THE EFFECT OF DIFFERENT TYPE OF CYTOPLASM ON SEED FRACTIONS IN MAIZE INBRED LINES

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Abstract

The seed in order to meet requirements of cropping practices and growing systems in the production of commercial maize. The aim of the present study was to determine the effects of The seed processing technology depends on the seed fraction. The introduction of new and improved solutions in maize seed processing contributes to the improvement of traits of different types of cytoplasm (cms-C, cms-S and fertile), and environmental factors on the medium large flat seeds of maize inbred lines. The study encompassed the 12 same maize inbred lines of each type of cytoplasm. The trial was set up according to the complete randomised block design in two locations with three replications. Statistical-biometrical data processing was based on means per replication and encompassed the analysis of variance. On the basis of this analysis, it was determined that there were significant differences among inbred lines regarding the medium large flat seed fraction (MLF) in dependence on the type of cytoplasm, year and the location. The average values of the seed fraction varied over inbreds from 0.4% to 16.3%. The highest, i.e. lowest value for this trait was expressed by sterile cytoplasm cms-C, i.e. fertile cytoplasm, respectively. Furthermore, a greater share of MLF seeds was recorded in the first year and the first location than in the second year and the second location. The inbred line × location interaction points out to very significant ($Lsd_{0.001}$) differences in the content of MLF seeds of maize inbred lines in dependence of observed locations. The years of investigation and locations significantly ($Lsd_{0.005}$) affected the content of MLF seeds in maize inbred lines. The analysis of obtained results points out to a significant effect of the type of cytoplasm on the medium large flat seed fraction.

Key words*:* *cytoplasmic male sterility, inbred lines, seed fractions*

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cultivated plant species in the world, due to high yields and also a possibility of its diverse utilisation in food, feed and industry. In Serbia, maize is also very important, and it is grown on more than a million hectares of cultivated areas. In developed countries, maize is primarily used as feed (78% of the total global production), then in the production of food and industrial products, such as starch, oil, sweeteners and alcohol. Furthermore, the production of bioethanol has been gaining in importance (Ragauskas et al., 2006; Farrell et al., 2006).

Maize is a plant very suitable for hybrid seed production on a large scale, because each maize plant has both male and female reproductive organs. Hybridisation can easily be accomplished by sowing parental components in alternative rows or by detasseling male flowers (tassels) from female plants, immediately after their occurrence. In such a way, only male component pollen is released in fields, and the seed produced on the female component is the hybrid seed.

In order to have heterosis expressed in the F_1 generation it is necessary to achieve the complete hybridisation between parental components. If complete hybridisation is not achieved, a self-pollinated female component occurs in produced seeds and heterosis will not be fully utilised which will result in the reduction of the total yield per area unit. In the production of maize seed hybrids, it is often not possible to achieve complete hybridisation between parental components, even with the bestorganised growers. In order to achieve complete hybridisation, it is necessary to remove all tassels from plants in the maternal rows before pollen is released. For this job, it is necessary to provide a large number of workers and to engage them in a relatively short period, from 10 to 30 days. Tassel removal is a difficult and tedious job for workers, and is usually done under adverse conditions during the hottest times of the year.

Mechanised topping of tassels, despite permanent efforts, has not given satisfactory results in practice. Experiments with machinery for mechanised topping of tassels were conducted by numerous researchers (Dungan and Wudworth, 1939; Borgeson, 1943; Kiesslbach, 1945; Bauman, 1959; Hunter et al., 1973 and others), while the results of these experiments were summarised by Huey (1971) and Trifunović (1975). Huey (1971) stated that mechanical detasselers - cutters did not solve the problem of the tassel removal from tillers as well as from plants lagging in growth, they were useless under poor weather conditions, and at the same time it was impossible to reduce the number of averagely lost leaves per plant bellow 2-3 even with the most careful work.

The possibility for an effective solution to the problem of detasseling in hybrid seed production has emerged with the discovery of cytoplasmic male sterility in maize. Using the sterile male version of the female component completely eliminates the need for detasseling, then the number of workers needed for control tasks is minimised, production quality is improved and costs and associated risks are significantly reduced, and finally, in this way, the seed production becomes very profitable for producers.

The first description of male sterility was given by Rhoades (1931). Further investigations showed that sterility was caused by cytoplasmic factors.

Kaeser et al. (2003) considered cytoplasmic male sterility (CMS) a trait interesting for the maize seed industry, because it led to lower costs of the hybrid seed production by eliminating of the labour-intensive mechanical emasculation of parental lines.

