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RESEARCH ARTICLE

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Effects of open- and self-pollination treatments on genetic estimations in maize diallel experiment

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Abstract

This study investigated the effects of open- and self-pollination treatments on genetic estimations and kernel biochemical content in a maize diallel experiment. A 7×7 complete reciprocal diallel set (7 parents and 42 hybrids) was used as plant material. Measured traits were: kernel weight per plant, protein content, oil content and carbohydrate content. General combining ability (GCA), specific combining ability (SCA), maternal effects (MAT), non-maternal effects (NMAT) and heterosis values were compared in open- and self-pollination treatments for measured traits. Results showed that the pollination treatments had a significant effect on all investigated traits. Parental lines and hybrid combinations gave different responses. Parents had relatively higher protein and oil content in self-pollination but hybrids had lower values in self-pollination compared with open-pollination. A considerable number of genotypes showed significant differences for genetic estimations (GCA, SCA, MAT, NMAT) and heterosis between open- and self-pollination treatments. Overall, findings suggest that evaluation of kernel quality traits should be made on selfed ear samples; however, evaluation for yield should be carried out on open-pollinated samples.

Additional key words: protein; oil; carbohydrate content; pollen effect; *Zea mays*

Abbreviations used: GCA (General Combining Ability); MAT (Maternal Effect); MPH (Midparent Heterosis); NMAT (Non-maternal Effect); SCA (Specific Combining Ability).

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Introduction

Diallel analysis is used for the evaluation of parents and hybrids in maize (*Zea mays* L.) breeding research in order to compare their combining abilities (Bertan *et al.,* 2007). The three most commonly-used methods in diallel analysis are those of Jinks-Hayman, Gardner-Eberhart and Griffing (Murray *et al.*, 2003). Although all these methods have similar goals, they use different computation procedures. Griffing (1956a) formulated four different methods for mating designs. These methods are still widely-used in grain quality-oriented breeding studies of maize.

Data for diallel analysis come from plants grown in adjacent small plots, where cross pollination can easily distort the evaluation of quality traits unless controlled pollination is practiced. Cross pollination can occur between genotypes that are situated nearby and have similar flowering characteristics (Thomison, 2013). As a result, changes may occur in kernel formation and structure due to the xenia effect (Letchworth & Lambert, 1998). To prevent xenia effect occurring as a result of pollen contamination, the genotypes planted can be either separated by distance (Setimela *et al.,* 2004) or else some form of controlled pollination may be applied (Abdin *et al.,* 1979). Controlled pollination (hand pollination) is preferable in experiments where numerous genetic materials are tested together, since distance control is not applicable in such experiments. Different practices of controlled pollination are prevalent in maize research, including selfing and bulking (multiple pollen source) (Kahrıman *et al.,* 2015),

and synchronous pollination (Cárcova *et al.*, 2000). The most commonly-used method is selfing, due to its ease of use.

Contradictory statements on the effect of pollination treatments on kernel biochemical constitution in maize have been reported in the literature. Letchworth & Lambert (1998) found significant differences between open- and self-pollination treatments for oil content. Krieger *et al.* (1998) speculated that starch thermal properties were altered by the pollination treatment and suggested the use of self-pollination when breeding for these traits. Hossain *et al.* (2008) reported that endosperm modification (opaqueness) was affected by the pollination method and self-pollination should be used in related breeding efforts. Conversely, other studies showed that open- and self-pollination treatments had no significant effect on the kernel biochemical components of temperate inbreds (Schaefer & Bernardo, 2013) or on the oil content of standard hybrids (Sulewska *et al.,* 2014). Although the effect of different pollination treatments on several biochemical traits in maize has been investigated, the effects on genetic calculations in diallel mating are still unclear. Almost all studies comparing genetic estimations in diallel analyses are based on contrasting statistical models and methods (Yao *et al.,* 2013; Fan *et al.,* 2014). However, a significant effect on the kernel structure resulting from the selected pollination treatment has been suggested as possible by Letchworth & Lambert (1998) and Krieger *et al.* (1998). Thus, the effect of pollination type on genetic calculations in diallel analysis requires more detailed evaluation.

Choice of pollination treatment in diallel experiments varies depending on whether the researcher takes the pollen effect into account or not. Oliveira *et al.* (2006) and Okporie *et al.* (2014) used controlled pollination to restrict pollen contamination in diallel experiments conducted for only kernel quality traits. In other studies, no information is provided about the pollination treatment or consideration was not given to pollen contamination (Balcı & Turgut, 2006; Abou-Deif *et al.,* 2012; Mahesh *et al.,* 2013; Werle *et al.,* 2014). Considering the conflicting results obtained from previous studies, and the presence of a variety of choices about pollination method, there is clearly a need for detailed investigation into the effect of different pollination methods in diallel experiments.

From this standpoint, the current study was intended i) to examine changes in kernel development and structure caused by two of the most widely-used pollination methods in breeding programs (open- and self-pollination); and ii) to investigate the effect of open- and self-pollination on genetic estimations in a maize diallel experiment.

