Journal of Pollination Ecology, 15(2), 2015, pp 6-14

THE ROLE OF ARTIFICIAL POLLINATION AND POLLEN EFFECT ON EAR DEVELOPMENT AND KERNEL STRUCTURE OF DIFFERENT MAIZE GENOTYPES

Fatih Kahrıman[1,*], Cem Ömer Egesel[2], Tuncay Aydın[1], Selinnur Subaşı[2]

[1]*Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Field Crops, 17020, Çanakkale, Turkey*
[2]*Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Agricultural Biotechnology, 17020, Çanakkale, Turkey*

Abstract—Pollen effect is important on several kernel traits in maize breeding and may vary under different pollination treatments. Our objectives in this study were i) to evaluate the effects of pollination treatments that are commonly used in maize breeding, on several ear and kernel traits, ii) to investigate if the genotypes so called "specialty corn" do have any different reaction to the pollen effect. A field trial was carried out at Dardanos Research and Application Center of Çanakkale Onsekiz Mart University, Turkey, in 2013. The experiment used a split plot design with three replicates. Four parents (three inbreds and one open pollinated landrace) were used as plant material. Three pollination treatments (open pollination, self-pollination and bulk pollination) were applied, and individual pollen effect of each parent on other parents was investigated. For this purpose, several ear and kernel traits (ear weight, kernel weight, kernel number, mean kernel weight) and biochemical features (protein, oil, carbohydrate and carotenoid content) were measured on harvested samples.

The results showed that pollination treatment affected the variation on all traits except for oil content ($P < 0.05$). Self-pollination caused a significant reduction in kernel development. Pollen effect was found significant for most traits and this effect was evident on the related genotypes with open pollinated landrace. Results indicate that pollen effect is an important factor on kernel and ear development in small plot trials, where different types of maize are grown together.

Keywords: Protein, Oil, Zea mays, *Pollination treatment*

INTRODUCTION

"Pollen effect" refers to the changes in female parent's phenotype caused by the pollen source (Focke1881). It has been well studied in maize and shown to be the cause of important differences in various traits, such as oil content, protein content, fatty acid composition and embryo/seed ratio (Letchworth & Lambert 1998; Tsai & Tsai 1990; Weingartner et al. 2004; Dong 2007; Tanaka et al. 2009). The findings about the pollen effect were variable in the literature. Some studies concluded that pollen parent had no significant impact on protein content (Gilbert 1960; Letchworth & Lambert 1998), whereas, some others showed the lysine and tryptophan content in maize were affected by the pollen source (Pixley & Bjarnason 1994). It was found that pollen from different individual plants of the same variety might have an impact on kernel volume and weight (Bulant & Gallais 1998; Balestre et al. 2007). These investigations enlarged the area of study on pollen effect in maize. Several methods have been developed in order to take the advantage of pollen effect. The best known example is TopCross Blend (Dupont ®), used to elevate oil level of a high yielding hybrid (Thomison et al. 2002). Plus Hybrid Effect is another application, designed to obtain high grain yield, where pollen effect and CMS (cytoplasmic male sterility) were collectively utilized (Weingartner 2002a; Weingartner 2002b).

Pollen must be under control in maize breeding studies and conventional seed production. To achieve this, artificial pollination methods are used in breeding, selfing being the most common one (Öz & Tuğay 2003). Selfing is accomplished by transferring the collected pollen from tassel to ear (pre-covered) in a controlled manner (Abdin et al. 1979). This method causes negative effects on many traits, known as inbreeding depression (Öz & Tuğay 2003). To avoid this, sib-pollination was introduced (Lindstorm 1939), which partly alleviated the ill effects of selfing. Another method, bulk pollination, is preferred in open pollinated varieties (OPV) to sustain the genetic constitution of OPV's, for which the other pollination methods have no use. In this method, pollen from a number of individual plants was bulked and then distributed to the same plants (Taba & Twumasi-Afriyie 2008). Natural pollination (open pollination) was also used in maize research in addition to the artificial pollination methods. Pollen contamination occurs in natural pollinated plants, resulting in undesired variations. Several researchers have studied the effects of pollination method on the kernel structure in maize. Open and self-pollinated genotypes (inbred and hybrids) were compared for protein, oil and starch content (Letchworth &

Received 31 July 2014, accepted 20 January 2015
*Corresponding author; email: fkahriman@hotmail.com

Lambert 1998; Schaefer & Bernardo 2013), fiber content (Şchiop et al. 2011) and starch properties (Krieger et al. 1998). Earlier studies exploring the effects of pollen and/or pollination treatment on the maize genotypes generally used a certain type of plant material, such as inbreds and hybrids. However, breeding nurseries may also contain genotypes with different characteristics, such as open pollinated varieties (OPVs). Use of plant material with different genetic composition such as OPVs, special types of inbreds or hybrids etc. in the studies on pollen and pollination effects could provide novel information. Additionally, evaluation of the traits rarely examined in previous studies, such as carotenoid content, could provide new findings about the pollen effect in maize.

The objectives of this study were: i) to evaluate the effects of pollination treatments that are commonly used in maize breeding, on several ear and kernel traits, ii) to investigate if the genotypes so called "specialty corn" do have any different reaction to the pollen effect.

