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STUDY ON THE ISONUCLEAR INBRED LINES REACTION UNDER NATURAL INFECTION CONDITIONS WITH *FUSARIUM* SPP.

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ABSTRACT

The fusarium ear rot caused by pathogens belonging to *Fusarium* genus is one of the most important diseases responsible for high economic losses, resulting from the decline of the production's quantity and quality. The objective of the present study was the identification of sources of cytoplasm that convey resistance to fusarium ear rot within certain inbred isonuclear lines. Twenty five maize inbred lines: 20 isonuclear inbreds (obtained by transferring the nucleus of five elite inbred lines on four different cytoplasms) and five lines using the original cytoplasm were studied in three experimental years 2012, 2013 and 2014. The analysis of variance for fusarium ear rot showed the very significant influence of factors years and nucleus and the significant action of the cytoplasm. The resistance to fusarium ear rot of each isonuclear line was compared to the line with original cytoplasm, over the three years of the experiment. Genetic variability within isonuclear groups regarding the resistance to fusarium ear rot was observed. The greatest difference in the degree of attack on the maize cob in comparison with the original cytoplasm (-4.92%), was noted for isoline TB 243 x TB 329, which showed a very significantly negative difference. Changing cytoplasm resulted in genotypes with a good resistance to the *Fusarium* spp. attack; therefore the use of these genotypes is recommended in creating of new hybrids.

Key words: *Fusarium*, maize, isonuclear inbred lines, cytoplasmic effects, cytoplasmic-nuclear interaction.

INTRODUCTION

In the case of maize, the cause of the epidemic outbreak with *Helmithosphorium maydis* (1969-1970) was the uniformization of the cytoplasm, respectively the use of a single androsterile Texas type (cms T) cytoplasm. The extent of over 80% of the maize cultivated area in USA affected by this cryptogamic disease resulted from the fact that the maize hybrid seed was produced based on Texas type (cms T) cytoplasmic androsterility, and in this way, all the hybrids having identical cytoplasm, increased the genetic vulnerability of maize to the *H. maydis* pathogen (Ullstrup, 1977). In this context, research for cytoplasm sources has been expanded, in order to identify cytoplasms that positively or negatively influence the characters of maize cobs, plants, kernels, as well as some characters of agronomical interest, such as resistance to disease and pests. The fusarium ear rot caused by pathogens belonging to *Fusarium* genus is one of the most important diseases responsible for high economic losses resulting from the decline of the production's quantity and quality (Nelson et al., 1983; Leslie and Summerell, 2006; Kvas et al., 2009).

The importance of these diseases has been recognized for many decades, but they are still difficult problems because high levels of genetic resistance have not been discovered and incorporated into agronomically desirable high-yielding hybrids.

The complexity of developing resistant maize hybrids is related to the many-faceted epidemiology of *Fusarium* diseases (Munkvold and Hellmich, 1997, 1999, Munkvold, 2003).

Research carried out by Rao and Fleming (1978, 1980), Nagy and Căbulea (1996), Nagy (1997), Haș et al. (2011) demonstrated the involvement of cytoplasms in the genetic determinism of some characters of the stems and kernel, such as resistance to *Fusarium*.

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The objective of this study was the identification of sources of cytoplasm that convey resistance to fusarium ear rot within certain inbred isonuclear lines.

MATERIAL AND METHODS

For the identification of differentiated cytoplasmic resistance sources to Fusarium ears rot, an experiment was set in place using the biological material consisting of 25 lines of the maize germplasm available at Agricultural Research & Development Station Turda: 20 isolines were obtained after crossing four cytoplasm donor inbred lines with five nucleus donor lines; additionally the five initial inbred lines were included in the study (Table 1). The nucleus donor inbred lines were used as recurrent parent in crosses, which were made over ten generations. The nuclei TC 209, TC 316, TC 243, TB 367 and D 105 were transferred into four cytoplasms, originating from T 248, TB 329, TC 177 and TC 221. The isonuclear inbred lines were studied in comparative plots (randomised), in five replications, in the 2012-2014 farming seasons. Each experimental plot consisted of two rows of 5 m length. The sowing density was 70000 plants ha^{-1}, each plot consisted of two rows: each 5 m long, 26 plants per row. The results of the experimental measurements were statistically processed using the variance analysis method. *Fusarium* degree of attack at harvest was transformed into arcsin √percent.