Material and methods

Plant material and field trials

Seven parental lines were used in this study, including high oil, high protein, quality protein maize and normal inbreds (Table 1). These inbreds were crossed and a 7×7 complete reciprocal diallel set was generated in 2012. The evaluation trial of the diallel set (7 parents and 42 hybrids) was carried out in 2013 at the Dardanos Agricultural Research Station of Çanakkale Onsekiz Mart University, Turkey. The experiment was conducted as a randomized block design with three replicates. Planting was made with a seed driller in May 2013. Plant density was about 71400 plants/ha. Each genotype was planted in 2-row plots (total 147 plots). One row was subjected to open-pollination and the other to self-pollination treatment. Plots were randomly distributed in the field. Self-pollination was practiced as indicated in Anonymous (2015) and silk tips were cut for even silk extrusion and a better seed set. In open-pollination, the plants were naturally pollinated without any treatment. Fertilization was carried out based on soil analysis and 170 kg/ha pure nitrogen (ENTEC Perfect, Germany) was applied. The plots were irrigated as necessary by drip irrigation. Ears were hand-harvested after physiological maturity and at least three ears were sampled per plot for each pollination treatment.

Observed traits

Two flowering events (days to silking and days to pollen shedding) were observed to detect possible pol-

Table 1. Parental genotypes used in this study

Parent[1]	General features	Source of material[2]
A680	Normal inbred line, dent type	COMU
B73	Normal inbred line, dent type	COMU
HYA	Inbred line with high oil and protein, dent-flint type	NCRPIS
IHO	Inbred line with high oil, dent-flint type	NCRPIS
IHP	Inbred line with protein, dent-flint type	NCRPIS
Mo17	Inbred line, dent type	COMU
Q2	Qpaque-2 inbred line, dent type	NCRPIS

[1] IHO: Illinois High Oil. IHP: Illinois High Protein. [2] COMU: Çanakkale Onsekiz Mart University, Çanakkale, Turkey. NCRPIS: North Central Regional Plant Introduction Station, Ames, IA, USA.

len contamination from different genotypes. For this purpose, field checks were carried out every day during the flowering stage. To determine the days to silking (DS) and days to pollen shedding (DP) values of the genotypes, we recorded the days from planting to 50% of the plants as they reached their respective stages.

Data were collected on kernel weight/plant (g), protein ratio (%), oil ratio (%) and carbohydrate ratio (%). Kernel weight/plant was determined by shelling the ear and weighing the kernels. The seed samples were ground with 0.5 mm sieves in a laboratory mill (Fritsch pulverisette 14, Germany). Kernel biochemical constituents were measured by NIR spectroscopy (Spectrastar 2400D, Unity Scientific, USA). The powder sample cup of the NIR instrument was used to load the samples for protein, oil, and carbohydrate analysis.

Statistical analysis

Data were analyzed in SAS V8 software (SAS Inst., 1999) using DIALLEL-SAS05 macro (Zhang *et al.,* 2005). Griffing's method 3, model 1 was applied for the diallel analyses (Griffing, 1956a,b), since it offers better estimation with less biased genetic calculations compared to other Griffing methods (Yao *et al.*, 2013). Statistical analyses were based on the following model:

$$Y_{ijkl} = \mu + \alpha_l + b_{kl} + v_{ij} + (\alpha v)_{ijl} + e_{ijkl}$$
$$v_{ij} = g_i + g_j + s_{ij} + r_{ij,} (av)_{ijl} =$$
$$= (ag)_{il} + (ag)_{jl} + (as)_{ijl} + (ar)_{ijl}$$
$$r_{ij} = m_i + m_j + n_{ij}$$
$$(ar)_{ijl} = (am)_{il} + (am)_{jl} + (an)_{ijl}$$

where, Y_{ijkl} = observed value from each experimental unit; μ = population mean; α_l = effect of pollination treatment; b_{kl} = block or replication effect within pollination treatment; v_{ij} = F1 hybrid effect; $(\alpha v)_{ijl}$ = interaction effect between ij^{th} F1 hybrid and pollination treatment; e_{ijkl} = random residual effect; g_i = general combining ability (GCA) for the i^{th} parent; g_j = GCA effect of j_{th} parent; s_{ij} = specific combining ability (SCA) for the ij^{th} F1 hybrid; r_{ij} = reciprocal effect (REC) for ij^{th} or jith F1 hybrid; $(ag)_{il}$ = interaction between GCA effect for i^{th} parent and pollination treatments; $(ag)_{jl}$ = interaction between GCA effect for jth parent and pollination treatments; $(as)_{ijl}$ = interaction between SCA effect for ij^{th} F1 hybrid and pollination treatments; $(ar)_{ijl}$ = interaction between reciprocal effect for ij^{th} or ji^{th} F1 hybrid and pollination treatments; m_i = maternal effect (MAT) of parental line i; m_j = maternal effect of parental line j; n_{ij} = non-maternal effect (NMAT) of ij^{th} or ji^{th} F1 hybrid; $(am)_{il}$ = interaction between pollination treatments and maternal effect of parental line i; $(am)_{jl}$ = interaction between pollination treatments and maternal effect of parental inbred j; and $(an)_{ijl}$ = interaction between pollination treatments and non-maternal effect of ij^{th} or ji^{th} F1 hybrid. The LSD (least significant difference) test was applied to compare the means in different treatments.

Midparent heterosis (MPH) was computed by dividing the difference between parental mean and hybrid value by parental mean (Falconer & Mackay, 1996). These calculations were made for each pollination treatment and the variation in MPH values from different pollination treatments was compared.

To evaluate the relationship between genotypic means, combining ability values (r_{GCA} and r_{SCA}) and other genetic components (r_{MAT} and r_{NMAT}) of the different pollination treatments were investigated using Proc CORR (SAS Inst., 1999). Spearman rank correlation was used for correlation analysis.

Results

Relation between means of open- and self-pollination treatments

The main effects (genotype, pollination treatment) and interaction components (G × T) were significant in the preliminary variance analysis (Table 2). Significant G × T interaction indicated that the responses of genotypes throughout the pollination treatments were different.

