GENIC, CYTOPLASMATIC AND NUCLEO-CYTOPLASMATIC INTERACTIONS INVOLVED IN PROTEIN CONTENT DETERMINISM IN A SERIES OF ISONUCLEAR INBRED CORN LINES

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Abstract: *This paper presents a study regarding the protein content in five groups of single cross corn hybrids-resulting from a cross between inbred tester lines and isonuclear inbred lines. Experiments were carried out at ARDS Turda during 2009 and 2010. These hybrids were also tested for their general and specific combining ability, in order to identify those isonuclear inbred lines that show potential use in breeding programs for protein content improvement. The experimental model was polifactorial and included five comparative cultures from which two TC 209 and TC 243 had 28 plots and TC 221, TB 367 and D 105 with 21 plots. For the chemical analysis 50 g of corn kernels were weighed from the average probes and were finely ground. The resulting corn flour was used to determine the protein content. The chemical analyses were performed on samples obtained from self-pollinated corn ears, and open-pollinated corn ears, with INSTALAB 600. In all five comparative groups used for testing there were differences between the experimental years 2009 and 2010 that were statistically assured. Influence of cytoplasms on the variance was between 15 and 67%. In all five experimental situations the interaction with the experimental years was statistically significant. The proportion of the factors involved in the genotypic variance of the protein content (open pollinated) is presented in figure 2. Cytoplasm proportion was between 5% for TC 221 and 17% for TC 209, while the proportion for testers was 45% for TC 221, 72% for TC 209 and 73% for D105. For the hybrid combinations, the specific combining ability was between – 0.71% for the hybrid combination TC 243(cit. A 665) x TC 344 and + 0.83% for the hybrid combination TC 243 x TC 344. The results of this comparative culture revealed the proportion of the cytoplasmatic effects, additive effects of the testers and of the interactions between cytoplasm x nucleuses are relatively close. Our findings revealed that nuclear genes played a great role in the variance of protein content, but the cytoplasmatic and nucleo-cytoplasmatic interactions are also important.*

Key words**:** *protein content, isonuclear inbred corn lines, genic, cytoplasmatic and nucleo-cytoplasmatic interactions*

INTRODUCTION

This paper presents the study made on five groups of single cross hybrids generated between inbred tester lines and isonuclear inbred lines of corn regarding protein content. The five groups of hybrids were also tested for their general and specific combining ability, in order to identify the isonuclear inbred lines which show potential use in breeding programs for protein content improvement.

Genetic determinism of protein and zein is significantly correlated at the additive genetic level, being controlled, in relation to the parental forms included in the cross breeding system, through additive gene and cytoplasmic actions (CĂBULEA and ZETEA, 1973) or only through gene interactions (CĂBULEA et al, 1984). The understanding of corn kernel quality determinism combined with improved kernel yield and other agronomic traits is very important

for the improvement of nutritional and technological values of corn (POLLAK and SCOTT, 2005; OSORNO and CARENA, 2008). Increasing nutritional value of corn kernels can be achieved with genetic and technological methods (HAŞ et al, 2004; HEGYI et al, 2007; HEGYI et al, 2008; IDIKUT at al, 2009).

MATERIAL AND METHODS

Experiments were carried out at ARDS Turda during 2009 and 2010. The experimental model was polifactorial and included five comparative cultures from which two TC 209 and TC 243 had 28 plots and TC 221, TB 367 and D 105 with 21 plots. Each plot consisted of two rows 5 m long, with a distance of 70 cm between rows, 23.7 cm distance between plants in a row, to obtain a density of 60000 plants/ hectare.

Ears were harvested from 10 plants in three repetitions from each observation plot. Each sample was placed in a cloth bag and dried in the corn drier. The procedure was the same for the self-pollinated corn ears used for the chemical analysis.

For the chemical analysis of corn kernels 50 g were weighted from the average probes and were finely ground. The resulting corn flour was used to determine the protein content. Chemical analyses were made with INSTALAB 600. The INSTALAB uses NIR technology and a statistical math treatment to predict the percent of constituent concentration within a sample. By bombarding a sample with a very narrow band of light at a specific wavelength of NIR light, analysis of a sample can be predicted. The light energy absorbed by the sample is inversely proportional to the reflected light (http://dikey-john.com/_media/1-1455.pdf).

RESULTS AND DISCUSSIONS

The protein content study was performed on five groups of isonuclear inbred test lines of corn, kernels were analyzed from self-pollinated ears and from open pollinated ears.