MATERIALS AND METHODS

Plant material and trial organization

Four parental genotypes were used in this study, each possessing a different kernel quality (Tab. 1). These genotypes were selected to study the pollen effect since they varied in terms of genetic, visual and biochemical features. Of these parents, IHO had high oil (~14%), low carotenoid and carbohydrate; Q2 had high carotenoid and low protein content; PR had high anthocyanin content; and OPV had normal values for all of the investigated traits. A 4 × 4 reciprocal full diallel mating design was generated by using these parental lines. A total of four parents and twelve hybrid combinations made up the plant material in this study. Except Q2 (dent), all of the parents were flint (Tab. 1). To characterize the parents for flowering features, we collected data on days to tasseling, days to anthesis and days to silking (number of the days from the sowing until 50% of number of plants in a plot had tassels, pollen shed, and silk emergence, respectively). Anthesis-silking interval (ASI) was also recorded as the number of days between 50% silking and 50% anthesis. Flowering data were collected only on open pollinated plots. Flowering features of the parental lines showed similar values except IHO, which was earlier for flowering as well as higher anthesis-silking interval than other genotypes. There was a good synchronization among the parental genotypes (Tab. 1).

The field trial was carried out at the Çanakkale Onsekiz Mart University, Dardanos Research and Application Center in Çanakkale, Turkey. Experimental design was a split plot design with three replicates. Genotypes were assigned to main plots (8 rows), and pollination methods to subplots (2 rows). Planting was accomplished with a mechanical seed driller at a plant density of about 71,400 plants ha^{-1},on May 17th, 2013. The soil of the experimental area was clay-loam, with a pH of 7.8, containing 12.7% lime, 1.27%, organic matter content 37.8 kg ha^{-1} phosphorus, and 549.9 kg ha^{-1} potassium. A total of 170 kg ha^{-1} nitrogen was applied in two occasions (80 kg ha^{-1} at planting, and 90 kg ha^{-1} at pre-flowering), based on the soil analysis. Plots were drip-irrigated as needed. Weeds were mechanically controlled.

Pollination treatments

Three different pollination methods were applied in this study. As a natural pollination treatment, open pollination was used; while, self-pollination and bulk pollination were artificial treatments (Fig. 1). To prevent pollen contamination among the genotypes, a controlled pollination method was used (Annonymous 2014). In the first step of this method, all of the ears on the plants were covered by shoot bags before the silk emerging to prevent pollen

TABLE 1. Plant materials used in the study.

	IHO	OPV	Q2	PR
				
Flowering Events (DT, DA, DS, ASI)	69,70,74, 4	74, 75, 76, 1	75, 75, 76, 1	72, 73, 75, 2
General Properties	High oil (14%), low carbohydrate, low carotenoid	Normal values for protein, oil and cabohydrate	Moderate oil, low protein, high carotenoid	High in anthocyanin; Normal values for protein, oil and cabohydrate
Source	NRPIC, USA	Trabzon, Turkey	NRPIC, USA	Çanakkale, Turkey
Hybrids made	IHO × Opaque-2 IHO × OPV IHO × PR	OPV × IHO OPV × Q2 OPV × PR	Q2 × IHO Q2 × OPV Q2 × PR	PR × IHO PR × OPV PR × Q2

NPRIC: North Central Plant Introduction Center. DT: Days to tasseling, DA: Days to anthesis, DS: Days to silking, ASI: Anthesis silking interval.

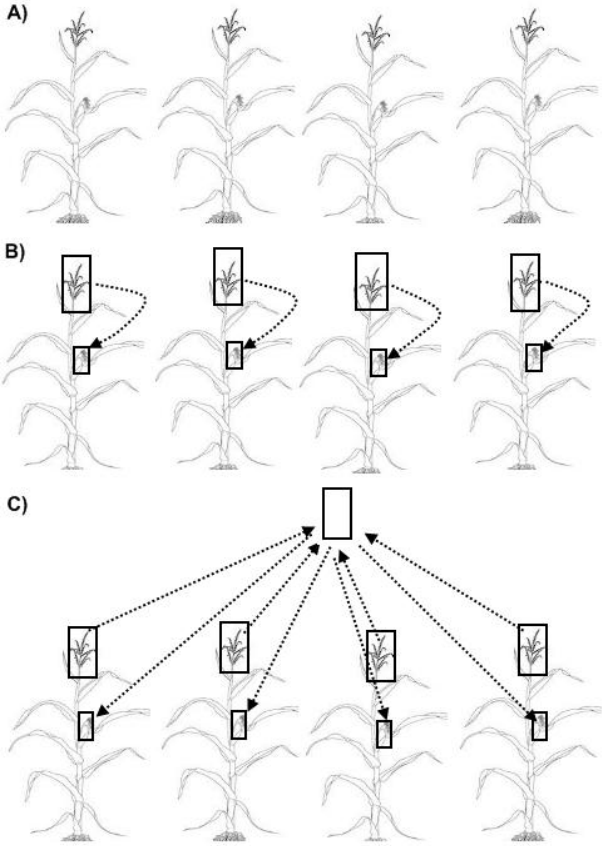

FIGURE 1. Graphical presentation of compared pollination methods: open pollination (A), self-pollination (B), and bulk pollination (C). Rectangular shapes indicate the pollination bags and shoot bags.

contamination. Emerging silk was truncated to get a satisfactory seed set. A tassel bag was carefully placed over the designated plants' tassels to collect pollen for the next day. Next morning (8:00-10:00 AM) the tassel bag was tapped to release pollen from the tassels. Then the tassel bag was brought onto the shoot exposing silk, and tapped gently so that the pollen was introduced to the fresh silk. Lastly, the flaps of the bag were tightly stapled against the stalk.