Table 1. Biological material - inbred lines heterotic group

Specification	Inbred line	Heterotic group
Nucleus	TC 209	BSSS
	TC 316	Lancaster
	TC 243	BSSS
	TB 367	Argentinean Flint
	D 105	Fv 2 Flint
Cytoplasm	T 248	Lancaster
	TB 329	Iodent
	TC 177	Fv 2 Flint
	TC 221	European Flint

RESULTS AND DISCUSSION

The climatic conditions influenced the growing and the developing of the maize, but also the emergence and the evolution of the pathogens. The year 2012, according to the recorded temperatures, was a very warm year and according to rainfall was totally unfavourable for maize crop.

The year 2013 was a warm year, with temperatures that were slightly above the average of 57 years. In terms of precipitations was normal, but in July we had a deficiency of 39 mm (compared to the 57 years average) which was reflected in the lower values degree of attack (Figure 1).

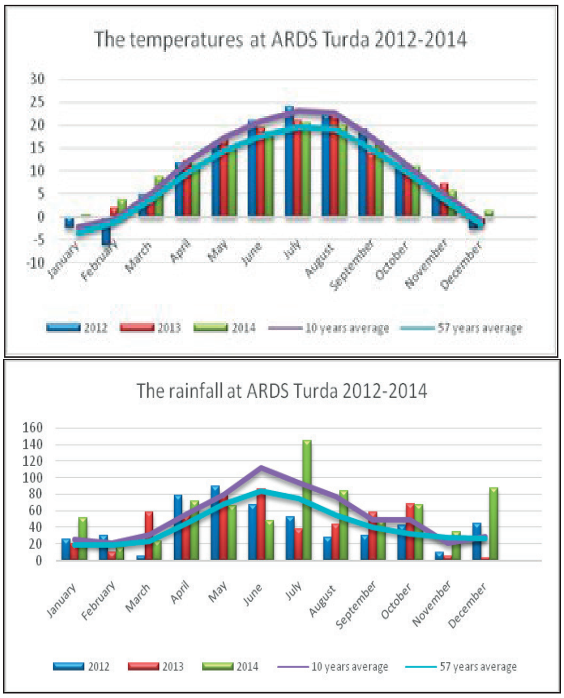

Figure 1. The temperatures and rainfall regime at ARDS Turda, 2012-2014
(Source: Weather Station Turda (longitude: 23° 47'; latitude 46°35'; altitude 427 m)

The temperatures recorded in 2014 were closer to the 57 years average, the year being characterized as normal in terms of temperatures. From the perspective of rainfall was rainy, but the rainfall was not evenly distributed, June was characterized as excessively dry and July as excessively rainy, with 67.7 mm extra rain in July (compared to 57 years average) which favored manifestation of fusarium ear rot (Figure 1).

The three experimental years were non-identical during maize growing season in terms of temperature and rainfall and influenced different the emergence and development of *Fusarium* spp.

Creating isonuclear lines involves a nucleus donor line and a line on whose cytoplasm the nucleus transfer is intended. Backcrossing with the nucleus donor line is carried out after the crossing of the two biological materials, until it is considered that the nucleus has been transferred at the rate of almost 100% (Zeng et al., 1998; Chicinaș et al., 2009, Has et al., 2011, Călugăr et al., 2016). The isonuclear lines considered within the study were obtained by means of the transfer of the nucleus to different types of cytoplasms, by 9-10 backcrossings, and the result was a theoretical 99.9% transfer rate (Racz et al., 2013). There were differences between the isonuclear lines of the same group, in terms of genetic factors positioned in the cytoplasm, as well as between the possible interactions among nuclear genetic factors and the cytoplasmic ones (Coste et al., 2011).

The analysis of variance for fusarium ear rot, according to data in Table 2, showed the very significant influence of factors years and nucleus and a significant influence of the cytoplasm. The interactions between the three factors had a very significant influence on the attack of *Fusarium* on the maize cob.

