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RESEARCH ARTICLE

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A comparison of controlled self-pollination and open pollination results based on maize grain quality

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Abstract

Maize (*Zea mays* L.) grain endosperm is triploid (3n), of which 2n come from the male (transferred by pollen) and only 1n from the female plant, thus a major impact of the male form can be expected on grain quality parameters. A good example of this relationship is the phenomenon of xenia. The aim of this study was to determine the effect of pollen on grain quality. The field experiment was conducted in 2011; seeds were harvested from eight cultivars: Bosman, Blask, Tur, Kozak, Bielik, Smok, SMH 220 and Kresowiak, derived from free pollination and controlled self-pollination of maize. Analyses of nutrient contents and starch content in the grain were conducted in the laboratory. In addition, 1,000 grain weight and the hectoliter weight of all grain samples were recorded. The results confirmed differences in grain quality of maize hybrids obtained by self-pollination and by open pollination. Grain of maize plants obtained by open-pollination was characterised by higher contents of N-free extract and starch, and lower protein content. Undertaking further studies on this subject may indicate specific recommendations for agricultural practice, such as mixtures of hybrids with good combining abilities, which will contribute to improved grain quality without additional costs.

Additional key words: chemical composition; hybrids; pollen; pollination.

Introduction

Maize (*Zea mays* L.) ranks as the third most important cereal grain in the world (Zilic *et al.*, 2011). In Poland, the cultivation of maize was introduced in the 1950's, during the communist era, when farmers not prepared for this task were ordered to grow this crop. During that period there were no varieties suitable for cultivation in Central and Eastern Europe, characterised by a short growing season, so this action was a failure and discouraged farmers from growing this crop. Early maize hybrids suitable for Polish conditions appeared later, only in the early 1970's (Adamczyk *et al.,* 2010).

Currently in Poland cultivation of maize accounts for about 10% of the sown area. In 2011, 760,000 ha of maize were cultivated and in 2012 there was an increase of the area to 1,056,000 ha, including 544,000 ha for grain (CSO, 2012). Such a large increase was due to the good situation on the cereal commodity market, but also the necessity of replanting damaged winter crops.

Progress in breeding allowed farmers to achieve higher yields in 1984-2011. According to a study of COBORU (2012), the average annual growth in grain yield for the three maturity groups of hybrids reached 156 kg ha^{-1} (2.29%), while in production it amounted to 80 kg ha^{-1}. Similarly, in the USA the average yield

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Abbreviations used: CV (coefficient of variation); DM (dry matter); NFE (N-free extract); PCA (principal component analysis); S (starch); SC (two way cross-breed); TC (three way cross-breed); TGW (thousand grain weight) increase between 1939 and 2004 was approximately 100 kg $ha^{-1} \cdot yr^{-1}$, *i.e.* 2% per year (Tollenar & Lee, 2006). Currently maize hybrids up to the so called FAO 300, grown in Polish agriculture, come from leading breeders, both domestic and from the EU and the USA.

In experiments on the assessment of individual hybrids, maize plants are sown close together. Maize plants as cross and wind pollinated species can be pollinated with pollen from considerable distances, according to Felsot (2002) from up to 300 meters. However, experiments with coexistence of conventional and genetically modified maize showed that the main pollination is done within a much shorter distance, up to 20 m (Brookes *et al.,* 2004; Arritt *et al.,* 2007).

Grain endosperm is triploid (3n), of which 2n come from the male (transferred by pollen) and only 1n from the female plant, so a major impact of the male form can be expected on grain quality parameters. A good example of this relationship is the phenomenon of xenia observed on sweet maize pollinated by fodder maize. The xenia effect in endosperm and embryo traits has been known for a long time (Davarynejad *et al.*, 1994; Seka *et al.,* 1995; Bulant *et al.,* 2000; David *et al.,* 2001).

Maize grain is harvested from the F2 generation of the grown hybrid, but only if it is pollinated with the pollen of the same cultivar, as happens in large fields. For small plots used in variety trials, free open pollination by pollen from varieties sown in close proximity is observed and the resulting kernels are hybrids from the neighbouring varieties. In the case of two-way hybrids grown, random four-way hybrids are obtained, which may show some effects of heterosis as well as the effect of splitting of the traits. Thus, grain from such pollination may differ in its chemical composition and other characteristics from that obtained from self-pollination of only one variety. The results of this evaluation will not be comparable with those collected from large fields. As it was observed by Mercer *et al.* (2002), there is a preference of other plant pollen (allopollen) over own pollen (autopollen) during fertilization of maize plants, with an average rate of 73.27% in the ear, which is close to the average frequency of 80.06% observed also by Balestre *et al.* (2007a).

