



Araştırma Makalesi/Research Article

## Comparison of Open and Hand Pollination Methods on Combining Ability Values for Kernel Quality Traits in a Maize Diallel Experiment

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### Abstract

This study was carried out to investigate the effect of pollination methods in maize experiments on the combining ability values calculated in breeding experiments. The field trial was carried out with three replications in a split plot design at Çanakkale Onsekiz Mart University, Faculty of Agriculture, Plant Production Research and Application Unit during 2016 growing season. A 4 × 4 half diallel set was used as the material in the study and four different pollination methods were tested on this material. These methods were open pollination, self pollination, sib-pollination and bulk pollination. Hand pollination methods were applied in two different ways as fully conservative (M1) and semi-conservative (M2). To investigate the effect of pollination methods, data were collected on total protein, oil, major fatty acids, tryptophan, and total carotenoid concentrations. The genetic parameters calculated for kernel quality traits were mostly changed by the genotype effect. The effect of pollination methods on kernel content was found to be insignificant, whereas it was observed that the genotypic ranking based on the calculated GCA and SCA values changed significantly across the pollination methods used herein. According to the results of this study, it was determined that controlled pollination methods prevent pollen contamination adequately. However, it has been understood that more extensive information can be provided with a broader set of materials for suggesting the best of pollination methods for maize researchers.

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

## Mısır Diallel Denemesinde Tane Kalite Özellikleri için Kombinasyon Yeteneği Değerleri Üzerine Açıkta ve Kontrollü Tozlama Yöntemlerinin Karşılaştırılması

### Öz

Bu çalışma mısır ıslah çalışmalarında hesaplanan kombinasyon yeteneği değerleri üzerine tozlama yöntemlerinin etkisini araştırmak amacıyla yürütülmüştür. Tarla denemesi 2016 yılı yetiştirme sezonunda Çanakkale Onsekiz Mart Üniversitesi Ziraat Fakültesi Çiftliği, Bitkisel Üretim Araştırma ve Uygulama Birimi'nde bölünmüş parseller denemese desenine göre üç tekerrürlü olarak yürütülmüştür. Deneme materyali olarak 4 × 4 yarım diallel dizaynına uygun bir set kullanılmış ve dört farklı tozlama yöntemi bu materyal üzerinde test edilmiştir. Bu metotlar açıkta tozlama kendileme, kardeşleme ve toplu tozlamadır. Kontrollü tozlama yöntemleri tam korumalı (M1) ve yarı korumalı (M2) olarak uygulanmıştır. Tozlama yöntemlerinin etkisini incelemek amacıyla, toplam protein, toplam yağ, başlıca yağ asitleri, triptofan ve karotenoid içerikleri hakkında ölçümler yapılmıştır. Tane kalite özellikleri için hesaplanan genetik parametreler genotip etkisiyle yüksek oranda değişmiştir. Tozlama yöntemlerinin tane içeriğine etkisi önemsiz bulunmasına karşın, bu çalışmada kullanılan tozlama yöntemleriyle elde edilen örnekler üzerinden hesaplanan GCA ve SCA değerlerine dayalı genotip sıralamasının değiştiği görülmüştür. Bu çalışma sonuçlarına göre, kontrollü tozlama yöntemlerinin polen bulaşımını yeterli düzeyde engellediği belirlenmiştir. Buna karşın, mısır araştırmacıları için en iyi tozlama yönteminin önerilmesi amacıyla daha geniş bir materyal setiyle daha kapsamlı sonuçlar sağlanabileceği anlaşılmıştır.

**Anahtar Kelimeler:** Protein, Yağ, *Zea mays*, Tozlama yöntemi.

### Introduction

Maize is a cross pollinated species with one of the largest pollen grains (90 to 125 x 85 μ) in cereals (Erdtman, 1952; Smith, 1990). Pollination occurs with the help of gravity, wind and insects (Percival, 1950; Purseglov, 1972). Cross-pollination occurs at a high rate because of the presence of