Climatic factors are decisive for causing infection and disease evolution. Most of the times, the optimal climatic conditions for plants are also optimal for phytopathogenic agents (Suciu et al., 2013).

Table 2. The variance analysis for fusarium ears rot in isonuclear inbred lines (2012-2014)

The source of variation	The sum of squares	Degrees of freedom	Mean square	F Test
Y (Year)	787.70070	2	393.85040	409.885***
N (Nucleus)	595.06880	4	148.76720	149.478***
Y x N	304.08690	8	38.01086	38.192***
C (Cytoplasm)	13.37700	4	3.34425	2.407*
Y x C	82.87078	8	10.35885	7.455***
N x C	77.59013	16	4.84938	3.490***
Y x N x C	111.78000	32	3.49312	2.514***
R	3.11616	1	3.11616	
Y x R	1.92176	2	0.96088	
N x R	4.50297	4	1.12574	
Y x N x R	7.44000	8	0.93000	
C xR	1.16333	4	0.29083	
Y x C x R	12.09418	8	1.51177	
N x C x R	14.57493	16	0.91093	
Y x N x C x R	55.53695	32	1.73553	
Error Y	1.92176	2	0.96088	
Error N	11.94297	12	0.99525	
Error C	83.36940	60	1.38949	
T o t a l	2072.82400	149		

During the three years of the experiment, the *Fusarium* spp. attack was influenced differently. As shown in Table 3, favourable conditions for the fusarium attack existed in 2012 and 2014, differences in the degree of attack during these years and the average of the three years of the experiment being significantly positive. Due to climatic conditions of 2013, the degree of *Fusarium* spp. attack was the lowest (0.25%), showing distinctly negative difference compared to the three year average.

In the case of maize, the most important disease-resistance genes are located near the centromere of chromosome 5, and on the long arms of chromosomes 6, 8 and 9 (Nagy, 1997), while Jinahion and Russel (1969) stated that a resistance gene to *Fusarium moniliforme* is located on the long arm of chromosome 10, identified in the inbred line 61 C, the second gene being located on the short arm of chromosome 7. It can be noted that during the three years of our experiments, in the case of nuclei TC 209 and

TB 367, the degree of attack of *Fusarium* spp. was lower than the average of the nuclei, with a very significant negative difference compared to the average. In the case of isolines with nucleus TC 243 the fusarium degree of attack was the highest, this being confirmed by the values of the Duncan test (Table 4).

Knowledge on the role of cytoplasm is important for establishing the hybridisation formulas, using as mother the lines which transmit the resistance in a favourable way via the cytoplasm (Nagy, 1996). Even if the cytoplasm does not affect significantly the resistance to the *Fusarium* spp. attack, it can be noted that in the case of isolines with TC 221 cytoplasm, was recorded the lowest degree of attack (0.99%) (Table 5).

Table 3. The influence of the climatic condition on the *Fusarium* spp. degree of attack (2012-2014)

No.	Year	Degree of attack (%)	Degree of attack (arcsin√%)	% to control	The difference to control	Duncan Test
1	Average	1.1	6.12	100.0	0.00	
2	2012	1.8	7.63	124.7	1.51*	B
3	2013	0.25	2.88	47.0	-3.24^{00}	A
4	2014	1.9	7.84	128.2	1.73*	B
LSD (p 5%)					0.84	DS = 0.84
LSD (p 1%)					1.95	
LSD (p 0.1%)					6.20	

Table 4. The influence of nucleus hereditary factors on the *Fusarium* spp. degree of attack (2012-2014)

No.	Nucleus	Degree of attack (%)	Degree of attack (arcsin√%)	% to control	The difference to control	Duncan Test
1	Average	1.1	6.12	100.0	0.00	
2	TC 209	0.32	3.26	53.2	-2.86^{000}	A
3	TC 316	1.6	7.37	120.6	1.26***	C
4	TC 243	2.1	8.25	135.0	2.14***	D
5	TB 367	0.54	4.21	68.8	-1.91^{000}	B
6	D 105	1.7	7.49	122.4	1.37***	C
LSD (p 5%)					0.56	DS = 0.56
LSD (p 1%)					0.79	
LSD (p 0.1%)					1.11	

Table 5. The influence cytoplasm hereditary factors on the *Fusarium* spp. degree of attack (2012-2014)

