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Influence of cytoplasmic male sterility on corn yield at different plant densities

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Abstract: This study investigates the effect of cytoplasmic male sterility on grain yield in maize hybrids. The experiment was conducted in 2024 at the Maize Research Institute – Knezha. Five sterile lines and their fertile analogs, crossed with two pollinators at different plant densities (5000 p/da and 6000 p/da), were analyzed. The results were obtained using ANOVA dispersion analysis, and the coefficient of variation (also known as Pearson's coefficient of variation) was calculated to measure the relative variability of the dataset. At all planting densities, F count > F table at the 5% and 1% significance levels, indicating that the differences between the variants were statistically significant. T-tests were applied to determine significant differences between genotypes at confidence levels of 0.1 – 0.05 – 0.01. Based on the conducted studies, we conclude that fertile analogs are more suitable for both high and low planting densities. In contrast, sterile analogs exhibit greater variability in results, making them less predictable and potentially requiring optimized growth conditions and lower planting densities. The aim of this study was to assess the statistical significance of the effect of cytoplasmic male sterility on yield at different planting densities. This analysis determines the impact of factors and their interactions on productivity. By calculating the coefficient of variation, we assessed the degree of variability in the results, which helps in understanding how different factors (such as plant type, pollinators, and planting density) influence productivity and how these variables can be optimized to achieve better results.

Keywords: maize; cytoplasmic male sterility; sterile analogs; fertile analogs; productivity

INTRODUCTION

Maize (*Zea mays L.*) is the most productive and one of the most significant cereal crops worldwide. It plays a key role in food, feed, and industrial sectors. Its productivity is influenced by multiple factors, including genetic characteristics, agronomic practices, and environmental conditions. Variety traits, cultivation technology, and growing conditions are essential for the expression of genetically determined productive potential and product quality (Stoyanov and Tonev, 2001; Delibaltova et al., 2009; Shafi et al., 2012; Mandi and Ismail, 2015; Mandic et al., 2016). Scientific research emphasizes that favorable climatic conditions such as moderate temperature and sufficient moisture during flowering and grain filling significantly improve fertility and yield in maize. High humidity and low temperatures are associated with increased fertility and extended flowering duration, which enhances pollination efficiency. Conversely, long photoperiods combined with high temperatures, especially during anthesis, can lead to accelerated anther aging, lower pollen viability, and ultimately reduced fertility and grain yield (Duvick, 1965). These factors become even more significant when working with sterile hybrids, where cross-pollination success is critically dependent on the synchrony between fertile and sterile components in the field.

One of the established and widely used methods for increasing yield and efficiency in hybrid production is the use of cytoplasmic male sterility (CMS). In the broadest sense, it refers to the inability to produce anthers, functional pollen, and viable male gametes. It was first observed by the German botanist Joseph Gottlieb Kölreuter in 1763 (Mayr, 1986) and has been reported in more than 610 plant species (Kaul, 1988). This biological system allows the production of hybrid seed without the need for manual detasseling, which significantly reduces costs and facilitates large-scale seed production.

Numerous studies on the influence of sterile cytoplasm on maize yield have been conducted in various geographical and climatic conditions. Although scientific literature presents different opinions, most authors report positive or neutral effects of CMS on yield when appropriate agronomic conditions are met. These include the works of Rogers and Edwardson (1952), Chinwuba et al. (1961), Vakhrusheva (1967), Kalman et al. (1986), and others, who report that sterile cytoplasm in most cases does not lead to reduced productivity but may even contribute to its increase with properly selected hybrid combinations. It is assumed that the positive effect of using CMS is due to better direction of assimilates to the grain, as the absence of anthers and tassels allows plants to use their resources more efficiently.

However, the choice of an appropriate CMS system (e.g., CMS-T, CMS-C, or CMS-S) is of fundamental importance, as some are associated with increased susceptibility to pathogens – a well-known example is the case of CMS-T and the leaf spot (*Helminthosporium maydis*) in the USA in the 1970s. Therefore, modern breeding and seed production rely on more resistant and safer systems such as CMS-C, which combine stable sterility with agronomic reliability.

Planting density is an important agronomic factor that directly affects both yield and quality characteristics of production. Optimal density depends on numerous conditions – the biological characteristics of the hybrid, soil structure and fertility, climatic factors (moisture, temperature, light regime), and the availability of nutrients. For sterile and fertile hybrids, the optimal density may differ, as sterile plants have the potential for better resource allocation to grain due to the absence of anther formation. At densities above optimal, plants begin to compete for light, water, and nutrients, leading to stem elongation, reduced photosynthetic activity, formation of smaller ears, and lower grain number. On the other hand, excessively low density limits the full use of available resources, which also adversely affects yield per unit area.

