrids (2014)

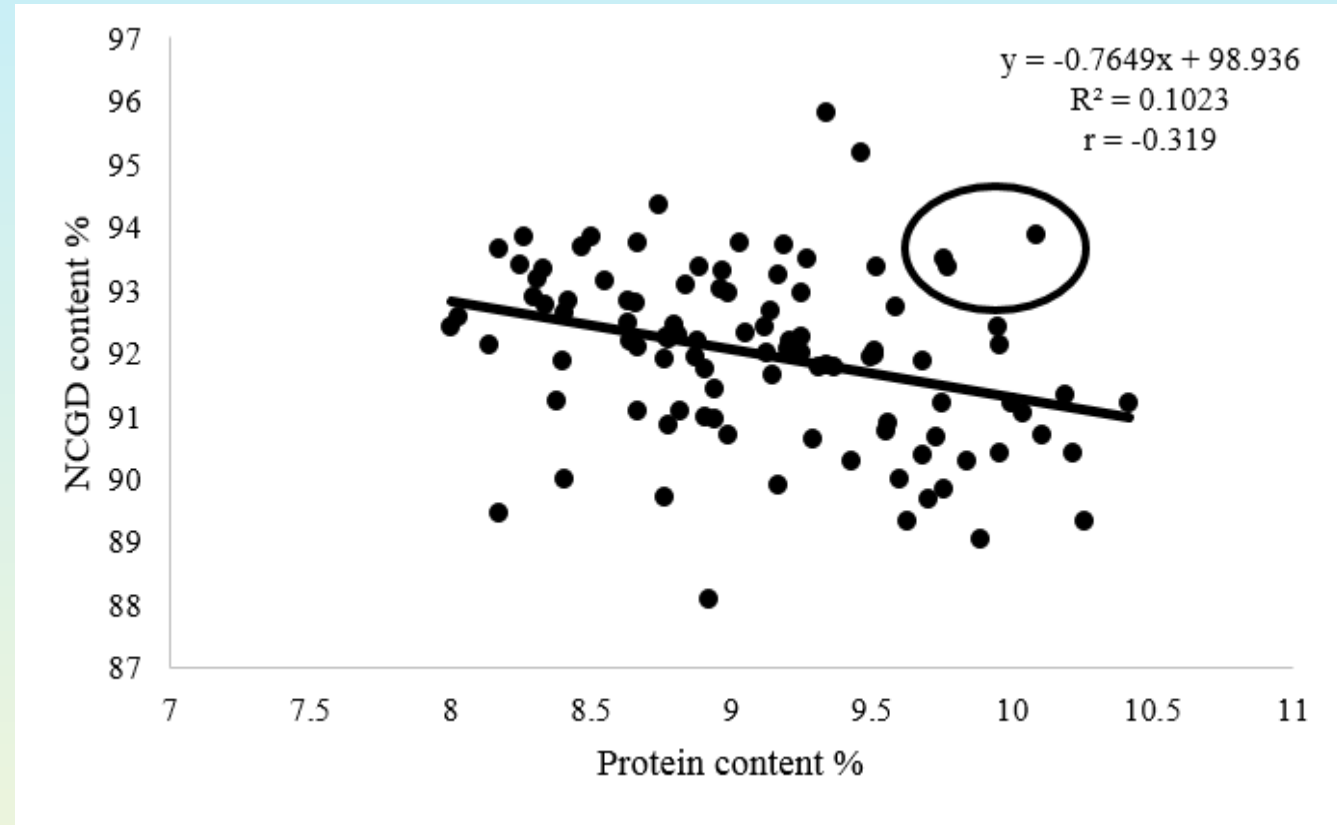