Genotype means obtained from different pollination treatments are shown in Fig. 1, while overall means and ranges are shown in Table 3. Looking at the means of pollination treatments (Table 3), kernel weight/plant and carbohydrate content were higher in open-pollination, while protein and oil content were lower. No significant difference was found between the treatment means in parents for any of the observed traits. Hybrids demonstrated significant differences between treatments except for oil content (Table 3). However, as indicated by the rank correlation values, genetic constitution (inbred *vs* hybrid) had an effect on these differences (Fig. 1). Nevertheless, selfing treatment yielded higher values than open-pollination in one parent and eight hybrids (Fig. 1). The rank correlation values for both hybrids (r=0.06) and parents (r=0.18) were quite low for kernel weight/plant. The kernel protein content was found to be higher in self-pollination (6.41-21.7%) than in the open-pollination treatment (6.25-20.5%).

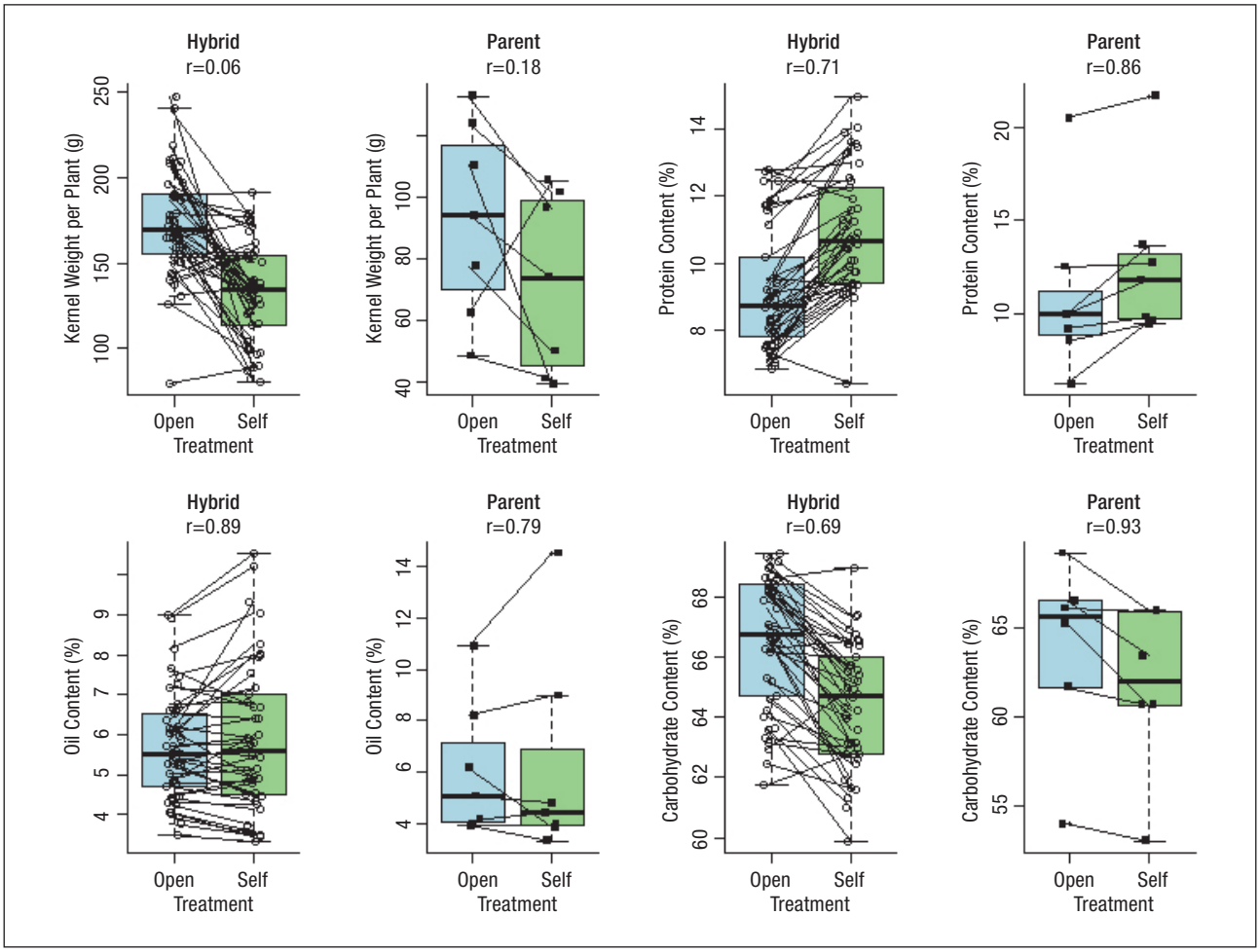

Figure 1. Relations of means from open (blue) *vs* self-pollinated (green) treatments by hybrids (circles) and parents (rectangles). Error bars indicate confidence interval at ±95%. Spearman rank correlation (*r*) values between pairs are shown above each plot.

Table 2. Means and ranges based on replicated data by pollination treatment for investigated traits

Trait	Type	Mean		Range	
		Open	Self	Open	Self
Kernel weight/plant (g)	Parent	92.9 ns	72.6 ns	48.4-133.1	39.4-105.5
	Hybrid	173.8 a	132.4 b	79.1-248.0	80.3-191.0
Protein content (%)	Parent	11.0 ns	12.7 ns	6.25-20.5	9.44-21.7
	Hybrid	9.17 b	11.0 a	6.86-12.8	6.41-14.9
Oil content (%)	Parent	6.05 ns	6.26 ns	3.92-10.9	3.33-14.5
	Hybrid	5.69 ns	5.91 ns	3.49-9.00	3.33-10.5
Carbohydrate content (%)	Parent	64.2 ns	62.3 ns	54.0-69.2	53.0-66.3
	Hybrid	66.5 a	64.4 b	61.8-69.4	59.9-69.0

Different letters show significant differences (LSD, α=0.05) within pollination treatments. ns: not significant.