The aim of this study was to determine the effect of pollen on grain quality. The research hypothesis assumes that maize grain produced from free pollination with pollen of different varieties grown next to each other on side-by-side plots varies in its chemical composition and other characteristics from that produced by controlled self-pollination.

Material and methods

Plant material and experimental design

Field experiment was conducted at the Plant Breeding Station in Smolice (Poland) in 2011. Seeds were harvested from eight cultivars diverse as regards of the length vegetation period expressed according to FAO classification: Bosman (C1, FAO 250; medium-early), Blask (C2, FAO 240-250; medium-early), Tur (C3, FAO 240; medium-early), Kozak (C4, FAO 250; medium-early), Bielik (C5, FAO 240; medium-early), Smok (C6, FAO 230-240; medium-early), SMH 220 (C7, FAO 230-240; medium-early) and Kresowiak (C8, FAO 240; medium-early), derived from free pollination and controlled self-pollination of maize. Tested cultivars come from two types of cross-breeding. One group of two way cross-breeds (SC) comprises the following: Bosman, Kozak, Smok and SMH 220. The other cultivars belong to the three way cross-breed group (TC).

The experiment was established in the split-plot design in one replication, which prevented analysis of variance in the split plot system. In the middle of each plot the hybrids were grown without any space isolation. Plots were established side by side, which facilitated free open pollination between plants without isolators. After ear formation in the other half of the plots according to the experimental design, paper isolators were introduced to protect the ears against foreign pollen, while tassels were covered with paper bags to collect the pollen. During the pollination period hand pollination was performed according to the trial design. Tassels were shaken to collect the pollen in bags, followed by removing the isolators from the ears and covering them with bags containing the pollen. All the tested hybrids were grown following recommendations of good agricultural practice for maize crop. After full maturity of the plants ears were harvested, threshed and samples were prepared for laboratory analyses.

Determination of physical traits

In addition, 1,000 grain weight (TGW) and the hectoliter weight of all grain samples were recorded. TGW was determined using a Kopciuszek seed counter by Sadkiewicz Instruments (Poland). Three samples for each treatment were weighed on a Sartorius balance (by Sartorius Poland Company) accurate to 0.01 g. The hectoliter weight was assessed three times, using an electronic moisture and grain density test weight (GMDM) meter by Dramiński Electronics in Agriculture from Olsztyn, Poland.

Quality attributes

Contents of nutrients and starch in the grain were analysed at the laboratory of the Department of Agronomy, Poznaƒ University of Life Sciences (Poland). Starch content was determined by the polarimetric method according to Ewers (Gugała, 2002) using 0.31 N HCl and 4% phosphotungstic acid to precipitate protein substances. Sample analysis was performed in triplicate, while the blank test was performed once. Starch (S) content in dry matter (DM) percentage was calculated using the following formula:

$$S = \frac{100 \times \left(P - P^1\right) \times 100}{\alpha_d \times l \times m \times \left(1 - \frac{W}{100}\right)}$$

where P: angle of rotation of plane polarised light in the sample tested; P^1: angle of rotation of plane polarised light in blank sample; α_d: specific value of starch rotation (184.6); l: polarimeter tube length (dm); m: test sample weight (g); W: moisture content of sample (%).

Contents of organic components in the grain were analysed according to the following methods: total protein was determined by examining nitrogen content in the sample by the Kjeldahl method (Persson, 2008) and multiplied by a conversion factor of 6.25; crude fat with the Soxhlet method (Virot *et al.*, 2008); crude fiber by hydrolysis of plant material residue; ash by dry incineration; and N-free extract (NFE) by subtracting from 100% the sum of the contents of the other components.

Statistical analysis

In the preliminary analysis of results recorded in laboratory assays mean values were calculated for parameters determining grain quality for the controlled and free open pollination variants. For the analysed traits the characteristics of variation were also determined, such as coefficient of variation, and minimum and maximum values.

