Stability and G × E Analysis of Oil Yield of Sunflower Single Cross Hybrids In Diverse Environments

Mehdi Ghaffari
AREEO: Agricultural Research Education and Extension Organization

Amir Gholizadeh (a.gholizadeh@areeo.ac.ir)
Agricultural Research Education and Extension Organization

Seyyed Abbasali Andarkhor
AREEO: Agricultural Research Education and Extension Organization

Asadolah Zareei Siahbidi
AREEO: Agricultural Research Education and Extension Organization

Seyed Ahmad Kalantar Ahmadi
AREEO: Agricultural Research Education and Extension Organization

Farnaz Shariati
AREEO: Agricultural Research Education and Extension Organization

Abbas Rezaeizad
AREEO: Agricultural Research Education and Extension Organization

Research Article

Keywords: Sunflower, Genotype × environment interaction, Stability analysis, GGE biplot

DOI: https://doi.org/10.21203/rs.3.rs-648764/v1

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Abstract

Multi-environment trials have a fundamental role in selection of the best genotypes across different environments before its commercial release. This study was carried out to identify high-yielding stable sunflower genotypes using the graphical method of the GGE biplot. For this purpose, 11 new hybrids along with four cultivars were evaluated in a randomized complete block design with four replications across 8 environments (combination of years and locations) during 2018–2020 growing seasons. The results indicated that genotype (G), environment (E) and genotype × environment (G×E) effects were significant for oil yield. The G, E and G×E interaction effects accounted for 51.94, 9.50 and 18.67% of the total variation, respectively. Results of biplot analysis showed that the first and second principle components accounted 45.9% and 20.4%, respectively, and in total 66.3% of oil yield variance. GGE biplot analysis indicated two major mega-environments of sunflower testing locations in Iran. Based on the hypothetical ideal genotype biplot, the genotypes G3 and G5 were better than the other genotypes for oil yield and stability, and had the high general adaptation to all environments. Ranking of genotypes based on the ideal genotype from the most appropriate to most inappropriate genotypes is as follows: G5  G3  G8  G14  G6  G2  G13  G12  G10  G11  G1  G7  G4  G15  G9. Furthermore, ranking the environments based on the ideal environment introduced Sari location as the best environment. Therefore, the Sari location can be used as suitable test location for selecting superior genotypes of sunflower in Iran. Generally, our results showed the efficiency of the graphical method of the GGE biplot for selection of the genotypes that are stable, high yielding, and responsive.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in the world (Ghaffari et al., 2019). Sunflower seeds contains protein, minerals, vitamin and oil that are used in nutritional and pharmaceutical industries (Darvishzadeh et al., 2011). The poly-unsaturated fatty acids including linoleic acid and oleic acid are the most important fatty acids in the oil of sunflower. The poly-unsaturated fatty acids plays a fundamental role in human nutrition, and they are important for reducing cholesterol and heart disease (Gholizadeh et al., 2018). Due to the economic importance of sunflower, the developments of effective hybrids are required with superior yield and quality traits. Increasing oil yield is the most important breeding goal in sunflower. The final goal of plant breeders is the development of new commercial genotypes, which can be adapted to a wide range of diverse environmental conditions. Genotypes with both stability and high yield are recognized using growing sets of different genotypes in different environmental conditions (Luquez et al., 2002). The obtained yield for each genotype in a set of genotypes is always affected by genotypes, environment and G×E interaction (Yan and Kang, 2003). The G×E interaction effect shows yield variability unexplained by individual genotypes and environment effects, and is important for breeders (Yan and Hunt, 2001; Munda et al., 2020). A genotype that has stable yield across diverse environments contributes little to G×E interaction. To identify of the genotypes that have general and specific adaptation is required modeling the G×E interaction in multi-environment trials (METs) (Aarthi et al., 2020).
There are a numerous numerical and graphical stability methods to analyze the extent of G×E interaction and determining the both high yield and stable genotypes under varying environmental conditions. Among the graphical stability methods, the GGE biplot (genotypes main effect plus G×E interaction) has previously been used to modeling of G×E interaction in multi-environment trials (METs) in different crops (Yan et al., 2000; Gauch, 2006; Rakshit et al., 2012; Mohammadi and Amri, 2013; Hassani et al., 2018; Dallo et al., 2019; Hamidou et al., 2019; Da Cruz et al., 2020; Jia et al., 2020). The GGE biplot analysis is a very useful graphical tool because provides graphic images and effective overview of the original data and results (Yan, 2015). A GGE biplot is helpful to: (i) identify the best performing genotype in an environment, (ii) determine the discriminating ability and representativeness of environments for genotypes evaluation, (iii) identify the most appropriate environment for a given genotype, (iv) identify the interrelations between environments, and (v) compare and rank genotypes by the average yield and stability (Yan and Tinker, 2006; Malla et al., 2010; Gerrish et al., 2019).