It was seen that selfing caused increases in the protein ratio in most genotypes (Fig. 1). This was more evident in parental lines. Parental lines also scored higher correlation values (r=0.86) compared to hybrids (r=0.71) in terms of protein content from different pollination treatments (Fig. 1). This indicates that parental lines had similar values for protein content across the pollination treatments. Self-pollinated samples had higher oil content (3.33-14.5%) than the open-pollinated samples (3.49-10.9%). As seen in Fig. 1, oil ratios

recorded in selfed samples were higher than those in open-pollinated ones for most of the genotypes. For oil content, the hybrids showed more constant changes (r=0.89) than parents (r=0.79) across the different pollination treatments (Fig. 1). Carbohydrate content among genotypes ranged from 54.0 to 69.4% in the open-pollination treatment and from 53.0 to 69.0% in self-pollination. In parents, carbohydrate content was lower in selfed samples (53.0-66.3%) than in open-pollinated ones (54.0-69.2%). Only five hybrids had higher carbohydrate content in selfed samples than in open-pollinated ones (Fig. 1). The rank correlation for carbohydrate content between pollination treatments was 0.93 in parents and 0.69 in hybrids.

Comparing genetic estimations in pollination treatments

We conducted a detailed analysis of combining abilities and other genetic effects because the preliminary variance analysis showed significant variation in genotypes and G × T interaction (Table 2). This analysis revealed that variances in combining abilities, reciprocal effects and other genetic components had a significant effect on all the measured traits. However, MAT × Treatment effect for kernel weight/plant was not significant (Table 4).

Figure 2 shows GCA and SCA values based on the data obtained from different pollination treatments. The genotypes in the upper left and lower right parts of the figure are those that received different signs (negative or positive), while genotypes in other parts had the same sign for genetic estimation. GCA and SCA values for kernel weight/plant were significantly affected by pollination treatment. Spearman rank correlation coefficients between these values (r_{GCA}=-0.14 and r_{SCA}=0.04) also indicated that there were significant deviations in genetic estimations (Fig. 2). The GCA values of parents were between -17.3 and 11.5 in open-pollination. The corresponding values were -13.8 and 9.5 in self-pollination. The range of SCA values for kernel weight/plant was -49.9 to 43.7, and -46.1 to 34.0 in open- and self-pollination, respec-

Table 3. Mean squares from preliminary variance analysis

Source of variation	df	Kernel weight per plant	Protein content	Oil content	Carbohydrate content
Treatment (T)	1	108333.3**	234.3**	3.49**	315.1**
Rep (Treatment)	4	1279.9	0.63	0.93	2.06
Genotype (G)	48	6901.9**	31.2**	20.9**	40.2**
G × T	48	2341.8**	2.41**	1.70**	3.96**
Error	192	744.2105	0.93	0.42	1.75
CV (%)		19.1	9.34	11.1	2.03

df: degrees of freedom, CV: coefficient of variation. *,**: statistically significant at 0.05 and 0.01 levels of probability, respectively.

Table 4. Mean squares from diallel analysis based on Griffing's Method 3, Model 1

Source of variation	df	Kernel weight per plant	Protein content	Oil content	Carbohydrate content
GCA	6	3492.7**	168.7**	332.0**	198.1**
SCA	14	3572.6**	6.28**	8.282**	8.00**
REC	21	2406.0**	6.44**	8.458**	12.5**
MAT	6	2333.3**	7.50**	3.665**	14.5**
NMAT	15	2435.1**	6.02**	10.4**	11.8**
GCA × Treatment	6	4058.0**	2.51*	10.3**	4.99**
SCA × Treatment	14	2451.7**	2.30**	2.40**	3.91**
REC × Treatment	21	1873.8**	2.55**	2.98**	4.05**
MAT × Treatment	6	1372.5	3.93**	3.48**	5.54**
NMAT × Treatment	15	2074.3**	1.99**	2.77**	3.45*

df: degrees of freedom, GCA: general combining ability, SCA: specific combining ability, REC: reciprocal effects, MAT: maternal effects, NMAT: non-maternal effects. *,**: statistically significant at 0.05 and 0.01 levels of probability, respectively.