Cytoplasmic male sterility (CMS) refers to the inability of the plant to produce functional pollen. This trait is conditioned by mutations in the mitochondrial genome, so it is transmitted through the cytoplasm, i.e. it is not transmitted by pollen and is not subjected to the Mendelian inheritance. CMS has found its application in the production of hybrid maize seeds, because detasseling of self-pollinated female component inbred plants is not necessary in seed production in hybrids based on sterility. Therefore, the production becomes cheaper, of better quality and less demanding than the conventional one. There are three types of cytoplasmic male sterility in maize, but only two, CMS-S and CMS-C, are used today, while the CMS-T type has been abandoned due to its susceptibility to a disease specific for this type of cytoplasmic sterility. Fertility restoration in CMS plants is done by an action of nuclear genes, so called restorers of fertility, so it can be said that this type of sterility is caused by the interaction between genes in nuclear and mitochondrial DNA. Maize hybrids developed on the sterile basis are derived by crossing of the female component with a sterile cytoplasm, and the male component with restorer genes in the nuclear genome for that type of sterility, so that male fertility would be restored in the F1 generation, i.e. in the hybrid. Along with the introduction of such a system in the hybrid production, studies on effects of CMS on traits of maize genotypes have been initiated. Many unrelated studies have shown a positive effect of cytoplasmic male sterility on maize grain yield, especially under adverse conditions of drought, deficit of water and nutrients. This is explained by the fact that the plant directs a part of nutrients and energy that would be spent on pollen production to the yield increase, and therefore limited amounts of nutrients would not need to be used for pollen production under unfavourable conditions. Nitrogen requirements of sterile plants are lower by approximately 10-30 kg ha^{-1} than of fertile plants, hence this amount of nutrients instead of being used to form pollen is directed into female reproductive organs, thus resulting in the grain yield increase. The sink strength of maize ears is great and they continuously import N assimilates during grain filling (Hirel et al., 2005). On the other hand, CMS plants may store and redirect nitrogen into the ear to contribute to a higher grain yield. Reduced consumption of nitrogen, water and energy for pollen development in sterile plants during the flowering period may result in a greater number of kernels per ear (Vega et al., 2001).

Since it is necessary to achieve as high yield as possible in seed maize production, as inexpensive production as possible, as high-quality and less risky as possible, it is necessary to observe effects of different CMS types on the percentage of medium large flat fractions (MLF) of seeds and to established which cytoplasm has the highest percentage of the given seed fraction.

2. MATERIAL AND METHODS

2.1. Material

Twelve maize inbred lines with three different types of cytoplasm were studied. The first group encompassed inbred lines with cytoplasmic male sterility - type C. The second group was composed of inbred lines with cytoplasmic sterility - type S, while the third group contained inbred lines with fertile cytoplasm. Table 1 presents used variants of inbred lines with different types of cytoplasm:

Table 1. Variants of inbred lines with different types of cytoplasm

cms-C type	cms-S type	Fertile	Origin
L_1C	L_1S	L_1	Local
L_2C	L_2S	L_2	Local
L_3C	L_3S	L_3	Local
L_4C	L_4S	L_4	Local
L_5C	L_5S	L_5	BSSS
L_6C	L_6S	L_6	BSSS
L_7C	L_7S	L_7	BSSS
L_8C	L_8S	L_8	BSSS
L_9C	L_9S	L_9	Lancaster
$L_{10}C$	$L_{10}S$	L_{10}	Lancaster
$L_{11}C$	$L_{11}S$	L_{11}	Lancaster
$L_{12}C$	$L_{12}S$	L_{12}	Lancaster

Two trails were set up according to the complete randomised block design within each of the three types of cytoplasm in three replications in two locations (Zemun Polje - Selekciono polje and Zemun Polje - Školsko dobro) during the 2017-2018 period. Each plot within a replication consisted of four rows. Fertile versions of inbred lines sown in two border rows had a role of a pollinator for its sterile counterparts. Each row encompassed 12 hills in which four seeds were sown. The within row hill distance amounted to 70 cm. The elementary plot size was 5.6m^2.

The trial was set up under rainfed conditions. Standard maize cropping practices were applied during the maize growing season.

The thinning to two plants per hill was done in the 5-leaf stage. Plants form the inner 10 hills were used for the analyses in order to avoid the effect of border plants.