The aim of this study was to evaluate the effect of cytoplasmic male sterility on maize yield depending on different planting densities by comparing hybrids with fertile cytoplasm and their analogs with different types of sterility (M and C). Specific emphasis is placed on the interaction between genotype, agroclimatic conditions, and possible mechanisms through which these factors affect productivity. The study aims to clarify the role of cytoplasmic sterility and genetic factors in the context of hybrid resilience under adverse climatic conditions and their response to stress factors such as heat and water deficit, characteristic of 2024.

MATERIALS AND METHODS

The aim of this study is to evaluate the effect of cytoplasmic male sterility on maize yield. It was conducted in the experimental field of the Maize Research Institute – Knezha in 2024. The experimental field is typical for the region, the soil type is chernozem, and the terrain is flat. Five sterile lines of M and C types and their fertile analogs were tested for productivity, crossed with two pollinators. The experiment was set up at two planting densities – 5000 p/da and 6000 p/da in two replications and under non-irrigated conditions. Planting was done manually using planters on April 10, 2024. The planting method was non-standard with an unsystematic arrangement of variants of the block type. Standard cultivation practices were applied during the vegetation period. The experiment was harvested manually at physiological maturity. Grain yield (kg/ha) was

recorded at a standard grain moisture of 14%. Analysis of variance was performed for all variants to assess the statistical significance of the results (Dimova and Marinkov, 1999). Average yields, varietal differences relative to the control, and statistical significance of differences were calculated.

RESULT AND DISCUSSION

The meteorological conditions during the year of study were characterized as extremely unfavorable for maize development due to pronounced high-temperature and water stress (Table 1). The first half of April was unusually warm and dry for the season, necessitating an earlier start to planting – on April 10. Plant emergence occurred on April 18, but there was a cooling period at the end of the month. May saw frequent, although insignificant in volume, precipitation (82.7 mm/m²), and the total precipitation for the vegetation period reached 214.9 mm. The most critical was the lack of moisture during key ontogenesis phases – tasseling, flowering, fertilization, and grain filling, when precipitation in the period June 29–September 10 amounted to only 44.4 mm, unevenly distributed and insufficient for the physiological needs of the crop. In July and August, extremely high average daily temperatures were recorded, with maximum values exceeding 35°C on 17 days, most of which had limited physiological benefit („ballast" temperatures). This led to depletion of soil moisture, leaf burn, and serious disturbances in reproductive processes – scorching of pollen and silks, which resulted in a high degree of non-fertilization and incomplete ear kernel formation. As a result, yields were extremely low and did not reflect the real productive potential of the hybrids studied.

When comparing the yield results between hybrids with fertile cytoplasm and their corresponding analogs in M and C-type sterilities, hybrids with normal cytoplasm exceed the productivity of those with sterile mothers (Table 2 and Table 4). These results are consistent with those of Has et al. (2025), according to whom, in years

Table 1. Meteorological data for the vegetation period (April-September) of maize in 2024

Month	Mean monthly temperatures (°C)	Relative air humidity (%)	Monthly precipitation (l/m^2)
April	14.3	66.0	35.0
May	16.3	78.2	82.7
June	24.9	61.5	25.6
July	26.1	55.4	42.3
August	26.0	50.3	2.1
September	19.0	63.7	27.2

Table 2. Grain yield (kg/da) from tested experimental hybrids with fertile mothers

Number	Hybrids	Grain yield, kg/da			Varietal difference	Significance
		5000 p/da	6000 p/da	Average		
1	2357ВзМНt× Mo17Rfc	427.9	380.1	404.1	118.1	***
2	2378ВзМ x ×Mo17Rfc	370.3	371.1	370.7	84.8	***
3	КБ11зС x ×Mo17 Rfc	326.5	392.9	359.7	73.8	**
4	К4640БзС ×Mo17Rfc	365.6	284.5	325.1	39.1	*
5	КБ11зС x ×ХМ9923	277.9	289.9	283.9	-2.1	0
6	КС4661зМ ×Mo17Rfc	220.5	312.5	266.5	-19.4	0
7	2357ВзМНt ×ХМ9923	226.9	232.2	229.5	-56.4	0
8	2378ВзМ ×ХМ9923	249.5	190.5	219.9	-65.9	0
9	К4640БзС ×ХМ9923	227.1	191.2	209.1	-76.8	0
10	КС 4661зМ ×ХМ9923	240.1	141.3	190.7	-95.2	0

with heat and water stress, hybrids with normal cytoplasm are higher yielding.