The t-Student test for dependent samples was applied to compare pairs of means for grain quality parameters between the controlled and free open pollination combinations. At this stage of research this model of the statistical analysis has been used because the aim was to identify differences in the quality of grain depending on pollination type. The test for bound variables was selected, which reduced the effect of variation between genotypes and experimental units on the experimental error. Linear regression was also used to illustrate the relationship between treatments from the controlled and open pollination variants. Multivariate analysis was also used to demonstrate the combined effects of the parameters on the variation of the experimental treatments. Treatments refer here to combinations of levels of factors, where one factor is the cultivar and the other is the pollination type. Each of the treatments is described by eight traits, parameters characterising grain quality.

The hierarchical clustering method was applied, which allows to group similar objects, characterised by many traits. In this method object means were used for tested traits. Clustering was performed by agglomeration with the linkage method according to Ward. This method consists in linking objects in a manner minimising variability in the resulting group (Marek, 1989).

The applied Principal Component Analysis (PCA) makes it possible to reduce the number of traits (dimensions) describing the objects and illustrates the distribution of object means on the plane. The principal components facilitate a more synthetic assessment of the phenomenon, replacing a group of interrelated traits with one component, which may often be interpretable (Morrison, 1990). Statistical analysis was performed using the STATISTICA 10 software package.

Results

Table 1 presents mean values for the studied traits under controlled and free open pollination, their variability characteristics and the probability of rejecting a true hypothesis of equal means for the t-Student statistics.

This test at a significance level of 0.01 showed significant differences between the means for the characteristics of the groups with controlled and free open

Table 1. Comparison of means and variability parameters in the groups of controlled and free open pollination for all studied traits

Trait	Self-pollinated (average)	Open-pollinated (average)	*p* value	Self-pollinated		Open-pollinated		Self-pollinated (% CV)	Open-pollinated (%CV)
				Min	Max	Min	Max		
1,000 grain weight (g)	391.0	328.0	0.000**	372.2	419.8	292.9	358.1	3.9%	6.1%
Hectoliter weight (kg hL^{-1})	85.0	83.2	0.009**	83.4	86.7	80.7	86.2	1.5%	2.6%
Protein (% DM)	11.5	9.0	0.000**	10.8	12.2	7.6	10.3	4.7%	9.3%
Fiber (% DM)	4.1	3.9	0.463	3.5	4.7	3.5	4.3	11.1%	5.9%
Ash (% DM)	1.4	1.4	0.278	1.2	1.7	1.2	1.6	12.6%	10.9%
Fat (% DM)	6.1	5.8	0.137	5.2	7.3	5.2	6.4	10.8%	7.1%
NFE (% DM)	76.9	79.9	0.000**	75.7	78.6	78.5	81.4	1.1%	1.2%
Starch (% DM)	63.5	67.9	0.000**	58.5	66.5	63.5	71.3	3.9%	3.4%

CV: coefficient of variation. DM: dry matter. ** Denotes significance at 0.01 level.

pollination for five of the studied traits (TGW, hectoliter weight, % protein in DM, % NFE in DM, % of starch DM). The assumption of homogeneity of variance in the compared groups was also verified while none of the tested hypotheses about the equality of variance was rejected.

Grain harvested from controlled self-pollination was characterised by a higher TGW and grain weight per hectoliter than that from the open pollination variant (Table 1). On average, the difference in the TGW between the self-pollination and open pollination combinations was 63 g and for the hectoliter weight it amounted to 1.8 kg hL^{-1}. Both differences were confirmed statistically. In addition, the use of self-pollination in this study resulted in the advantageous lower variability of TGW and hectoliter weight, at higher minimum and maximum values of the two traits in comparison with the open pollination variant.

In our study pollination type determined starch content in grain of all maize hybrids compared (Fig. 1). The difference in starch content ranged from 1.7% (Smok) to 6.0% (Bosman), at an average 4.4% in favour of open pollination, which was statistically significant. Starch content in grain produced by open pollination was a more stable trait (CV = 3.4) than in the controlled self-pollination (CV = 3.9). In addition, open pollination resulted in a favourable increase in both the minimum and maximum values of this trait.