Breeding efforts in sunflower have concentrated on production of stable genotypes with good oil yield in different environmental conditions. Sunflower is a relatively new crop in Iran and little information is available to describe genotype performance across multi-environment trials (METs). Therefore, the objectives of this study were (1) to analyze G×E interaction on oil yield of sunflower genotypes (2) to identify genotype(s) that have stable performance and high oil yield (3) determine representativeness and discriminating ability of the environments, and (4) to study the relationship among environments.

**Materials And Methods**

**Plant materials and field evaluation**

Fifteen genotypes, including 11 new hybrids along with four cultivars (Table 1) tested for oil yield in four locations with different climates for two growing seasons (2018–2019, 2019–2020). Table 2 shows more description on these locations. Randomized complete block designs with four replications were applied as experimental design for all environments (combination of years and locations). The plots consisted of three rows 0.6 m apart and 3 m in length in each genotype for all environments. Plants were harvested at maturity, and then the seed yield was record for each genotype at each test environments. The oil content was measured according to the method used by Balalić et al. (2012). Oil yield was computed multiplying seed yield by seed oil content.
Table 1
Genotypic code, name and origin of the tested sunflower genotypes.

| No | Code | Name/pedigree       | Origin | Mean oil yield (kg ha$^{-1}$) |
|----|------|---------------------|--------|------------------------------|
| 1  | G1   | RGK25×AGK330        | Iran   | 1255                         |
| 2  | G2   | RGK15×AGK376        | Iran   | 1224                         |
| 3  | G3   | RGK15×AGK370        | Iran   | 1374                         |
| 4  | G4   | RGK15×AGK358        | Iran   | 1190                         |
| 5  | G5   | RGK111×AGK32        | Iran   | 1555                         |
| 6  | G6   | RGK21×AGK2          | Iran   | 1257                         |
| 7  | G7   | RGKo54×AGKo60       | Iran   | 1138                         |
| 8  | G8   | RGK15×AGK1221       | Iran   | 1565                         |
| 9  | G9   | RGK21×AGKo42        | Iran   | 1085                         |
| 10 | G10  | RGK111×AGK78        | Iran   | 1245                         |
| 11 | G11  | RGK24×AGK370        | Iran   | 1211                         |
| 12 | G12  | Golsa               | Iran   | 1277                         |
| 13 | G13  | Ghasem              | Iran   | 1228                         |
| 14 | G14  | Shams               | Iran   | 1361                         |
| 15 | G15  | Farrokh             | Iran   | 1145                         |
Table 2
Agro-climatic characteristics of the environments studied in this research.

| Code | Location   | Cropping season | Longitude (E) | Latitude (N) | Altitude (m) | Rainfall (mm) | Mean oil yield (kg ha\(^{-1}\)) |
|------|------------|-----------------|---------------|--------------|--------------|---------------|-----------------------------|
| Krj19| Karaj      | 2018–2019       | 50° 54’       | 35° 56’      | 1312         | 300           | 1152                        |
| Krj20| Karaj      | 2019–2020       |               |              |              |               | 1578                        |
| Sa19 | Sari       | 2018–2019       | 53° 10’       | 36° 41’      | 29           | 650           | 848                         |
| Sa20 | Sari       | 2019–2020       |               |              |              |               | 833                         |
| Krm19| Kermanshah | 2018–2019       | 47° 26’       | 34° 08’      | 1346         | 468           | 1766                        |
| Krm20| Kermanshah | 2019–2020       |               |              |              |               | 1177                        |
| Dez19| Dezful     | 2018–2019       | 48° 32’       | 32° 22’      | 82           | 319           | 1485                        |
| Dez20| Dezful     | 2019–2020       |               |              |              |               | 1353                        |