Sterility was monitored and evaluated on the 1-5 scale during the growing season:

1. 100% sterile
2. Empty anthers exsertion on the central tassel stalk
3. Anthers exsertion on the central tassel stalk and lateral tassel branches as well as occurrence of visible pollen, but not on the entire tassel

4. Anthers exsertion and visible pollen throughout tassel branches
5. 100% fertile

A greater part of maize germplasm used in the seed production at the Maize Research Institute is contained in 12 observed inbreds. Comparison of their probable breakthrough in the two observed CMS types can indicate the CMS type more suitable for seed production under our conditions.

Immediately prior to harvest, the total number of plants and the number of lodged and broken plants in all replications, whereby the broken plants were those broken below the node bearing the upper ear were established. Lodged plants were those in which angle between the stalk and the ground was less than 45º.

Harvest was done in the stage of full maturity. Yields of fresh ears were measured at harvest for each inbred per replication of each elementary plot. A submitted sample consisting of 20 ears was measured on the technical balance in the laboratory. After shelling of the submitter sample, the cobs were measured and the kernel moisture percentage was determined with a moisture meter for all replications.

2.2. Methods

The statistical and biometrical data processing was based on the mean values per a replication. The factorial analysis of variance was performed to determine the difference among maize inbred lines of different types of cytoplasm (C, S and fertile) used in the trial set up according to the complete randomised block design in two locations performed in two years. Moreover, the LSD test at the probability levels of 5% and 1% was also applied (Hadživuković 1991). Homogeneity of variances was tested with the aim to draw objective conclusions on the effects of observed factors on studied traits of maize inbred lines, and the possibility to apply parametric tests (ANOVA and LSD test).

3. RESULTS AND DISCUSSION

The grain yield is an important and complex trait consisting of a greater number of components of quantitative nature whose genetic basis is polygenic.

The seed material processing is performed in order to uniform seeds up to their condition suitable for sowing.

Data presented in Table 2 show that the inbred lines L_2 and. L_6 had the highest (16.3%) and the lowest (0.4%). percentage of the MLF seed fraction, respectively. The highest average value for this property was expressed by sterile cytoplasm cms-C type (7.0%). On the other hand, the lowest value was recorded in fertile cytoplasm (6.5%). Average values of the MLF seed fraction of inbred lines very significantly ($P \leq 1\%$) varied in dependence on the year of investigation and the location. The average value of the MLF seed fraction was higher in inbred lines in 2017 than in 2018 (7.75% vs. 5.69%). This average value was higher in the first location (Selekciono polje, 7.17%) than in the second location (Školsko dobro, 6.28%), (Table 2).

The trait - medium large flat (MLF) seed fraction of maize inbred lines very significantly ($Lsd_{0.01}$) varied depending on the type of cytoplasm. Inbred lines L_2 and L_{12} differed in the MLF percentage in dependence on the type of cytoplasm (*cms*-C, *cms*-S and fertile), while there were no significant differences among remaining 10 inbred lines (Table 3).

The average values of the MLF seed fraction of inbred lines very significantly ($Lsd_{0.01}$) varied over years in L_2, L_{10} and L_{12}, while significant differences were not established in remaining nine inbred lines (Table 3).

Depending on the type of cytoplasm and the year, significant differences ($Lsd_{0.05}$) were determined in L_2, L_3, L_9, L_{10} and L_{12} (Table 3).

The inbred line × location interaction points out to the existence of a very significant ($Lsd_{0.01}$) difference in the MLF seed fraction in inbred lines L_2, L_3, L_9 and L_{11} in dependence on the observed location (Table 3).

The MLF seed fraction of inbred lines with the same type of cytoplasm varied significantly ($Lsd_{0.05}$) over locations. Inbred lines with sterile cytoplasm *cms*-S type and fertile cytoplasm produced greater percentage of the MLF seed fraction in the first than in the second location. There were no differences in the MLF seed fraction in inbred lines with sterile cytoplasm *cms*-C type between observed locations (Table 3).

Years and locations significantly ($Lsd_{0.05}$) affected the MLF seed fraction percentage. The highest value of the MLF seed fraction of inbred lines was achieved in the first location during the first year of investigation. In the second year, there were no differences in the average MLF seed fraction values between locations (Table 3).

According to stated it can be concluded that soil quality and climate conditions had the crucial importance in the given location.

A comparison the obtained average values of MLF of inbred lines with values gained by Tabaković et al. (2013, 2015, 2016) shows that the both the genotype and the location affected the varying of this trait.

