

# Multivariate analysis of interactive effects of sowing dates and sesame genotypes

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Six divergent genotypes of sesame (*Sesamum indicum* L.) were crossed using a half diallel mating design according to Griffing (1956) excluding reciprocal crosses, to estimate stability for studied traits under three sowing dates in the experimental farm at Agricultural Research Station in Ismailia Governorate during summer season 2018. The 15 hybrids and their 6 parents were grown in field experiments in two geographical locations (Ismailia and El-Sharkia Governorates) and two seasons (2019 and 2020) under three sowing dates (April 1<sup>st</sup> and 2<sup>nd</sup> (SD1), May 1<sup>st</sup> and 2<sup>nd</sup> (SD2) and June 1<sup>st</sup> and 2<sup>nd</sup> (SD3) in Ismailia and Sharkia, respectively. A split plot design was used in randomized complete blocks with four replications, the main plots included planting dates while the 21 genotypes were in sub-plots. Combined analysis of variance over locations and seasons gave highly significant differences. Most of the interactions between the studied factors showed high significance for most of the traits under study. Additive Main effects and Multiplicative Interaction (AMMI) analysis for seed yield for 12 environments (two locations, two seasons and three sowing dates) showed significant effect of environments, genotypes and their interactions. The E2, E3, E8 and E9 had high mean seed yield above the grand mean environments values and are considered suitable and favorable for sesame seed production due to its low interactive effects. Sesame genotypes 7, 18, 10, 19, 12, 13, 15 were nearly close to the origin and thus the most stable and less responsive to the GEI (Genotype-by-Environment Interaction). In contrast, genotypes 1, 20, 8, 11 and 2 were far from the origin, sensitive to environmental changes and their yields are unstable.

**Keywords:** Sesame, half diallel, sowing dates, AMMI, biplot

## INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the oldest cultivated and most important oil crops in the world. China and India followed by Burma, Sudan, Nigeria, Uganda, Venezuela, Mexico, Turkey and Ethiopia are the most important sesame producers (Hakeem et al., 2020). The global cultivated area of sesame crop reached 13 million ha, producing about six million metric tons (FAOSTAT, 2020). The cultivated area of sesame in Egypt is 4,200 ha with an average productivity of 250 kg/ha. The cultivated area of sesame in Egypt is 100,055 feddan with an average productivity of 600 kg/fed. Total cultivated areas in Ismailia and El-Sharkia governorates are 4,579 and 20,315 Fed, respectively, producing about 3.5 and 4.8 Ardab/Fed, respectively (ASBAE, 2022).

Sesame seeds contain 45–60% oil and 25% protein, and constitute a rich source of linoleic acid, vitamins A, B1, B2, E and minerals including calcium and phosphorus. It is used in a wide variety of

foods such as confectioneries, cakes, and pastries. While high-quality oil is utilized in cooking, margarine manufacture, and pharmaceutical industries, lower-quality oil is used in soap production (El Naim et al., 2010). Despite the high nutritional value, health benefits and economic importance of sesame in many countries, it is largely ignored by plant breeders (Bedigian, 2010).

The genotype by environment interaction ( $G \times E$ ) refers to the deviation in performance of any attribute of genotypes within the various growing environments.  $G \times E$  interaction makes varietal selection more difficult because it lessens the utility of genotypes by confusing their yield and performance by reducing the correlation between genotypic and phenotypic parameters (Farshadfar et al., 2012). However, it is possible to develop genotypes with low  $G \times E$  interaction via sub-division of heterogeneous area into smaller-more homogeneous sub-regions and by selecting genotypes with a better stability across a wide range of environments (Farshadfar et al., 2011). So,  $G \times E$  interaction may be considered as both an opportunity and a challenge for breeders. Additive Main Effect and Multiplicative Interaction (AMMI) is important to analyze multi-environment trials data to interpret the effect of the genotype ( $G$ ) and environments ( $E$ ) as additive effects and the  $G \times E$  as a multiplicative component and submits it to principal component analysis. The AMMI approach has been proven to improve estimation accuracy since it uses an ordinary ANOVA procedure to fit additive main effects for genotypes and environments and then applies Principal Component Analysis (PCA) to the matrix of residuals that remain after fitting main effects (Singh and Bisen, 2020). In AMMI model, the Genotype by Environment Interaction (GEI) can be decomposed into several Interaction Principal Component Axes (IPCA) using PCA. GGE biplot is a data visualization tool, displays graphically a  $G \times E$  interaction in a two-way table (Yan, 2000). It is an effective tool for recommending specific genotypes to a specific mega-environment (Yan and Kang, 2002), determining stability and performance of genotypes and discriminating among genotypes in target environments. Singh and Bisen (2020) and Movahedi et al. (2020) are among the many authors who used GGE biplot to identify mega environments, to evaluate the genotypes and to test the environments. The present study was designed with the following objectives: i) Find out promising combinations of genotypes at different sowing dates across locations and years and ii) Apply AMMI and bi-plot models to visualize the GEI and summarize patterns and relationships of sesame genotypes and tested environment.

