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**EFFECTS OF POLLEN CONTAMINATION AND KERNEL WEIGHT ON KERNEL STRUCTURE OF MAIZE IN OPEN AND SELF POLLINATION TREATMENTS**

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**ABSTRACT**

In small plot experiments conducted in maize, the mostly used pollination methods are open and self-pollination treatments. Comparative studies using these treatments are abundant in scientific literature; however studies on the effect of cross pollination and kernel weight on kernel biochemical properties in different treatments are limited. In this study, we conducted a comparative experiment to investigate the effect of pollen contamination and mean kernel weight on kernel biochemical composition of ten different maize genotypes by using two different pollination methods. Open and self-pollination treatments were applied as pollination methods. Eight different traits; kernel weight, cross pollination rate, protein, oil, carbohydrate, oleic acid linoleic acid and carotenoid content were measured. Regression analyses were performed to understand the effects of cross pollination and mean kernel weight on biochemical constituents of maize kernel in different treatments. Results showed that the major biochemical traits, such as protein, oil and carbohydrate content were significantly affected by pollen contamination but minor traits were not. When data were combined (n=60) the effect of pollen contamination in different treatments was not clearly understood. When data (n=30) of each treatment were separately analyzed, it was found that cross pollination rate had significant effect on the most of biochemical constituents in open pollination. Overall, results suggested that pollen contamination had an effect on major biochemical traits in maize and hand pollination could be used for preventing of unwanted effect of pollen contamination in small plot experiments. However, it should be considered that the effects of hand pollination on kernel weight affect the some biochemical traits in maize.

**Keywords:** *Xenia, quality traits, Zea mays, pollen contamination.*

## INTRODUCTION

Maize (*Zea mays* L.) is used in both human and animal nutrition and it has important place in the World's cereal production. Normal maize contains 8-11% protein, 3-18% oil, and 72-73% carbohydrates (Khan et al., 2104). These are major components that directly affect grain quality in maize kernel. In addition to major components, minor constituents such as amino acids, secondary metabolites and fatty acids are available and they are present in relatively small proportions within the kernel but which have significant grain quality effects,.

In recent years, the development of kernel quality has become an important breeding goal in maize. Maize breeding experiments are carried out in breeding nurseries where a large number of materials are grown together in the same area. Due to maize is a cross pollination species, different methods of pollination are used to prevent pollen transmission among different breeding materials in this species (Kahrman, 2016). In the scientific literature, the mostly used pollination treatments are self- pollination and open pollination techniques. The effect of the pollination methods on major kernel quality components has been the subject of different studies. It was reported that the biochemical structure of the kernel has been changed by pollen transmission (Letchworth and Lambert, 1998; Kahrman *et al.*, 2015a). Another factor affecting kernel structure is the size and weight of the kernel. The single kernel weight is also an important variable affecting final yield (Prado *et al.*, 2014). In different studies, the relationship between major kernel quality traits and grain weight characteristics has been discussed (Aliu *et al.*, 2012; Chuckwu *et al.*, 2013; Scrob *et al.*, 2014). It has been shown that open and self-pollination have an effect on kernel structure (Sulewska *et al.*, 2014) and on calculated parameters in breeding studies (Kahrman *et al.*, 2015b). However, no comparative study subjected to the relationship between kernel composition and pollen transmission and mean kernel weight had been conducted in open or self-pollination treatments. A detailed study in this context was thought to give beneficial results in the understanding of this relationship. From this point, this study was conducted to investigate the relationships between kernel quality features and cross pollination rate and mean kernel weight in open and self-pollinated samples of the half-diallel set consisting of 4 parents and 6 hybrids.

## MATERIAL AND METHODS

In this study, six hybrids (Q2xIHO, Q2xIHP, Q2xPR, IHOxIHP, IHOxPR, IHPxPR) and their 4 parents (IHO, IHP, Q2 and PR) were used as plant material. IHO has high oil content, IHP has high protein content, PR high anthocyanin content and Q2 opaque genotypes.