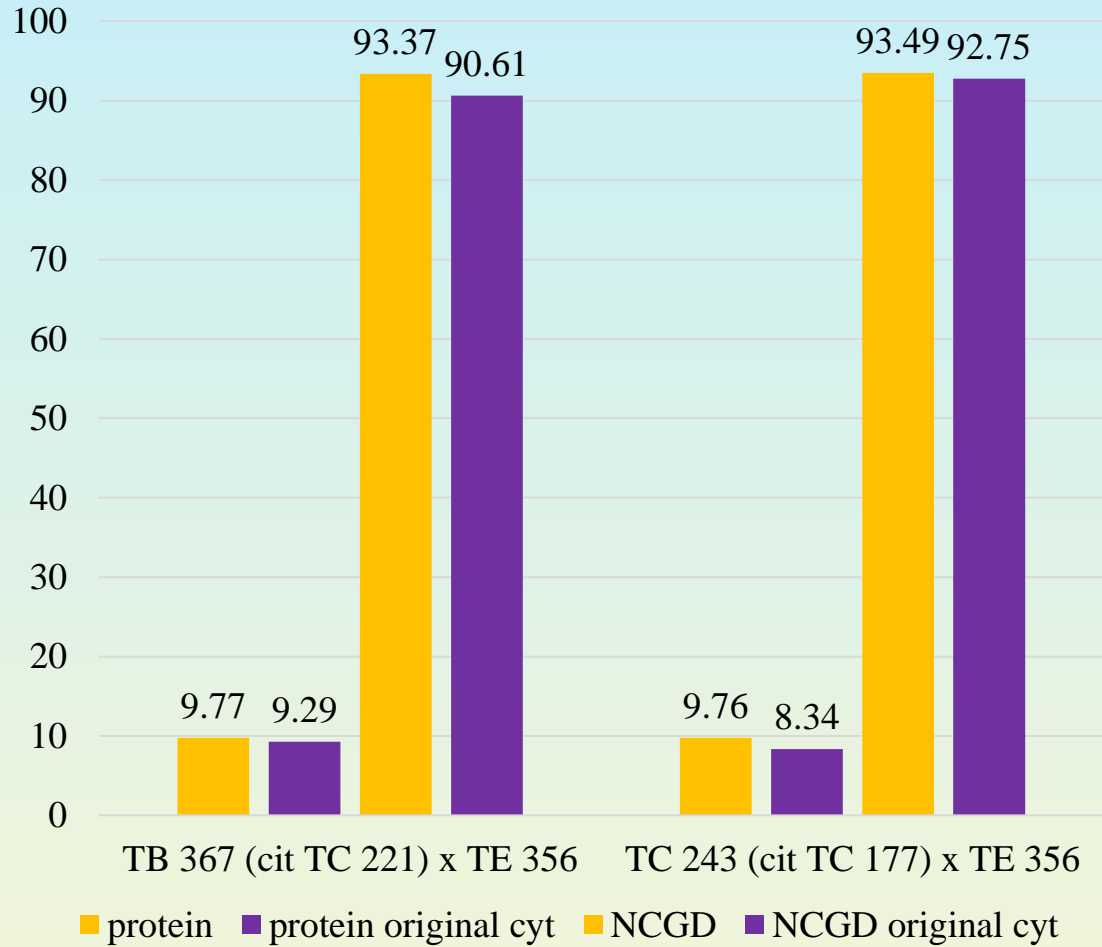
Hybrids with high protein and fat content