## **MATERIALS AND METHODS**

The present study has been coordinated by Agronomy Department, Faculty of Agriculture, SCU and Institute of Oil Crops Research, ARC, Giza and continued during the period from 2018 to 2020.

### **Description and experimental locations**

The field experiments were conducted in two locations during two summer growing seasons. The first location is the experimental farm of Agricultural Research Station, ARC, Ismailia Governorate. The second location is a farmer's field in El-Sharkia Governorate. To analyze physical composition and chemical properties of the experimental sites, soil samples were collected from 0-20 cm depth at random before sowing. Analysis were performed at the Laboratory of Soil and Water Department, Agric. Res. Station, Ismailia according to Kilmer and Alexander (1949) and are presented in Table 1. Monthly meteorological data during growing seasons including temperature, RH% and rainfall (Table 2) was obtained from Egyptian Meteorological Authority (EMA).

### **Genetic materials**

Six sesame genotypes were selected and provided by Research Institute of Oil Crops, Agricultural Research Center, Giza and constituted the parents in this study. The genotypes comprised variability in origin, plant and yield characteristics (Table 3). Sesame genotypes were crossed in a half diallel mating system (excluding reciprocals) to produce 15 F1 crosses according to Griffing (1956) during 2019 summer season at the experimental farm of Agricultural Research Station,

Ismailia.

### Experimental design and trial management

Four field experiments were established comprising two growing seasons (2019 and 2020) and two locations (Ismailia and El-sharkia governorates). For each experiment, six sesame parents along with their 15 F1 crosses were arranged in split-plot in three randomized complete block design. Three sowing dates namely, 1 and 2 April (SD1), 1 and 2 May (SD2) and, 1 and 2 June (SD3) in Ismailia and El-sharkia locations, respectively comprised main plots and 21 sesame genotypes comprised sub-plots. Each genotype was sown in a plot size of 3.6 m, consisting of three rows of 2 m in length. The spacing between rows and plants was 60 and 20 cm, respectively resulting in 30 plants plot<sup>-1</sup>. Fertilizer in the form of Calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) at rate of 200 kg/fed and potassium sulphate (48% K<sub>2</sub>O) at rate of 100 kg/fed were added during soil preparation. Nitrogen in the form of ammonium nitrate (33%) at rate of 150 kg was manually side dressed into three portions, at sowing, after thinning and at flowering stage. All agricultural practices were adopted as recommended for each location.

### Data collection

From each harvestable plot, 10 representative plants were selected randomly and tagged to collect all the under mentioned data for each genotype at each test environment. The data collection was represented by each of days to 1st flower (d), plant height (cm), height to 1st capsule (cm), fruiting zone length (cm), capsules plant<sup>-1</sup>, 1000-seed weight (g), seed yield plant<sup>-1</sup> (g) and oil content (%), Oil content (%): Extraction and analysis procedures were applied using Soxhlet system HT, apparatus according to the method of AOAC (1980).

### Physiological and quality traits

**SPAD value:** Estimated by using chlorophyll meter (SPAD - 502, soil plant analysis development (SPAD) Minolta, Japan) according to Castelli et al. (1996).

**Relative water content (%):** Leaf relative water content (LRWC) was estimated according to the procedure of Meher et al. (2018). Briefly, about 0.5 g of leaf sample was incubated in 100 mL of distilled water for 4 h. After that, the turgid weights of leaf samples were taken. The leaf samples were oven-dried at 80°C for 24 h. The dry weights of the samples were taken till a constant weight was achieved.

$$\text{LRWC (\%)} = [(\text{Fresh wt} - \text{Dry wt}) / (\text{Turgid wt} - \text{Dry wt})] \times 100$$

### Peroxidase activity ( $\mu\text{ mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}\text{ FM}$ )

Fresh leaves (0.5 g) were freeze in (-20°C) and then homogenized with a prechilled mortar and pestle under ice cold-conditions in 4 ml 50 mM potassium phosphate buffer (KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>), pH 7.0, with the addition of 1 mM Na<sub>2</sub> EDTA. The homogenate was centrifuged at 15000 rpm, at 4°C for 20 min. The supernatant was stored at -20°C and used for the assay of enzyme activity. Peroxidase (POD) activity was determined as described by Liu and Huang (2000). The POD reaction solution (3 ml) contained 50 mM potassium phosphate buffer (pH 7.8), 20 mM guaiacol, 40 mM H<sub>2</sub>O<sub>2</sub>, and 100  $\mu\text{l}$  enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 30 sec. One unit of peroxidase activity was defined as an absorbance change of mg fresh weight per min.

