EFFECTS OF CYTOPLASMIC MALE STERILITY ON MAIZE HYBRIDS YIELD

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Abstract

The principal aim of breeding and seed production in Serbia is to provide sufficient amounts of all seed categories of the most productive hybrids for national needs and export. Climate conditions in Serbia are favourable for maize seed production (soil quality, effective precipitation sum, long freeze-free periods, etc.). The introduction of sterile forms of parental lines in the development of seed material of maize hybrids has resulted in the improvement of maize growing practices and provided easier control of seed crops. Therefore, the objective of the present study was to observe the optimum ratio of sterile to fertile hybrid variants for the commercial maize production and their effects on yields. The trial was set up according to the randomise complete block design with three replications. The elementary plot size amounted to 5.18m². The used material consisted of seed mixtures of 0, 5, 10,… to 100% fertile plants mixed with the sterile variant of the observed hybrid. Upon processing of obtained results, the highest yield of 17.341 t ha^{-1} was recorded in the fertile to sterile variant ratio of 80%: 20%. The coefficient of correlation points out to a low correlation of yields on fertility percentage (rxy=0.164). Furthermore, based on the coefficient of determination, a low percentage of dependence of yields on fertile plants percentage was established (R^2=0.027).

Key words: *cytoplasmic male sterility, maize, yield*

1. INTRODUCTION

Maize, due to its morphology, is a plant very suitable for the production of hybrid seed in large quantities, because hybridisation is relatively easily achieved by sowing parental components in alternate rows and by detasseling, i.e. removal of pollen-producing flowers (tassels) from female plants immediately after their exsertion. In such a way, the following is achieved: pollen of solely male parents (which are not detasseled) circulates in the field, and hybrid seed is produced on female (detasseled) plants.

The advantages of using hybrids in agricultural production are known and numerous. The increase of yields and product quality are the most important. However, the production of hybrid maize seed faces many obstacles. In order to accomplish total hybridisation between two parents, it is very important not to allow self-pollination of the female component. Failure to achieve the complete hybridisation, the female component, which, as a rule has low yield, occurs in produced seeds and reduces the total yield per area unit, and, consequently results in incomplete utilisation of heterosis.

Control-pollination of the female component plants in the production of hybrid seed can be achieved in one of the following ways: by detasseling (manually or mechanically), by the application of chemicals that prevent pollen formation or its dispersal and by the use of male sterility.

Manual detasseling is a very hard work that requires the engagement of a large number of labourers in a relatively short period of time (10-30 days). In addition to labour force, it is necessary to provide the adequate control and super control of quality of performed activities, which involves the engagement of a high number of professionally trained workers. Detasseling is a difficult and tedious job for labourers, and it is usually done under unfavourable conditions during the hottest time of the year. Unfavourable weather conditions, i.e. occurrence of long-lasting rainfalls during pollination, may particularly complicate detasseling, which usually does not tolerate delays, which most often leads to a decline in the quality of achieved production, and even to its rejection for seed crops. Plots for the seed maize production are mainly located far from settlements from which the labour force is engaged and transport and distribution of a large number of labourers to various locations is a problem.

Bearing in mind the abovementioned difficulties related to detasseling in the maize hybrid seed production, it is obvious that this production is linked with great risks, whose most important source is detasseling. The high level of risk accompanying this, otherwise, very expensive production, discourages producers so they unwillingly accept it, especially when the price of commercial maize is high and ensures an equally high income per hectare without previously encountered difficulties and risks.

Despite constant efforts, mechanised detasseling did not provide satisfactory results in practice, mainly because mechanical topping of tassels leads to the loss of the large leaf area, which results in economically significant reduction in yield. In addition, machinery is unusable under unfavourable weather conditions.

Experiments with detasseling machines, cutters, had been performed by many researchers (Dungan and Wudworth, 1939; Borgeson, 1943; Kiesslbach, 1945; Bauman, 1959; Hunter et al., 1973 and others), and obtained results were summarised by Huey (1971) and Trifunović (1975). Huey (1971) stated that mechanical cutters of tassels were not usable under poor weather conditions, did not solve the problem of removing tassels on tillers and plants lagging in growth, and at the same time it was not possible to reduce the average number of leaves lost per plant bellow 2-3 even with the most careful work.