At least four plants were pollinated this way in each subplot. For self-pollination, the pollen collected from a tassel was used to pollinate the ear on the same plant. For bulk pollination, the pollen collected and bulked from at least four plants was distributed to the plants that provided this pollen bulk (Fig. 1). Unlike selfing, bulk pollination was made by pouring the pollen collected in the tassel bags onto the silks. The open pollinated plants were not bagged. To evaluate pollen effect of each genotype, all possible crossing combinations among the parental genotypes were made by artificial pollination. Harvest was done upon physiological maturity (black layer formation). All of the hand pollinated ears were harvested for the artificial pollination treatments, while four ears were sampled from the open pollinated plots ($N = 288$).

Observed traits

Data were collected on four ear traits and four biochemical traits to compare the pollination methods and to evaluate the pollen effect of parents. On the harvested ear samples, ear weight (g), kernel weight per ear (g), and kernel number per ear were measured. Mean kernel weight was determined by dividing kernel weight to kernel number for each sample. After collecting the data on ear traits, the kernels were grounded using a laboratory mill (Fritsch pulverisette 14) with 0.5 mm sieving. Grounded samples were analyzed to determine protein (%), oil (%), carbohydrate (%) and carotenoid contents (μg g^{-1}). Protein, oil and carbohydrate ratios were estimated with a Near Infrared Reflectance Spectroscopy (Spectrastar 2400D, Unity Scientific, USA). For this purpose, each sample was scanned within 1200-2400 nm interval, using the powder cup of the instrument. Carotenoid concentration was determined according to Rodriguez-Amaya & Kimura (2004). For this assay, two grams of sample weighed into a glassine tube. Then, 5mL of pure water added on the samples and incubated at 4 C° overnight. Samples were subjected to a series of pure acetone (15 mL two times) and acetone:hexane (25 mL one times) solutions. After each application, tubes were shaken (2 min) and liquid phase in tubes was collected. The upper phases of extracts that collected in 3 occasions were gathered in a glass funnel. About 300 mL cold water was added and the upper phase was transferred into a 25 mL flask. Three mL of extract was measured at 450 nm by using a quartz cuvette with 1 cm pathlenght in a pre-conditioned UV-VIS spectrophotometer (PG Instrument, England). Total caroteniod content (TCC) of each sample was determined by the following formula:

$$TCC\ (\mu g\ g^{-1}) = \frac{25\ x\ A1\ x\ 10^4}{2500\ x\ W}$$

where, $A1$ was the absorbance value of the sample at 450 nm and W was the sample weight.

Statistical analyses

Two different statistical methods were used to analyze the data. Firstly; analysis of variance was performed based on the following statistical model, using Proc GLM procedure of SAS (SAS Institute, 1999) to test the effect of the pollination methods on the parent traits.

$$yijk = \mu + \tau i + \beta j + (\tau\beta)ij + \gamma k + (\beta\gamma)jk + \varepsilon ijk$$

where; μ is the population mean, τi is replication effect, βj is genotype effect (main plot), (τβ) ij is whole plot error, γk is pollination treatment effect (sub-plot effect), (βγ) jk is interaction term between whole and sub-plot effect, εijk is sub-plot error term. Pollination treatment was assigned to subplots and genotypes to main plots since we need a more accurate evaluation of the pollination effect. Type III error was used in ANOVA to calculate the significance levels of the sources of variation. LSD test was performed to compare the treatment means.

Second analysis of the data was performed to determine the effect of the male parent on the female parent. This was described as "pollen effect" and computed according to Bulant et al (2000) for each parental genotype. These computations give the deviation of hybrid values from the average of female parents. To compute the pollen effect of parental lines, samples from self-pollination treatment were used for all of the genotypes. Pollen effect of each parental genotype was equal to differences of least squares means in Proc MIXED procedure in SAS. REML based computations were made for each parental group, including one female parent and its related hybrids (e.g., IHO and the hybrids in which IHO was the female parent were collectively referred to as 'IHO and its Related Hybrids'). In each group, individual pollen effects of male parents over the female parents were compared by t test.

RESULTS AND DISCUSSION

Comparison of pollination treatments

Variance analysis revealed significant differences between the pollination treatments for ear and kernel development traits. Except the oil content, other biochemical constituents showed significant differences by the pollination treatment. Genotype × Treatment interaction was found significant for all biochemical features, while the genotype effect was significant for all of the evaluated traits (Tab. 2).