No.	Cytoplasm	Degree of attack (%)	Degree of attack (arcsin√%)	% to control	The difference to control	Duncan Test
1	Original cytoplasm	1.2	6.21	100.0	0.00	AB
2	T 248	1.3	6.44	103.7	0.23	B
3	TB 329	1.3	6.41	103.3	0.20	B
4	TC 177	1.0	5.81	93.6	-0.40	AB
5	TC 221	0.99	5.72	92.2	-0.48	A
LSD (p 5%)					0.61	DS = 0.61
LSD (p 1%)					0.81	
LSD (p 0.1%)					1.05	

The noticeable climatic conditions of three years of the experiment have influenced differently the degree of *Fusarium* spp. attack in the tested isolines. In the climatic conditions of year 2012, the highest degree of attack was recorded for isolines with TC 243 nucleus, the difference relative to the control being very significantly positive (Table 6).

Table 6. The influence of climatic conditions and nucleus on the *Fusarium* spp. degree of attack (2012-2014)

No.	Year	Nucleus	Degree of attack (%)	Degree of attack (arcsin√%)	% to control	The difference to control	Duncan Test
1	2012	Average	1.8	7.63	100.0	0.00	
2		TC 209	1.4	6.80	89.2	-0.83	C
3		TC 316	2.7	9.40	123.2	1.77**	F
4		TC243	2.9	9.78	128.1	2.15***	FG
5		TB 367	0.5	4.05	53.1	-3.58^{000}	B
6		D 105	2	8.12	106.4	0.49	DE
7	2013	Average	0.25	2.88	100.0	0.00	
8		TC 209	0.06	1.35	46.9	-1.53^{00}	A
9		TC 316	0.58	4.37	152.0	1.50**	B
10		TC 243	0.35	3.38	117.6	0.51	B
11		TB 367	0.06	1.40	48.5	-1.48^{00}	A
12		D 105	0.46	3.88	135.0	1.01*	B
13	2014	Average	1.9	7.84	100.0	0.00	
14		TC 209	0.08	1.62	20.6	-6.22^{000}	A
15		TC 316	2.1	8.35	106.5	0.51	E
16		TC 243	4.1	11.60	148.0	3.76***	H
17		TB 367	1.6	7.19	91.6	-0.66	CD
18		D 105	3.3	10.45	133.3	2.61***	G
LSD (p 5%)						0.97	DS = 0.97
LSD (p 1%)						1.37	
LSD (p 0.1%)						1.93	

Although 2013 was a year during which the pathogens attack was less important, due to a lack of rainfall, in the studied isolines a fusarium attack was recorded, but not greater than 0.60%. In 2014 the highest degree of attack of *Fusarium* spp. was recorded.

During this year also, the most susceptible isolines where those with the TC 243 nucleus. In the three years of the experiment the best behaviour was noted in isolines with nuclei TC 209 367 and TB 367, as having the cobs the least affected by fusariosis (Table 6).

The degree of attack on the cob was influenced by the interaction of the climatic conditions with the cytoplasm. During the three years of the experiment, the isolines with cytoplasms TC 177 and TC 221 had the least degree of attack in year 2013-2014 (Table 7).

Based on data shown in Table 7, the different behaviour of isolines with cytoplasm TB 329 can be noted. In the first year of the survey these lines showed a low risk of attack, the difference compared with the average being significantly negative; but in 2014, these isolines were the most susceptible, with the highest degree of attack, the difference from the control being very significantly positive.

Table 7. The influence of climatic conditions and cytoplasm on the *Fusarium* spp. degree of attack (2012-2014)