It was also verified whether there is a regression relationship between the observations of individual parameters in the controlled pollination and open pollination variants. This relationship proved to be significant only for starch content and hectoliter weight. The regression equation of starch content in grain of the same hybrid from open pollination versus that from controlled pollination takes the form $y = 19.95 + 0.76x$, where the x variable explains about 68% of the variation of the variable y. The hectoliter weight equation has the form of $y = ^{-}-39.20 + 1.44x$, and the coefficient of determination is about 67%.

The method of pollination determined total protein content in maize grain. Grain produced by controlled

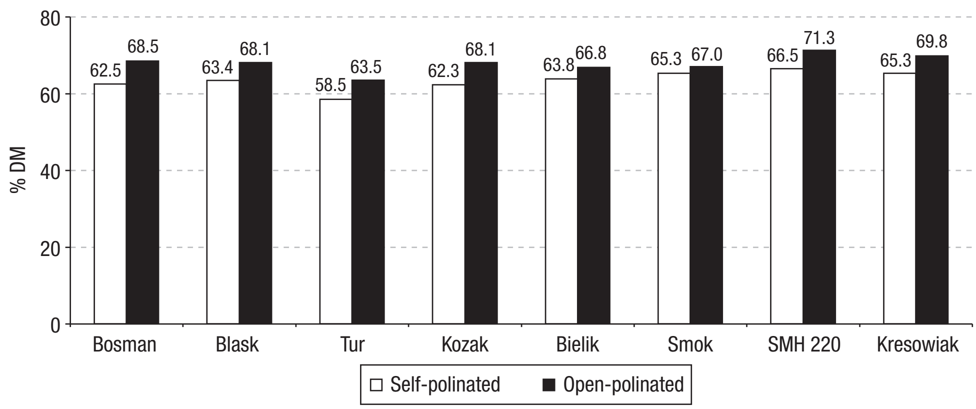

Figure 1. Starch content of maize grain, depending on the cultivar and pollination type (% DM).

self-pollination contained 2.5% more protein than open pollinated maize and this difference was statistically significant. This relationship was found for all tested maize hybrids. Furthermore, protein content in DM of grain from self-pollinated hybrids was characterised by lesser variability and both higher minimum and maximum values in comparison with the grain harvested from openly pollinated plants.

The content of NFE, similarly to starch content of grain produced by open pollination, was significantly higher than in the self-pollinated hybrids, with the difference being on average 3%. Furthermore, in the case of open pollinated plants, this character showed more variability with a favourable increase in its minimum and maximum values.

Similarly, fat content in grain from the controlled self-pollination variant was on average higher by 0.3% than in grain produced by open pollination. However, this difference was not confirmed statistically (Table 1).

Also fiber content in DM of grain produced in the self-pollination combination showed only a trend towards higher values of these parameters as compared to grain produced by open pollination and these differences amounted to 0.2%. No differences were observed in ash content in DM of grain from either in the self- or open pollination variants.

Multivariate analysis

Sixteen medium-sized objects described by the eight studied traits were grouped using the clustering method and the methods of agglomeration at Ward branches. The result of the agglomeration is shown in a dendrogram (Fig. 2). It shows a clear distinction between the two experimental object groups. At the ends of the two major branches there are objects numbered C1 to C8 at the first branch (controlled pollination) and C11 and at the second branch there are objects from C9 to C16 (open pollination). All the cultivars in this analysis, except for no. C11, are grouped depending on the pollination type, which shows the extent to which this factor determines the value of produced grain.

Similar findings were obtained using PCA for medium-sized objects and 8 traits, in this study identified with their dimensions. 100% of variation between treatments is represented by eight principal components. The two first components explain 72.8% of variation and have values higher than 1 (Suppl. Fig. S1