Isonuclear lines (seven from each group) were obtained by transferring the nucleus on different types of cytoplasm through 9-10 backcrosses. It was thought that in this way the transfer between the isonuclear lines from the same group was about 99.9%, with differences only regarding the genetic factors that are found in the cytoplasm and maybe between the interactions of nuclear and cytoplasmic factors.

Given that corn proteins are located both in the embryo and endosperm, there could be some differences between self-pollinated and open-pollinated corn kernels.

In table 1 the analysis of protein content variance is presented for the five groups tested for the self-pollinated ears. There are differences among the five tester groups regarding the degrees of freedom. These differences are due to the fact that testing for TC 209 and TC 243 isonuclear lines was performed with four testers while for TB 367, TC 221 and D 105 isonulear lines, was made only with three testers.

In all five comparative groups used for testing there were differences between the experimental years 2009 and 2010 that were statistically assured.

For all five groups of testing there were significant statistical differences between genotypes.

Cytoplasms provided significant statistical differences in all of the five groups of testing, highlighting the role of cytoplasm in the differentiation of the genotypes. The highest values of variation were registered for the testers. Lower, statistically significant and distinct values were also registered for nucleo-cytoplasmatic interactions.

In all five experimental situations the interaction with the experimental years was statistically significant.

The proportion of the factors involved in the variance of protein (CEAPOIU, 1968) content regarding the self-pollinated ears in presented in figure 1.

Influence of cytoplasms on the variance was between 15 and 67%. As expected the proportion of the testers was between 14% for TB 367 and 63% for D 105. High values were also registered for most situations regarding cytoplasm x nucleus interactions, with values between 16% for D 105 and 43% for TC 209.

Table 1

The variance analyses of protein content for the isonuclear inbred lines (self-pollinated) tested at ARDS Turda (2009-2010)

Source of variability	DF	TC209 isonuclear line		TC243 isonuclear line		DF	TC221 isonuclear line		TB367 isonuclear line		D105 isonuclear line	
		SP	s^2	SP	s^2		SP	s^2	SP	s^2	SP	s^2
Experimental years (Y)	1	68.15	68.15**	21.07	21.07**	1	0.09	0.09*	8.49	8.49*	104.60	104.60**
Genotype	27	39.69	1.47**	55.48	2.05**	20	31.65	1.58**	17.91	0.90**	42.65	2.13**
Cytoplasm (C)	(6)	8.66	1.44**	10.92	1.82**	(6)	4.76	0.79**	11.96	1.99**	9.12	1.52**
Tester (T)	(3)	13.83	4.61**	25.87	8.62**	(2)	14.78	7.39**	2.56	1.28**	26.68	13.34**
(CxT) interaction	(18)	17.21	0.96**	18.69	1.04**	(12)	12.11	1.01**	3.38	0.28**	6.84	0.57**
(YxT) interaction	3	7.86	2.62**	26.32	8.77**	2	3.05	1.52**	8.90	4.45**	12.37	6.19**
(YxC) interaction	6	6.59	1.10**	2.64	0.44**	6	26.79	4.47**	7.08	1.18**	7.11	1.18**
(YxTxC) interaction	18	11.65	0.65**	6.79	0.38**	12	15.18	1.27**	4.81	0.40**	6.45	0.54**
Repetition (R)	2	0.06	0.03	0.16	0.08	2	0.18	0.09	0.19	0.10	0.02	0.01
Error Y	2	0.03	0.01	0.16	0.08	2	0.01	0.00	0.49	0.25	0.08	0.04
Error T	12	0.70	0.06	1.42	0.12	8	1.49	0.19	0.35	0.04	0.28	0.04
Error C	96	4.65	0.05	5.50	0.06	72	4.89	0.07	7.80	0.11	4.86	0.07

