

THE YIELD CAPACITY OF MAIZE ISONUCLEAR INBRED LINES (*ZEA MAYS* L.)

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ABSTRACT

This paper presents the yield capacity, kernel dry matter and grain quality for five maize isonuclear inbred lines groups. The isonuclear inbred lines were created at ARDS Turda, by nucleus transfer from elite maize inbred lines (early and intermediate maturing inbred lines) on various types of cytoplasm of American and European inbred lines origin. The nucleus transfer was realized through 10 back-cross-breeding procedures, considering that over 99.8% of the nucleus genes were transferred in this way.

The grain yield, kernel dry matter at harvest and percentage of unbroken plants at harvest for five maize isonuclear inbred lines groups, each group obtained by nucleus transfer on six different cytoplasm types, are presented. The elite inbred lines used as recurrent parents (nucleus genes donors) have been: TC 209, TC 243, TC 221, TB 367 and D105.

The plasmagenes and the interaction between the cytoplasm genes and the nucleus genes influenced in a few situations the isonuclear lines yield capacity and the kernel dry matter accumulation and had no influence on the resistance to plant breaking at harvest.

The cytoplasm diversification could bring a variability source for maize inbred lines improvement.

Key words: isonuclear inbred lines, yield capacity, maize quality, cytoplasm diversity.

INTRODUCTION

In the last 20 years, huge progress in the genetic maize improvement has been achieved; the progress has been based both on genes accumulation with additional effects in parental forms and the heterotic effect between the genetically differenced parents. The best hybrid combinations have been identified both through classical methods, by cyclic or diallel crossbreeds, and modern methods, like molecular genetic markers (Căbulea, 2004; Sarca, 2004; Hegyi et al., 2008; Bernardo and Yu, 2007; Bernardo, 2008).

Phenotype and genotype differences between isonuclear inbred lines have been observed short time after introducing the cytoplasm andro-sterile inbred lines (obtained through nucleus transfer from maternal hybrid lines on different andro-sterile cytoplasm sources) in maize hybrid seeds production (Duvick, 1965; Gracen et al., 1979; Gracen, 1982; Haș et al., 1989; Nagy et al., 1996;

Nagy, 1997). Recent molecular genetic researches have described the role of mitochondrial genome of maize (Fauron and Gasper, 1994; Fauron et al., 1995 a,b; Allen et al., 2007). Fauron et al., (1995 a,b) underlined that genes from the mitochondrial genome of maize have high variability among different cytotypes and that the mitochondrial genome size is evolving in relatively short time, being able to increase or decrease, the mechanism of this phenomenon being yet unclarified. The most common mitochondrial genome in most fertile varieties of commercial maize has been named NB; its sequence was reported by Clifton et al. (2004) and contains 58 coding genes, 33 known proteins, 3 ribosomal RNAs and 21 tRNAs which are able to recognize 14 amino acids.

Allen et al. (2007) proved that mitochondrial genomes NB and NA (fertile) and CMS-C, CMS-S, CMS-T main genome consists of 51 genes, plus one attached to a linear plasmid. These mitochondrial genomes have similarities and differences which lead to

variability induced by these cytoplasmic genes.

The phenotype and genotype differences observed between the isonuclear lines with normal cytoplasm and those with androsterile cytoplasm have lead us to the assumption that differences could exist among different cytoplasm sources.

MATERIAL AND METHODS

The nucleus transfer procedures of 12 elite inbred lines on the five cytoplasm types which were analysed in this paper were conducted since 1992 at the ARDS Turda by the Maize Breeding Laboratory, starting from the hypothesis that among cytoplasm of different origin could exist differences in genetic value, taking into account previous results showing differences between the androsterile lines and the fertile analogous (Gracen, 1982; Haş et al., 1992). The transfer was realized through 10 retro cross-breeding

procedures with the nucleus donor line, in the 1992-2004 period. After that period, considering that 99.9 % of the recurrent line nucleus had been transferred, the isonuclear inbred lines maintenance has been realized through self-pollination and SIB pollination alternation (Chicinaş et al., 2009) (Figure 1).

The cytoplasm source inbred lines are presented in Table 1; the nucleus donor inbred lines are presented in Table 2.

Each inbred line generating a group of isonuclear lines was studied on six different cytoplasm sources, the group generating line (the nucleus genes donor recurrent line) being assumed as check line. The name assignment for the newly created isolines was based on the name of the recurrent parent inbred line, the cytoplasm source being mentioned in brackets: TC 209 (cyt. A 665), TC 243 (cyt. K 2051), TC 221 (cyt. TC 243), TB 367 (cyt. B 329), D 105 (cyt. T 291).