### Statistical analysis

A combined analysis of variance over sites and seasons based on split-plot was performed for each season to test the significance effect of locations, genotypes, sowing dates and their interactions on

the responses of sesame attributes. The data obtained were analyzed using the program Agri-stat software according to Mather and Jinks (1982), and mean differences among tested factors were calculated using LSD at 0.05% level of significance.

### **Multivariate analysis of G×E interaction**

The seed yield data were subjected to combined analysis of variance and AMMI analysis which is a combination of analysis of variance and multiplication effect analysis. Analysis of variance is used to partition variance into three components: genotype deviations from the grand mean, environment deviations from the grand mean, and GE deviations from the grand mean. Afterward, multiplication effect analysis is used to partition GE deviations into different interaction principal component axes (IPCA), which can be tested for statistical significance through ANOVA. Statistical analyses were performed using GENSTAT software version 17.

### **AMMI biplot analysis**

To understand specific GEI combination and the general pattern of adaptation, a biplot of genotypes and environments was performed. The AMMI biplot is developed by placing both genotype and environment values on the abscissa (X- axis) and the respective PCA axis, Eigen vector on the Y- axis. XLSTAT software was used to perform analysis and biplot visualization.

### **AMMI Stability Value (ASV)**

AMMI Stability Values were calculated for each genotype to rank them in terms of stability according to the relative contribution of IPCA1 and IPCA2 to the interaction SS.

The AMMI stability value (ASV) as described by Purchase et al. (2000) was calculated as follows.

IPCA score=

Where  $SS_{IPCA1}/SS_{IPCA2}$  is the weight given to the IPCA1 value by dividing the IPCA1 SS by the IPCA2 SS; and the IPCA1 and IPCA2 scores are the genotypic scores in the AMMI model.

IPCA score is the weight given to the IPCA1-value by dividing the IPCA1 sum of squares by the IPCA2 sum of squares. The larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller ASV scores indicate a more stable genotype across environments.

## **RESULTS**

### **Multivariate analysis of G×E interaction**

Combined analysis of variance for seed yields over 12 environments (2 years, 2 locations, 3 sowing dates, table 4) showed significant effect of environments, genotypes, and their interactions (Table 5). Sum squares due to environments explained 74.8 of the total variation for seed yield. In contrast, variation due to genotypes and G×E interaction constituted less contribution (2.26 and 22.3), respectively. The genotypes by environments interaction as a measure of environmental diversity was very small.

### **AMMI analysis**

The presence of significant GEI confirmed the diverse responses of genotypes among tested environments, therefore GEI analysis through AMMI analysis (Table 6) is presented. The AMMI analysis demonstrated the presence of significant  $G \times E$  interactions, and this has been partitioned

among the first and second Interaction Principal Component Axes (IPCA's). Both components showed significant effects and accounted for a total of 63.6 of GEI for seed yield. IPCA1 had a higher significant contribution to GEI (41.7 %) than IPCA2 (21.9 %) for seed yield.

Means, IPCA1 and IPCA2 scores and ASV for seed yield of 21 sesame genotypes are shown in Table 6. Genotypes 7 and 19 had low values for IPC1(1.49 and 0.09), ASV (7.15 and 6.39) and high mean seed yield (707 and 705 kg/fed) above the grand value, considered the most stable genotypes. While G20 and G1 that considered the least stable genotype, had the highest ASV (38.3 and 38.3), and IPC1 values (-18.5 and 19.1); and low mean yield (655.2 and 634.5 kg/fed) below the grand mean value.

### AMMI biplot

The AMMI graph is used to visualize the adaptability (average performance across locations) and stability (consistent performance across environments) of various genotypes. In the AMMI graph the genotypes that are located close to the origin are relatively stable, while the ones that are located far away from the origin are unstable because they interact with the environments.

E2, E3, E8 and E9 had high mean seed yield above the grand mean values and is considered as suitable and favorable for sesame seed production due to its low interactive effects. The six lowest yielding environments (4, 5, 6, 10, 11 and 12) were separated from other environments and grouped together. It showed rather less interactive effect for seed yield and considered the least favorable environments for almost genotypes for sesame yield production.