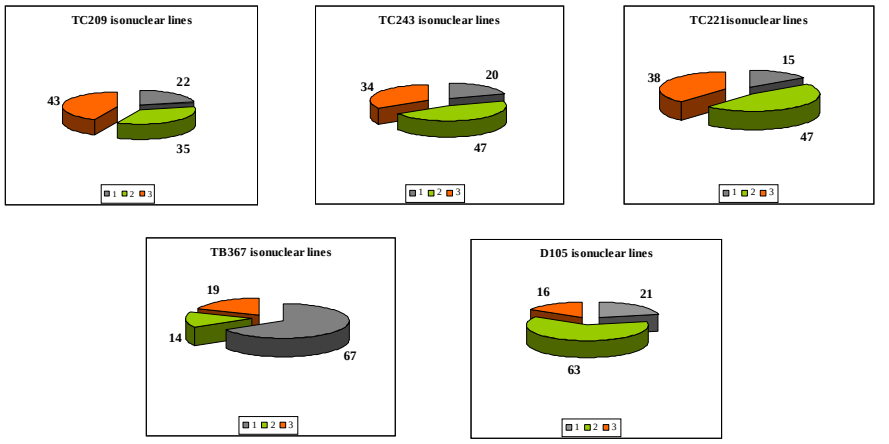

Figure 1: The proportion of each factor involved in genetic variance of protein content (self-pollinated)

1. cytoplasm variance
2. tester variance
3. interaction between cytoplasm x testers

The analyses for the variance of protein content in the tested isonuclear lines, obtained from open-pollinated ears, are presented in table 2. Also in this case the differences between protein content for the two experimental years are statistically significant, revealing the importance of climatic conditions in the manifestation of this character. Interactions between

the experimental years also revealed the influence of the experimental years conditions on the differences in protein content. Statistically significant values were also registered for the open pollinated ears between the genotypes that were studied in the testing of the five groups of isonuclear lines.

Although the values for cytoplasm variance were quite low in absolute terms, they were statistically significant in all five comparative test cultures studied. Variance for the influence of the testers had the highest absolute values, being significant for all studied situations. Interaction between cytoplasm x nucleus also had statistically significant values.

The proportion of the factors involved in the genotypic variance of the protein content (open pollinated) is presented in figure 2. Cytoplasm proportion was between 5% for TC 221 and 17% for TC 209, while the proportion for testers was 45% for TC 221, 72% for TC 209 and 73% for D105.

Table 2

The variance analyses of protein content for the isonuclear inbred lines (open-pollinated) tested at ARDS Turda (2009-2010)

Source of variability	DF	TC209 isonuclear line		TC243 isonuclear line		DF	TC221 isonuclear line		TB367 isonuclear line		D105 isonuclear line	
		SP	s^2	SP	s^2		SP	s^2	SP	s^2	SP	s^2
Experimental years (Y)	1	108.32	108.32**	126.01	126.01*	1	92.91	92.91**	49.91	49.91**	52.46	52.46**
Genotype	27	54.62	2.02 **	64.71	2.4**	20	63.83	3.19**	23.39	1.17**	67.05	3.35**
Cytoplasm (C)	(6)	9.2	1.53 **	5.76	0.96**	(6)	3.34	0.56**	2.39	0.40**	4.44	0.74**
Tester (T)	(3)	37.18	12.39 **	46.36	15.45**	(2)	27.64	13.82**	10.88	5.44**	49.2	24.6**
(CxT) interaction	(18)	8.23	0.46 **	12.59	0.7**	(12)	32.84	2.74**	10.12	0.84**	13.41	1.12**
(YxT) interaction	3	0.81	0.27**	2.53	0.84**	2	6.05	3.02**	11.67	5.84**	5	2.50**
(YxC) interaction	6	8.23	1.37 **	3.75	0.62*	6	9.18	1.53 **	3.18	0.53**	0.92	0.15**
(YxTxC) interaction	18	14.8	0.82 **	16.35	0.91**	12	16.06	1.34**	4.89	0.41**	16.86	1.41**
Repetition (R)	2	0.03	0.02	0.01	0.00	2	0.17	0.08	0.14	0.07	0.07	0.04
Error Y	2	0.02	0.01	0.26	0.13	2	0.16	0.08	0.08	0.04	0.06	0.03
Error T	12	0.57	0.05	0.63	0.05	8	0.49	0.06	3.07	0.38	0.42	0.05
Error C	96	2.82	0.03	3.19	0.03	72	4.65	0.06	6.74	0.09	3.61	0.05

