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Original scientific paper

THE PLUS-HYBRID EFFECT ON THE GRAIN YIELD OF TWO ZP MAIZE HYBRIDS

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The combined effect of cytoplasmic male sterility and xenia on maize hybrid traits is referred to as the plus-hybrid effect. Two studied ZP hybrids differently responded to this effect for grain yield. All plus-hybrid combinations of the firstly observed hybrid had a higher yield than their fertile counterparts, but not significantly, while only one combination of the second hybrid positively responded, also without statistical significance. It seems that the observed effect mostly depended on the genotype of the female component.

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INTRODUCTION

The effect of male sterility on the maize grain yield has been considerably studied since the beginning of the cms use. Different results can be found in the available literature. The positive cms effect on grain yield of maize hybrids was revealed by KALMAN *et al*. (1985). Studies carried out by STAMP *et al*. (2000) showed increased yields of sterile hybrids when different nitrogen rates, different water rates supply and different cropping densities were applied. This is mainly explained by the indirect effect of the sterility. Namely, male sterile plants do not consume nutrients and energy to form and shed pollen, but to form the grain. The fertile pollen is a great recipient of mineral nitrogen, much more than any other part of the plant. It was calculated that sterile plants can save about 10-30 kg nitrogen ha^{-1}, which is, instead of being used for the pollen production, directed into female reproductive organs, thereby resulting in higher grain yields. Under drought conditions, cytoplasmic male sterile plants of maize absorb more water form the soil than fertile plants (VINCENT and WOOLEY, 1972), especially during florescence. Therefore, a reduced consumption of nitrogen, water and energy for the pollen formation during the flowering time can result in the increase of the number of kernels per ear (VEGA *et al.,* 2001).

On the other hand, some researchers published that there was neither grain yield increase, nor it was regular in sterile hybrids (LIM *et al*., 1974). In any case, the cms effect on grain yield has been modified by natural and agronomic stress conditions, but also by a genetic origin of the used material.

The fact that male sterile maize plants have a higher yield by approximately 5-10% than their fertile counterparts, especially under stress conditions, and also that certain cms-based hybrids have increased yields, has stimulated growers to sow the hybrid seed with partially recovered fertility, that is to sow a mixture of sterile and fertile variants of the same hybrid in the commercial production (VIDAKOVIĆ and VANČETOVIĆ, 1994). The share of male sterile plants usually ranges from 50 to 70%, while there must not be less than 25% of fertile plants in the crop fertilisation of high quality.

Xenia is a direct cross fertilisation effect on the grain traits of the female component in the year of crossing. The embryo, i.e. the endosperm, receives one half, i.e. one third of a genome of a male component, respectively. The embryo contributes 11%, and the endosperm 83% of the dry matter of the kernel (TOLLENAAR and DWYER, 1999), pointing out to a potential positive effect of xenia on the grain yield. Hence, TSAI and TSAI (1990), WEILAND (1992) and WESTGATE *et al.* (1999) found out a significant grain yield increase in some hybrids crossed to different hybrid pollinators.

The conventional cultivation of single cross hybrids leads to a pronounced inbreeding depression; kernels produced in the F1 generation plants are actually kernels of the F2 generation; hence, cross-fertilisation with another hybrid increases grain heterozygosity, what probably affects the grain yield increase.

A new approach dating from the end of the 20th century is the mixture of the sterile version of one yielding hybrid and the fertile version of another hybrid. Namely, it was observed that cms and xenia had a positive common effect on grain yields of sterile hybrids, which was obtained by increasing 1000-kernel weight and the number of kernels per area unit without grain quality deterioration (WEINGARTNER *et al*., (2004), VANČETOVIĆ *et al*., (2009)). Also, combined cms and xenia effect on grain yield components differed for two sterile hybrids (BOŽINOVIĆ and VANČETOVIĆ, 2009). Such a system of the maize production is referred to as the plus-hybrid system (WEINGARTNER *et al.,* 2002a). These authors observed the plus-hybrid effect in seven single cross hybrids and it amounted, on the average, to 9.1%.