Moreover, attempts to prevent pollen formation and dispersal in seed-bearing plants by the application of appropriate chemicals, did not yield in satisfactory results so far, despite the large number of performed experiments and tested substances.

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.

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.

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.

Today, in practice, many hybrid seeds are based on male sterile inbred lines and are produced by applying the main cms types, cms-C and cms-S (the type cms-T is susceptible to maize leaf pathogens).

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 attractive for producers.

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 for that type of sterility in the nuclear genome, 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.

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 so as to contribute to a higher grain yield. Reduced consumption of nitrogen, water and energy for pollen formation in sterile plants during the flowering period may result in a greater number of kernels per ear (Vega et al., 2001).

Moreover, the cultivation of commercial crops in this way can prevent contamination by genetically modified (GMO) pollen, in case the sterile hybrid is genetically modified. Considering that the production of sterile hybrids is not more complicated than the production of fertile ones, the proposed production system may be a simple answer to the constant requirements for increasing maize yield without increasing the cultivation areas.

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 male sterility on grain yield of one of the leading hybrids developed at the Maize Research Institute, Zemun Polje, ZPSC 341 and to establish the optimum ratio of sterile to fertile component for needs of the commercial production of this hybrid.

2. MATERIALS AND METHODS

2.1. Materials

In order to observe the optimum ratio of sterile to fertile variant of the hybrid ZPSC 341 for the commercial production, 21 mixtures with 0, 5, 10... to 100% of fertile plants were mixed with the sterile variant of this hybrid. The original fertile hybrid ZPSC 341 was included three times into the experiment as a check (ZPSC 341 from hand pollination, ZPSC 341F1 and ZPSC 341 from reciprocal crossing), to control reliability of the experiment. Table 1 shows the list of used variants of the commercial hybrid ZPSC 341. It has been assumed that male sterile plants gave a higher yield than their fertile counterparts di. Therefore, the mixture of male sterile and male fertile hybrid should give a higher yield than an exclusively fertile hybrid, taking into account the minimum percentage of fertile plants necessary for complete pollination in the crop.

Based on the stated, it is expected to determine the optimum ratio of sterile to fertile component of the hybrid ZPSC 341 that would be the most suitable for the commercial production.

The trial was set up according to the complete randomised block design with three replications in the location of Školsko dobro. The trial was conducted under conditions of dry land farming. Sowing was performed manually on the optimum dates (the second half of April). The elementary plot consisted of two rows, with the row distance of 0.7 m, 10 hills per row, with the hill distance of 0.37 m and 2 plants per hill. The elementary plot size amounted to 5.18m². Cropping practices standard for maize cultivation were applied.

During the growing season, when plants reached full pollination, the total number of plants, including fertile and sterile ones, was established for each elementary plot.

The following parameters were observed prior to harvest: total number of plants, number of lodged plants (whereby the lodged plant is the one whose angle between the stalk and the ground is less than 45º), number of broken plants (whereby the criterion for this parameter is a broken node below the upper ear).

Harvest was done in the stage of full (physiological) maturity. Yields of fresh ears were measured at harvest for each hybrid per replication of each elementary plot. A submitted sample consisting of five ears was measured on the technical balance in the laboratory.

After shelling of the submitted sample, the cobs were measured and kernel moisture percentage was determined by the moisture meter

Table 1. Variants of the commercial hybrid ZPSC 341

Ordinal number	% Fertility	Fertile to sterile plants ratio
1	0% Ft	0Ft + 40St
2	5% Ft	2Ft + 38St
3	10% Ft	4Ft + 36St
4	15% Ft	6Ft + 34St
5	20% Ft	8Ft + 32St
6	25% Ft	10Ft + 30St
7	30% Ft	12Ft + 28St
8	35% Ft	14Ft + 26St
9	40%Ft	16Ft + 24St
10	45% Ft	18Ft + 22St
11	50% Ft	20Ft + 20St
12	55% Ft	22Ft + 18St
13	60%Ft	24Ft + 16St
14	65% Ft	26Ft + 14St
15	70% Ft	28Ft + 12St
16	75% Ft	30Ft + 10St
17	80% F	32Ft + 8St
18	85% Ft	34Ft + 6St
19	90% Ft	36Ft + 4St
20	95% Ft	38Ft + 2St
21	100% Ft	40Ft + 0St