male and female flowers in different parts of the maize plant. While this is normal in a common corn field, it is necessary to prevent cross-pollination among different genotypes in breeding experiments. For this purpose, different “hand pollination methods” have been developed. They have some differences in their application and purpose of use. The main methods are self pollination, sib-pollination and bulk pollination. Hand pollination methods require a lot of labor, including covering the ears of the plants to be pollinated, carrying out field inspections the day before the pollination, attaching the tassel bag in order to collect the pollen, and pollinating the suitable plant in the early hours of the next day (Abdin et al., 1979). Hand pollination techniques can be applied in a fully conservative or semi conservative manner (Kahrıman, 2016). Fully conservative application (called as M1 in this paper) is managed by bending the upper 15-20 cm of the tassel bag onto the shootbag, without exposing the silk, to minimize the risk of contamination with foreign pollen. The shootbag is removed properly while under the folded upper part of the tassel bag, and the pollen is then poured onto silks by lifting the lower portion of the tassel bag. In semi conservative application (called as M2 in this paper); first, the shootbag is removed, the shoot is exposed for a brief moment of time, before pouring the collected pollen onto the silk. Then the shoot is covered with the tassel bag and it is stapled around the stalk. These applications look like similar but they differ in terms of the amount of pollen grains reached onto the silks of protected ears and they have a variability in the risk of pollen contamination from the neighboring genotypes. These differences are not taken into consideration by the researchers in most studies.

Open-pollination and self pollination techniques are the most commonly mentioned techniques in maize breeding literature. Open-pollination is favorable in the experiments on yield related traits (Abou-Deif et al., 2012; Mahesh et al., 2013; Werle et al., 2014), while hand pollination methods are more appropriate for the studies targeted kernel quality traits. Comparative studies were conducted to evaluate the effects of open and hand pollination on several kernel quality traits, such as protein, oil and carbohydrate content in maize kernel (Letchwort and Lambert, 1998; Kahrıman et al., 2015a). General and specific combining ability values are the key parameters to determine the superior parents and hybrids for target traits in breeding experiments. Therefore, these estimations should be made accurately in such studies. There have been limited studies investigated the effects of pollination methods on the genetic estimations in maize breeding experiments. Kahrıman et al. (2015b) evaluated the effects of open- and self pollination treatments on genetic calculations for single ear yield, protein ratio, oil content and carbohydrate content in a  $7 \times 7$  full diallel set of maize genotypes. They found an important effect on the results of genetic estimations such as combining ability values.

Related research studies up-to-date have some shortcomings in that they merely compared open- and self-pollination methods, and they focused only on major quality traits. In fact, the scientific literature lacks well-rounded studies investigated the effects of different pollination methods on a variety of valuable traits. From this standpoint, the objectives of this study was to evaluate the variation in the combining ability values estimated for several major and minor grain quality traits of maize as affected by 7 different pollination methods in a 4x4 half diallel experiment.

## **Material and Methods**








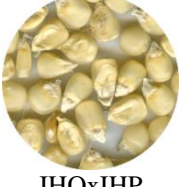
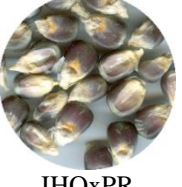

### **Plant Material and Experimental Organization**

In this study, 2 white kernelled (high oil, high protein), 1 yellow kernelled (opaque-2 endosperm), and 1 purple kernelled (normal) genotypes were used as parents. The high oil, high protein, and opaque-2 genotypes had been obtained from North Regional Central Plant Introduction Station in 2009, and had been increased and maintained by selfing since then. To be able to investigate pollen effect, we preferred to use genotypes that differed in kernel color and biochemical content but nearly synchronized in terms of flowering, except for IHP (Table 1). Planting date of IHP was arranged accordingly to nick pollen shedding. The parents used in the experiment were grown in a 4x4 diallel mating design in 2015, to yield a material set consisting of 4 parents and 6 hybrids, totaling 10 different genotypes (Table 1).

The field trial was carried out at Dardanos Research and Experimental Station of Çanakkale Onsekiz Mart University in 2016. Planting was done in the first week of June by hand, into 2-row plots, 2 m in length, and spaced 70 cm. Within row spacing was adjusted as 20 cm. The late flowering parent IHP was planted 7 days earlier so that the genotypes synchronize. A split plot experimental design was used with 3 replicates. The genotypes were randomly distributed to main plots, while the

pollination treatments were assigned to subplots with the same order. Fertilization was done based on soil analysis, with 180 kg/ha nitrogen, and 80 kg/ha phosphorus. The phosphorus and a quarter of the nitrogen were incorporated before planting, while the rest of the nitrogen was given with drip irrigation in three occasions (when the plants were 30-35 cm, just before flowering, and seed filling periods). The amount of water applied with the drip irrigation was recorded at each time of application. First irrigation after the planting was done in order for the soil to reach field capacity. Weed control was managed mechanically. Harvest was done by hand, following physiological maturity.