Statistical analysis

Analysis of variance

Data from each environment (combination of years and locations) were first analyzed separately and then the combined analysis of variance for oil yield was performed to determine the effects of genotype, environment and G×E interaction. The combined analysis of variance was carried out with “genotype” as a fixed effect and “environment” as a random effect by General Linear Model (GLM) procedure of Statistical Analysis System. Statistical tests of significance for genotype, environment and G×E interaction effects were determined using F-tests.

GGE biplot methodology

The data were graphically analyzed using a genotype main effect plus genotype × environment interaction (GGE) biplot based on the principal component analysis (PCA) of environment-centered data (Yan et al., 2000). The GGE biplots were performed by GGE biplot software (Yan, 2001) using the first two principal components derived from singular value decomposition (SVD). The detailed description and further information on GGE biplot methodology are available in the review of Yan and Tinker (2006).
results obtained from this analysis were used to (1) identify the best performing genotype in an environment, (2) identify the most appropriate environment for a given genotype, (3) determine the discriminating ability and representativeness of environments for genotypes evaluation, (4) compare and rank genotypes by the average yield and stability, and (5) determine the interrelations between environments.

Results

Analysis of variance and partitioning of the GE interactions

The combined analysis of variance (ANOVA) indicated that oil yield was significantly affected by main effects of genotype, environment and G×E interaction (Table 3). The results of ANOVA showed that the genotype, environment and G×E interaction effects accounted for 51.94, 9.50 and 18.67% of the total variation, respectively (Table 3). The mean oil yield of environments varied from 833 kg ha\(^{-1}\) in Sa20 to 1565 kg ha\(^{-1}\) in Krm19 (Table 2) and oil yield of genotypes ranged from 1085 kg ha\(^{-1}\) in genotype G9 to 1565 kg ha\(^{-1}\) in genotype G8 (Table 1).

| Source of variation | df | Sum of squares | Mean square | %TSS\(^{a}\) |
|---------------------|----|---------------|-------------|--------------|
| Environment (E)     | 7  | 47107634      | 6729662**   | 51.94        |
| Replication/E       | 24 | 8645142       | 360214      |              |
| Genotype (G)        | 14 | 8618388       | 615599**    | 9.50         |
| G × E               | 98 | 16938390      | 172841**    | 18.67        |
| Error               | 336| 9392715       | 27955       |              |
| Total               | 479| 90702268      |             |              |

\(^{a}\)Total sum of squares.

** Significant at 0.01 probability level.

GGE biplot analysis

The biplot polygon view for grouping the genotypes and environments
The GGE biplot has a different applications. The polygon view is one of the most important applications of GGE biplot to the mega-environments identification. In the polygon view, the vertex genotype in each sector is the best genotype in the test environment(s) that falls within that particular sector. According to Fig. 1, two mega-environment identified and the vertex genotypes were G9, G12, G8, G5, G3, G14 and G1. The first mega-environment (M-I) consisted Sa19, Sa20, Krj19, Krj20 and Krm19 with winning genotypes G8 and G5 (see Table 1 and Table 2 for full genotypes and environments names). The second mega-environment (M-II) consisted Krm20, Dez19 and Dez20 with winning genotypes G3 and G14.