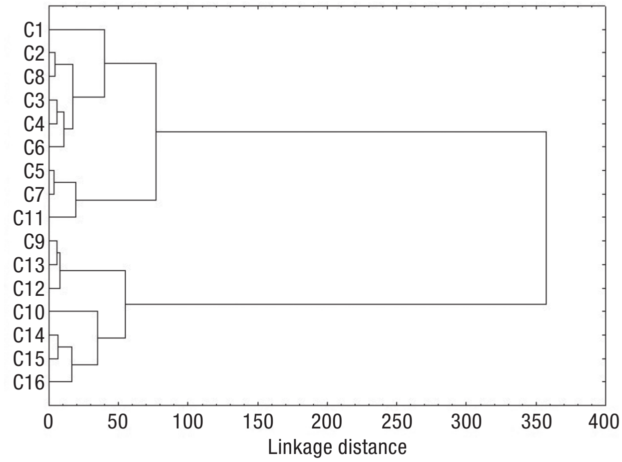

Figure 2. Dendrogram grouping objects depending on quality characteristics. C1: Bosman ⊗. C2: Blask ⊗. C3: Tur ⊗. C4: Kozak ⊗. C5: Bielik ⊗. C6: Smok ⊗. C7: SMH 220 ⊗. C8: Kresowiak ⊗. C9: Bosman OP. C10: Blask OP. C11: Tur OP. C12: Kozak OP. C13: Bielik OP. C14: Smok OP. C15: SMH 220 OP. C16: Kresowiak OP.

[pdf online]), which allows us to reduce the number of traits of the objects and to illustrate the distribution of medium-sized objects on the plane.

The first principal component is composed in equal proportions of the following traits: NFE content in % of grain DM, TGW, protein % in DM and starch content in % of DM. The largest share in the second principal component (factor 2) is ash % of DM and fat % in DM, as shown in Table 2. We can say that our treatments were first of all differentiated by the traits that make up the first principal component.

Fig. 3 shows how the object means are placed after projection of the first two principal components. It can

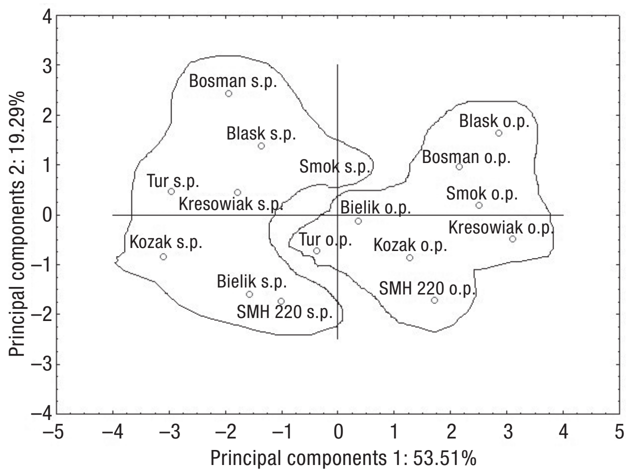

Figure 3. Projection of objects on the plane of the first two principal components.

Table 2. Percentage shares of observed variables for the first two principal components (based on correlations)

Variable	Principal component 1		Principal component 2	
	Variable contribution	Factor loadings	Variable contribution	Factor loadings
Starch (% DM)	17.2%	0.86	4.28%	–0.26
Protein (% DM)	19.2%	–0.91	0.5%	0.09
Fiber (% DM)	4.2%	–0.42	3.1%	0.22
Ash (% DM)	0.5%	–0.15	37.7%	0.76
Fat (% DM)	4.9%	–0.46	39.5%	–0.78
NFE (% DM)	22.1%	0.97	0.2%	0.06
1,000 grain weight (g)	19.2%	–0.91	3.5%	0.23
Hectoliter weight (kg hL^{-1})	12.7%	–0.74	11.2%	–0.42

DM: dry matter. NFE: N-free extract.

be seen that the objects were split into two groups, those with the letter k (average for the controlled pollination cultivars) are arranged on the left side of the vertical axis and the other group of objects is on the right (except for the open pollinated cv. Tur). This confirms the conclusion provided by the clustering method.

Discussion

Maize germ and especially endosperm, which is more than 95% of kernel mass, presents the phenomenon of xenia, which is a direct expression of pollination. The xenia effect can be interpreted as an early expression of heterosis, which increases the ability of the endosperm, modified by the effect of foreign pollen. Cross pollination directly modifies the accumulation of photoassimilates and thus affects kernel weight. The greater the genetic difference between the allelopollen receiver and the donor plant, the greater the chances are that the phenomenon will occur (Denney, 1992).

Numerous studies have shown that pollen of different origin, namely originating from other than the male genotype (allopollen) increases the weight of the resulting kernels (Lopes & Larkins, 1993; Seka & Cross, 1995; Bulant & Gallais, 1998; Mercer *at al.*, 2002; Balestre *et al.,* 2007b).