p 5% = 0.20
 p 1% = 0.26

Regression analysis for protein and NCGD percent for some maize hybrids (2014)

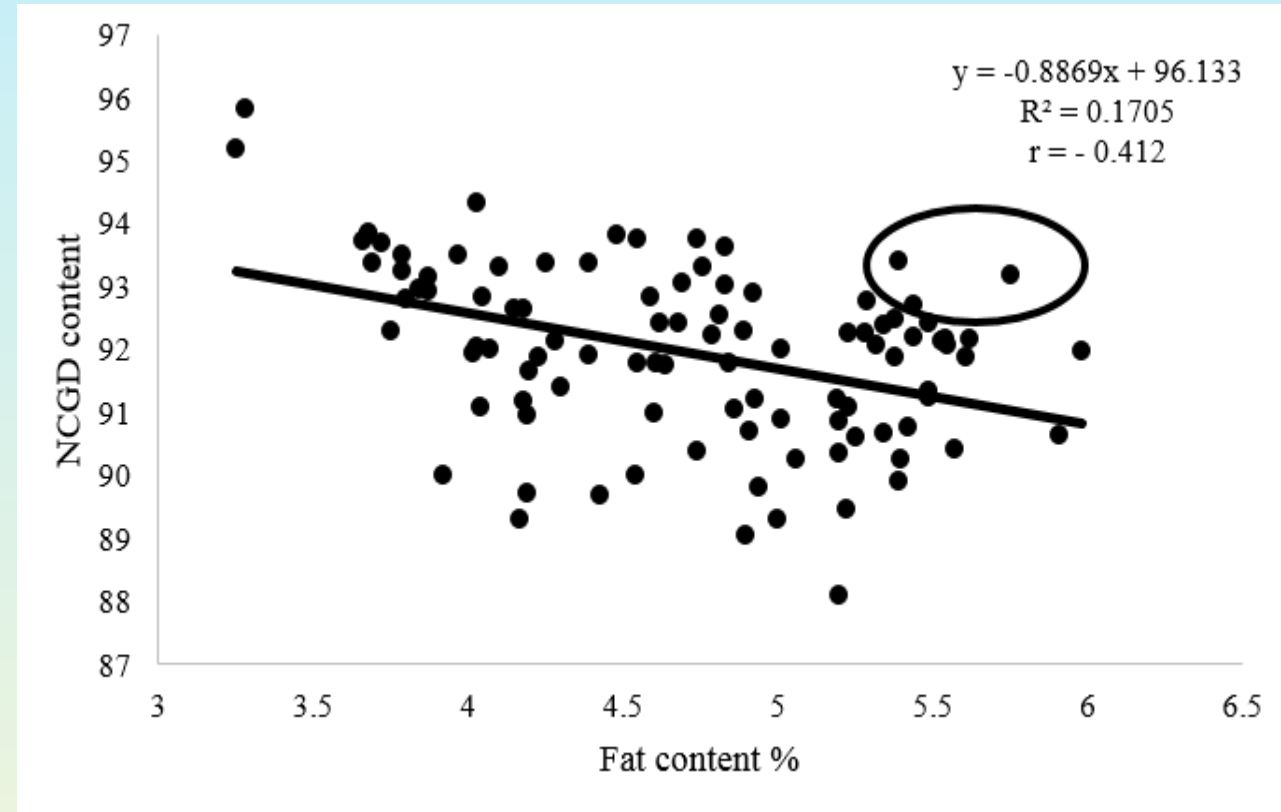
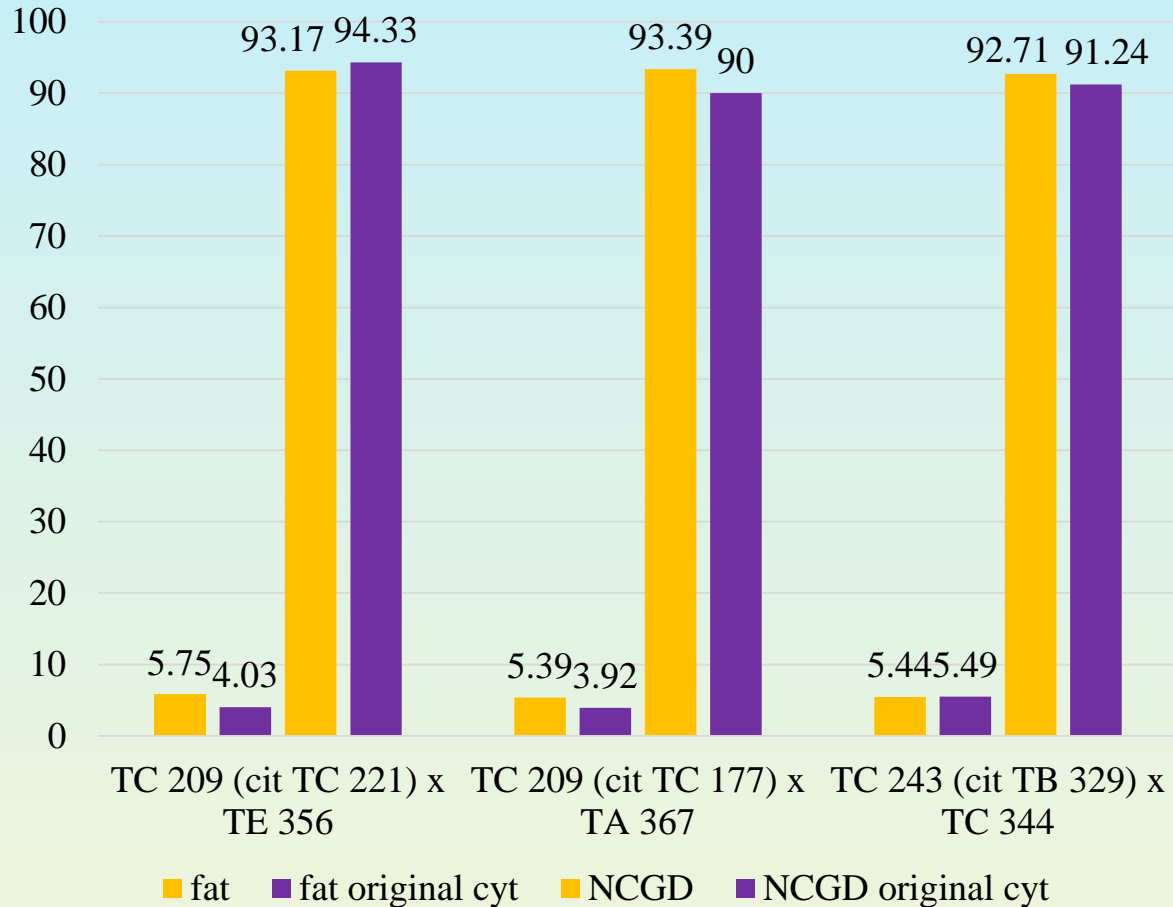
Hybrids with high protein and NCGD percent