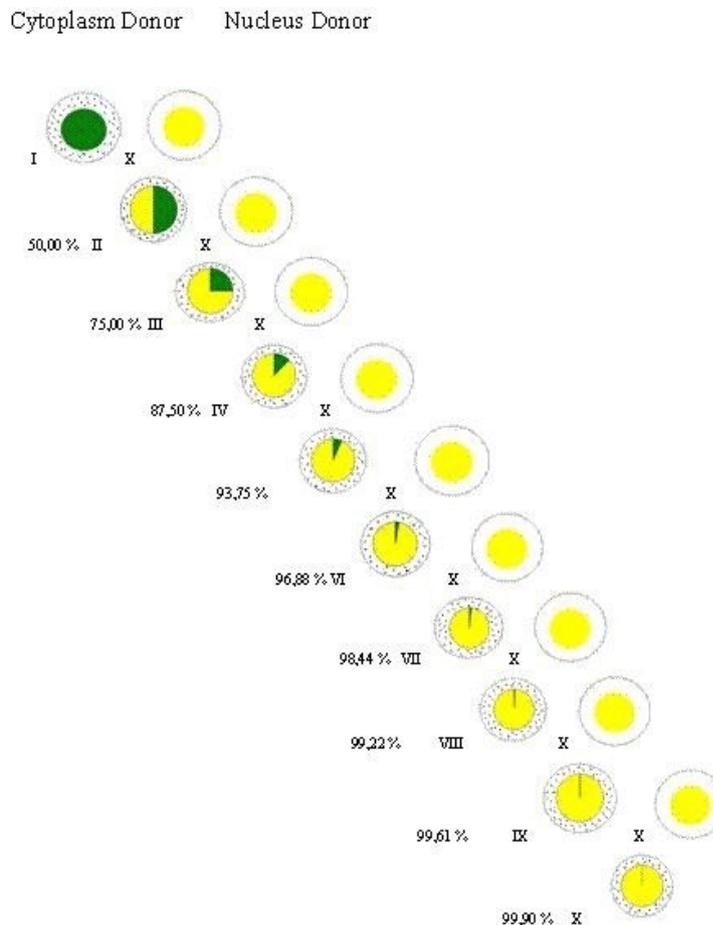


Figure 1. The nucleus on cytoplasm transfer scheme

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Table 1. Inbred lines used as cytoplasm sources

No.	Inbred line	Genealogy (germplasm)	Breeding institution
1.	T 248	Hibrid comercial KC 3	ARDS Turda
2.	TC 243	A 654 X TC 209	ARDS Turda
3.	TC 298	WF 9 X A 116	ARDS Turda
4.	TC 209	BSSS	ARDS Turda
5.	K 1080	Iodent Early	Nordsaat, Germania
6.	TC 316	S54 X Mo 17	ARDS Turda
7.	TB 329	Iodent (MBS 847)	ARDS Turda
8.	TC 221	Lo3 Berg. X Josenii Bârgăului	ARDS Turda
9.	K 2051	European Early Indurata	Nordsaat, Germania
10.	T 291	Pop. Ungheni- Mureş	ARDS Turda
11.	A 665	Stiff Stalk Synthetic	Univ. Minnesota – SUA
12.	W 633	Reid Yellow Dent	Univ. Wisconsin – SUA
13.	TC 177	Sel. Fv. 2	ARDS Turda

Table 2. Nucleus donor inbred lines

No.	Inbred line	Genealogy (germplasm)	Breeding institution
1.	TC 209	BSSS	ARDS Turda
2.	TC 243	A 654 X TC 209	ARDS Turda
3.	TC 221	Lo3 Berg. X Josenii Bârgăului	ARDS Turda
4.	TB 367	PI 187 X T 248 I	ARDS Turda
5.	D 105	European Early Indurata	Univ. Hohenheim, Germania

The isonuclear inbred lines were studied in comparative plots (randomised), in three replications, in the 2009 and 2010 farming seasons. Each experimental plot consisted of two rows of 5 m length. The in-row distance was 23.7 cm, and between rows distance was 70 cm, which result in a plant population of 60,000 plants ha⁻¹.