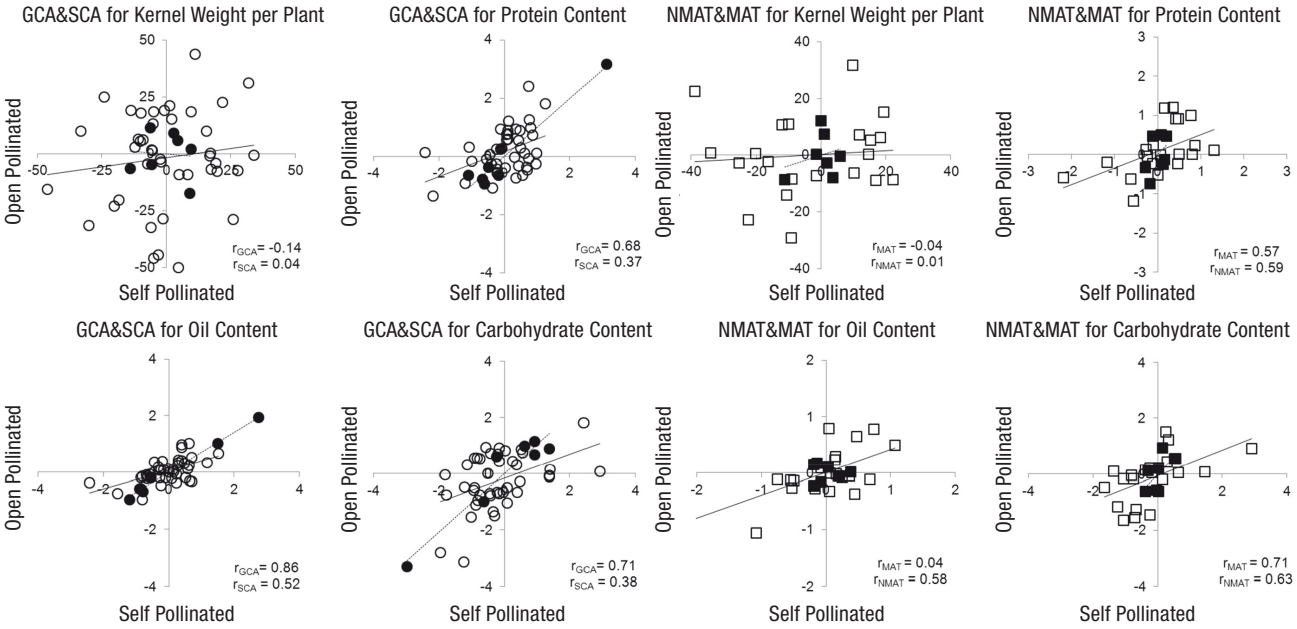

Figure 2. Diagrams showing genetic parameter estimations based on open- and self-pollination treatments in 7×7 diallel set. Null circles = SCA values for F1s and their reciprocals; filled circles = GCA values; filled squares = MAT effects; null squares = NMAT effects. Genotype names not included for sake of clarity. rGCA, rSCA, rMAT and rNMAT indicate Spearman rank correlation coefficients for each genetic estimation between the values calculated from open- and self-pollination treatments.

tively. The range of GCA values from different pollination treatments was similar for the protein ratio (-0.95 to 3.15 in open-pollination, -1.10 to 3.11 in self-pollination), oil ratio (-0.96 to 1.94 in open-pollination, -1.20 to 2.74 in self-pollination), and carbohydrate ratio (-3.30 to 1.15 in open-pollination, -2.97 to 1.37 in self-pollination) (Fig. 2). Low correlation coefficients between SCA values (r_{SCA} for protein: 0.37, for oil: 0.52, for carbohydrate: 0.38) indicated that there were significant differences between the genetic calculations of genotypes as affected by pollination treatment. These results are verified by the fact that the symbols in the upper left and lower right sides of Fig. 2 mostly belong to hybrids.

Differences between maternal (MAT) and non-maternal (NMAT) effects in the two pollination treatments are summarized in Fig. 2. Both MAT and NMAT estimations showed significant differences for kernel weight/plant per pollination treatment (Fig. 2). Maternal effects for protein content in open-pollination ranged between -0.74 and 0.50, while the range for non-maternal effects was -1.19 to 1.20. When the ears were selfed, MAT was between -0.19 and 0.38, and NMAT was between -2.09 and 1.07. The ranges of MAT and NMAT effects also varied by pollination treatment regarding oil and carbohydrate content (Fig. 2). MAT effects ranged from -0.19 to 0.38 for oil content in self-pollination, whereas the corresponding values were -0.23 and 0.17 in open-pollination.

Similarly, NMAT effects were also affected by pollination treatment. We looked at the correlations between MAT and NMAT effects obtained from selfed and open-pollinated samples to evaluate the effect of pollination treatment on estimation of MAT and NMAT effects. The correlation coefficient between the values obtained from different pollination treatments was quite low for kernel weight/plant (Fig. 2). They were generally moderate for protein (r_{MAT}=0.57 and r_{NMAT}=0.59) and carbohydrate content (r_{MAT}=0.71 and r_{NMAT}=0.63). For oil, the type of pollination treatment had a great impact on the estimation of maternal effects (r_{MAT}=0.04), but the r_{NMAT} value of 0.58 suggests that non-maternal effects were not greatly affected by pollination treatment (Fig. 2).

Figure 3 shows the midparent heterosis values. Hybrids generally had positive heterosis for kernel weight/plant and carbohydrate content, whereas heterosis values for protein and oil content were mostly negative. This was true for both pollination treatments. In addition, we detected remarkable differences in heterosis values in terms of ranges as well as direction (*i.e.* positive or negative) from a certain genotype when comparing the two pollination treatments. For example, heterosis values were between -15.2% and 200.8% for kernel yield/plant when the ears were open-pollinated; this range was -14.1% to 319.4% in the selfing treatment. Only one hybrid (IHO×Q2) out of 42 had negative heterosis for kernel weight/plant in both treatments.