No.	Year	Cytoplasm	Degree of attack (%)	Degree of attack (arcsin√%)	% to control	The difference to control	Duncan Test
1.	2012	Original cytoplasm	2.0	8.14	100.0	0.00	Control
2.		T 248	1.8	7.66	94.0	-0.49	BC
3.		TB 329	1.3	6.64	81.5	-1.51^{00}	B
4.		TC 177	1.7	7.42	91.1	-0.72	BC
5.		TC 221	2.1	8.29	101.8	0.14	CD
6.	2013	Original cyt.	0.38	3.55	100.0	0.00	Control
7.		T 248	0.18	2.46	69.3	-1.09^{0}	A
8.		TB 329	0.34	3.31	93.3	-0.24	A
9.		TC 177	0.22	2.68	75.5	-0.87	A
10.		TC 221	0.17	2.39	67.2	-1.17^{0}	A
11.	2014	Original cyt.	1.5	6.92	100.0	0.00	Control
12.		T 248	2.6	9.20	132.9	2.27***	D
13.		TB 329	2.6	9.27	134.0	2.35***	D
14.		TC 177	1.6	7.32	105.8	0.40	BC
15.		TC 221	1.3	6.50	93.8	-0.43	B
LSD (p 5%)						1.05	DS = 1.05
LSD (p 1%)						1.40	
LSD (p 0.1%)						1.82	

The resistance to fusarium ear rot of each isonuclear line was compared to the original cytoplasm, over the three years of the experiment. Under the test conditions in 2012, in case of three groups of isolines, changing the cytoplasm led to the reduction of the degree of attack of by *Fusarium* spp. The highest difference in the degree of attack on the maize cob in comparison with the original cytoplasm (-4.92%), was found for isoline TB 243 x TB 329, which showed a very significant negative difference. Within the TC 209 group of isolines, isoline TCB 209 x TB 329 is noticeable, with an increase of the degree of attack of 3.07% compared to the original cytoplasm (Figure 2).

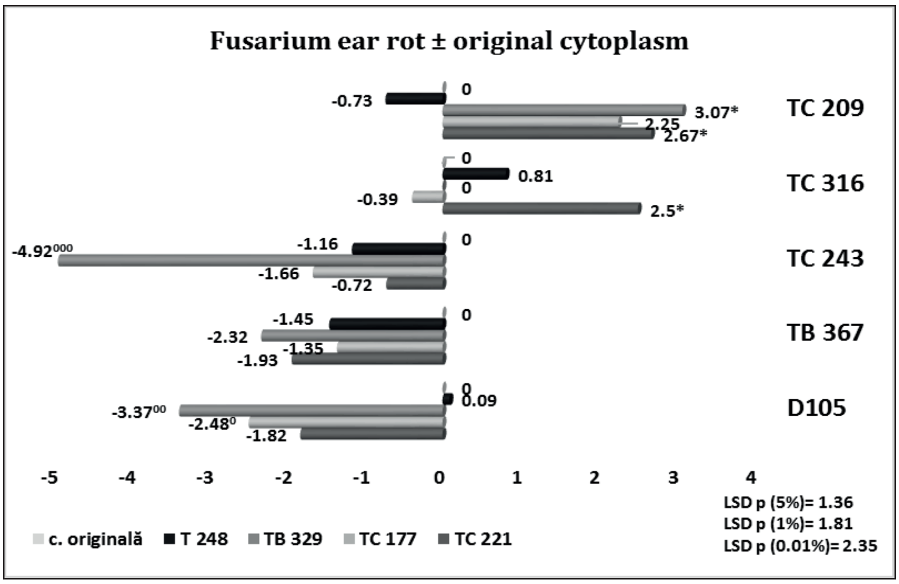

Figure 2. Influence of nuclear-cytoplasmatic interaction on the *Fusarium* spp. degree of attack in a set of isonuclear inbred lines (2012)

In 2013, the situation was slightly different in four of the five isoline groups; the cytoplasm change induced the decrease of the degree of Fusarium attack. The group of isolines TB 367 is to be noted, as the change of cytoplasm within it lead to an increased vulnerability to *Fusarium* spp. and thus to an increase in the degree of attack (Figure 3).

The year 2014 was a favourable year for the cultivation of maize and as well for the occurrence of fusarium ear rot. As can be seen in Figure 4, the groups of isolines behaved differently from each other, but also within the group.

The most sensitive isolines to the attack where those of the TC 316 group, showing the largest differences of degree of attack relative to the original cytoplasm.

A significant reduction of the degree of attack was recorded for isolines D 105 x TC 221, D 105 x TC 177 and TB 367 x TC 221 (Figure 4).