E1 and E7 also exhibited the highest mean yield above the grand value and represented high and negative interactive effects. Those environments considered not suitable to select for high seed yield in the present set of genetic materials. They negatively interacting with high yielding genotypes (G8, G11, G13 and G14).

Genotypes 7, 18, 10, 19, 12, 13, 15 were nearly close to the origin and the most stable with less responsive to the GEI (Figure 1). In contrast, genotypes, 1, 20, 8, 11 and 2 were far from the origin, sensitive to environmental changes and be unstable. Therefore, the genotypes, 8, 13, 11 and 2 are considered the best and could be grown in wide environments.

### Association of traits and classification of sesame genotypes using PCA

PCA for 13 traits and 21 genotypes at three sowing dates (SD's) averaged across two sites and two growing seasons was performed to identify the principal components of measured parameters that best described the response to SD's (Figures 2, 3 and 4). Results showed that 57.5, 49.8 and 56.2% of the total variation were contributed by the first two PCs when evaluating sesame genotypes at SD1, SD2 and SD3, respectively. Generally, contribution of PC1 for the 13 traits was more than two times that of PC2. The first PC was positively correlated with SYF, OYF, PH, SYP and SW, whereas, SYF, OYF and RWC were the most important contributing traits to PC2 under SD1 condition. Under SD2, the PC1 can be interpreted as representing higher values for SYF, OYF, SW, NOB and NFC. At SD3, SYF, OYF, SYP, NOB and FZ contributed with PC1, while FZ and OC contribution were the most important in PC2 variation. Positive and high loadings values of variables are related to the diversity of sesame genotypes. Biplot of PC1 vs. PC2 should separate the tested genotypes based on differences in responses to different sowing dates and are placed in the upper-right and lower-left corner of the graph. At SD1, the hybrids, H8, H4, H12, parent Imported 188 are located in the 1st quadrant and performed the best and have higher values for NOB, NOC, FZ, PH and DTF. The parents H2, H5, H10 and H14, performed less and possessed high values for SW, HFC, SYF. The least performed genotypes (Shandaweel3, Local122 and H102 F18-2) placed in the 4th quadrant and do not possess desirable yield traits. For the 2nd sowing date, one parent Import188 and three hybrids (H2, H 7 and H14) revealed high values for NOB, HFC, OYF, FF, SW and SYF. Under late sowing condition, higher values for variables SW, PH, FF, RWC and Oil% were presented in three

parents (Local133, Imported282 and imported188) and the hybrid H3 possessed.

Under late sowing, selection for SYF may be practice for FZ, SYP, PH, SW and NOC, as those variables showed acute angle with SY. SPAD values were the traits that represented negative correlation with SY under the three sowing dates and does not consider as a criterion for selection under varying planting dates.

**The first Sowing date (SD1)**

**The second sowing date (SD2)**

**The third sowing date (SD3)**

## **DISCUSSION**

### **Multivariate analysis of G×E interaction**

Combined analysis of variance for seed yields over 12 environments showed significant effect of environments, genotypes, and their interactions (Table 5). This result indicates the differential responses of genotypes to sowing dates and locations in both years. The large sum square for environments reveals the diversity of the tested environments that caused the most variation in sesame yield. The genotypes by environments interaction as a measure of environmental diversity was very small. That was to be expected because the locations in the study were not geographically distinct. It seems that although significant, GEI is generally of less importance than effects of genotypes and environments. Similar results were reported for seed yield of sixteen sesame genotypes and 12 environments (4 seasons and 3 location), where environment and GEI contributed 73.4% and 20.3% of the total variation, respectively (Okello-Anyanga et al., 2016). In another study carried out by Baraki . 2020 to analyze GEI in thirteen sesame genotypes under eight environments results recorded environment, genotype, and their interactions 42.6%, 6.22% and 25.1% of the total sum of squares. In contrast, variation due to genotypes explained the maximum contribution to sesame seed yield (37.4%) followed by environment (30.2%) and GEI (11.2%) (Sedeck et al., 2018).

### **AMMI analysis**

Both components showed significant effects of GEI for seed yield. IPCA1 had a higher significant contribution to GEI for seed yield (Table 6). This suggested that the other interaction principal components were not effective predictors in explaining observed GEI. Using the first two IPCAs to predict AMMI model accuracy was confirmed by many investigators (Gauch and Zobel 1996).

The AMMI model does not provide a measure of quantitative stability. But quantitative stability measure is essential in order to quantify and rank genotypes according to yield stability. For this reason, ASV was proposed by Purchase (1997). In this model, genotypes with least ASV were considered the most stable, whereas those which had highest ASV were considered unstable.