Table 1. Maize genotypes used in the study

PARENTS			
			
Opaque-2* Moderate oil, low protein, high carotenoid	IHO* High oil (14%), low carbohydrate, low carotenoid	IHP* High protein (22%), low carbohydrate, low carotenoid	PR High in anthocyanin; Normal values for protein, oil and carbohydrate
HYBRIDS			
			
	Opaque-2xIHO	Opaque-2xIHP	Opaque-2xPR
			
		IHOxIHP	IHOxPR
			
			IHPxPR

\*Obtained from NPRIC (North Central Regional Plant Introduction Station, USDA/ARS & Iowa State University State and Mortensen Rd. Ames, IA, USA).

### Pollination Treatments

We compared 3 different controlled pollination methods along with open pollination. These are selfing, sibbing, and bulk pollination. Each of these pollination methods was applied in two different manners, conservative and semi-conservative way, so 7 different treatments were used totally, as following:

- 1- Open pollination
- 2- Selfing-M1
- 3- Selfing-M2
- 4- Sibbing-M1
- 5- Sibbing-M2
- 6- Bulk pollination-M1
- 7- Bulk pollination-M2

The plants in the open pollination plots were not interfered in any way. Controlled pollination applications were carried out as described by Abdin et al. (1979). Based on this method, the ears are covered prior to silking during daily field controls. Pollens collected from the tassel(s) are given to the ear shoot(s) in the morning (7-10 am). In selfing, the pollen collected from a plant goes to the silk of the same plant, and in sibbing to a different plant of the same genotype. In bulk pollination, the pollens are collected from a number of plants of the same genotype and distributed to the silks of the very same plants (Kahrıman et al. 2015a).. Transfer of the pollen is achieved either in a conservative way (Method1) or in a semi-conservative way (Method2). In the conservative method (M1) tassel bag containing the pollen is directly covered onto the shoot, while in the semi-conservative method (M2), first the shootbag is removed from the ear shoot and the pollen is poured onto the silk (Kahrıman, 2016). At least 5 plants were pollinated as described for each of the plots assigned with controlled pollination treatments. These plants were randomly chosen from among the plants located in the middle parts of the plots.



Figure 1. Application of compared pollination methods: Method 1 (A), Method 2 (B).

### Investigated Traits

We collected data on 6 different kernel traits. For this purpose, harvested ears were shelled and the seeds were grinded with a laboratory mill. The flour samples were kept +4 °C until the analyses. Protein and oil concentrations were estimated through NIR spectroscopy (Spectrastar 2400D, USA). The spectra taken from the flour samples within the range of 1200-2400 nm were used for this purpose by means of a local calibration model generated formerly in our laboratory (Egesel and Kahrıman, 2012). For estimation of oleic and linoleic acid ratios we used a transreflectans cup (liquid sample cup). The oil samples were extracted from the flour samples by keeping them within diethyl ether and separating the solvent-sample mixture in an evaporator (Çavdar et al., 2017). Extracted oil samples were analyzed in NIR instrument and the fatty acid ratios were estimated, utilizing a calibration model developed earlier in our laboratory (Egesel et al., 2015). Carotenoid concentrations were determined following the method of Rodriguez-Amaya & Kimura (2004). For this, 2 g (W) flour and 5 mL distilled water were kept in refrigerator (4 °C) overnight within a glass tube. Then, the samples were treated with pure acetone (15 mL, twice), and acetone:hexane (25 mL, once). The sample was shaken for 2 minutes each time, and the liquid phase was collected into a separation funnel, where 300 mL cold water was added. The separated the upper phases from each sample were taken into a volumetric flask and added up to 25 mL with cold hexane. Three mL sample was taken into quartz cuvette and absorbance value (A1) was read at 450 nm in a UV-VIS spectrophotometer (PG Instrument, England). Total carotenoid content was calculated based on the following formula:

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

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

Tryptophan concentrations were determined according to the method by Galicia et al. (2009). For tryptophan analysis, 80 mg oil sample was taken to a 15 mL Falcon tube. Onto this, 3 mL papain solution was added. The samples were hold at 64 °C for 16 hours, 1 hour before and after which they were vortexed. Then, they were cooled down to room temperature, and centrifuged at 3600 rpm for 5 minutes. One mL of upper phase was taken into a clean tube, on which 3 mL colorimetric solution was added. They were vortexed, then incubated at 64 °C for 30 minutes, and waited to cool down to room temperature. Absorbance values of the samples were recorded at 560 nm in a preconditioned UV-VIS spectrophotometer. Tryptophan concentrations of the samples were determined using the standard curve prepared with tryptophan standard (Sigma Aldrich).