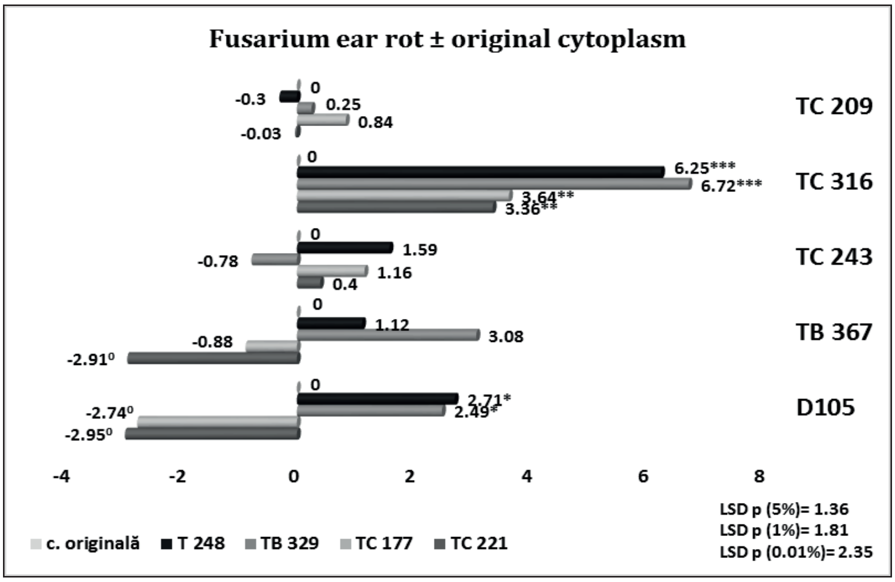

Figure 3. Influence of nuclear-cytoplasmatic interaction on the *Fusarium* spp. degree of attack in a set of isonuclear inbred lines (2013)

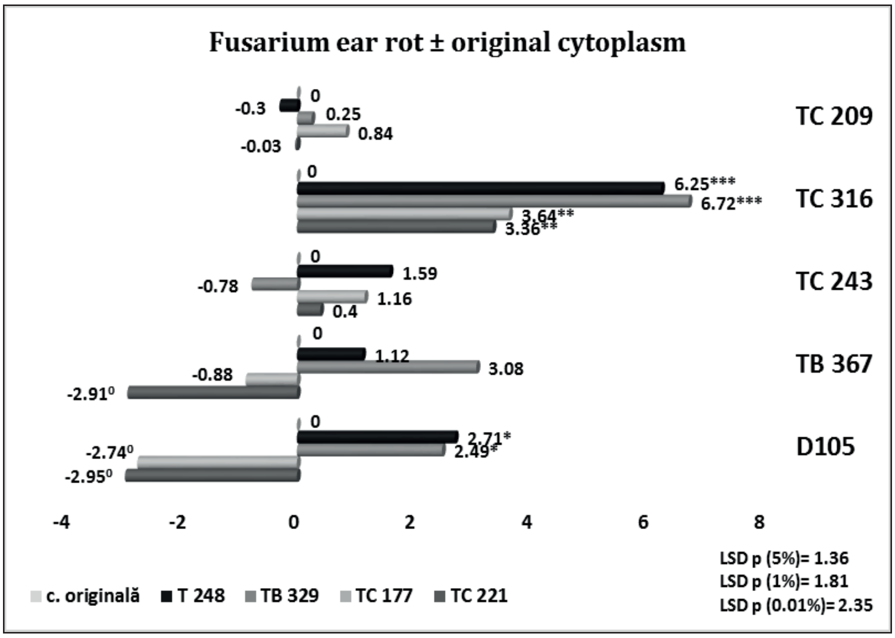

Figure 4. Influence of nuclear-cytoplasmatic interaction on the *Fusarium* spp. degree of attack in a set of isonuclear inbred lines (2014)

CONCLUSIONS

The result of our research showed that there is genetic variability within isonuclear groups regarding the resistance to fusarium ear rot.

In different climatic conditions, the interaction between cytoplasm and nucleus influenced differently the resistance to fusarium.

Changing the cytoplasm resulted in the creation of genotypes with a better resistance to the *Fusarium* spp. attack, therefore when creating new hybrids, their use is recommended.

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