The biplot view for simultaneous selection of yield and stability

The mean yield versus stability view biplot (Fig. 2) was applied to assess oil yield and stability of the 15 genotypes across all test environments. In this biplot, the single-arrowed red line that passes through the biplot origin and the average environment is considered as the average environment axis or the axis of the average environment coordinate (AEC) abscissa. Furthermore, the axis of the AEC ordinate is a blue vertical double-arrow line that passes through the biplot origin and is perpendicular to the AEC abscissa. The axis of the AEC ordinate separates genotypes into two groups including genotypes with a higher mean oil yield than the overall mean at the right-hand side and genotypes with a lower mean oil yield than the overall mean at the left. Based on this, the genotypes G8, G5, G3 and G14 produced yields higher than the mean oil yield of all genotypes. Genotypes G9, G15, G7 and G4 had the lowest oil yield across test environments. Furthermore, a vector is used to connect each genotype to the average environment axis which shows the stability of each genotype. Genotypes with a short vector are stable whereas those with long vectors are not stable across all test environments. On this basis, genotypes G3, G5 and G14 were the most stable. Among the stable genotypes, G3 and G5 were the high-yielding performance across 12 test environments. Genotypes G8 were high yielding but less stable, suggesting that this genotype may be has specific adaptation to some of the environment.

The biplot view to compare the studied genotypes with the ideal genotype

Figure 3 is ranking biplot for comparison of the genotypes with the ideal genotype. This figure identifies the genotype(s) based on the concepts of high yield and stability. Based on this, the best genotype is the one that has the closest distance from the ideal genotypes (concentric circles) and the most undesired one is the genotype with the furthest distance to the ideal genotype. According to Fig. 3, genotypes G5 with minimum distance to the hypothetical ideal genotype was identified as the best genotype and genotype G9 due to its furthest distance to the hypothetical ideal genotype is identified as the most inappropriate genotype. Ranking of genotypes based on the ideal genotype from the most appropriate to most inappropriate genotypes is as follows: G5 ≈ G3 ≈ G14 ≈ G12 ≈ G6 ≈ G2 ≈ G13 ≈ G10 ≈ G11 ≈ G1 ≈ G7 ≈ G4 ≈ G15 ≈ G9.
The biplot view to compare the studied environments with the ideal environment

According to the representativeness versus discriminating power view of the GGE biplot, the discriminative ability and representativeness of test environments are shown based on the position of the vectors and the length of the vectors formed by each environment in relation to the “ideal” environment (Fig. 4). An “ideal” test environment is the environment that has the longest vector of all test environments (most discriminating) and is located on the average environment axis (AEA) (most representative). The AEA passes through the biplot origin and the point defined by the average coordinates of all test environments. Based on the representativeness versus discriminating power view of the GGE biplot (Fig. 4), the test environments can be grouped into three types. Type I consisted environments with short vectors that are little (or not) informative on the genotype difference, and hence these environments are not suitable for evaluating the genotypes. Type II environments have long vectors and small angles with the ideal environment, so are ideal for evaluating and selecting the genotypes. Type III environments have long vectors and large angles with the ideal environment, so are not ideal for evaluating the genotypes (Yan et al., 2007). The results indicated that the environments Dez19 and Dez20 is a Type I environment and should not be used for evaluating the genotypes. The environments Sa19 and Sa20 due to their high discriminating power and representativeness are ideal environments (Type II) for evaluating and selecting the genotypes (see Table 2 for full environments names).

The biplot view for displaying the relationships among the environments

The vector view of the GGE biplot was also used to examine interrelationships between environments (Fig. 5). Each environment in the biplot is connected to the origin with a line, called the vector. The correlation coefficients between environments approximate by the angle between the vectors of environments. If the angle between the vectors of the two environments is > 90° or < 90°, it indicated a negative and positive correlation, respectively. Also, if the angle is near 90°, it indicated that is no relationship. Therefore, the most prominent interrelationships were: (i) positive correlations between Dez19, Dez20 and Krm20, between Sa19 and Sa20, and between Krj19 and Krj20; and (ii) near-zero correlations between Krj20 and Dez19, and between Krj19 and Dez20 (see Table 2 for full environments names).