### Statistical Analyses

In order to compare the variation originated from the use of different pollination methods, we utilized a general variance analysis. The data were analyzed in SAS with GLM procedure (SAS Institute, 1999). Statistically significant differences were determined by an LSD test. We followed Griffing's method to estimate genetic parameters (general and specific combining abilities- GCA and SCA) with a diallel analysis (Griffing 1956). In this, GCA and SCA values of the genotypes were calculated for each pollination method. Genotype was considered as fixed effect, and the parents were included in the diallel analysis (Fixed model, Method 2). We utilized DIALLEL-SAS05 macro generated by Zang and Kang (2005). Combination ability values based on genotypes were compared with t test as within and between years (Steel and Torrie, 1980). Diallel analysis was based on the following model:

$$Y_{ijkl} = \mu + \alpha l + b_{kl} + v_{ij} + (\alpha v)_{ijl} + e_{ijkl},$$

$$v_{ij} = g_i + g_j + s_{ij}, (\alpha v)_{ijl} = (ag)_{il} + (ag)_{jl} + (as)_{ijl}$$

where,  $Y_{ijkl}$  = observed value;  $\mu$  = population mean;  $\alpha l$  = effect of pollination treatment;  $b_{kl}$  = block effect within pollination treatment;  $v_{ij} = F_1$  hybrid effect =  $g_i + g_j + s_{ij}$  ( $g_i$ =GCA effect of  $i_{th}$  parent;  $g_j$  = GCA effect of  $j_{th}$  parent;  $s_{ij}$  = SCA effect of  $ij_{th}$  hybrid;  $(\alpha v)_{ijl}$  = interaction effect between  $ij_{th}$   $F_1$  hybrid and pollination treatment;  $e_{ijkl}$  = random error;  $(ag)_{il}$  = interaction effect between the GCA of  $i_{th}$  parent and pollination treatment;  $(ag)_{jl}$  = interaction effect between the GCA of  $j_{th}$  parent and pollination treatment;  $(as)_{ijl}$  = interaction effect between the SCA of  $ij_{th}$   $F_1$  hybrid and pollination treatment. The values obtained from diallel analysis to see if the combination abilities vary across the pollination methods were presented as radar plots.

### Results and Discussion

#### Comparison of Pollination Treatments

Variance analysis results were presented in Table 2. Genotype effect was significant for all of the investigated traits, while the effect of pollination treatment was significant for only tryptophan concentration. GCA was a significant source of variation for all traits, and so was SCA, with the exclusion of carotenoid content. GCA variance exceeded SCA variance for all traits, except for linoleic acid ratio. No interaction term was found to be significant between combining abilities and pollination treatment, excluding a GCA x P interaction for tryptophan concentration. These findings suggest that pollination treatment did not have a significant effect on the concentrations of the investigated biochemical variables. Nevertheless, in order to confirm this suggestion, GCA values of the parental lines as well as SCA values of the hybrids should be similar across the pollination types. This matter is thoroughly discussed under the following subtitles.

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

Source of Variation	df	Protein Content	Oil Content	Oleic Acid
Pollination (P)	6	2.66	0.96	32.9
Replication (R)	14	2.37	0.49	17.1
Genotype (G)	9	121.4**	116.2**	1215.7**
P×G	54	2.37	0.98	32.3
GCA	3	324.1**	290.5**	3110.1**
SCA	6	21.8**	2.56*	340.6**
GCA×P	18	2.28	1.08	48.9
SCA×P	36	2.67	0.88	22.5
Error	126	2.14	0.95	34.4
Source of Variation	df	Linoleic Acid	Carotenoid Content	Tryptophan Content
Pollination (P)	6	31.7	19.1	0.0004**
Replication (R)	14	16.5	5.10	0.0001
Genotype (G)	9	1388.7**	110.6**	0.0046**
PxG	54	31.0	10.1	0.0002**
GCA	3	3270.3**	304.2**	0.0113**
SCA	6	363.0**	13.8	0.0012**
GCA×P	18	38.8	13.4	0.0003*
SCA×P	36	26.9	8.43	0.0001
Error	126	33.1	11.8	0.0001