Open pollination produced higher ear weight, kernel number and total kernel weight compared to the artificial pollination methods. Selfing yielded lower values within the artificial methods for ear traits (Fig. 2). This variation may be due to the difference in the degree of pollen availability among the pollination treatments. In open pollination, great amount of pollen grain can reach the silk surface. In artificial pollination, however, the amount of viable pollen is limited. Thus, the amount of pollen available for the silks would be much more with the open and bulk pollination methods than it is with selfing. This is probably the main reason for getting more kernels from the open or bulk pollinated ears. This idea is supported by earlier research which showed restricted pollination resulted in fewer seeds in maize (Borrás et al. 2002; Borrás et al. 2003). Nevertheless, the same researchers argued hand pollination would yield a better seed set compared to open pollination and restricted pollination. This is a contradiction with our results, probably due to the effect of synchronous pollination method used in those studies (Cárcova et al. 2000). The lower values for the mean kernel weight in open pollination could be attributed to the higher number of kernels per ear in open pollination. Borrás and Otegui (2001) reported a decrease in seed weight as the number of seeds increased on an ear. Bulant et al. (2000) argued that increased activity of ADPGPPase enzyme was associated with greater kernel size in the case of cross pollination. Our results from bulk pollination treatment agreed with this argument, whereas the results from open pollination did not. Having more kernels in open pollination may have masked the role of pollen effect in this case. Letchworth & Lambert (1998) reported that there was no significant difference for average mean kernel weight between open (30.7 mg) and self-pollination (30.9 mg) treatments. Similarly, we found no significant differences between these treatments for average of kernel weight (Fig. 2). A comparison within artificial pollination methods showed that kernel weight was slightly lower in selfing (pollen from the same plant) than in bulk pollination (pollen from different plants) (Fig. 2). In terms of kernel and ear development, the genotypes had similar reactions to different pollination treatments (Tab. 3), resulting in a non-significant G × Y interaction effect for ear traits (Tab. 2).

Artificial pollination had also significant effect on kernel biochemical structure. Protein, oil and carotenoid were found to be higher in self-pollination than the other methods. The lowest figures were obtained from open pollination treatment for these traits. The carbohydrate content significantly decreased with artificial pollination in our study. Oil content had no significant variation among the pollination treatments (Fig. 2). Our results for protein and carbohydrate contents were in agreement with Letchworth & Lambert (1998). Sulewska et al. (2014) also reported non-significant differences for oil content between

TABLE 2. Means squares from the ANOVA for the investigated traits.

Source of Variation	df	Ear Weight	Kernel Weight	Kernel Number	Mean Kernel Weight
Replication (R)	2	79.2	434.7	13364.6	0.0001
Genotype (G)	3	20746.3**	15121.1**	171391.4**	0.0294**
Error 1	6	2598.6	1403.8	8841.6	0.0023
Pollination (P)	2	12530.7*	9085.7*	117315.7**	0.0048
PxG	6	3003.2	2423.4	10871.9	0.0037
Error 2	12	1287.2	1035.9	5267.1	0.0019
Source of Variation	df	Protein Content	Oil Content	Carbohydrate Content	Carotenoid Content
Replication (R)	2	2.25	7.24	3.93	0.50
Genotype (G)	3	31.3**	70.1**	34.7**	22.9**
Error 1	6	1.92	2.91	3.51*	1.52
Pollination (P)	2	5.13*	1.77	9.33**	12.1**
PxG	6	4.84*	4.72*	7.61**	52.2*
Error 2	12	1.28	2.78	1.63	1.56

df: Degrees of freedom. *, ** statistically significant at 0.05 and 0.01, respectively.