The field experiment was carried out at the Dardanos Research and Application Center of Çanakkale Onsekiz Mart University, Faculty of Agriculture, during the summer growing season of 2016. The experiment was conducted according to the split plot design and genotypes were located in main plots whereas pollination methods were in sub-plots. Drip irrigation was applied according to the state of the plants. Fertilization was applied by taking into consideration the results of soil

analysis with account of 18 kg / da pure N. Self-pollination treatment was performed the method proposed by Kahrman *et al* (2015a).

In the harvest, six ear samples were taken open and self-pollinated plants. These samples were shelled and kernel number of each sample was determined. Afterwards, kernel weights of the samples were determined by a laboratory scale. Cross pollination rate was calculated by the ratio of foreign kernel number to total kernel number in each sample. The mean kernel weight (g) was determined by dividing the value for total kernel weight to value for total kernel number in each sample. Kernel samples were grounded using a laboratory mill (Fritsch pulverisette 14, Germany) then subjected to laboratory analyses. Protein, oil and carbohydrate ratios and oleic and linoleic acid contents were determined using NIR spectroscopy apparatus (Spectrastar 2400D, Unity Scientific, USA) with local calibration models (Egesel and Kahrman, 2012; Egesel *et al.*, 2016). Carotenoid content was determined according to the method recommended by Rodriguez-Amaya and Kimura (2005).

The obtained data were analyzed in the R 3.0.3 package program (R Development Core Team, 2012) using the *stargazer* package. In the regression analysis, each quality trait was assigned as a dependent variable and mean kernel weight (MKW) and cross pollination rate (CPR) were assigned as estimators. In order to compare the results of three different models, intercept, slope and  $R^2$  values of the model were used.

## RESULTS AND DISCUSSION

The mean values for the investigated traits in the study are shown in Table 1. As clearly seen in this table, cross pollination rate is higher in the open-pollination than self-pollination treatment. The mean percentage of protein content, oil content, oleic acid content, and carotenoid content were found to be numerically higher in the self-pollination treatment than in the open-pollination (Table 1).

Table 1. Mean values for measured traits in open-pollinated samples (n=30), self-pollinated samples (n=30) and combined data (n=60)

	n	MKW (g)	CPR (%)	Protein (%)	Oil (%)	Carb (%)	Oleic (%)	Linoleic (%)	Carotenoid ( $\mu\text{g/g}$ )
Open	30	0.21	17.71	12.12	5.78	74.2	30.96	49.67	7.80
Self	30	0.22	0.14	13.04	6.39	72.9	31.85	48.78	8.27
Combined	60	0.21	8.92	12.53	6.05	73.5	31.32	49.38	8.03

The  $R^2$  values of the models formed from the open pollinated samples varied between 0.001 and 0.309, between 0.018 and 0.216 in the self-pollinated samples, and between 0 and 0.162 in the combined dataset. It has been understood that the  $R^2$  values vary considerably in different pollination treatments. For all features, it was determined that the regression coefficient for CPR was higher than the other sets for CPR in open pollination.  $R^2$  values were dropped with the effect of self-pollination and they were similar in combined dataset. It has been understood that the  $R^2$  values of the models was high in which the CPR and MKW variables were

included, but this increase did not available for all measured traits. It is noteworthy that this increase was particularly pronounced in open-pollination treatment. In the models where CPR and MKW variables coexist as predictors in the samples obtained from open pollination treatment had higher  $R^2$  values compared to the others. Indeed, it was determined that the 15.4% of total variation for protein content, 30.9% for oil content, 25.9% for carbohydrate content, 23.8% for oleic acid content and 25.6% for linoleic acid content can be explained when two variables was used as predictors together (Table 2).

Table 2. The  $R^2$  values of the regression models for predicting grain quality characteristics based on the CPR and MKW.