The influence of sterile cytoplasm on yield in maize has been the subject of numerous studies, but conclusions on the topic remain ambiguous. Some authors (Ilchovska, 1991; Stamp et al., 2000; Stevanovic et al., 2016; Jovanović et al., 2018) reported that sterile forms outperform their fertile parental lines in yield, citing competition between the tassel and ear for assimilates as the reason. According to Valkova (2012), Vulchinkov et al. (2014), and Khan et al. (2023), factors such as genotype, agroclimatic conditions, and genotype-environment interaction often prevail over the effect of cytoplasmic sterility itself.

Yields from the obtained crosses are extremely low at both cultivation densities and range from 141.3 kg/da to 427.9 kg/da for hybrids in fertile cytoplasm and from 115.6 kg/da to 448.6 kg/da for hybrids with restored fertility. It is noteworthy that the crosses of tester Mo 17 Rfc with lines 2357B, КБ11, and 2378 in normal and sterile cytoplasm are the highest yielding in both trials, indicating the leading role of genotype in yield formation.

Fertile analogs are characterized by a significantly lower coefficient of variation (CV) – 7.80%, compared to sterile analogs, where the value reaches 10.3%. The reduced variation coefficient in fertile forms indicates more stable growth and development of plants under the expressed abiotic stress during the year of study. This is an indication of higher physiological stability and consistency in the phenotypic expression of traits related to fertility. Conversely, the higher coefficient of variation in sterile analogs reflects greater variability in growth and development, which is likely due to the weaker adaptive ability of sterile cytoplasm to stress conditions, such as high temperatures and moisture deficit. This instability may result from impaired functionality of mitochondria in sterile plants, leading to poorer pollination, lower grain set, and more unpredictable productivity results. The dispersion of traits in sterile forms emphasizes the need for careful selection of parental components and fertility restorers to ensure greater hybrid stability under adverse conditions.

Crosses with tester XM 9923 showed significantly lower yields compared to those with Mo17Rfc. This shows that Mo17Rfc is more productive and better suited to the test conditions, while XM9923 is more responsive to better cultivation conditions.

The results of the multifactor analysis of variance (ANOVA) show the influence of individual factors - lines (G), testers (L), densities (S), and replications (REP), as well as interactions between them on the studied trait (Table 3).

Factor L – tester has a pronounced effect on yield, with a statistically highly significant val-

Table 3. ANOVA results for grain yield (kg/da) in fertile analogs

Source of variation	SS	df	MS	F-count	F-table		P-value
					5%	1%	
S - Density	2144.76	1	2144.76	4.32	4.49	8.53	0.055
L - Tester	140552.88	1	140552.88	282.98	4.49	8.53	<0.0001
S*L	4270.42	1	4270.42	8.60	4.49	8.53	0.0098
REP*S*L	9035.60	4	2258.90	4.55	3.01	4.77	0.0126
G - Lines	47740.07	4	11935.02	24.03	3.01	4.77	0.0000014
G*S	10403.45	4	2600.76	5.24	3.01	4.77	0.0071
G*L	15653.40	4	3913.35	7.88	3.01	4.77	0.0010
G*S*L	19628.60	4	4907.15	9.88	3.01	4.77	0.0003
Error	7946.94	16	496.68				
Corrected Total	257376.13	39					

CV=7.80%

ue (F = 282.98; $p < 0.0001$), which emphasizes the substantial influence of male parental components on hybrid productivity. Also, G-lines (F = 24.03; $p < 0.001$) have a significant influence, which is consistent with the importance of female parental forms in yield formation.

Factor S – planting density alone does not show a statistically significant effect at the 5% significance level ($p = 0.055$), although it approaches the threshold, suggesting some tendency towards influence. However, the interaction S × L (density × tester) is statistically significant ($p < 0.01$), indicating that the effect of density on yield depends on the specific tester. Similarly, all interactions involving female and male parental components – G × S, G × L, and G × S × L – show statistically significant differences (respectively $p = 0.0071$, 0.0010, and 0.0003). This confirms the complex nature of genotype-environment interaction (including planting density), which directly affects the heterosis effect and adaptive ability of hybrids.