The objective of this study was to determinate both, individual and combined (plus hybrid) effects of cms and xenia on grain yield of the two ZP maize hybrids. Furthermore, results, can possibly point out to hybrids of good general pollinator abilities (GPA; they result in higher values of the observed traits with female components in the trail), as well as, to combination of hybrids with good specific pollinator abilities (combination that is better than expected on the basis of GAP of the male component and the average values of the female component's traits).

MATERIALS AND METHODS

Two sterile hybrids, ZP 341st and ZP 360st, and their fertile counterparts were used in the study. Also, the following five fertile hybrids with normal (N) cytoplasm: ZP 341, ZP 360, ZP 42A, ZP 434 and ZP 488 were used as pollinators. The selected hybrids belong to the FAO maturity group 300-400 and therefore they avoid drought under conditions of our country. Also, a special attention was paid to their synchronous flowering.

The three-replicate experiment was carried out according to the randomised complete-block (RCB) split-plot design in the location of Zemun Polje during 2008. The observed female components represented the main plots (sterile and fertile versions of the hybrids ZP 341 and ZP 360), provided that detasseling was performed on fertile female components and they were used only for the estimation of cms effects in the trial. Subplots were made of fertile pollinator hybrids. Each subplot consisted of 14 rows, each 18 m long. The row distance was 0.75 m and the crop density amounted to 77,220 plants ha^{-1}. The female component was sown in the middle two rows, with a particular pollinator, on the distance of 3.7 m from the upper and the bottom side of the plot. The error occurred at harvest of the fourth block sown with the female-fertile hybrid ZP 360, hence the data obtained from this block were not considered when traits were analysed. The trial was sown mechanically and harvested manually.

The grain yield (t ha^{-1} with 14% moisture) was determined for female and male components within subplots. The male component traits were related to the plus-hybrid effects, and based on it, it was determined whether they could be used for the prediction of these effects. In practice, a mixture of cms hybrids and pollinator hybrids is grown, hence the traits of the fertile hybrid in the blend affect the value of the grown hybrid mixture. The following three effects were monitored for grain yield:

1. cms effect: the difference between the average value of the fertile and isogenically pollinated sterile hybrid (pollinated by its fertile counterpart) for hybrids *per se*, that is a difference between the average value of a non-isogenically pollinated fertile hybrid (pollinated by unrelated genotype) and a non-isogenically pollinated sterile hybrid with the same pollinator for combinations of sterile hybrids and non-isogenic pollinators. The cms effect was the only effect possible to estimate for the hybrid ZP 360 *per se*, and instead of the average value of the performance of the fertile isogenically pollinated ZP 360 hybrid, the average value of the performance of this hybrid was taken form the subplots where this hybrid was a pollinator, i.e. the male component (the average values within each block, then the total average of all three blocks)
2. xenia effect: the difference between the average values of a nonisogenically pollinated hybrid and isogenically pollinated hybrid.
3. plus-hybrid effect: the difference between the average value of a fertile isogenically pollinated hybrid and a sterile non-isogenically pollinated hybrid.

The general pollinator ability (GPA) was observed by measuring the average value of the performance under the influence of a certain pollinator. Moreover, the specific pollinator ability (SPA) was also observed. The SPA is related to the combinations of the female component and the pollinator that had a better average performance than would be expected based on the average performance of the two components involved.

The analysis of variance (ANOVA) for a randomised complete block (RCB) split-plot design was applied (MSTAT-C). A *t*-test was performed to test the significance of differences between the relevant means.

RESULTS AND DISCUSSION

Results of ANOVA (Table 1) indicate a significant effect of the female hybrid on the grain yield of two observed hybrids, while the effects of the pollinator, as well as, a hybrid × pollinator interaction were not significant.

Table 1. ANOVA of grain yield

Source of variation	Degrees of freedom	F values
Pollinator (P)	4	0.8766
Hybrid (H)	2	10.3956**
P × H	8	1.5027

P – hybrids used as pollinators., H – hybrids used as mothers.