	Predictors	Dependent Variables					
		Protein	Oil	Carbohydrate	Oleic	Linoleic	Carotenoid
Open Pollination (n=30)	CPR	0.138	0.180	0.252	0.049	0.049	0.078
	MKW	0.001	0.209	0.027	0.228	0.246	0.027
	CPR+MKW	0.154	0.309	0.253	0.238	0.256	0.087
Self Pollination (n=30)	CPR	0.110	0.054	0.174	0.0001	0.032	0.020
	MKW	0.079	0.062	0.018	0.091	0.100	0.025
	CPR+MKW	0.216	0.104	0.209	0.092	0.120	0.040
Combined Data (n=60)	CPR	0.007	0.026	0.016	0.011	0.009	0.033
	MKW	0.025	0.123	0.000	0.152	0.162	0.027
	CPR+MKW	0.039	0.132	0.016	0.153	0.163	0.050

For all three models, the regression constant for protein to oil ratio was found to be lower in open pollination than the combined dataset and self-pollination. On the contrary, the exact opposite case was observed for carbohydrate content (Figure 1). The effect of cross pollination rate on protein and oil ratio was negative while its effect on carbohydrate ratio was positive. Considering the effect of mean kernel weight alone, it was observed that one unit increase in the weight of kernel resulted in a decrease in oil ratio and an increase in protein ratio. This effect was similar in open and self-pollination treatments. It has been reported that there was a negative correlation between oil content and kernel weight in some studies, whereas this relationship was found to be positive in some others. Therefore, it can be stated that the relationship between kernel weight and oil ratio may change depending on the material used.

The effect of mean kernel weight on carbohydrate ratio was significantly different from that of open pollination and self-pollination (Figure 1). This effect was found to be negative in the open pollination while it was negative in the self-pollination treatment. This can be attributed to the fact that the number of kernels in the ears was higher in the open pollination treatment. This is because the increase in the number of kernels may have reduced the mean kernel weight, thus causing the carbohydrate content to be low in the open pollination treatment. Indeed, this conclusion was confirmed by previous studies where there is a positive relationship between the kernel weight and the carbohydrate content.

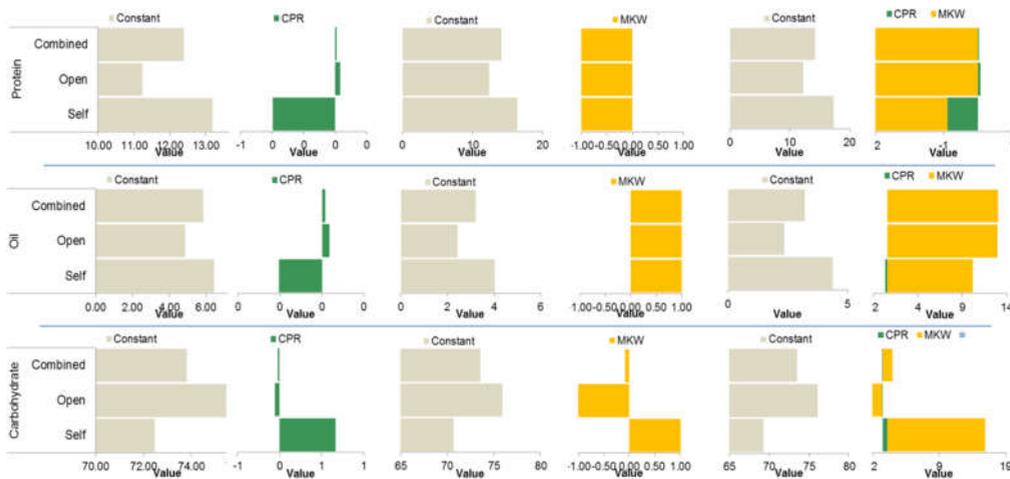


Figure 1. Graphical representation of constant and intercept values of regression models for protein, oil and carbohydrate ratio.