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



### **Changes in GCA Values by Pollination Treatments**

Changes in the GCA values of the investigated parents based on the pollination treatments were presented in Figure 2. IHP and IHO parents, in line with their breeding objective, had the highest GCA values for protein and oil concentrations, respectively, in all pollination treatments. Q2 was notable in that, in all of the pollination methods, it had the highest GCA values for carotenoid concentration, while having the lowest ones for protein. Oleic acid data highlighted IHO, whereas for linoleic acid and tryptophan, GCA values for the parents were variable. Variation of GCA values indicates that the parental lines superior for a certain trait possess high GCA levels for that trait. This, in turn, means that the hybrids having a special parent in their lineage would have high concentrations of that certain trait. Among the traits of interest in this study, protein concentration is the most stable one when the GCA values were ranked for all genotypes across the pollination treatments. For the other traits, ranking of the GCA values showed differences from one pollination method to other, at least for three of the parental lines.

GCA values are under the control of additive gene effects (Sprague and Tatum, 1942). They are known to be influenced to a lesser extent by environmental factors. In this study the data suggest that the genotypes with considerably high GCA values seem to be not affected much by the pollination treatments. Significance of genotype and GCA effects in ANOVA indicates that the variance detected among the parental lines is mainly arisen from the effects of homozygous alleles. The specialty genotypes IHO, IHP, and Q2 possess remarkable differences from the other genotypes of the experimental material, thereby masking the variation due to the pollination treatments. This may have resulted in obtaining a nonsignificant GCA×P interaction effect. Having a nonsignificant GCA×P interaction effect despite the fact that GCA values varied across the pollination treatments supports this argument. Also, the limited number of parents (4) may be another factor contributing to the lack of interaction between GCA and treatments.

### **Changes in SCA Values by Pollination Treatments**

The changes in SCA values of the investigated hybrids across the pollination treatments were presented in Fig 3. IHP×PR combination showed the highest SCA values for protein in the pollination treatments excluding bulk pollination, for which IHO×PR and IHO×IHP were leading. For oil concentration, the ranking based on SCA values was relatively stable across the pollination methods. IHO×PR was consistently top hybrid while the other combinations showed different levels of SCA values across pollination methods for oleic acid. IHO×PR was also the only genotype consistent across the treatments for linoleic acid, though having the lowest values this time. For tryptophan concentration, IHO×IHP hybrid had high SCA values when selfing-M1, selfing-M2, and sibbing-M1 methods were used; IHP×PR came to the fore with bulk pollination and sibbing-M2 methods. Genotype SCA ranking was quite changeable for carotenoid concentration, highlighting IHO×IHP and Q2×PR hybrids for open pollination. Among the investigated traits; none, except oleic acid ratio, had a certain genotype ranked first for all of the variables.

SCA values are considered to be under the influence of dominance gene action (Sprague and Tatum, 1942). Environment and cultural applications can greatly affect this type of genes. Although this was a single-environment experiment, use of different pollination applications was adequate to create changes in SCA values. Nevertheless, the data indicated a nonsignificant SCA×P interaction, suggesting that the genetic variation of the hybrids were much more important than the treatment effects.

Upon the evaluation of results, detection of nonsignificant interaction terms for GCA×P and SCA×P despite both GCA and SCA values varied across different pollination methods could be related with two other issues along with the ones discussed above. In the first place, the relatively limited number of parents is one of the factors. Earlier studies commenting on the optimum number of parents in diallel analyses have mentioned the minimum acceptable number as about four parent for combining ability analysis (Suhroue, 1995).