**- significant at the 0,01 level.

It was observed that the cms effect on ZP 341 *per se* was positive and that grain yield increased by 8.53% at the level of significance of P = 0.05. This hybrid in the combination with the pollinator ZP 488 had significantly higher yield, even by 10.76% (P = 0.01), than its fertile counterpart (Table 2) pollinated by the same pollinator. Moreover, cms positively affected all ZP 341 × pollinator combinations, in contrast to the hybrid ZP 360, which had a yield reduction of 7.53% under the cms effect (P = 0.1). The average yield increase of the first hybrid affected by cms amounted to 5.16%.

Table 2. Cms effect on grain yield

Genotype	Grain yield
ZP 341st × ZP 341	8,53*
ZP 341st × ZP 360	0,2
ZP 341st × ZP 488	10,76**
ZP 341st × ZP 434	1,34
ZP 341st × ZP 42A	4,95†
ZP 360st × ZP 360	-7,53†

Values (%) of grain yield indicate changes relative to the male-fertile hybrid, isogenically pollinated.
†- significant at the 0,1 probability level, * - significant at the 0,05 probability level., ** - significant at the 0,01 probability level.

Effects of xenia on the grain yield of the hybrid ZP 341 *per se* were negative at the level of significance of P = 0.1, while the differences between different ZP 341 × pollinator combinations were not significant (Table 3). Hence, this hybrid had the highest grain yield when it had been isogenically pollinated, which is in agreement with its general pollinator ability (Table 4). The greatest grain yield reduction was detected under effects of the pollinator ZP 42A, which at the same time, had the worst general pollinator ability for this trait. In contrast to ZP 341, the hybrid ZP 360 positively responded to foreign pollinators (Table 5). Effects of xenia on the hybrid *per se* resulted in the increase of the yield at the level of significance of P = 0.1, while the greatest increase amounted to 10.53% when ZP 434 was a pollinator. The comparison of different ZP 360 × pollinator combinations shows that ZP 360 × ZP 434 was the best specific combination, as it resulted in the highest grain yield, although ZP 341 was the best general pollinator for this trait. The average grain yield increase of this hybrid under xenia effects amounted to 6.28%.

The combined (plus-hybrid) effect on observed hybrids varied, but it was observed that none of them were significant (Table 6). The plus-hybrid effect was positive for ZP 341, while ZP 341st × ZP 360 was the best plus-hybrid combination, as the yield was higher by 4.05% than in its fertile counterpart. Only one plus-hybrid combination for the hybrid ZP 360 had increased yield (by 2.79%), while the ZP 360st × ZP 42A combination even had a reduced yield (by 6.03%), although this reduction was not statistically significant.

Table 3. Xenia effect on grain yield for hybrid ZP 341 per se and for different ZP 341× pollinator combinations.

Poliinator 1	Pollinator 2	Grain yield
ZP 341	ZP 360	-4,34
	ZP 488	-4,3†
	ZP 434	-5,5†
	ZP 42A	-5,9†
ZP 360	ZP 488	0,15
	ZP 434	1,1
	ZP 42A	1,5
ZP 488	ZP 434	0,97
	ZP 42A	1,35
ZP 434	ZP 42A	0,39

Values (%) for grain yield indicate differences between ZP 341 hybrid pollinated with pollinator 1 and 2.

†- significant at the 0,1 probability level.

Table 4. General pollinator ability for grain yield of five hybrids used as a pollinators.

Pollinator	Grain yield
ZP 341	13,138
ZP 360	12,835
ZP 488	12,643
ZP 434	13,102
ZP 42A	12,561

Table 5. Xenia effect on grain yield for hybrid ZP 360 per se and for different ZP 360× pollinator combinations.

Pollinator 1	Pollinator 2	Grain yield
ZP 360	ZP 341	6,92†
	ZP 488	6,23†
	ZP 434	10,53†
	ZP 42A	1,42†
ZP 341	ZP 488	0,65
	ZP 434	-3,38
	ZP 42A	5,43
ZP 488	ZP 434	-4,05
	ZP 42A	4,75
ZP 434	ZP 42A	9,0†

Values (%) for grain yield indicate differences between ZP 360 hybrid pollinated with pollinator 1 and 2.

†- significant at the 0,1 probability level.

Table 6. Plus-hybrid effect on grain yield.