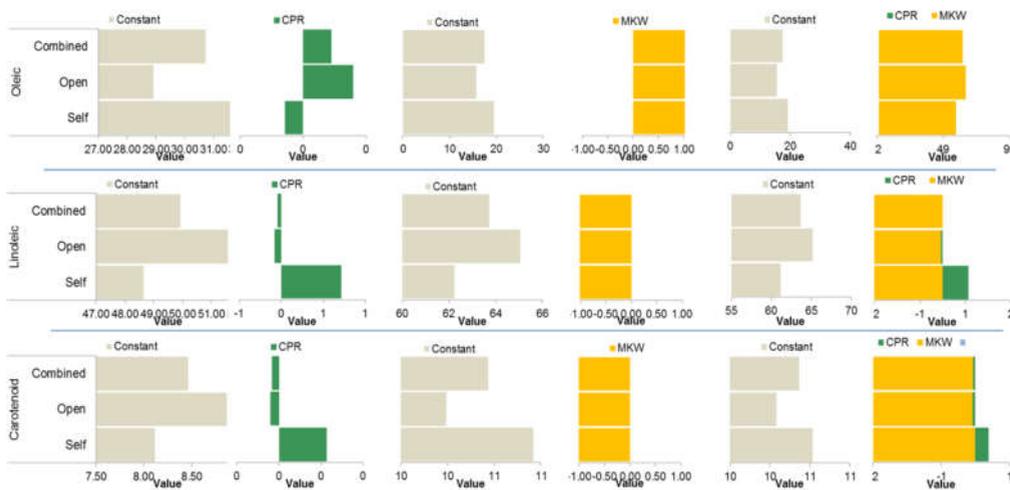


Figure 2. Graphical representation of constant and intercept values of regression models for oleic acid ratio, linoleic acid ratio and carotenoid content.

For oleic acid, the effect of cross pollination rate was positive in the open pollination and negative in the self-pollination. The effect of mean kernel weight on this trait was found to be positive in all data sets. In contrast to these findings, the effect of cross pollination rate on linoleic acid and carotenoid contents was positive in self-pollination treatment. Mean kernel weight had a negative effect on both traits. It was understood that the effect of mean kernel weight and cross pollination rate were also effective in changing of linoleic acid and carotenoid content. In this study, the effect of mean kernel weight and cross pollination rate on the oleic and linoleic acid contents differed according to the pollination treatments.

This result can be attributed to the synthesis mechanism of the respective components. Because linoleic acid is a fatty acid synthesized from oleic acid (Huang *et al.*, 2016), and for this reason, the proportional increase of one of these components causes the decrease in other. It is not possible to discuss the findings of the study in a comprehensive way, since the relationships between major components and mean weight are generally considered in the scientific literature and minor components are not. However, it is understood that oleic and linoleic acid were highly affected by changes in mean kernel weight change and cross pollination rate, while carotenoid content was not affected much by these variables (Figure 2). This result is attributable to the fact that carotenoid content is a property controlled by maternal effects (Egesel *et al.*, 2003).

### CONCLUSION

The results of this study showed that major and minor components of kernel quality characteristics changed with the effect of pollen transmission and mean kernel weight. This variation was affected by open and self-pollination treatments. Cross pollination rate and mean kernel weight can explain the variation in the quality traits examined. According to the results of the regression analysis, it was seen that a significant part (30.9%) of the variation in oil content was explained by mean kernel weight and cross pollination rate in open pollination treatment. Except the carotenoid content, there is a need to be careful in selecting pollination treatments that have the potential to affect pollen transmission risk and weight of the kernel. In this study, pollen contamination was examined depending on kernel color. In the future studies, more detailed results may be possible to obtain using different methods in determining of pollen contamination (such as molecular markers) and sensitive reference analyzes for kernel quality traits.

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