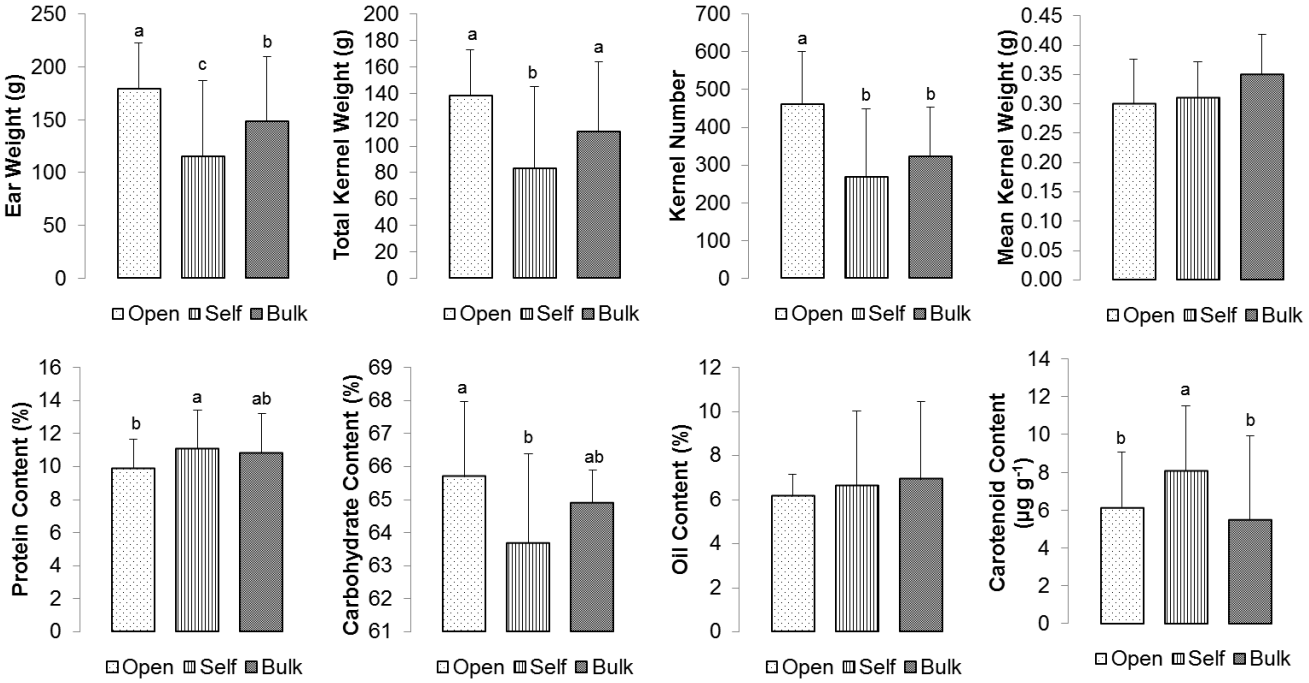

FIGURE 2. Differences among the pollination treatments (Open, Self, Bulk) for the investigated traits. Different letters among pollination treatments indicate the statistically significant differences at $P < 0.05$. Standard deviations are shown above the bars.

TABLE 3. Genotype means from the different pollination treatments for the investigated traits.

Trait	Treatment	IHO	PR	OPV	Q2
Ear Weight (g)	Open	147.2	231.6	155.1	185.7
	Self	103.2	156.4	31.6	170.1
	Bulk	114.1	236.3	111.1	133.3
Kernel Weight (g)	Open	114.9	178.2	112.3	147.5
	Self	77.9	116.2	6.7	132.1
	Bulk	82.4	186.2	78.6	97.1
Kernel Number	Open	477.2	572.3	261.8	536.0
	Self	308.6	361.0	20.7	391.0
	Bulk	316.3	493.5	185.5	298.7
Mean Kernel Weight (g)	Open	0.24	0.31	0.42	0.27
	Self	0.25	0.33	0.32	0.32
	Bulk	0.26	0.38	0.42	0.33
Protein Content (%)	Open	11.6 b	9.98 a	9.70 b	8.13 a
	Self	12.8 ab	9.48 a	13.33 a	8.94 a
	Bulk	13.7 a	11.58 a	10.28 ab	7.56 a
Oil Content (%)	Open	9.7 a	5.56 a	6.64 a	4.60 a
	Self	10.7 a	4.95 a	5.33 a	5.10 a
	Bulk	11.9 a	4.64 a	4.56 a	3.76 b
Carbohydrate Content (%)	Open	62.9 a	66.2 a	66.6 a	67.2 a
	Self	61.9 a	66.3 a	61.6 b	65.1 b
	Bulk	61.7 a	64.3 a	66.8 a	67.0 a
Carotenoid Content (mg g^{-1})	Open	1.82 a	7.07 a	7.12 b	8.63 a
	Self	1.53 a	8.47 a	6.90 b	9.73 a
	Bulk	1.00 a	6.60 a	12.65 a	7.49 a

Note: Different letters in a column indicate significant differences at 0.05 alpha level.

open and self-pollination. The differences in chemical composition by the pollination methods may be a result of inbreeding effect. Inbreeding increases the homozygosity in maize, normally a cross-pollinated species. Combining recessive genes results in the expression of the respective phenotype that would be masked by the dominant alleles under heterozygote condition (Jalal et al. 2006). Our results suggest that selfing increased the frequency of favorable alleles for oil and protein content since self-pollination treatment resulted in elevated levels of these components. The differences in biochemical constituents among the pollination treatments are also related to kernel size. It is expected that higher kernel number per ear results in smaller kernels, thereby lower carbohydrate content per kernel. Negative relation between the carbohydrate and other constituents (Dado 1999) was also apparent in our data (Fig. 2). In contrary to our results, Schaefer & Bernardo (2013) obtained similar values from self and open pollination treatments for biochemical features. They concluded that there was no significant effect of pollination method on biochemical structure in maize kernel, based on their data collected on temperate inbreds. Our study includes more treatments and different specialty genotypes. The difference between the results of these studies indicates that the plant material may be a significant factor, as well as the pollination treatments tested, when investigating the pollen effect in maize. The genotypes used gave different responses to pollination treatments for kernel biochemical components while the effects on ear traits were similar (Tab. 3). As a result, we detected a significant G × T interaction effect on kernel biochemical properties. IHO had higher protein content in bulked samples, while selfed ears of OPV had higher levels. Genotypes had similar values for oil content in different pollination treatments, except the Q2 which had lower oil in bulk pollination compared to other treatments. For carbohydrate content, IHO and PR had similar values for pollination treatments, while, Q2 and OPV had significantly lower values in self-pollination treatments. Carotenoid content significantly increased by the bulk pollination in OPV. Other genotypes had similar values for carotenoid content in samples with different pollination treatments. Based on these data, pollination treatment caused the variation of kernel quality traits and this situation was closely related to genetic specialties of the genotypes used.

Assessment of pollen effect

Results of variance analysis to assess the pollen effect by the genotypes were summarized in Tab. 4. Individual pollen effect varied by the genotypes for evaluated traits and it was found to be most significant for open pollinated landrace. In different studies, it was speculated that the pollen effect could be varied by year, genotype and climate conditions. There were also some studies reporting this effect was similar in different conditions for certain genotypes (Bulant et al. 2000).