Discussion

A main part of vegetable oil in Iran is imported to the country, and so cultivation and breeding of oil seeds to increase yield is necessary. Due to the adaptation of sunflower to different environmental conditions, its cultivation area and production has been the center of attention in Iran. Sunflower is a relatively new crop in Iran and the improvement of oil yield is very important in breeding of this crop. The lack of
genotypes which consistently perform well across diverse growing environments is as the main problem of oil yield improvement programs in sunflower. Therefore, the development of new high-yielding hybrids with a low G×E interaction effect is one of the most important breeding objectives of sunflower. The G×E interaction effect reduces the relation between phenotypic and genotypic values and confuses the plant breeders to the selection of new improved genotypes (Ebdon and Gauch, 2002; Dia et al., 2016). There are two strategies to reducing the G×E interaction effects: (1) the sub-division of different environments into smaller, relatively homogeneous environments to develop genotypes for specific environments, and (2) identifying genotypes with high stability across a diverse range of environments (Eberhart and Russell, 1966; Tai, 1971). Multi-environment trials (MET) play a main role in selecting the best genotypes for diverse environments and even specific genotypes for specific environmental conditions.

In this study, we evaluated the stability and oil yield of 12 new hybrids along with four cultivars under the different environmental conditions in Iran. The combined ANOVA indicated that oil yield was significantly affected by genotype, environment and G×E interaction. Based on the results, environment was the major source of variability (51.94% of TSS). The environment is the sum of all external conditions affecting genotype development and growth. The test locations cover a relatively wide range of land and soil types, pH, depth, fertility, organic matter, insects, diseases, growing seasons and climatic conditions. Hence, a large variation explained by environments is due to large differences among environmental means causing most of the variation in oil yield. Previous reports in Iran also showed that the environment was the major source of the total variation in sunflower genotypes under different environmental conditions (Ullah et al., 2007; Jockovic et al., 2019). The magnitude of sum of squares for G×E interaction was higher than genotypic effect suggesting considerable differences in genotypic response of sunflower genotypes across the test environments. In other words, the responses of the sunflower genotypes changed depending on the environmental conditions. Therefore, we can estimate the adaptation patterns and stability of sunflower genotypes. In the present study, the GGE biplot methodology was used to study the stability of sunflower genotypes. This method for selecting stable genotypes was used in different crops by Jamshidmoghaddam and Pourdad (2013) in safflower, Dehghani et al. (2016) in tall fescue and Vaezi et al. (2019) in barley.

When evaluating some genotypes across several locations and years, it is often difficult to identify the pattern of genotypic response across different environments without the help of graphical display of the data. The GGE biplot analysis is a very powerful multivariate analytical method and contains a set of biplot interpretation models that graphically displays the relationships between genotypes, environments, and G×E interactions (Yan and Kang, 2003). The ‘which-won-where’ pattern polygon view is an important graphical pattern of GGE biplot for recognizing the different mega-environments and to identification of the best genotype in each mega-environment (Yan, 2001). The mega-environments have different high yielding genotypes and it shows crossover G×E interaction. The information on G×E interaction will be useful in grouping the target environment into different mega-environments and deploying different genotypes in different mega-environments (Gauch and Zobel, 1997; Yan et al., 2007). The polygon plot in this study identified two mega-environments with different winning genotypes, that shows there are specific adaptations of a genotype to a mega-environment and positive utilization of the G×E interaction.
G8 and G5 were the best specific adapted genotypes for oil yield under Sa19, Sa20, Krj19, Krj20 and Krm19. The second mega-environment consisted Krm20, Dez19 and Dez20 that G3 and G14 were identified as the best specific adapted genotypes for oil yield. Generally, based on the GGE biplot mega-environment analysis, the test environments separated into two groups of different genotypic performances. The reasons for this may be related to the amount of variation accounted for by environments. In this study, the environment contributed 51.94% of the total variation in the data. A similar trend was reported by Jamshidmoghaddam and Pourdad (2013) in saflower (*Carthamus tinctorius* L.), Hassani et al. (2018) in sugar beet (*Beta vulgaris* L.) and Vaezi et al. (2019) in barley (*Hordeum vulgare*).