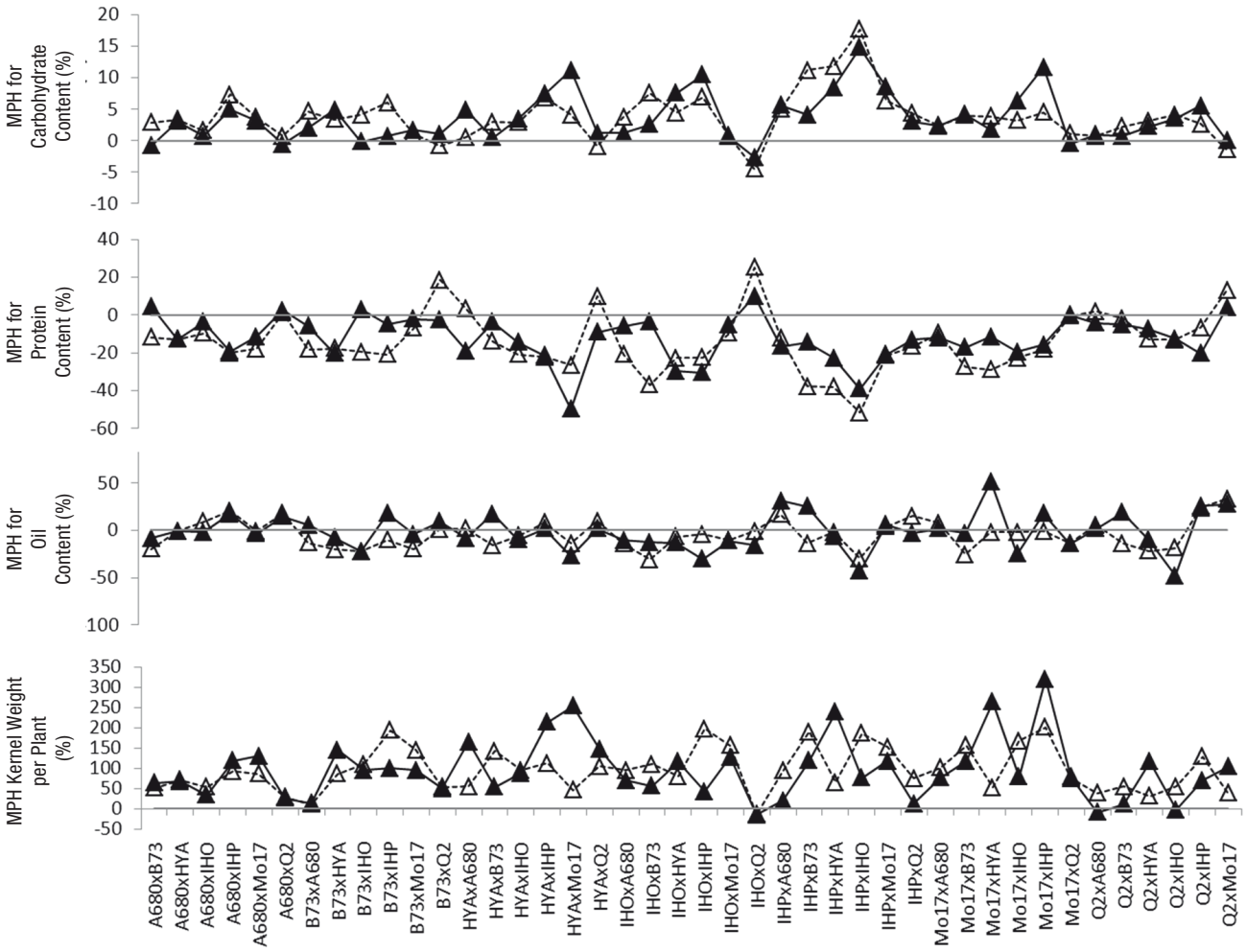

Figure 3. Midparent heterosis (MPH) values for variables from open (Δ) and self (▲) pollination treatments.

Additionally, two hybrids (Q2×A680, Q2×IHO) had opposite heterosis values from different pollination treatments.

The heterosis values calculated for protein ratio ranged between -51.6 and 25.5% in open-pollination and between -49.7% and 9.9% in self-pollination. For this trait, 3 hybrid combinations had positive heterosis in both pollination treatments whereas 9 hybrids had opposite signs. The heterosis values from open-pollinated plots were between -31.0% and 33.4% for oil content. When the ears were selfed, heterosis values had a greater range (-48.0% to 51.6%). Eleven hybrid combinations showed positive heterosis for oil content in both pollination treatments. The treatments yielded heterosis values with opposite signs in 6 hybrids. Heterosis values for carbohydrate in open-pollinated hybrids ranged between -4.6% and 17.7%. The corresponding values were between -2.7% and 14.8% in self-pollinated samples. Six hybrids had heterosis values with opposite signs, whereas 34 had positive values (Fig. 3). The above conclusions were made based solely on the sign of the heterosis values. Deviation of values carrying the same sign was disregarded (Fig. 3).

Changes in flowering events

To evaluate the effect of pollen contamination among the genotypes, we observed two flowering events. Based on these data, we grouped the genotypes into three zones, where they were separated by five-day intervals, as presented in Fig. 4. We made this grouping based on Thomison (2003), who reported that normal maize plants shed pollen for 5-6 days after 50% of anthesis. Also shown in Fig. 4 are the genotypes in which we detected changes in genetic estimations/heterosis values in terms of sign (*i.e.* + or – values). Twenty genotypes had different GCA, SCA and REC values in regard to their varying pollination treatments