Fig. 3 shows the differences between hybrids and their female parents for each variable. It was found that four crosses had significant differences for ear weight and total kernel weight, while three crosses did so for total kernel number and mean kernel weight (Fig. 3). OPV had significant increases for ear and kernel development when pollinated with other genotypes. IHO × OPV cross also had a significant pollen effect for ear weight and kernel weight per ear. Selfing in OPV resulted in a low ear weight, and cross pollination caused significant increases. Therefore, we concluded that pollen effect of the other genotypes on OPV was significant. In fact, inbreeding depression was reported to be higher in open pollinated landraces compared with the inbreds or hybrids (Öz & Tuğay 2003). Total kernel weight

TABLE 4. F values for the investigated traits in the variance analysis by mixed model for the parental genotypes and their respective hybrids.

Trait	Source of Variation	Df	IHO and Related F1s	PR and Related F1s	OPV and Related F1s	Q2 and Related F1s
Ear Weight	Replication	2	2.31*	1.26	0.70	3.41
	Genotype	3	5.03*	0.89	17.6**	0.38
Kernel Weight	Replication	2	2.13	1.15	2.39	4.52
	Genotype	3	3.56	0.69	24.5**	0.39
Kernel Number	Replication	2	1.66	1.94	1.67	2.74
	Genotype	3	0.78	1.42	28.4**	0.30
Mean Kernel Weight	Replication	2	0.34	0.86	2.49	0.15
	Genotype	3	2.06	1.86	13.8**	0.51
Protein Content	Replication	2	0.00	1.89	0.43	0.92
	Genotype	3	4.51*	2.04	2.75	1.93
Oil Content	Replication	2	2.01	0.44	1.90	3.85
	Genotype	3	1.79	1.32	0.48	2.46
Carbohydrate Content	Replication	2	0.35	1.81	2.83	1.97
	Genotype	3	3.50	0.52	6.06*	3.53
Carotenoid Content	Replication	2	0.38	0.93	2.67	3.30
	Genotype	3	16.6**	11.2**	76.2**	47.3**

*,** statistically significant at 0.05 and 0.01, respectively.