The results of the experimental measurements were statistically processed through the variance analysis method. Before processing data on unbroken plants at harvest (percentage, calculated by dividing the number of unbroken plants to the total number of plants, multiplied by 100) the figures have been transformed into $\arcsin \sqrt{\text{percent}}$.

The two experimental years (2009 and 2010) were completely different in terms of climatic conditions. The maize vegetation period in 2009 farming season could be characterized as warm and dry. The effective temperature sum was above normal for the 1st of April – 30th of September period by +334.2°C, and the recorded rainfall for the same period was below normal by -118.6 mm.

On the contrary, the 2010 vegetation period was moist and less warm than normal. The effective temperature sum was above normal for the 1st of April – 30th of September period by 120.3°C, and the rainfall above normal by 183.8 mm.

RESULTS AND DISCUSSION

The grain yield (kg ha⁻¹), kernel dry matter at harvest (%) and $\arcsin \sqrt{\%}$ figures for unbroken plants at harvest are presented in Tables 3-7. In the tables, the group generating line of isonuclear inbred lines was assumed as check line when using the ANOVA test method; when using the Duncan test method, isolines resulted from the same inbred line were compared inside the group.

Because for the five groups of isonuclear inbred lines the cytoplasm source on which the nucleus was transferred was different, results are presented for all the five groups.

The grain yield for the seven isonuclear lines from the group generated by TC 209 line (Table 3) ranged from 3331 kg ha⁻¹, for TC

209 (cyt. T 291) line to 4388 kg ha⁻¹ for TC 209 (cyt. TC 177) line. The difference between the two isolines was statistically significant when using the Duncan test.

The kernel dry matter at harvest was influenced only by the T 291 inbred line cytoplasm source; the dry matter at harvest difference was very significant below the inbred check line dry matter content. In the case of the other isonuclear lines generated by TC 209 line, no significant differences were recorded for the two statistical analysis methods applied. The highest percentage of unbroken plants at harvest was registered for TC 209 (cyt. TC 177) line; but the differences

were not significant neither compared to the check line or to the inbred line with the lowest percentage of unbroken plants at harvest – TC 209 (cyt. W 633). The grain yield of the isonuclear inbred lines for the group generated by TC 243 (control) line ranged from 3655 kg ha⁻¹ for TC 243 (cyt. TC 221) line to 4428 kg ha⁻¹ for TC 243 (cyt. T 248) line (Table 4). The difference between the check line and TC 243 (cyt. T 248) line was statistically significant in favour of the line generated by the inbred line T 248 cytoplasm source. The Duncan test highlighted significant differences in yield among isolines in this group.

Table 3. Grain yield, dry matter content in grain and erect plants in isonuclear inbred lines group TC 209 (Turda, 2009-2010)

Isonuclear inbred lines	Grain yield		Dry matter content in grains		Erect plants	
	kg ha ⁻¹	Duncan test	%	Duncan test	arcsin√%	Duncan test
TC 209 (control)	3694	AB	82.02	B	60.62	A
TC 209 (cyt. A 665)	4102	AB	82.88	B	61.58	A
TC 209 (cyt. T 291)	3331	A	77.6 ⁰⁰⁰	A	63.38	A
TC 209 (cyt. T 248)	3440	A	82.55	B	61.39	A
TC 209 (cyt. W 633)	3937	AB	82.68	B	58.55	A
TC 209 (cyt. TC 177)	4388	B	83.08	B	69.22	A
TC 209 (cyt. D 105)	3895	AB	82.60	B	63.05	A
LSD (P 5%)	771		1.10		10.01	
LSD (P 1%)	1048		1.49		13.60	
LSD (P 0.1%)	1403		2.00		18.21	
Theoretical CD		773		1.10		10.03

Table 4. Grain yield, dry matter content in grain and erect plants in isonuclear inbred lines group TC 243 (Turda, 2009-2010)

Isonuclear inbred lines	Grain yield		Dry matter content in grains		Erect plants	
	kg ha ⁻¹	Duncan test	%	Duncan test	arcsin√%	Duncan test
TC 243 (control)	3757	A	78.52	A	70.34	A
TC 243 (cyt. A 665)	4047	B	81.98 ^{***}	C	74.55	A
TC 243 (cyt. T 248)	4428 ^{**}	C	79.88	AB	74.45	A
TC 243 (cyt. TC 208)	3706	A	81.42 ^{**}	BC	72.06	A
TC 243 (cyt. TC 221)	3655	A	80.05	AB	66.96	
TC 243 (cyt. K 1080)	3963	AB	81.60 ^{***}	BC	69.32	A
TC 243 (cyt. K 2051)	3751	A	80.60 [*]	BC	74.33	A
LSD (P 5%)	486		1.69		8.07	
LSD (P 1%)	662		2.30		10.97	
LSD (P 0.1%)	886		3.08		14.69	
Theoretical CD		488		1.69		8.09

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Very significant differences were recorded among the isonuclear lines in this group for kernel dry matter at harvest; the low kernel dry matter at harvest content for the group generating inbred line must be pointed out.