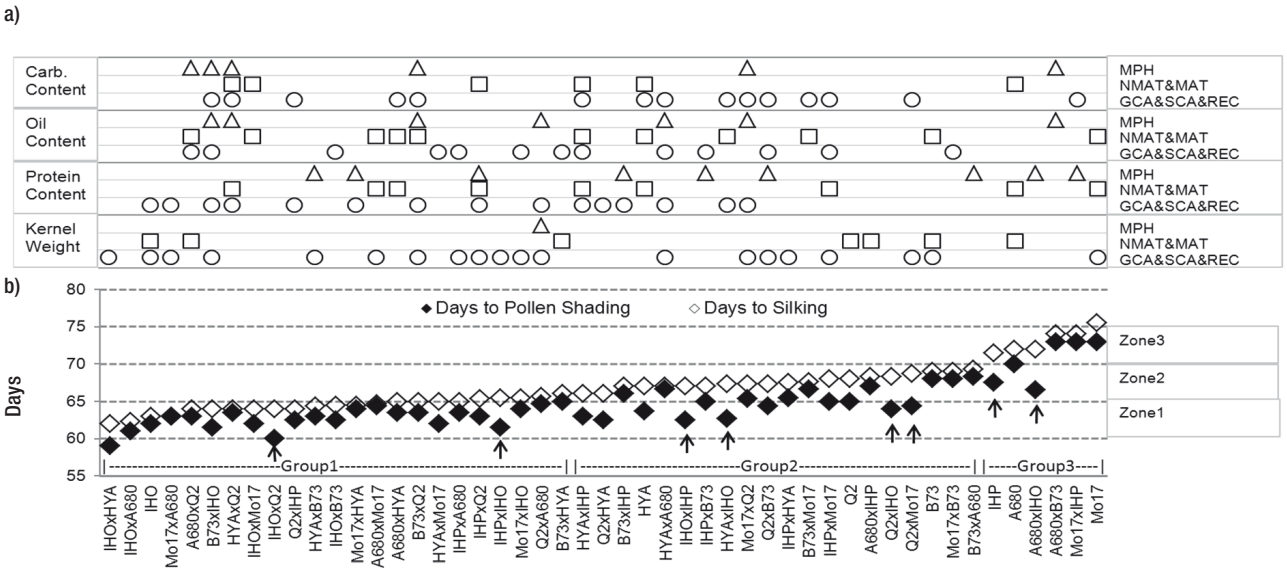

Figure 4. Combined plot showing differences for sign of genetic estimations in different pollination treatments (a) and flowering events (b) of genotypes used. Symbols ○, □ and Δ represent genotypes having opposite sign of estimations for GCA&SCA&REC, MAT&NMAT and MPH, respectively. Generative stage values are partitioned into three zones in lower part of plot, each with 5-day intervals. Arrows indicate genotypes with anthesis-silking interval values of more than 4 days.

for kernel yield/plant. Interestingly, few parents had greatly different GCA values for kernel biochemical traits (Fig. 4a). The number of genotypes whose genetic calculations for GCA, SCA and REC effects showed opposite signs in different pollination treatments was 20 for kernel weight, 15 for protein, 13 for oil, and 15 for carbohydrate (Fig. 4a).

The changes in MAT and NMAT effects across pollination treatments were remarkable. In genotypes 6, 11, 9 and 7 out of 49 genotypes, we detected NMAT and MAT effects with opposite signs for kernel weight/plant, protein, oil and carbohydrate, respectively (Fig. 4a). Changes in these values for quality traits were more evident in the hybrids, where B73, HYA, IHP, IHO and Q2 were female parents. This suggests that these particular hybrids were cross-pollinated with other genotypes having different characteristics in their biochemical features. Midparent heterosis values had opposite signs for carbohydrate content in 6 hybrids, for oil content in 7 hybrids, for protein content in 9 hybrids, and for kernel weight/plant in one hybrid, when subjected to different pollination treatments (Fig. 4a). This also validates the hypothesis that pollen contamination may cause changes in seed composition at a level that can affect genetic estimations as well as heterosis values. These results indicate that major errors may arise due to changes resulting from pollen contamination if genotypes are left to open-pollination in research investigating quality traits.

According to the flowering events, number 23, 20 and 6 genotypes were grouped into the first, second and third zones, respectively (Fig. 4b). In the open-pollination treatment, there was no possibility of cross-pollination between genotypes from the first and third zones due to large differences between their days to silking and pollen-shading values. However, pollen exchange might have occurred between genotypes from the first and second zones, especially genotypes planted relatively closely (Fig. 4b). Nine genotypes had a longer anthesis silking interval (≥4 days), increasing the possibility of pollination with genotypes from other zones. IHO, HYA, Q2 and B73 might have received more foreign pollen than the other parental lines because they were located in the first and second zones.

Discussion

Data suggest that the pollination treatment had various effects on the means of the investigated traits. These effects appeared differently in the parents and hybrids. Rankings of the genotypes also varied according to their treatment. Rank correlation was used to learn whether the ranking of genotypes changed according to pollination treatment. High correlation indicates a similar ranking of genotypes as well as similar values for a given trait. We observed a high rank correlation for protein and carbohydrate in parental lines. Similarly, hybrids showed a high rank correlation for oil content. Our numbers were in the range of those reported by Schaefer & Bernardo (2013) for proteins and carbohydrates, but lower for oil content. Use of

solely temperate inbreds by these researchers may be one reason for the difference.

The results showed that pollination treatment had a significant effect on kernel yield/ear. Self-pollination yielded lower values for both hybrids and parents. Bulant *et al.* (2000) reported that pollination of a genotype with its own pollen resulted in smaller kernels than pollination from a different genotype. In our study, the differences detected between pollination treatments regarding kernel yield/plant are thought to have originated not only from kernel size but also the number of kernels/ear. Kahrıman *et al.* (2015) found that selfing resulted in a lower seed set as compared to bulking and open-pollination; due to the fact that the silks received a lower amount of viable pollen in selfing. This conclusion is supported by results from other studies that used open and restricted pollination treatments (Borrás *et al.,* 2003).