2.2. Methods

The statistical data processing encompassed the analysis of variance in accordance with the complete block design, regression and correlation analysis of grain yield and the percentage of fertile plants in the hybrid ZPSC 341, in order to establish the changes in grain yield in relation to the percentage ratio of sterile to fertile plants (after Hadživuković, 1991). The following scale was conventionally used for the interpretation of the values of coefficients of correlation:

- $0.00 < (rxy) \leq 0.50$ - low correlation
- $0.50 < (rxy) \leq 0.75$ - medium correlation
- $0.75 < (rxy) \leq 0.90$ - high correlation
- $0.90 < (rxy) \leq 1.00$ - very high correlation

The estimation of quadric regression was performed also.

It was assumed that this analysis would give an answer to questions set in the working hypothesis.

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.

According to data presented in Table 2 it is obvious that the most yielding hybrid was the one with 80% of fertile plants (17.341 t ha^{-1}), while the least yielding was the hybrid with fertility of 5% (16.004 t ha^{-1}).

According to the above stated it can be concluded that the soil quality and climate conditions in the given location were crucially significant.

Table 2. Average yield and its variation interval for the check and different levels of fertility percentage

Ordinal number	%Fertility	Average yield (t ha^{-1})	95% interval of confidence for mean yield	
			Lower limit	Upper limit
1	ZP341Hand	17.043	12.879	21.208
2	ZP341F1	17.411	17.057	17.766
3	ZP341Rec.	17.324	15.832	18.815
4	0%	16.113	13.685	18.541
5	5%	16.004	15.426	16.583
6	10%	16.981	15.132	18.830
7	15%	16.638	14.088	19.189
8	20%	16.345	15.366	17.324
9	25%	17.163	16.434	17.892
10	30%	16.150	11.346	20.954
11	35%	17.000	14.449	19.552
12	40%	17.060	13.713	20.408
13	45%	16.861	15.014	18.708
14	50%	17.054	16.154	17.954
15	55%	17.134	14.561	19.708
16	60%	16.840	13.792	19.889
17	65%	16.867	15.085	18.649
18	70%	16.373	15.039	17.706
19	75%	16.563	13.354	19.772
20	80%	17.341	15.173	19.508
21	85%	16.967	15.782	18.151
22	90%	16.987	13.469	20.505
23	95%	16.460	11.354	21.565
24	100%	17.096	15.301	18.891

If the average yield gained in the location of Školsko dobro (16.824 t ha^{-1}) is compared with the yield of the hybrids ZP 360 (14.160 t ha^{-1}) and ZP 434 (14.260 t ha^{-1}) recorded by Videnović et al. (2000) in the location of Sombor it can be concluded that the yields recorded in the location of Školsko dobro were higher by over 2.0 t ha^{-1}.

Furthermore, studies of the most recent the 5th and the 6th generation of ZP hybrids carried out by Jovanović et al. (2007) show that the highest yields in Serbia were recorded in the following hybrids: ZP 684 (9.50 t ha^{-1}), ZP 544 (9.23 t ha^{-1}) and ZP 434 (9.21 t ha^{-1}). The hybrids ZP 341 (10.02 t ha^{-1}) and ZP 434 (9.50 t ha^{-1}) were the most yielding in the region of Banat, while the highest yield in the region of Srem was achieved with the hybrid ZP 434 (11.34 t ha^{-1}). Moreover, based on long-term studies on medium late maturity hybrids with a shorter growing season carried out by the group of researches, it was concluded that given hybrids had significantly lower grain moisture content (16-18%).

It may be concluded that the 5th generation of ZP hybrids (FAO 300-400) expressed exceptional yielding and yield stability. Additionally, these hybrids are characterised by a shorter growing period and a significantly lower grain moisture at harvest, which is great advantage due to reduced costs of maize drying and storage (Tabaković et al., 2015, Pavlov et al., 2015).

Figure 1 presents the trend of average yield over levels of the share of fertile plants for the given location.