This increase may result from the activity of the enzyme ADPGPPase (EC2.7.7.27) generally responsible for the synthesis of starch in the endosperm of maize kernels (Bulant *et al.,* 2000). According to those authors, ADPGPPase activity in grain from foreign pollination was higher, which resulted in increased grain weight. The activity of this enzyme in kernels produced by foreign pollination (allelopollen) was by 19% higher 14 days after pollination, and the difference decreased to 8% after 74 days, compared with the kernels formed as a result of self-pollination.

A study by Balestre *et al.* (2007b) showed that foreign pollen (allopollen) caused the average kernel weight to increase by 16.25 mg when compared to self-pollination, which was statistically confirmed at 95% probability. According to the authors, this increase is caused by several factors. One of them may be inbreeding depression, which occurs when the plant is pollinated with its own pollen. In other words, lethal genes (deleterious alleles), whose expression is masked by heterozygous loci in hybrids, have a chance to be revealed at self-pollination. This is likely to occur especially in single cross hybrids, which have the strongest effect of heterosis in the F1 generation, and plants are assumed to have the same genotype. In such a situation on the field (cultivation of SC hybrids), even with cross pollination, plants do not differ from self-pollinated plants (the same genotype). The combinations of dominant or partly dominant alleles can also affect specific phases or grain development processes that reduce the final weight (Leng, 1949).

In a study of Balestre *et al.* (2007a) the heterosis effect caused by foreign pollination (allopollen) was 4.65%, compared to self-pollination (autopollen). This result was lower than in another study of Balestre *et al.* (2007b), where it amounted to 11.90%.

The results of our studies carried out on Polish hybrid varieties did not confirm the results mentioned above, as TGW resulting from self-pollination was higher by an average of 63 g compared with those produced by open-pollination. Similar results were obtained by Tsai & Tsai (1990), Weiland (1992) and Andrade & Pereira (2005), who conducted research on single, double and triple cross hybrids, and also did not observe the impact of allopollen on reciprocal crosses. The probable reason, which explains the different response of hybrids to pollination method observed by researchers, *i.e.* an interaction with the heterosis effect in the embryo and endosperm, is connected with a hybrid or line combining ability. It is known that hybrids and lines differ in their general and specific combining ability. In other words, there are differences in heterosis, which are associated with dominance and divergence in lines and then in hybrids derived from them. Thus, in the most contrasting pairs an increase in grain weight is observed. Changes in grain weight depend on the specific combining ability of these hybrids. The effect of changing kernel weight may vary, depending on the crossing involved between the hybrids (Hoekstra *et al.,* 1985; Pinter *et al.,* 1987).

The effect of self-pollination may be different, since the proportion between the maternal and paternal genomes differs in the triploid endosperm.

Significant differences in grain weight, their size and test weight between maize hybrids depending on the method of pollination were also reported in a study by Letchworth & Lambert (1998). Test weight (average for 12 hybrids) was higher in the case of self-pollination compared with kernels produced by open pollination. The authors cited the result obtained for ten hybrids; in two cases the result was opposite. Similar relations as for test weight were reported by those researchers for kernel weight and kernel size, which corresponds with our own results, in which both of these traits have higher values in the case of self-pollination.

Based on the observations of an increase in grain weight as a result of open-pollination, studies were undertaken to obtain increased grain yield on production fields by sowing mixtures of cultivars (Bulant & Gallais, 1998; Mercer *et al*., 2002; Balestre *et al.,* 2007b). In a study by Andrade & Pereira (2005) the effect of changes in kernel weight as a result of foreign pollination did not occur. While in the study with other species, researchers observed that berries of blueberry were larger after cross-pollination than after self-pollination. All fertility parameters were reduced for self-pollination, including a lower number of seeds per berry that contributed to a smaller berry size (Chavez & Lyrene, 2009). Significant differences in kernel weight of *Prunus dulcis*, depending on the method of pollination were also reported in a study by Dicenta *et al.* (2002). These authors observed obvious differences between pollination treatments, kernels from cross-pollination being heavier (mean weight 1.48 g) than those from self-pollination (1.37 g).