A seed size is genetically regulated, but it is also controlled by environmental factors. Depending on genetic factors, biochemical and physiological abilities of the plant, as well as temperature, moisture and the presence of utilisable nitrogen in the soil, the duration and the degree of seed filling differ and therefore the seed size varies (Sadras and Egli, 2008).

Đukanović et al. (2008) established that small seeds had lower germination than medium small, but that the difference was not statistically significant.

Table 2. Average values of the MLF seed fraction over inbred lines, years, type of cytoplasm and locations

Year	Location	Cytoplasm (C)	Inbred lines (I)												LSD test	
(Y)	(L)		1	2	3	4	5	6	7	8	9	10	11	12	5%	1%
Y_1	L_1	C_1	8.7	21.4	8.3	1.8	1.4	1.1	1.0	1.5	5.7	12.9	9.3	17.9		
		C_2	14.3	21.5	8.8	1.2	3.0	1.3	1.8	0.9	5.3	12.0	10.3	25.1		
		C_3	16.7	24.9	12.0	1.5	2.0	0.6	0.9	1.0	7.9	14.4	10.1	19.1		
	L_2	C_1	15.8	16.7	3.5	0.4	1.1	0.5	0.5	0.5	4.1	15.2	14.1	22.3		
		C_2	16.2	10.3	3.6	0.2	0.8	0.3	0.4	0.2	2.8	12.6	12.0	21.6		
		C_3	11.0	12.1	5.4	0.7	1.3	0.4	0.5	0.2	2.6	7.4	13.4	19.7		
Y_2	L_1	C_1	14.7	22.6	3.8	0.1	0.3	0.1	0.2	0.1	7.0	4.8	11.6	8.1		
		C_2	16.0	12.2	7.8	0.1	0.1	0.1	0.1	0.5	9.0	7.6	7.2	10.2		
		C_3	13.3	13.0	9.6	0.1	0.3	0.1	0.1	0.2	2.8	8.7	11.8	3.8		
	L_2	C_1	15.9	20.7	6.7	0.1	0.2	0.4	0.1	0.1	5.1	4.7	13.0	7.6		
		C_2	15.0	8.0	8.5	0.2	0.1	0.1	0.2	0.1	3.6	7.4	14.8	8.0		
		C_3	12.5	12.4	6.1	0.1	0.5	0.1	0.1	0.2	1.4	8.8	11.6	7.2		
Average for inbreds			14.2	16.3	7.0	0.5	0.9	0.4	0.5	0.5	4.8	9.7	11.6	14.2	1.35	1.78
Average for cytoplasm			C_1	7.0			C_2	6.7			C_3	6.5				
															F test	
Average for years			G_1			7.75**			G_2			5.69			** P≤1%	
Average for locations			L_1			7.17**			L_2			6.28				

C_1 -cms-C cytoplasm; C_2 -cms-S cytoplasm; C_3 -fertile (N) cytoplasm

* ≤0,05; **≤ 0,01

Table 3. Average values of the MLF seed fraction over inbred lines, years, type of cytoplasm and locations