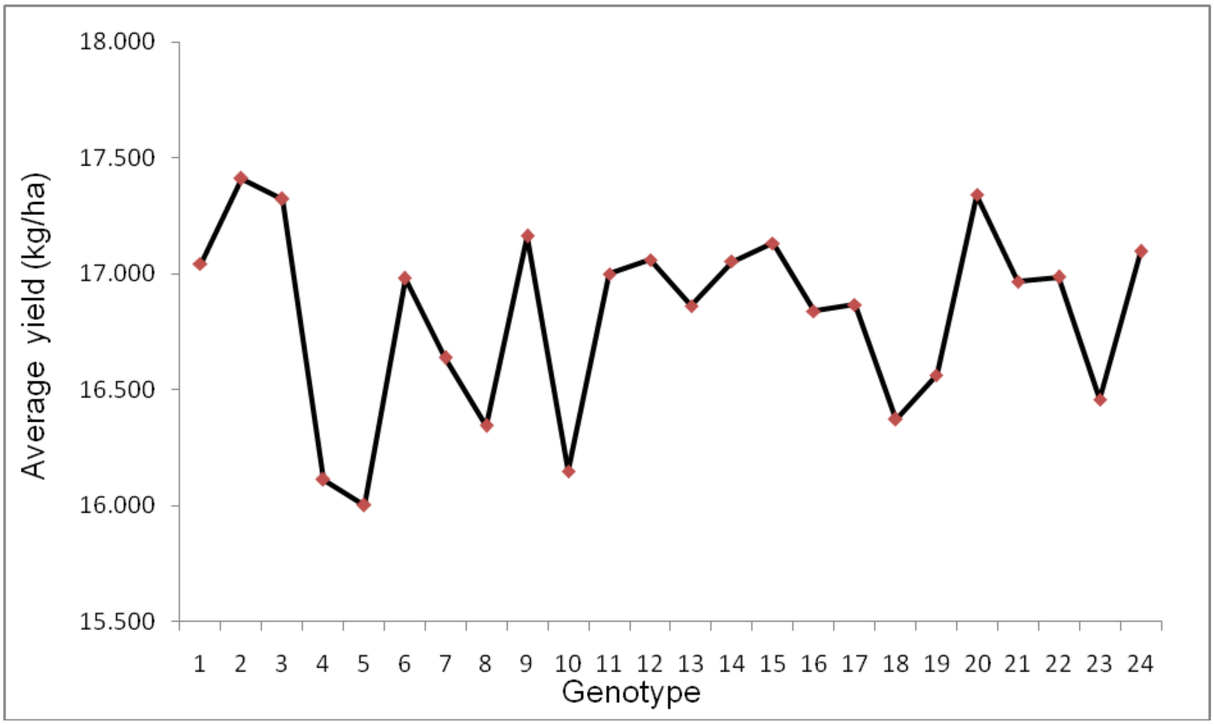

Figure 1. Variation of average yield of the hybrid ZPSC 341 with different levels of the percentage share of fertile plants

Results presented in Table 3 indicate that different ratios of fertile to sterile components in the seed mixture used for sowing significantly affected the level of yields (rxy =0.164).

The value of coefficients of correlation ranges from -1 to +1, but the obtained coefficient of correlation tends to or is close to 0 it can be said that yields and percentages of fertility are not interrelated.

Table 3. Correlation coefficient of yield and fertility percentage

Location	rxy
Školsko dobro	0.164

Based on the interpretation of the value of the coefficient of correlation it can be concluded that there is a low functional dependence between the yield and the fertility percentage.

By observing previously presented mean values and the ratio of the share of fertile plants, it may be assumed that the correlation would be less significant, i.e. that it would insignificantly affect the fertility percentage.

The yield of hybrids is a consequence of effects of soil and precipitation distribution during the growing season. This statement is also confirmed by results of the analysis of variance that shows statistical significance of the location.

Furthermore, it is impossible to determine the relative importance of each independent variable on the dependent variable - yield (Table 4). The small influence of different ratios of fertile to sterile component is observable through small coefficients of regression (ß). Their contribution to the change in yields is only 0.027 % (R^2).