However in a study by Derin & Eti (2001) the effect of changes in pomegranate fruit quality as a result of foreign pollination did not occur. Different sources of pollen did not play an important role in the quality parameters, namely, juice percentage, total soluble solids (TSS) and titrable acidity contents. On the other hand, fruit weight and 100 aril weight were increased considerably by cross-pollination with pollen taken from other cultivars' male flowers.

The method of pollination differentiated chemical composition of maize grain. Maize hybrids differ in protein and starch contents in the grain, and the dependence of the concentration of these components in the grain is inverse. Similarly, an inverse relation is observed between protein content and grain yield (Idikut *et al.,* 2009). In a study by Letchworth & Lambert (1998) grain produced by self-pollination contained more protein than the open-pollinated variant and the ranking of hybrids in terms of protein content did not change. The evaluation of reverse crossing shows a strong maternal effect on protein content and a lack of the pollen effect. In our studies, as in the cited literature, grain produced by controlled self-pollination contained more protein than the DM produced by a mixture of pollen of the variety and other varieties growing nearby.

Also fat content in grain from controlled self-pollinated plants was higher than in the case of open pollination. This result does not correspond to that reported by Letchworth & Lambert (1998), who showed significantly higher fat contents in grain produced by open pollination than self-pollination. In the study of Letchworth & Lambert (1998), grain produced by open pollination had higher starch content than that from self-pollinated plants. Reverse crosses showed the maternal effect on starch content and the absence of the effect of pollen. Usually the result of allopollen in reciprocal crosses is not significant and inversion of the female plant does not affect kernels (Tsai & Tsai, 1990; Weiland, 1992; Andrade & Pereira, 2005).

Open pollination by a pollen mixture from varieties grown nearby led, similarly as in the cited literature, to formation of greater amounts of starch in the kernels than in controlled self-pollination. Also NFE content in the grain produced by open pollination was significantly higher than in the case of self-pollination.

Our studies showed that contents of fat, fibre and ash in the grain produced by open pollination with a mixture of pollen from varieties grown in close proximity were slightly lower than in kernels derived from self-pollination. These differences, however, were not confirmed statistically. Opposite observations were obtained by Letchworth & Lambert (1998), who showed a higher fat content in the kernels resulting from open-pollinated than from self-pollinated plants. Significant differences between the hybrids evaluated in the cited studies indicate the maternal effect, and the data obtained for reciprocal crosses indicated the pollen effect. Fat content in the grain of tested hybrids varied within a narrow range of 41-55 g kg^{-1} and the ranking of the three hybrids with the highest and lowest fat contents did not change depending on the method of pollination. According to these authors, detailed studies on fat content should be conducted in self-pollination combinations. Analyzing other species in the literature we can also find data that sunflowers pollination by bees improves seed weight and seed oil content. The ability of honey bees to forage on many sunflower heads improves their pollination potential. This study showed that bee pollination increased sunflower seed number by 59% (Nderitu *et al.,* 2008). Also interesting results were obtained by Oukabli *et al*. (2002) on *Prunus dulcis* over a two years study. The average fruit set after cross-pollination (25.6%) was higher than that obtained by self-pollination (19.2%). The low fruit set after self-pollination suggests the existence of inbreeding effects.

According to David *et al.* (2001), maize hybrids grown in the vicinity of other hybrids, produce higher quality grain yield than in the case of self-pollination. Our own studies confirmed that open pollination was a factor increasing starch concentration in the grain.

The results of this study and literature data indicate a need to clarify the methodology of variety testing, especially in the allocation of the experimental varieties on plots. When it is difficult to meet the isolation requirement, the solution might be to use isolators and forced self-pollination to produce grain, which will be subjected to chemical analyses. Kernels obtained in this way will reflect the quality of the grain specific to the tested hybrid. In addition, these results will correspond with the results obtained in farming practice when hybrids are grown in large fields.

In conclusion, the study confirmed differences in the quality of grain of maize hybrids obtained by self-pollination or open pollination. Kernels obtained by open-pollination were characterised by higher contents of N-free extract and starch, and lower protein contents. To ensure that the results of variety testing are in line with field production conditions, it is necessary to verify methodology of maize variety testing. Further such studies may indicate specific recommendations for agricultural practice, such as mixtures consisting of selected hybrids with good combining abilities, which will contribute to improved grain quality with no additional costs incurred.

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