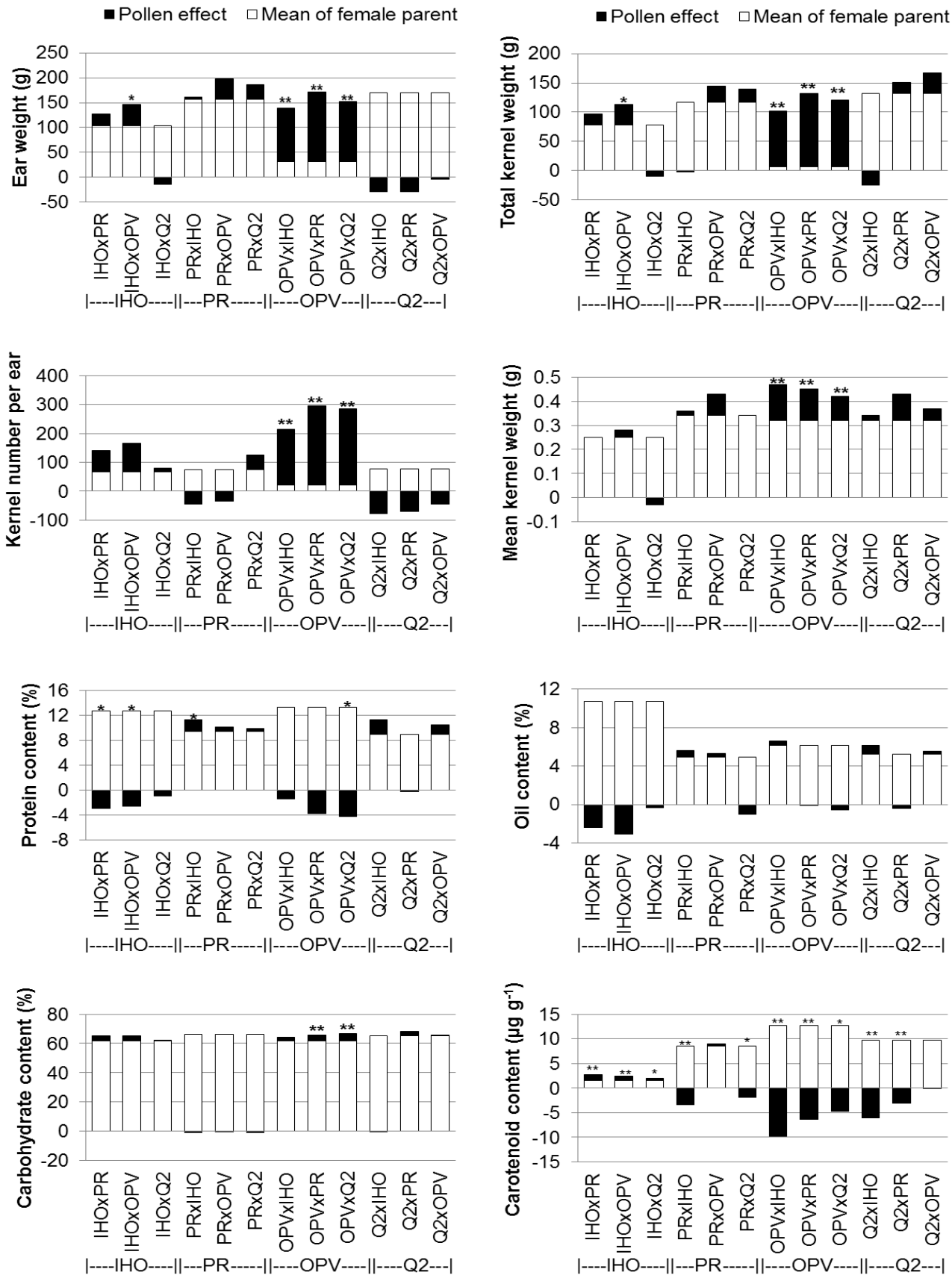

FIGURE 3. Pollen effect of the genotypes on female parent.

*,** statistically significant at 0.05 and 0.01, respectively. Black portions of the bars represent the pollen effect (the difference between the pollen parent and the hybrid), while white portions indicate the mean value belong to the female parent.

in ear is a product of mean kernel weight and kernel number per ear. Increase in total kernel weight directly affects the ear weight. The increase in ear weight in OPV when pollinated with the other parents is a product of the increase in mean kernel weight and total kernel number per ear. It was reported that hybrids produced more kernels when pollinated by a different hybrid (Weingartner et al. 2002a; Weingartner et al. 2002b; Bozonovic et al. 2010). Pollen effect could not be determined when hybrids with similar kernel weight are crossed (Kannenberg & Hunter 1972). In our study, OPV hybrids had higher mean kernel weight than their parents, although OPV parent had similar values for mean kernel weight with those of pollen sources. Seka & Cross (1995) found that small kernel hybrids could bear bigger kernels when crossed with a large kernel hybrid. IHO has relatively small kernels within the set of genotypes used here. Interestingly, we did not observe any increase in kernel size when IHO was pollinated with the parents with larger kernel (Fig. 3). These results did not agree with earlier reports, where, unlike our study, mostly hybrids were tested. These results suggest that inbreds and landraces could display different responses to pollen source than the hybrids would. Moreover, carrying a special type endosperm (such as IHO) could be a factor on how the pollen effect would appear in a maize genotype.

Four of our crosses had significant differences for protein and carbohydrate content, while six crosses showed differences for carotenoid content (Fig. 3). Protein and carbohydrate contents were negatively affected by pollen source in IHO and OPV parents. These parents had higher protein and carbohydrate content than the others. In fact, pollination of these genotypes with lower parents resulted in decreases in protein and carbohydrate values. The low × high or high × low parent combinations gave similar results in earlier studies for protein and oil content (Curtis et al. 1956; Letchworth & Lambert 1998). The oil contents of crosses in our study were close to the pollen parent. However, the differences between the hybrids and their parents were not significant. The non-significant differences in our study may due to small number of genotypes ($N = 4$) to compare. All comparisons between crosses and parents for carotenoid concentration showed that pollen effect had a negative impact on this variable (Fig. 3). Vancetovic et al. (2014) found that antioxidant properties, including yellow pigments (i.e., carotenoids), were significantly affected the pollen parent. They concluded that when the hybrids with low pigment were pollinated by the high pigment hybrids, the pigment content increased. Our results did not agree their findings, probably because we used inbreds and OPV as female parent, while they used hybrids. Moreover, we observed that when the parent with low carotenoid content (IHO) was pollinated by the high carotenoid genotype, carotenoid content of the low parent had a very small increase. This indicates that maternal effects are more important than pollen effect for carotenoid content (Fig. 3).

In conclusion, results of this study showed that there were important differences among the pollination methods in terms of their effect on some kernel traits. Pollen effect played a significant role for ear and kernel development in the investigated genotypes. This case was very distinct in open pollinated landrace. The change by pollen effect in carotenoid content was different than those in the other biochemical constituents. More accurate methods of chemical analysis (e.g., Reference Labs, HPLC methods) may provide a better picture for the biochemical constituents discussed here. Multi-year and multi-location trials would yield more informative results on pollen effect for these variables.

REFERENCES

Abdin Z, İdris M, Banta MGT (1979) A pollinating technique in maize. Pertenika 2:62-65.

Annonymous (2014) Controlled Pollination in Maize, Schnable Lab Protocols. [online] URL: http://schnablelab.plantgenomics.iastate.edu/resources/pollination/ (accessed December 2014).

Balestre M, Desouza JC, Dos Santos JB, Luders RR, Lima IJ (2007) Effect of self-pollination monitored by microsatellite markers on maize kernel weight. Crop Breeding and Applied Biotechnology 7:340-344.

Borrás L, Otegui ME (2001) Maize kernel weight response to post flowering source–sink ratio. Crop Science 41:1816–1822.

Borrás L, Cura JA, Otegui, ME (2002) Maize kernel composition and post flowering source-sink ratio. Crop Science 42:781-790.

Borrás L, Westgate ME, Otegui ME (2003) Control of kernel weight and kernel water relations by post flowering source-sink ratio. Annals of Botany 91:857-867.