Most lines in this group registered higher kernel dry matter at harvest (differences were statistically significant to very significant compared to the group generating inbred line).

For the isonuclear lines group generated by TC 221 inbred line, no significant differences in grain yield were recorded; but

the kernel dry matter at harvest for isonuclear TC 221 (cyt. T 248), TC 221 (cyt. TC 243) and TC 221 (cyt. D 105) lines was significantly lower compared to the check line. Significant differences were registered among isolines in this group for unbroken plants at harvest percentage (when using DUNCAN test) (Table 5). The most favourable interaction between the nucleus and cytoplasm genetic features for the plants breaking resistance was registered for TC 221 (cyt. TC 208) isonuclear line, and the most unfavourable one for TC 221 (cyt. T 248).

Table 5. Grain yield, dry matter content in grain and erect plants in isonuclear inbred lines group TC 221 (Turda, 2009-2010)

Isonuclear inbred lines	Grain yield		Dry matter content in grains		Erect plants	
	kg ha ⁻¹	Duncan test	%	Duncan test	arcsin√%	Duncan test
TC 221 (control)	2809	A	77.67	D	54.33	AB
TC 221 (cyt. T 248)	2951	A	76.20 ⁰⁰	BC	50.83	A
TC 221 (cyt. TC 243)	2751	A	74.08 ⁰⁰⁰	A	51.89	A
TC 221 (cyt. TC 208)	2972	A	78.12	D	62.08	B
TC 221 (cyt. TC 209)	2718	A	78.03	D	53.06	AB
TC 221 (cyt. K 1080)	2597	A	77.02	CD	51.04	A
TC 221 (cyt. TC 316)	2678	A	75.23 ⁰⁰⁰	B	52.84	AB
<i>LSD (P 5%)</i>	456		1.02		8.72	
<i>LSD (P 1%)</i>	620		1.38		11.86	
<i>LSD (P 0.1%)</i>	830		1.85		15.88	
<i>Theoretical CD</i>		457		1.02		8.74

Two of the isonuclear lines (TB 367 (cyt. TB 329) and TB 367 (cyt. K 2051) from the group generated by nucleus transfer from the inbred line TB 367 significantly exceeded the group generating inbred line. Regarding the kernel dry matter content at harvest, the highest value was recorded for the isonuclear line TB 367 (cyt. K 2051); this result was somehow predictable because the cytoplasm source was the earliest maturing of the inbred lines used as cytoplasm source. On the contrary, the TC 221 line used as cytoplasm donor determined significantly lower dry matter content at harvest, compared to the group generating inbred line (Table 6).

For the isonuclear lines generated by the inbred line D105 (Table 7), the differences in yield were statistically significant (Duncan test); a distinctive significant difference compared to the check line was recorded for D 105 (cyt. TB 329) line. Among the groups generating inbred lines, the D 105 line had the shortest vegetation period; the fact that for the isonuclear lines D 105 (cyt. TC 243), D 105 (cyt. TC 209), D 105 (cyt. K 1080) and D 105 (cyt. TB 329) significant lower kernel dry matter contents at harvest, compared to the group generating line were recorded is considered normal, taking into account that all these lines belong to FAO 300-400 maturity group.

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Table 6. Grain yield, dry matter content in grain and erect plants in isonuclear inbred lines group TC 367 (Turda, 2009-2010)

Isonuclear inbred lines	Grain yield		Dry matter content in grains		Erect plants	
	kg ha ⁻¹	Duncan test	%	Duncan test	arcsin√%	Duncan test
TB 367 (control)	2659	AB	77.68	BC	66.38	A
TB 367 (cyt. T 248)	2948	BC	78.08	C	64.27	A
TB 367 (cyt. TB 329)	3221**	C	77.23	ABC	69.60	A
TB 367 (cyt. TC 208)	2551	A	76.62	AB	66.12	A
TB 367 (cyt. TC 221)	2996	BC	76.35 ⁰	A	70.49	A
TB 367 (cyt. TC 209)	2967	BC	76.90	ABC	72.05	A
TB 367 (cyt. K 2051)	3056*	C	79.43**	D	66.26	A
LSD (P 5%)	353		1.10		8.58	
LSD (P 1%)	480		1.50		11.66	
LSD (P 0.1%)	643		2.00		15.62	
Theoretical CD		354		1.10		8.60