Genotypes had low values for IPC1 and high mean seed yield above the grand value, considered the most stable genotypes. While the genotypes had the highest ASV, IPC1 values and low mean yield below the grand mean value was considered the least stable genotype,. This result is confirmed by the study of Movahedi et al. (2020) who affirmed that the lowest values of ASV refer to the most stable genotypes.

### **AMMI biplot**

The AMMI graph is used to visualize the adaptability (average performance across locations) and stability (consistent performance across environments) of various genotypes. In the AMMI graph



the genotypes that are located close to the origin are relatively stable, while the ones that are located far away from the origin are unstable because they interact with the environments.

Graphical presentation of GEI using AMMI parameters is known as biplot, it is used to study the pattern of response of G, E and GEI using main effect of means vs the IPCA1 and identify genotypes with broad or specific adaptation to target environments (Amiri et al., 2013).

According to Weikai (1999), yield stability is an important issue in genotype testing and selection, but stability is meaningful for cultivar evaluation only when the genotypes are comparable in average yield. There for stability alone is meaningless, that means a less stable cultivar that performs well on average is better than a cultivar that stable and performs consistently poor.

### **Association of traits and classification of sesame genotypes using PCA**

PCA gives two important pictures of association among traits and classification of tested genotypes based on their tolerance/sensitivity to environmental conditions. Since it is not sufficient to consider one of yield-correlated traits as indicative for stress resistance, all traits together as indicators must be considered.

The cosine of the angles between vectors shows the magnitude of correlation between traits. The acute angles represent positive correlations, and the wide obtuse angles show a negative correlation. On the other hand, the length of the vectors connecting traits to the origin shows the extent of variability.

Our results showed that the total variation was contributed by the first two PCs when evaluating sesame genotypes at SD1, SD2 and SD3, respectively (Figures 2, 3 and 4). Baraki et al. (2020) extracted about seven PCs, which are with greater than a unity although they have less than 80% of the total variance. The importance and relationship between variables within a component are determined by the magnitude and direction of a factor loadings within a PC (Abate et al., 2015). Positive and high loadings values of variables are related to the diversity of sesame genotypes. Biplot of PC1 vs. PC2 should separate the tested genotypes based on differences in responses to different sowing dates and are placed in the upper-right and lower-left corner of the graph. Baraki et al. (2015) reported that G1 and G4 had the largest scores in the first PC (PC1) and located in the positive direction of this PC were highly associated with grain yield, oil content, length of capsule bearing zone and number of capsules. In study in maize by Omar et al. (2022) found that sowing date on 10 April displayed the highest agronomic performance compared to the late date on 28 May. The sowing delay negatively impacted the plant height, the number of rows ear-1, the number of kernels row-1, 100-kernel weight, and the grain yield. Although late sowing decreased the grain yield by 18.3% compared to timely sowing. Baraki et al. (2020) used the PCA for sketching biplots and to assess the association of sesame genotypes and their agronomic traits under two locations  $\times$  two seasons  $\times$  two irrigations. Accordingly, G5 is positively associated with plant height and seeds capsules -1 while G12 and G13 are associated with grain yield, capsules Plant-1, primary branches plant-1 and 100 seed weight indicating these traits may be important to develop high yielding sesame.

## **CONCLUSION**

AMMI analysis of variance for seed yield over 12 environments showed significant effect of environments, genotypes and their interactions. Sum squares due to environments explained 74.85% of the total variation for seed yield. In contrast, variation due to sesame genotypes and G $\times$ E interaction constituted less contribution (2.26 and 22.3) respectively. The AMMI analysis demonstrated the presence of significant G  $\times$  E interactions, and this has been partitioned among the first and second Interaction Principal Component Axes (IPCA). Both components showed significant effects and accounted for a total of 63.6 of GEI for seed yield IPCA1 had higher

significant contribution to GEI (41.7 %) than IPCA2 (21.9 %) for seed yield. The E2, E3, E8 and E9 had high mean seed yield above the grand mean values and is considered as suitable and favorable for sesame seed production due to its low interactive effects. The E1 and E7 also exhibited the highest mean yield above the grand value and represented high and negative interactive effects.

Sesame genotypes 7, 18, 10, 19, 12, 13, 15 were nearly close to the origin and the most stable with less responsive to the GEI. In contrast, genotypes, 1, 20, 8, 11 and 2 were far from the origin, sensitive to environmental changes and be unstable. Therefore, the sesame genotypes, 8, 13, 11 and 2 are considered the best and could be grown in wide environments.

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