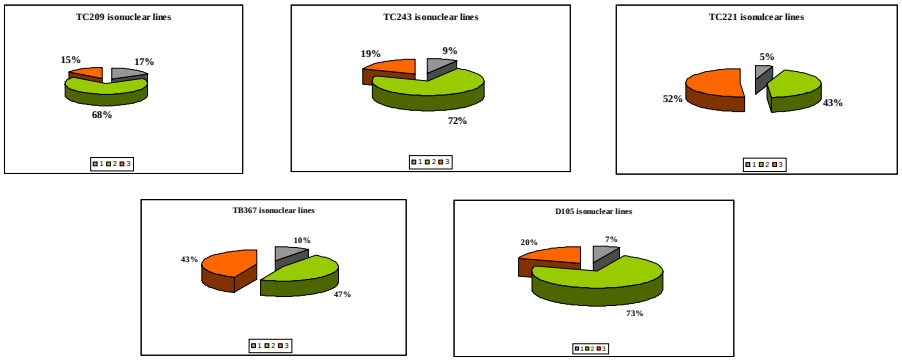

Figure 2: The proportion of each factors involved in genetic variance of protein content (open-pollinated)

1. cytoplasm variance
2. tester variance
3. interaction between cytoplasm x testers

The effect of general and specific combining ability of „cytoplasms x testers", was studied for both pollination types and for all five tester groups. Because of the limited space, in this paper are presented only those results regarding the protein content analysis of ears for the TC 243 testing group lines in the case of self-pollinated ears (table 3) and TC 221 for open-pollinated ears (table 4) .

Average protein content in the isonuclear lines that generated the TC 243 (self pollinated) group of inbred lines (table 3), was 11.6%. Hybrids from the testing of the inbred lines that generated the group realized mean protein content of 11.7% ($\hat{g}_{cit}$= +0.06%). Significantly higher statistical values for mean protein content and implicitly higher values of transmission at a cytoplasmatic level were presented by the following isolines: TC 243(cit TC 221) - $\hat{g}_{cit}$= +0.29% and TC 243 - $\hat{g}_{cit}$= +0.25%; in both cases the differences from $\hat{g}_{cit}$ registered in the testing of check is statistically significant. With much lower values for the transmission of protein content were noted for TC 243(cit. A 665) - $\hat{g}_{cit}$= - 0.40% and TC 243(cit TC 208) - $\hat{g}_{cit}$= -0.34%.

Among the isonuclear tester inbred lines the highest capacity of combining at an additive level was realized by tester line from the indurate corn variety, Lo_3Rf with a general combining ability of + 0.55%, and the lowest value – 0.56% was registered by TC 344.

For the hybrid combinations, the specific combining ability was between – 0.71% for the hybrid combination TC 243(cit. A 665) x TC 344 and + 0.83% for the hybrid combination TC 243 x TC 344. The results of this comparative culture revealed the proportion of the cytoplasmatic effects, additive effects of the testers and of the interactions between cytoplasm x nucleuses are relatively close, but the cytoplasm and testers influence would be decisive in the determinism of the hybrids with the highest values of protein content:

- TC243 (cit K1080) x Lo_3Rf = 12.6% = μ(+11.8%) + $\hat{g}_{cit}$(+0.18%) + $\hat{g}_{test}$(+0.55%)+$\hat{s}_{cit\ x\ test}$(0.26%...)
- TC243 (cit TC221) x Lo_3Rf = 12.5% = μ(+11.8%) + $\hat{g}_{cit}$(+0.29%)+ $\hat{g}_{test}$(+0.55%)+$\hat{s}_{cit\ x\ test}$(0.10%)

Table 3

The general an specific combining ability involved in the transmission of the protein content in the tested isonuclear inbred lines of corn TC 243 (self-pollinated) (ARDS Turda 2009-2010)

cytoplasm/ testers	TC 344		Lo3 Rf		TB 329		TD 233		x	$\hat{g}_{cit}$
	%	$\hat{s}_{cit}$ x test	%	$\hat{s}_{cit}$ x test	%	$\hat{s}_{cit}$ x test	%	$\hat{s}_{cit}$ x test		
TC243	11.9	0.83	12.2	0.02	11.1	-0.51	11.3	-0.34	11.7	0.06
TC243(cit. A665)	9.9	-0.71	12.0	0.26	11.6	0.40	11.3	0.04	11.2	-0.40
TC243(cit.T248)	11.4	0.43	11.8	-0.33	11.3	-0.30	11.8	0.20	11.6	-0.03
TC243(cit.TC208)	10.6	-0.13	11.8	-0.01	11.3	0.09	11.3	0.06	11.2	-0.34
TC243(cit.TC221)	11.0	-0.36	12.5	0.10	12.0	0.13	12.0	0.13	11.9	0.29
TC243(cit. K1080)	11.1	-0.14	12.6	0.26	12.1	0.34	11.3	-0.47	11.8	0.18
T 243(cit. K2051)	11.4	0.08	12.1	-0.31	11.7	-0.15	12.2	0.38	11.8	0.25
Testers average	11.0		12.1		11.6		11.6		11.6	0.00
$\hat{g}_{test}$	-0.56		0.55		-0.01		0.02		11.6	0.00
LDS (P= 5%) $\hat{g}_{cit}$	0.14									
LDS (P=5%) $\hat{g}_{tester\,i}$	0.16									
LDS (P= 5%) interactions c x t	0.27									