The negative effect of selfing on the seed set and carbohydrate content is apparent in our results. Nevertheless, selfing yielded higher oil and protein ratios in most of the genotypes. It is well-known that oil and protein concentrations have a negative correlation with carbohydrate in the maize kernel (Dado, 1999). A decrease of carbohydrate level and thus kernel weight in genotypes whose oil and protein values increased due to the selfing treatment may be attributed to this fact. A general tendency was that protein and oil content increased with self-pollination, while carbohydrate content decreased (Fig. 1). This result is in agreement with previous studies (Letchworth & Lambert, 1998; Sulewska *et al.,* 2014). East & Jones (1920) argued that protein content in self-pollinated kernels was higher than in open-pollinated kernels, stating that this phenomenon occurred for two reasons: open-pollinated ears had some degree of heterosis, and they also had more kernels than self-pollinated ones. Our results support this conclusion for both protein and oil content. Undoubtedly, changes caused by pollination treatment had an impact on genetic estimations.

Few parents had opposite signs for GCA values. High similarity in GCAs obtained from different pollination treatments indicated that the average performance of parental lines was not significantly affected by the pollination treatment. These findings imply that parental genotypes could be evaluated using both open- and self-pollination in genetic experiments; whereas hybrids should not because SCA values changed considerably according to the pollination treatment. From a breeding standpoint, GCA values of parental lines refer to additive gene actions, while SCA values are related to non-additive gene actions (Falconer & Mackay, 1996). Thus, it could be argued that additive type gene actions were not affected to any great extent by the pollination treatments; whereas non-additive gene actions showed significant changes in some of the tested plant material. Our figures for GCA and SCA were in agreement with other studies for kernel yield/plant, while they were beyond the limit for protein, oil and carbohydrate content (Khadzhihov *et al.,* 1978; Lorencetti *et al.,* 2005; Balcı & Turgut, 2006; Aliu *et al.,* 2008; Werle *et al.,* 2014). This is because the parents we used, such as IHO, IHP and HYA, had higher values for oil and protein than parents used in those studies.

Maternal (MAT) and non-maternal (NMAT) effects have rarely been taken into account in diallel experiments. These values are calculated based on reciprocal effects. In particular, NMAT effects are associated with nuclear and cytoplasmic gene interactions (Fan *et al.,* 2014). Assessment of these calculations is limited to grain yield in maize research. Interaction of nuclear and cytoplasmic genes may also have an effect on kernel biochemical traits (Han *et al.,* 2008). Moderately high correlation coefficients between different pollination treatments for proteins and carbohydrates suggest that the treatment may have an effect on such interactions (Fig. 2). Our data clearly demonstrates that pollination treatment significantly affected the MAT effects for oil content. Furthermore, both MAT and NMAT effect values differed significantly between different pollination treatments for kernel weight/plant. Such differences should be taken into account in future studies targeting the enhancement of kernel weight/plant and/or oil content.

Another important issue is that the heterosis values of the hybrids were significantly affected by the pollination treatments. High heterosis values are common in maize species for grain yield, whereas kernel quality traits, such as oil and protein content, generally have much lower, if not negative, numbers. Nevertheless, positive and high heterosis values have also been reported for protein and oil content (Oliveira *et al.,* 2006; Drinić *et al.,* 2012). In fact, we also observed remarkable heterosis values for the investigated kernel quality traits. The heterosis values from a certain hybrid differed across pollination treatments. These differences, stemming from either the parents or the hybrid itself, may alter the breeder's decision on hybrid performance. Such variation due to pollination method would also affect genetic calculations, such as GCA, SCA, REC, MAT and NMAT. In addition to the discussion on the effect of pollination methods given so far, we offer here a possible reason for these changes, that of pollen contamination.

There is variation among the open-pollinated samples in the present study caused by pollen contamina-

tion from different genotypes. To achieve pollen contamination between two genotypes; (i) they should be simultaneously flowering, and (ii) they must have been planted in close proximity. In this case, pollen from a certain genotype can reach others in uncontrolled conditions. The results on genetic estimations showed that parental lines underwent constant change for kernel biochemical traits due to pollen contamination from other genotypes, while hybrids did not. This finding was supported by differences for genetic estimations between the different pollination treatments in our study (Fig. 2). We had no means of knowing which genotype was cross-pollinated by which other genotype's pollen. Furthermore, it is rather difficult to distinguish variation caused by the "pollen effect" from the effect of the pollination treatment itself. The methodology used in this study is unable to make such a distinction. Therefore, future research needs another means of differentiating variation caused by genotypes from variation caused by pollination methods. Advanced analysis techniques such as molecular assays may offer more detailed data. Indeed, molecular tools have already been used to determine pollen source and cross-pollination rates in maize (Balestre *et al.,* 2007).

In conclusion, researchers should be aware that the pollination method used in studies will have a significant effect on the precision of calculations for genetic parameters (*e.g*., GCA, SCA, MAT, NMAT) and heterosis. The method of choice should simulate open-pollination while preventing pollen contamination. We compared a method that could minimize pollen contamination (*i.e.* self-pollination) with open-pollination. Our data suggest that genetic estimations should be made using self-pollination in diallel experiments for protein, oil and carbohydrate content, while yield evaluations can be carried out on open-pollinated samples. More specifically, selfing can be done by transferring pollen collected from different plants of the same genotype without pollen contamination from other genotypes. Hand pollination using bulked pollen from individual plants from the same genotype would help in this respect. Taking these results into account may improve the accuracy of breeding efforts and research studies which include genotypes having different kernel characteristics. A better evaluation of this topic may be possible by designing experiments that could differentiate the pollen effect caused by different pollination treatments (bulk pollination, synchronous pollination, etc.), and by using other quality traits that were not investigated here. Also, more informative methods, such as molecular tools, may be used in future studies for more detailed conclusions.

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