Table 4. Values of parameters of quadric regression model and coefficient of determination

Location	β_0	β_1	β_2	R^2
Školsko dobro	3E-05X^2	3E-05X	11.155	0.027

The coefficient of determination describes the proportion of the dependent variable explained by the variation of the independent variable. Its supplement to 1 is called the coefficient of nondetermination and describes the proportion of the total variation of the dependent variable that is not explained by the impact of the independent variable.

Based on the value of the coefficient of determination (0.027), a small percentage dependency is observed, which indicates that a great percentage of variability of factors affecting the yield variability was not encompassed.

The equation of the estimated quadric regression is schematically presented in Figure 2, where the abscise is the percentage of fertile plants, while the ordinate is maize yield. Moreover, Figure 2 also presents to which extent the variation of maize yield affects the variation of the percentage of fertile plants.

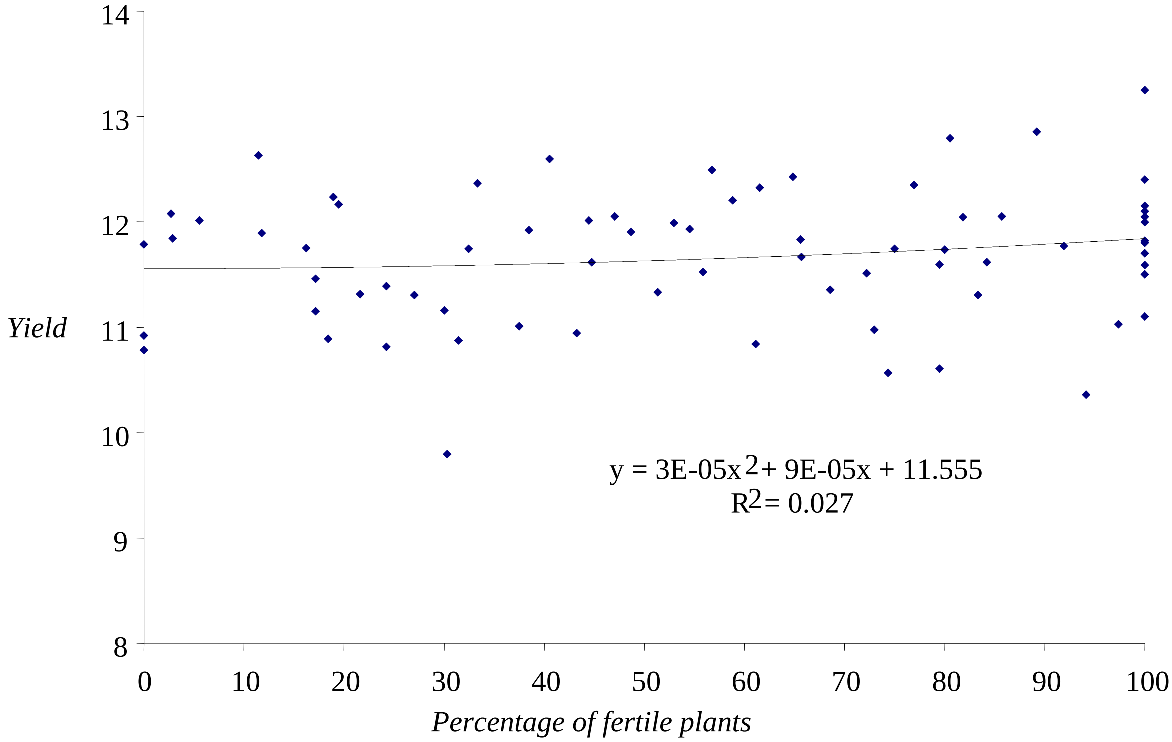

Figure 2. Equation of the estimated quadratic regression for the location of Školsko dobro

The regularity of effects of the percentage of fertility on yield is not observable in Figure 2, which indicates the possibility of their independence.