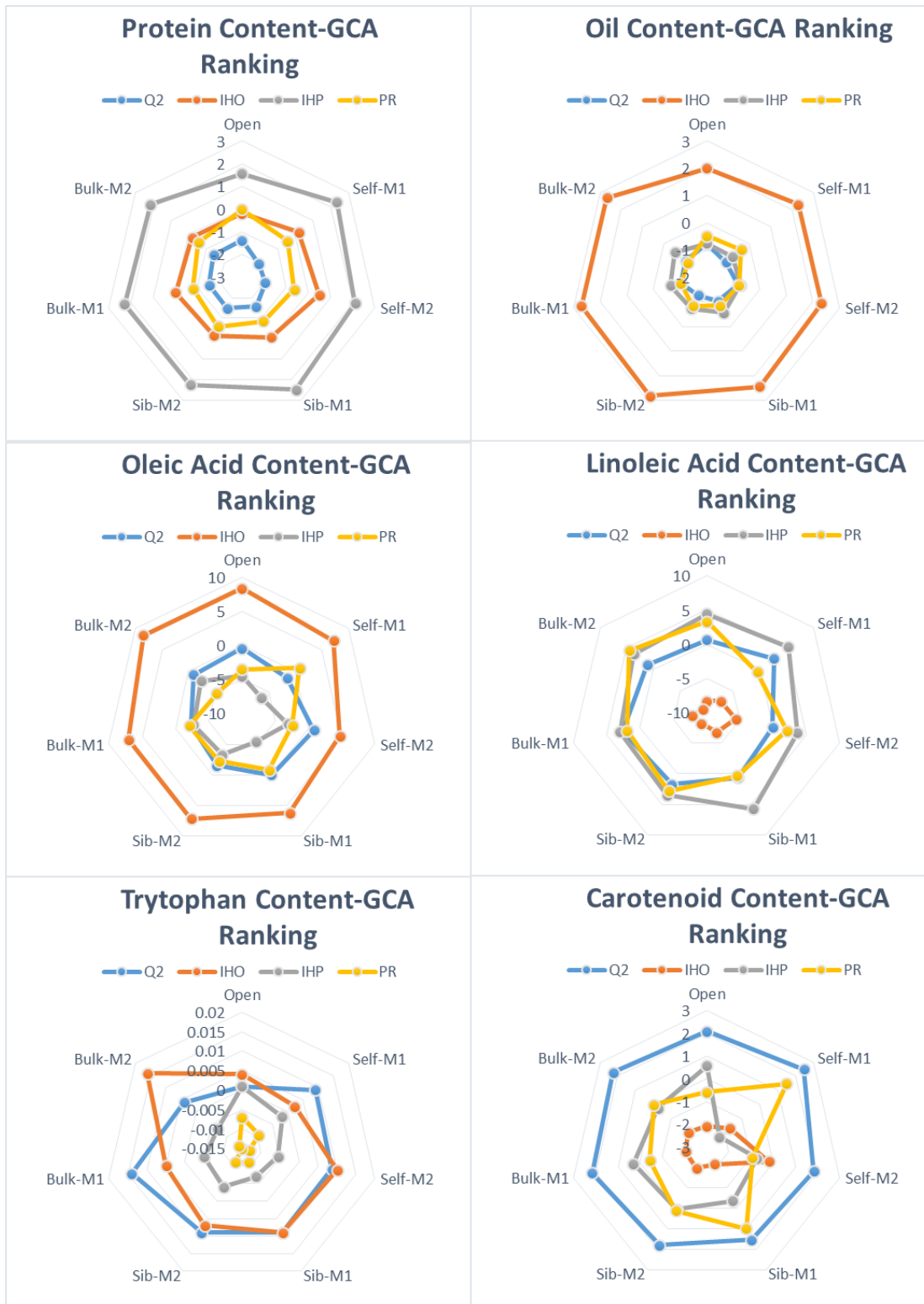


Figure 2. GCA values of parental lines in different pollination treatments for the investigated traits.