ear(G), Location (L)	Inbred lines (I)												LSD test	
Cytoplasm (C)	1	2	3	4	5	6	7	8	9	10	11	12	5%	1%
I x C_1 x G_1	12.3	19.0	5.9	1.1	1.2	0.8	0.8	1.0	4.9	14.1	11.7	20.1		
I x C_2 x G_1	15.2	15.9	6.2	0.7	1.9	0.8	1.1	0.6	4.1	12.3	11.2	23.4		
I x C_3 x G_1	13.9	18.5	8.7	1.1	1.7	0.5	0.7	0.6	5.2	10.9	11.8	19.4		
I x C_1 x G_2	15.3	21.6	5.3	0.1	0.3	0.2	0.1	0.1	6.1	4.7	12.3	7.9		
I x C_2 x G_2	15.5	10.1	8.1	0.2	0.1	0.1	0.2	0.3	6.3	7.5	11.0	9.1		
I x C_3 x G_2	12.9	12.7	7.8	0.1	0.4	0.1	0.1	0.2	2.1	8.8	11.7	5.5	3.30	4.35
I x C_1 x L_1	11.7	22.0	6.1	1.0	0.8	0.6	0.6	0.8	6.4	8.9	10.4	13.0		
I x C_2 x L_1	15.2	16.8	8.3	0.7	1.6	0.7	1.0	0.7	7.2	9.8	8.8	17.6		
I x C_3 x L_1	15.0	18.9	10.8	0.8	1.2	0.3	0.5	0.6	5.4	11.6	11.0	11.5		
I x C_1 x L_2	15.8	18.7	5.1	0.3	0.7	0.4	0.3	0.3	4.6	9.9	13.6	15.0		
I x C_2 x L_2	15.6	9.1	6.1	0.2	0.4	0.2	0.3	0.2	3.2	10.0	13.4	14.8		
I x C_3 x L_2	11.8	12.3	5.7	0.4	0.9	0.3	0.3	0.2	2.0	8.1	12.5	13.5		
I x C_1	13.8	20.3	5.6	0.6	0.8	0.5	0.5	0.6	5.5	9.4	12.0	14.0		
I x C_2	15.4	13.0	7.2	0.4	1.0	0.5	0.6	0.4	5.2	9.9	11.1	16.2	2.33	3.08
I x C_3	13.4	15.6	8.2	0.6	1.0	0.3	0.4	0.4	3.7	9.8	11.7	12.5		
I x G_1	13.8	17.8	6.9	1.0	1.6	0.7	0.9	0.7	4.8	12.4	11.5	21.0		
I x G_2	14.6	14.8	7.1	0.1	0.3	0.1	0.1	0.2	4.8	7.0	11.7	7.5	1.91	2.51
I x L_1	14.0	19.2	8.4	0.8	1.2	0.6	0.7	0.7	6.3	10.1	10.1	14.0		
I x L_2	14.4	13.4	5.6	0.3	0.7	0.3	0.3	0.2	3.3	9.3	13.2	14.4		
C x G	C_1xG_1	7.7	C_1xG	6.2	C_2xG	7.8	C_2xG	5.7	C_3xG_1	7.7	C_3xG	5.2	0.95	1.26
C x L	C_1xL_1	6.9	C_1xL	7.1	C_2xL	7,4	C_2xL	6,1	C_3xL_1	7,3	C_3xL	5,7		
G x L	G_1 x L_1	8.6		G_1 x L_2	7.0		G_2 x L_1	5.8		G_2 x L_2	5.6		0.78	1.03
I x G_1 x L_1	13.2	22.6	9.7	1.5	2.1	1.0	1.3	1.1	6.3	13.1	9.9	20.7		
I x G_1 x L_2	14.3	13.0	4.2	0.4	1.0	0.4	0.5	0.3	3.2	11.7	13.2	21.2		
I x G_2 x L_1	14.7	15.9	7.0	0.1	0.2	0.1	0.1	0.3	6.3	7.1	10.2	7.3		
I x G_2 x L_2	14.5	13.7	7.1	0.1	0.3	0.2	0.1	0.1	3.4	6.9	13.1	7.6		
C x G x L	C_1 x G_1 x L_1 7.6			C_1 x G_1 x L_2 7.9			C_1 x G_2 x L_1 6.1			C_1 x G_2 x L_2 6.2				
	C_2 x G_1 x L_1 8.8			C_2 x G_1 x L_2 6.8			C_2 x G_2 x L_1 5.9			C_2 x G_2 x L_2 5.5				
	C_3 x G_1 x L_1 9.3			C_3 x G_1 x L_2 6.2			C_3 x G_2 x L_1 5.3			C_3 x G_2 x L_2 5.1				

4. CONCLUSION

According to obtained results of the two-year study of maize inbred lines with different types of cytoplasm it can be concluded that the analysis of variance indicates to highly significant differences among observed genotypes regarding to the MLF seed fraction and the significant impact of the year and the locations as well as their interaction.

The average values of the seed fraction varied over inbred lines from 0.4% to 16.3%. The highest average value for this trait was determined in sterile cytoplasm cms-C type (7.0%), while the lowest value was recorded in fertile cytoplasm (6.5%). Moreover, a higher percentage of MLF was established in inbred lines in the first year of investigation (7.75%) and in the first location (7.17%) than in the second year (5.69%) and the second location (6.28%). The inbred line × location interaction points out to the existence of a very significant ($Lsd_{0.01}$) difference in the MLF seed fraction in inbred lines in dependence on the observed locations. Years of investigation and locations significantly ($Lsd_{0.005}$) affected the percentage of the MLF seed fraction of inbred lines. The analysis of gained results indicates a significant effect of the type of cytoplasm on the medium large flat seed fraction.

Therefore, based on everything stated it can be concluded that the MLF seed fraction is an important seed trait that varies under effects of genetic factors, i.e. depends on the genetic background of the trait, environmental conditions to a lesser extent and on their interaction.

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