The results for the system of testers belonging to the group of isonulcear inbred lines TC 221 (open pollinated) are presented in table 4. The average of the experimental system was 11.8% for the inbred line that created the group TC 221, testing average at a cytoplasmatic level was 11.6% ($\hat{g}_{cit}$ = - 0.20%). The highest capacity of transmitting at a citoplasmatic level was registered for the cytoplasm TC 208 (+ 0.30%), followed by cytoplasm TC 243 (+0.08%) both values are significant in comparison to the cytoplasm of the line that created the group. It can be concluded that these cytoplasms can be used to improve the transmission of protein content by the TC 221 line.

The tester that transmits the highest content of protein at the level of general combining ability (additive level) was T 291 (+0.41%), the lowest value for protein content was transmitted by TC 209 (-0.66%). Results from this comparative culture highlight the larger importance of transmitting protein content at the level of testers in comparison to the value of the protein content transmitted at cytoplasmatic level.

In some situations the levels of nucleo-citoplasmatic interactions were pretty high:

- TC 221(cit. K1080) x T 291 = $\hat{s}_{cit\ x\ test}$ = - 1.17%
- TC221 (cit. K1080) x TC 209 = $\hat{s}_{cit\ x\ test}$ = + 1.14%

The contribution for protein content in the hydrides with the highest values was determined in the first place by the average effects, but equally important are also the effects of the cytoplasms, nucleus and nucleo- cytoplasmatic interactions.

- TC 221(cit. TC208) x TD233=13.1%= μ(11.8%)+ $\hat{g}_{cit}$(0.30%) + $\hat{g}_{test}$(0.25%) + $\hat{s}_{cit\ x\ test}$(0.74%)
- TC 221(cit. T243) x T291=13%= μ(11.8%)+ $\hat{g}_{cit}$(0.08%) + $\hat{g}_{test}$(0.41%) + $\hat{s}_{cit\ x\ test}$(0.70%)

Table 4

The general an specific combining ability involved in the transmission of the protein content in the tested isonuclear inbred lines of corn TC 221 (open-pollinated) (ARDS Turda 2009-2010)

cytoplasm/ testers	T 291		TC 209		TD233		x	$\hat{g}_{cit}$
	%	$\hat{s}_{cit}$ x test	%	$\hat{s}_{cit}$ x test	%	$\hat{s}_{cit}$ x test		
TC221	12.3	0.25	11.2	0.28	11.3	-0.53	11.6	-0.20
TC 221(cit. T 248)	12.2	0.00	10.8	-0.34	12.4	0.34	11.8	-0.02
TC 221(cit. TC 243)	13.0	0.70	11.1	-0.11	11.6	-0.59	11.9	0.08
TC 221(cit. TC 208)	12.3	-0.25	11.0	-0.49	13.1	0.74	12.1	0.30
TC221(cit. TC 209)	12.3	0.12	10.4	-0.51	12.1	0.20	11.6	-0.21
TC221(cit. K 1080)	11.0	-1.17	12.3	1.14	12.1	0.03	11.8	-0.02
T 221(cit. TC 316)	12.4	0.15	11.2	0.02	12.0	-0.17	11.9	0.06
Testers average	12.2		11.2		12.1		11.8	0.00
$\hat{g}_{test}$	0.41		-0.66		0.25		11.8	0.00
LDS (P= 5%) $\hat{g}_{cit}$	0.17							
LDS (P=5%) $\hat{g}_{tester}$	0.13							
LDS (P= 5%) interactions c x t	0.29							

CONCLUSIONS

In the determinism of corn protein content, in a system in which isonuclear lines were tested, the following variances are involved: cytoplasms, testers, and also nucleo-cytoplasmatic interactions.

It seems like nuclear genes have the most important role in the variance of protein

content, but the cytoplasm and the nucleo-cytoplasmatic interactions are also important.

In the determinism of protein content for the tested isonuclear lines the most important are the average values, followed by the nucleo-cytoplasmatic interactions, the additive gene actions because of the testers, and also gene actions with cytoplasmatic localization.

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