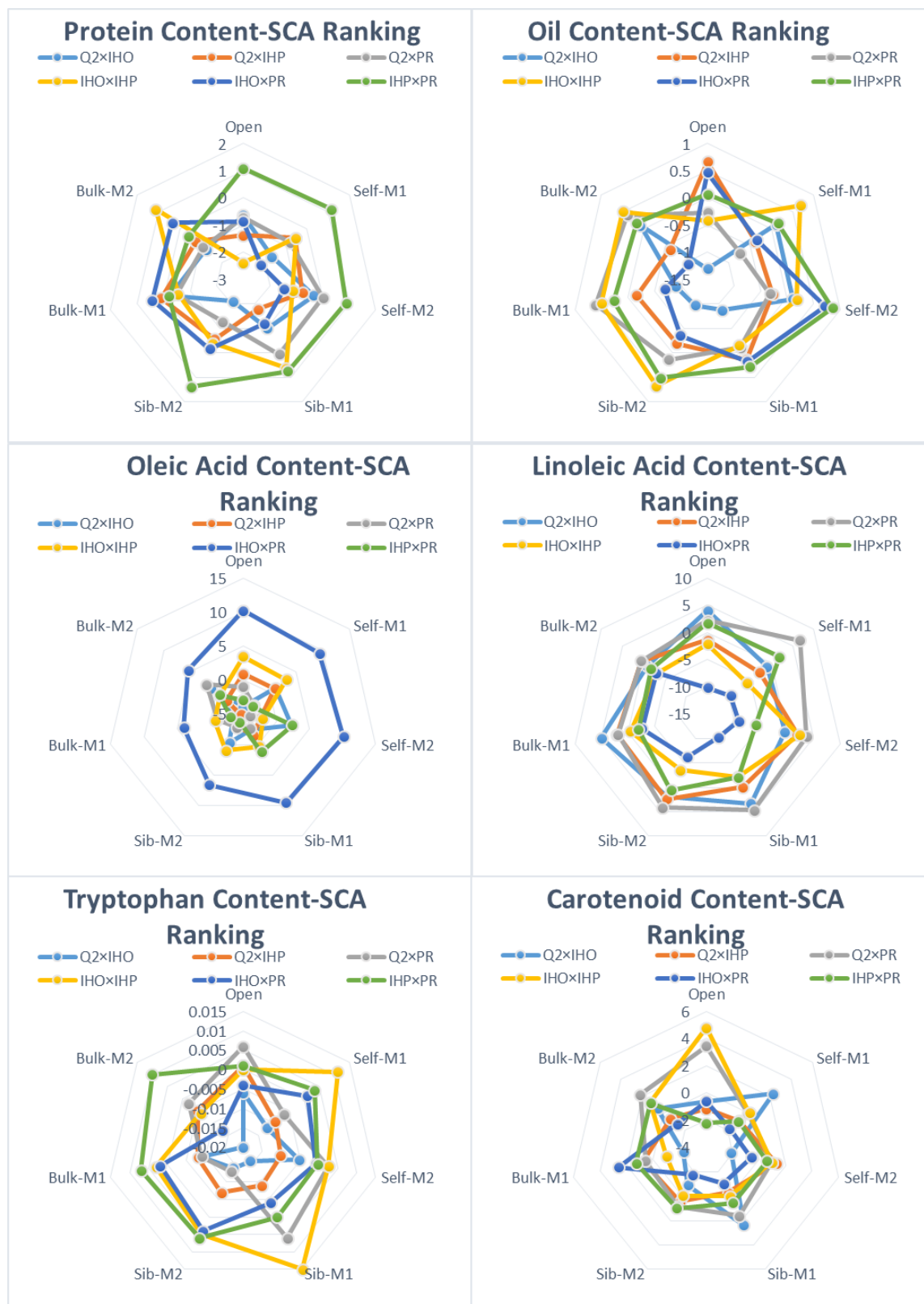


Figure 3. SCA values of hybrid combinations in different pollination treatments for the investigated traits.

Another point is that, the statistical method used in this study is not compatible with the experimental design. DIALLEL-SAS05 macro used herein is originally developed based on RCDB experimental design, and does not comply very well with split plot design. In diallel analyses the calculations of genetic parameters could possibly be quantified by utilizing approaches such as ANOVA (Griffing, 1956a), sequential model fitting based on ordinary least squares (Gardner and





Eberhart, 1966), MINQUE (Zhu and Weir, 1996), and REML (Xiang and Li, 2001). Relatively newer approaches offer more options in terms of model construction for variance analysis. Future studies taking advantage of such paradigm would enhance the effectiveness of the analyses. Better understanding the effect of pollination method may be possible if further studies on this area consider including a higher number of parents in a complete diallel design and use novel statistical tools. The observation that the ranking of genotypes based on their GCA and SCA values changed across the different pollination methods whereas that change was not reflected on results of variance analysis may cause misinterpretation of the genetic merit of parental lines or hybrids depending on the pollination method used in breeding programs.

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