Table 7. Grain yield, dry matter content in grain and erect plants in isonuclear inbred lines group D 105 (Turda, 2009-2010)

Isonuclear inbred lines	Grain yield		Dry matter content in grains		Erect plants	
	kg ha ⁻¹	Duncan test	%	Duncan test	arcsin√%	Duncan test
D 105 (control)	1921	AB	80.18	D	57.24	A
D 105 (cyt. T 291)	1922	AB	80.30	D	56.92	A
D 105 (cyt. T 248)	2134	ABC	79.53	CD	57.99	A
D 105 (cyt. TB 329)	2334*	C	78.80 ⁰	ABC	58.03	A
D 105 (cyt. TC 243)	1789	A	78.03 ⁰⁰⁰	AB	57.18	A
D 105 (cyt. TC 209)	2261	BC	77.68 ⁰⁰⁰	A	65.84	A
D 105 (cyt. K 1080)	2091	ABC	78.93 ⁰	BC	62.44	A
LSD (P 5%)	349		1.13		9.16	
LSD (P 1%)	474		1.54		12.45	
LSD (P 0.1%)	634		2.06		16.68	
Theoretical CD		349		1.14		9.18

A synthesis of the results obtained for the agricultural important features of the isonuclear lines from the five tested groups is presented in Table 8. Significant differences in yield as compared to the check line were registered for three groups, and among isonuclear lines inside groups in four cases; for the kernel dry matter at harvest content significant differences were obtained for all the five groups (on both statistical analysis methods), while for the percentage of unbroken plants at harvest significant differences were recorded only in the case of the group generated by the inbred line TC 221. The differences in yield among the isonuclear lines are probably generated by the

genetic factors contained in the cytoplasm, taking into account the role of cytoplasm in the photosynthesis process; that is why it is most likely that the cytoplasm genes had an active role in generating these differences.

The differences in kernel dry matter content at harvest among the isonuclear lines were generated by the fact that the largest part of the kernel weight is made up of endosperm; the monocotyledonous plants endosperm development is due to maternal polar nuclei fecundation by one of the paternal nuclei. Taking into account that from the maternal side both cytoplasm and nucleus genes are participating in endosperm formation the differences in kernel dry matter content at

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harvest among the isonuclear lines could be explained.

From the inbred lines where the cytoplasm generated a statistically significant increase in grain yield we underline the line TB 329 (in interaction with the nucleus from the inbred lines D 105 and TB 367).

The inbred lines for which the cytoplasm contributed in the kernel dry matter

accumulation were: A 665, TC 208 and K 2051, in interaction with the TC 243 line nucleus. For the inbred line TB 367 the nucleus transfer on the K 2051 line cytoplasm generated kernel dry matter increase at harvest. On the contrary, the TC 243 line cytoplasm, in interaction with the group generating TC 221 and D 105 lines nucleus, generated the kernel dry matter decrease.

Table 8. A synthesis of the results obtained for the agricultural important features of the isonuclear lines from the five tested groups (Turda, 2009-2010)

Isonuclear inbred lines group	Yield capacity		Dry matter content in graines		Errect plants	
	ANOVA test	Duncan test	ANOVA test	Duncan test	ANOVA test	Duncan test
TC 209		*	***	*		
TC 243	**	*	***	*		
TC 221			***	*		*
TB 367	**	*	**	*		
D 105	*	*	***	*		

CONCLUSIONS

Our results proved that there is a genetic variability inside the isonuclear lines groups in grain yield and kernel dry matter content at harvest.

The interaction between the cytoplasm source and the nucleus generated an increase in grain yield, in some isonuclear inbred lines cases.

In all the studied isonuclear lines groups the interaction between the cytoplasm and the group generating line nucleus determined statistically significant differences in the kernel dry matter content at harvest.

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REFERENCES

Allen, J., Fauron, C., Minx, P., Roak, L., Oddiraju, S., Lin, G.N., Meyer, L., Sun, H., Kim, K., Wang, C., Du, F., Xu, D., Gibson, M., Cifresse, J., Clifton, S.W., Newton, K. J., 2007. *Comparisons among Two Fertile and Three Male – Sterile*

Mitochondrial Genomes of Maize. Genetics, 177: 1173-1192. DOI: 10.1534/genetics.107.073312.