The error in the model has a relatively low value, suggesting good precision of the experimental setup. The statistical significance of the term REP × S × L ($p = 0.0126$) also indicates that replications and their interaction with factors have an influence and should be taken into account in the interpretation of results.

A similar pattern is observed in the trial with crosses with sterile analogs (Table 4). Lines tested with Mo17Rfc have higher yields compared to those with HM 9923 at both planting densities. Crosses of lines 2357BMHt and KB11C with Mo17Rfc stand out significantly from the others at a reliability indicator of P=1.0%.

The results of the analysis of variance for grain yield reveal that genetic factors are the main sources of variation in the experiment, having a highly statistically significant influence (Table 5).

Factor L – tester shows high significance ($F = 77.62$; $p < 0.0001$), which emphasizes the key role of the male parental component in hybrid yield formation. Even more pronounced is the influence of G-lines, with $F = 63.14$ ($p < 0.0001$), which testifies to the significant contribution of maternal parental forms to the yield potential of the hybrid. This corresponds to the established practice in breeding, according to which both parental components must be carefully selected for optimal heterosis effect.

The high significance of interactions G × S ($F = 12.24$), G × L ($F = 13.27$), and especially G × S × L ($F = 9.045$) at a significance level of $p < 0.001$ reveals that grain yield is significantly influenced by complex interactions between genotype, planting density, and the tester used. This confirms the need for a comprehensive approach

Table 4. Grain yield (kg/da) from tested experimental hybrids with sterile mothers

Number	Hybrids	Grain yield, kg/da			Varietal difference	Significance
		5000 plants/da	6000 plants/da	Average		
1	2357BMHt×Mo17Rfc	280.3	448.6	364.5	129.9	***
2	КБ11С ×Mo17 Rfc	397.5	286.9	342.2	107.6	***
3	2378BM × Mo17Rfc	296.2	256.1	276.2	41.6	**
4	2378BM x XM9923	272.4	272.4	272.4	37.8	**
5	2357BMHt×XM9923	237.8	237.8	237.8	3.2	0
6	КБ11С × XM9923	219.7	189.6	204.6	-29.9	0
7	К4640БС x Mo17Rfc	140.2	226.5	183.3	-51.3	0
8	КС4661М×Mo17Rfc	191.6	158.5	175.1	-59.5	0
9	К4640БС× XM9923	169.1	169.1	169.1	-65.5	0
10	КС4661М× XM9923	115.6	126.1	120.8	-113.8	0

Table 5. ANOVA results for grain yield (kg/da) in sterile analogs

Sources of variation	SS	df	MS	F-count	F-table		P-value
					5%	1%	
S - Density	262,14	1	262,14	0,45	4.49	8.53	0.513
L - Tester	45306,36	1	45306,36	77,62	4.49	8.53	<0.0001
S*L	819,03	1	819,03	1,40	4.49	8.53	0.255
REP*S*L	1333,97	4	333,49	0,57	3.01	4.77	0.686
G - Lines	147418,72	4	36854,68	63,14	3.01	4.77	<0.0001
G*S	28581,32	4	7145,33	12,24	3.01	4.77	<0.001
G*L	30991,66	4	7747,91	13,27	3.01	4.77	<0.001
G*S*L	22062,17	4	5515,54	9045	3.01	4.77	<0.001
Error	9339,50	16	583,72				
Corrected Total	286114,86	39					

CV=10.30%

in evaluating hybrids under different conditions, taking into account not only the basic yield potential but also the stability of productivity under different agronomic conditions.

During the year of study, factor S – density alone did not have a significant effect on grain yield ($F = 0.45$; $p = 0.513$), suggesting that the densities used were within the optimal ranges for the hybrids grown and did not cause a significant change in yields. A similar trend is observed for the interaction S × L ($p = 0.255$), as well as for REP × S × L, which does not show a statistically significant effect.

The overall error is low, indicating high reliability of the experimental data.

CONCLUSION

Based on the results obtained, the following conclusion can be made:

Grain yield in the studied hybrids is determined mainly by genetic factors, while specific interactions between genotype and agronomic conditions also play an important role. Testers and lines have a strong effect on yield, with genotypes that include specific combinations of parental forms showing higher productivity. It is important to note that the influence of these factors is not linear but depends on their interactions.

Planting density itself does not have a significant effect on the magnitude of yield, but interactions between genotype, density, and tester are key to yield stability.

Fertile analogs have a lower coefficient of variation, making them more stable and predictable under the study conditions.

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