p 5% = 0.20
 p 1% = 0.26

Regression analysis for fat and NCGD percent for some maize hybrids (2014)

Hybrids with high fat and NCGD percent



p 5% = 0.20
 p 1% = 0.26

Conclusions

- The transfer of elite inbreds nucleus on different cytoplasms influences the biochemical content, by increasing or decreasing the percent of protein, fat or NCGD.
- Both cytoplasms, the interactions between the nucleus and cytoplasm of the maternal inbred line, and the testers are involved in the determinism of the three analyzed constituents.
- The isonuclear inbreds of TC 209 group have significant differences compared to conventional inbred for all interactions with the testers for NCGD content.
- Significant negative differences for protein content were calculated for the interactions between TB 367 and D 105 groups, and all testers.
- Cytoplasmic nuclear interactions have a very high influence on NCGD, the differences between hybrids obtained by using the original maternal cytoplasm and those with isonuclear maternal lines being up to 5.84%.
- There were identified hybrids using isonuclear maternal inbred lines that have higher values compared to hybrids with the original cytoplasm for at least two biochemical components.

References

- Berardo, N., Mazzinelli, G., Valoti, P., Lagana, P., and R. Redaelli, (2009): Characterization of maize germplasm for the chemical composition of the grain. *Journal of Agricultural Food Chemistry* 57, 2378–2384.
- Coste, I.D., Haş, I., Şchiop, T., Haş, V., Tritean, N. and C. Chicinaş (2011): Phenotypic and Genetic Value of some Isonuclear Inbred Lines of Maize IV. Phenotypic and Genetic Study of Oil Content. *An. INCDA Fundulea* 79 (2), 211-223
- Haiyan, C., and H. Yong (2007): Theory and application of near infrared reflectance spectroscopy in determination of food quality, *Trends Food Sci Tech* 18(2), 72–83.
- Haş, I., Chicinaş, C., Haş, V., Stan, C., Şchiop, T., Coste, I.D. and N. Tritean (2011): Phenotypic and Genetic Values of some Isonuclear Inbred Lines in Maize. I. The Role of Cytoplasmic Diversity on the Behavior Inbred Lines. *AN. I.N.C.D.A. Fundulea* 79 (1), 31-48
- Haş, V., Haş, I., Pamfil, D., Copandean, A. and S. Campean (2009): Evaluation of “Turda” Maize Germplasm for Phenotypic Variability in Grain Chemical Composition. *Maydica* 54, 313-320.
- Pajic, Z. (2007): Breeding of maize types with specific traits at the Maize Research Institute. *Zemun Polje. Genetika* 39 (2), 167-180.
- Şchiop, T., Haş, I., Haş, V., Coste, I.D., Racz, C. and N. Tritean (2011): Phenotypic and Genetic Value of Isonuclear Inbred Lines of Maize. V. Phenotypic and Genetic Study of Starch Content. *AN. INCDA Fundulea*, 79 (2), 225-236
- Scrob, S., Muste, S., Haş, I., Mureşan, C., Socaci, S. and A Fărcaş (2014): The Biochemical Composition and Correlation Estimates for Grain Quality in Maize, *Journal of Agroalimentary Processes and Technologies*, 20 (2), 150-155.

Thank you for your attention!