Bozonovic S, Vancetovic J, Babic M, Filipovic M, Delic N (2010) The plus-hybrid effect on the grain yield of two ZP maize hybrids. Genetika 42:475-484.

Bulant C, Gallais A (1998) Xenia effects in maize with normal endosperm: I importance and stability. Crop Science 38:1517-1525.

Bulant C, Gallais A, Matthys-Rochon E, Priul JL (2000) Xenia in maize with normal endorsperm: II kernel growth and enzyme activities grain filling. Crop Science 40:182-189.

Cárcova J, Uribelarrea M, Borrás L, Otegui ME, Westgate ME (2000) Synchronous pollination within and between ears improves kernel set in maize. Crop Science 40:1056-1061.

Curtis JJ, Brunson AM, Hubbard JE, Earle FR (1956) Effect of the pollen parent on oil content of the corn kernel. Agronomy Journal 48:551-555.

Dado RG (1999) Nutritional benefits of specialty corn grain hybrids in dairy diets. Journal of Animal Science 77:197-207.

Dong H (2007) Effect of high oil corn pollinator on kernel quality of common corn and their physiological and biochemical basis. Shandong Agricultural University, Master Thesis.

Gilbert N (1960) Xenia in quantitative characters. Journal of Genetics 57: 327-328.

Jalal A, Rahman H, Khan MS, Maqbool K, Khan S (2006) Inbreeding depression for reproductive and yield related traits in S1 lines of maize (*Zea mays* L.). Songklanakarin Journal of Science Technology 28:1170-1173.

Kannenberg LW, Hunter RB (1972) Yielding ability and competitive influence in hybrid mixtures of maize. Crop Science 12:274-277.

Krieger KM, Pollak LM, Brumm TJ, White PJ (1998) Effects of pollination method and growing location on starch thermal properties of corn hybrids. Cereal Chemistry 75:656-659.

Letchworth MB, Lambert R.J (1998) Pollen parent effects on oil, protein, and starch concentration in maize kernels. Crop Science 38:363-367.

Lindstorm EW (1939) Analysis of modern maize breeding principles and methods, International Congress of Genetics, Proceedings 7:191-197.

Öz A, Tuğay ME (2003) Variation on some agronomic characters in selfing generations in corn (*Zea mays indendata* Sturt). Journal of Agricultural Faculty of Gaziosmanpasa University 20:123-132.

Pixley KV, Bjarnason MS (1994) Pollen-parent effects on protein quality and endosperm modification of quality protein maize. Crop Science 34:404-409.

Rodriguez-Amaya DB, Kimura M (2004) HarvestPlus handbook for carotenoid analysis. HarvestPlus Technical Monograph Series 2.IFPRI, Washington, D.C., and CIAT, Cali.

SAS Institute (1999) SAS V8 User Manual, Cary, NC.

Schaefer CM, Bernardo R (2013) Pollen control and spatial and temporal adjustment in evaluation of kernel composition of maize inbreds. Maydica 58:135-140.

Seka D, Cross HZ (1995) Xenia and maternal effects on maize kernel development. Crop Science 35:80-85.

Sulewska H, Adamczyk J., Gygert H, Rogacki J, Szymanska G, Smiatacz K, Panasiewicz K, Tomaszyk K (2014) A comparison of controlled self-pollination and open pollination results based on maize grain quality. Spanish Journal of Agricultural Research 12:492-500.

Şchiop T, Haş I, Haş V, Coste I, Racz C (2011) Gene actions, cytoplasmic actions and cytoplasmicnuclear interactions involved in the determination of fiber content in a series of isonuclear maize lines. Research Journal of Agricultural Science, 43:282-288.

Taba S, Twumasi-Arfiyie S (2008) Regeneration Guidelines: Maize. Crop specific regeneration guidelines (CD-ROM), In: Dulloo ME, Thormann I, Jorge MA, Hanson J (eds) CGIAR System-wide Genetic Resource Programme, Rome, Italy.

Tanaka W, Matese AI, Maddoni GA (2009) Pollen source effects on growth of kernel structures and embryo chemical compounds in maize. Annals of Botany 104:325-334.

Thomison PR, Geyer AB, Lotz LD, Siegrist HJ, Dobbels TL (2002) TopCross High-Oil Corn Production: Agronomic Performance. Agronomy Journal 94:290-299.

Tsai CL, Tsai CY (1990) Endosperm modified by cross-pollinating maize to induce changes in dry-matter and nitrogen accumulation. Crop Science 30: 804-808.

Vancetovic J, Zilic S, Bozinovic S, Micic DI (2014) Simulating of Top-Cross system for enhancement of antioxidants in maize grain. Spanish Journal of Agricultural Research 12:467-476.

Weingartner U, Camp K-H, Stamp P (2004) Impact of male sterility and xenia on grain quality traits of maize. European Journal of Agronomy 21:239-247.

Weingartner U, Kaeser O, Long M, Stamp P (2002a) Combining male sterility and xenia increases grain yield of maize hybrids. Crop Science 42:1848-1856.

Weingartner U, Prest TJ, Camp K-H, Stamp P (2002b) The plus-hybrid system: A method to increase grain yield by combined cytoplasmic male sterility and xenia. Maydica 47:127-134.