According to described recommendation by Yan (2001), an ideal environment should have the most discriminating and representative abilities. In other world, an ideal test environment should be most discriminating of the genotypes and representative of all environments (Yan and Kang, 2002). Environments that have the most discriminating and representative abilities are an important in breeding programs. These types of environments can assist breeders in assessing new varieties for their full yield potential as well as be a suitable place for growing seed increase plots in preparation for the release of a variety. In this study, the representativeness versus discriminating power view of the GGE biplot indicated that the location Dezful is a Type I environment. A Type I environment, or a redundant environment should be removed to reduce the costs of field testing. The location of Sari had a high discriminating power and representativeness. Therefore, this location can be used as suitable and ideal test location (Type II environment) for selecting superior genotypes of winter sunflower in Iran. It is possible that fewer but better test locations can provide equally or more informative data for cultivar evaluation (Yan and Kang, 2003). GGE biplot analysis was also applied to identifying the interrelationships between environments. The correlation between the environments was determined by the angle between the environmental vectors. In this study, the correlations among Sari and Karaj locations were high and positively significant, suggesting that these locations are very similar. Therefore, we suggest that one of the two locations be dropped to reduce the cost of testing increase breeding efficiency in multi-environment trials (MET) of sunflower.

In this study we used the Mean versus Stability view of the GGE biplot to identification of ideal genotype(s). Based on Yan and Kang's theory, an ideal genotype should have both high mean yield and high stability across test environments (Yan and Kang, 2002). Genotypes G3 and G5 with high mean yield and high stability were identified as the ideal genotypes than other genotypes in this study. The GGE biplot method is preferred compared with the other methods to evaluate genotype, environment and their interactions. The GGE biplot method has some advantages compared with the other methods of data analysis in multi-environment trials (MET). The first advantage of this method is graphical display of data, which largely enhances our ability to understand the patterns of the data. The second is that it is the best method for recognizing the different mega-environments and to identification of the specific adaptations of a genotype to a mega-environment. The third advantage of this method is that it is an excellent tool for visual evaluation of ideal genotypes and environments, and also to studying relationship among environments. The GGE biplot method for evaluation of genotypes, environments and
their interactions was used in different crops by Rakshit et al. (2012) in sorghum, Dehghani et al. (2016) in tall fescue, Omoigui et al. (2017) in cowpea, Hassani et al. (2018) in sugar beet, Sserumaga et al. (2018) in maize, Vaezi et al. (2019) in barley, Gerrish et al. (2019) in winter wheat and Dallo´ et al. (2019) in soybean.

**Conclusion**

Generally, according to results in this study, the important results obtained could be listed follows: 1. The oil yield of sunflower is strongly influenced by the genotype, environment and G×E interaction effects. The environment and G×E interaction were the most important with 51.94 and 18.67% of the variation, respectively. 2. The studied genotypes showed both non-crossover and crossover types of G×E interaction. 3. The GGE biplot analysis showed that among the four tested locations, Sari had high discriminating power and representativeness. So, this location can be considered as ideal test locations for selecting superior genotypes of sunflower in Iran. 4. Based on our results, the genotypes G3 and G5 with high oil yield and stability are the most recommended as promising genotype for commercial release in Iran and other similar environments. 5. In the absence of the numerical method, the GGE biplot method could be a useful alternative for selecting ideal genotypes with high-yielding and stable performance in a graphical manner.

**Declarations**

**Acknowledgments**

This study was supported by grant and genetic material provision from the Seed and Plant Improvement Institute (SPII), Karaj, Iran. We would like to thank all members of the project who contributed to the implementation of the field work.

**Conflicts of interests**

The authors declare that they have no conflict of interest.

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**Figures**

![GGE Biplot](image)

**Figure 1**

‘Which-won-where’ view of the GGE biplot to show mega-environments and their winning genotypes for oil yield. Referee to Tables 1 and 2 for genotypes and environments name.
Figure 2

Biplot for simultaneous selection of oil yield and stability of the studied genotypes. Refer to Tables 1 and 2 for genotypes and environments name
Figure 3

Biplot view to compare the studied genotypes with the ideal genotype. Refer to Table 1 for environments name.
Figure 4

Biplot view to compare the studied environments with the ideal environment. Refer to Table 2 for environments name.
Figure 5

Biplot view for displaying the relationships among the studied environments. Refer to Table 2 for environments name.