Hybrid	Pollinator	Grain yield
ZP 341st	ZP 360	4,05
	ZP 488	3,86
	ZP 434	2,87
	ZP 42A	2,47
ZP 360st	ZP 341	-0,57
	ZP 488	-1,22
	ZP 434	2,79
	ZP 42A	-6,03

Values (%) on grain yield indicate changes relative to the male-fertile hybrid, isogenically pollinated.

The highest grain yield in male hybrids was recorded in ZP 341. This hybrid also was the best general pollinator. However, the ZP 360 × ZP 434 combination was the best plus-hybrid combination in the hybrid ZP 360 (Table 7). The yield recorded in the hybrid ZP 434 is lower by a tonne than the yield detected in the hybrid ZP 341, but it still ranked second best among observed hybrids. It cannot indubitably be stated that the traits of the male component could be used to predict the plus-hybrid effect, although results showed (not presented in a table in this paper) that the highest yield was recorded in the ZP 341st × ZP 341 combination, which was not the plus-hybrid combination, but it was in agreement with results presented in Table 7.

Table 7. Grain yield of hybrids used as pollinators

Pollinator	Grain yield
ZP 341	13,781
ZP 360	12,678
ZP 488	11,265
ZP 434	12,787
ZP 42A	9,954

WEINGARTNER *et al.* (2002b) studied the plus-hybrid effect in micro-trials by using two single cross cms hybrids (European flints and dents) and three fertile pollinators in Switzerland, then two single cross cms hybrids (dents of the US maize belt) and four pollinators, as well as, one cms hybrid with five pollinators. Effects of cms and xenia were greater in experiments carried out in Switzerland than in those performed in the USA, which was explained by the difference in the origin of the used germplasm, i.e. genotype. Furthermore, different types of sterile cytoplasm were used: T type in Switzerland and S and C types in the USA. Considering that our experiments encompassed only S type of sterile cytoplasm, the effect of germplasm on the expression of cms and xenia effects, both, individually and combined was confirmed. Also, hybrids observed in Switzerland belong to the FAO maturity group 100-200, which is of a broader genetic base in comparison to our hybrids belonging to the FAO maturity group 300-400. According to BULANT and GALLAIS (1998) a greater gain is obtained from xenia if a genetically distant material is crossed. According to the pedigree, ZP 42A out of all used pollinators is genetically the most distant with all observed hybrids. However, it was the worst pollinator for the observed trait in both hybrids. The xenia effect was the best in the observed hybrid ZP 360 when ZP 434, significantly close by the pedigree, was used as a pollinator. The analysis of molecular markers for the genetic distance between observed hybrids is in progress, hence the effect of the genetic origin of the material on the plus-hybrid effect will be determined.

CONCLUSION

According to presented results, the plus-hybrid effect differed over observed hybrids, but it was not significant. Considering that each plus-hybrid combination of the first hybrid was better than its fertile variant, it can be concluded that the genotype of a sterile female hybrid in the combination is crucial in the expression of the plus-hybrid combination. Furthermore, for the time being, it can be claimed that the genetic distance is related to this effect.

Although the grain yield increase is not significant, all the same, both hybrids, at least in some combinations, positively responded to the combined effect of cms and xenia. The study will be continued for another two years, and results of the molecular marker analysis will be considered. According to this, the best combination, in order to finally confirm the possibly obtained plus-hybrid effect, could be set in the strip trials.

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PLUS-HIBRID EFEKAT NA PRINOS ZRNA KUKURUZA DVA ZP HIBRIDA

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I z v o d

Plus-hibrid efekat odnosi se na kombinovani uticaj citoplazmatične muške sterilnosti i ksenija na svojstva hibrida kukruza. Dva ispitivana ZP hibrida različito su reagovala ovaj efekat za prinos zrna. Sve Plus-hibrid kombinacije prvog ispitivanog hibrida su imale veći prinos od svojih fertilnih analoga, ali ne značajno, dok je samo jedna kombinacija drugog hibrida pozitivno odreagovala, takođe bez statističke značajnosti. Ispitivani efekat izgleda zavisi najviše od genotipa majke.

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