4. CONCLUSION

According to results obtained on the seed production of the commercial hybrid ZPSC 341, i.e. on effects of different percentages of fertile and sterile plants on the yield of the hybrid ZPSC 341, the following may be concluded:

- The effect of the location on maize grain yield was significant;
- Environmental conditions significantly affected variation of yields over location;
- The highest average yield was 17.341 t ha^{-1};
- The lowest average yield was 16.004 t ha^{-1};
- 80% of fertile plants was the most favourable fertile to sterile variant ratio;
- 5% fertility was the least favourable ratio;
- Coefficients of correlations were positive, but there were no statistical significances of yield and the fertility percentage.

Although obtained results do not provide sufficient information to establish the optimum ratio of sterile to fertile variant of the hybrid ZPSC 341 for the purposes of its commercial production, there are a lot of reasons to assume that the previously applied fertile to sterile variant ratio of 75% to 25% is most likely the optimum ratio for the commercial production of the hybrid ZPSC 341.

REFERENCES

Bauman, L. F. (1959): Progress report on genetic control of male sterility *Proc of 6th Ann. Hybrid corn Industry-Res. Conf.*, pp. 13-18.

Borgeson, C., (1943): Methods of detasseling and yield of hybrid seed corn. *Jour. Amer. Soc. Agron.*, 35: 919-922.

Dungan, G. H. and Woodworth, C. M. (1939): Loss resulting from pulling leaves with tassels in detasseling corn. *Ag J.*, 31: 872-875.

Hadživuković, S. (1991): Statistički metodi s primenom u poljoprivrednim i biološkim istraživanjima. Drugo izdanje. Poljoprivredni fakultet, Novi Sad.

Hirel, B., Martin, A., Terce-Laforgue, T., Gonzalez-Moro, M.-B., Estavillo, J-M. (2005): Physiology of maize I: A comprehensive and integrated view of nitrogen metabolism in a C4 plant. *Physiologia Plantarum* 124: 167–177.

Huey, J. R. (1971): Experiences and results of mechanical topping versus detasseling in 1971. *Proc. of 26th Corn. Res. Conf.* pp. 144-147. Amer. Seed Trade Assoc.

Hunter, R. B., Mortimore, C. G., and Kannenberg, L. W. (1973): Inbred maize performance following tassel and leaf removal. *Agronomy Journal* 65: 471-472.

Jovanović, Ž., Tolimir, M., Kaitović, Ž., (2007): ZP hibridi kukuruza u proizvodnim ogledima 2006. godine. *Zbornik naučnih radova* 2007. Vol. 13. br. 1-2. str. 53-60.

Kaeser, O., Weingartner, U., Camp, K.-H., Chowchong, S., Stamp, P. (2003): Impact of different cms types on grain yield of dent x flint hybrids of maize (*Zea mays* L.). *Maydica* 48: 15-20.

Kiesselbach, T. A. (1945): The detasseling hazard of hybrid seed corn production. *Jour. Amer. Soc. Agron.* 37: 806-811.

Rhoades, M. M., (1931): The cytoplasmic inheritance of male sterility in Zea mays. *J. Genet*. 27:71-93.

Trifunović, V., (1975): Proučavanje sterilnosti polena materinskih linija kukuruza s obzirom na dobijanje hibridnog semena. Arhiv za poljoprivredne nauke. 28 (104): 59-107.

Vega, C.R.C., Andrade, F.H., Sadras, V.O., Uhart, S.A., Valentinuz, O. (2001): Seed number as a function of growth. A comparative study in soybean, sunflower, and maize. *Crop Science* 41: 748-754.

Videnović, Ž., Jovanović, Ž., Kresović, B., Tolimir, M. (2000): Effects of agroecological on zp maize hybrid yield in Serbia. *Genetika*, Vol. 32, No. 3. str. 397-405.

Pavlov. M., Todorović, G., Crevar, M., Tolimir, M. (2015). Influence of hybrid combinations on the maize seed, *PTEP-Journal on processing and energy in agriculture*, Novi Sad, Serbia, vol.19, No 5, 233-240.

Tabaković, M, S. Jovanović, G. Todorović, M. Mišović (2015). Effect of insecticides on physiological characteristics of seeds in storage. Fourth International conference sustainable postharvest and food technologies – INOPTEP 2015 and XXVII National conference processing and energy in agriculture – *PTEP*, *Book of proceedings*, April, 19st-24th, Divčibare, Serbia, 269-273.