Bernardo, R., 2008. *Molecular markers and selection for complex traits: Learning from the last 20 years*. Crop Sci., 48: 1649-1664. DOI: 10.2135/cropsci2008.03.0131.

Bernardo, R. and Yu, J., 2007. *Prospects for genomewide selection for quantitative traits in maize*. Crop Sci., 47: 1082-1090. DOI: 10.2135/cropsci2006.11.0690

Căbulea, I., 2004. *Genetica porumbului*, În: Butnaru, Gallia, Căbulea, I., Cristea M., Haş I., Haş, Voichița, Malschi, Dana, Mureşan, Felicia, Nagy, Elena, Perju, T., Sarca, T., Sarca, Vasilichia, Scurtu, D., Porumbul - Studiu monografic, Edit. Academiei Române, Bucureşti.

Chicinaş, Camelia, 2010. *Studiul fenotipic și genotipic al liniilor consangvinizate de porumb diferențiate citoplasmatic*. Teză de doctorat, Universitatea de Științe Agricole și Medicină Veterinară Cluj-Napoca.

Chicinaş, Camelia, Haş, I., Haş, Voichița, 2009. *Phenotypic characterization of maize inbred lines differentiated through cytoplasm*. Research Journal of Agricultural Science, 41(2): 239-244, Ed. Agroprint, Timișoara.

Clifton, S.W., Minx, P., Fauron, C.M.R., Gibson, M., Allen, J.O., Sun, H., Thompson, M., Barbazuk, W.B., Kanuganti, S., Tayloe, C., Meyer, L, Wilson, R., Newton, K.J., 2004. *Sequence and comparative Analysis of the Maize NB Mitochondrial Genome*. Plant Physiology, 136(3): 3486-3503. DOI: 10.1104/pp.104.044602.

Duvick, D.N., 1965. *Cytoplasmic pollen sterility in corn*. Adv. Gen., 13: 1-56.

- Fauron, C.M., Gasper, M., 1994. *A second type of normal maize mitochondrial genome; an evolutionary link*. Genetics, 137: 875-882.
- Fauron, C., Gasper, M., Gao, Y., Moore, B., 1995a. *The maize mitochondrial genome: dynamic, yet functional*. Trends Genetics, 11: 228-235.
- Fauron, C., Moore, B., Gasper, M., 1995b. *Maize as a model of higher plant plasticity*. Plant Science, 112: 11-32.
- Gracen, V. E., 1982. *Types and availability of male sterile cytoplasms*. In: Maize for Biological Research, Sheridan W.F. (Ed.): 221-224.
- Gracen, V.E., Kheyr-Pour, A., Earle, E.D., Gregory, P., 1979. *Cytoplasmic inheritance of male sterility and post resistance*. Proc. 34th Ann. Corn, Sorg. Res. Conf.: 76-91.
- Hegy, Z., Pok, T., Berzy, J., Pinter, L., Marton, C., 2008. *Comparison of the grain yield and quality potential of maize hybrids in different FAO maturity groups*. Acta Agron. Hung., 56: 161-167.
- Haş, Voichiţa, Grecu, C., Căbulea, I., Haş, I., 1989. *Efectul unor citoplasme mascul sterile asupra stabilităţii comportării hibridilor de porumb*. Probleme de Genetică Teoretică şi Aplicată, XXI, 3: 149-170.
- Nagy, Elena, 1997. *Cercetări privind rolul genotipului gazdă în cadrul patosistemului Zea mays x Fusarium spp.* Teză de doctorat, ASAS Bucureşti.
- Nagy, Elena, Căbulea, I., Haş, Voichiţa, Haş, I., 1996. *Reaction of the maize hybrids with the different types of cytoplasmic sterility to the attack of Fusarium spp.* (In Romanian). Analele ICCPT Fundulea, LXIII: 219-229.
- Sarca, T., 2004. *Ameliorarea porumbului*. In: Butnaru Gallia, Căbulea I., Cristea M., Haş I., Haş Voichiţa, Malschi Dana, Mureşan Felicia, Nagy Elena, Perju T., Sarca T., Sarca Vasilichia, Scurtu D., Porumbul - Studiu monografic, Edit. Academiei Române, Bucureşti.