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Genotype by Trait Biplot Analysis of Trait Relations in Safflower

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ABSTRACT

In the present investigation, 81 safflower genotypes were studied in a 9×9 simple lattice design for several plants per plot (NPP), plant height (PH), the height of the first lateral branch (HFL), the height of the first lateral capitulum (HFC), stem diameter (SD), number of lateral branches per plant (NLB), number of main branches per plant (NMB), number of capitula per plant (NCP), number of seeds per main capitulum (SMC), number of seeds per lateral capitulum (SLC), seed yield (SY) and thousand seed weight (TSW). The genotype by trait (G×T) interaction biplot tool was used to indicate the pattern of G×T interaction two-way interaction data in a graph with 73% description of observed variation whereas the first principal component (PC) effect explained 49%, and the second PC, 24%, of the observed interaction variability. The vector view displayed that NCP with NMB, and SMC with SY were positively associated while there was a negative association between HFC with TSW, and between NLB with NPP. The polygon-view graph is divided into eleven sectors, and the sector of genotype G80 was a winner for most traits. Genotype G58 followed by genotypes G30, G33 and G72, were the most favorable genotypes in regard to SY while regarding this trait as a reference, SMC was identified as the most related trait which is followed by SLC, SD. Applying G×T biplot to the safflower multiple trait data demonstrated that this model visually showed the associations among seed yield with the number of seeds per main and lateral capitula followed by the number of capitula per plant and thousand seed weight, and provide ease of visual genotype comparisons and choosing. We found that choose of seed yield alone was not only dependent on the number of seeds per main and lateral capitula but also related to the other traits in safflower breeding.

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1. Introduction

Safflower (*Carthamus tinctorius* L.), Asteraceae, diploid (2n=24), annual herbaceous oilseed crop, is originated from the Middle East region and cultivated thousands of years ago. The genus *Carthamus* has 25 species and is well adapted to hot and dry climate conditions of arid and semi-arid environments while only *C. tinctorius* is cultivated as a crop worldwide (Rathnakumar and Sujatha, 2022). However, safflower is a water-stress-tolerant plant and can be well adopted in rain-fed agriculture systems. Kazakhstan is the largest safflower producer (35%), following to Russian Federation (24%) and the United States (10%) in the world with the highest acreage (43%) to the Russian Federation (28%) and United States (6%) and seed yield is for the vegetable oil market (FAOSTAT, 2021). According to statistics of FAOSTAT (2021), the highest mean yield performance is belonging to the United States (5463 kg ha^{-1}), India (4647 kg ha^{-1}) and Mexico (3110 kg ha^{-1}). Safflower is cultivated on 3700 hectares in Iran, average yielding 1320 kg ha^{-1} and a quantity of 4889 tons (FAOSTAT, 2021). Regarding the global mean yield of safflower (1600 kg ha^{-1}), the performance of this crop is low (about 20% lower) in Iran, and considering the highest mean yield in the world is very low (about 75% lower), thus it must be improved through a strong breeding program.

Safflower is a good valuable crop that contains flavonoids, lignins, triterpene alcohols and polysaccharides as well as anticoagulant, antioxidant and neuroprotective (Adeleke and Babalola, 2020). It is rich in magnitudes of oleic acid as well as linoleic acid

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and is very similar to olive oil, and is considered one of the most ancient oilseed plants worldwide (Joshan *et al.*, 2019). The press cake or oil cake after industrial oil extraction has a good protein content that is used as animal feed while its seed oil yield is about 40% similar to sunflower and olive oil yield performance. Safflower is produced for many targets such as bird feed seed, medicinal plants with special uses, livestock forage, instead of saffron in foods, tea bags, and ornamental cut flowers. In most breeding programs, the sunflower germplasm is measured to identify the most important lines because the efficiency of a selection project depends on the degree of genetic diversity level. Therefore, genetic variation is evaluated by measuring some characteristics like morphological traits (Gholami *et al.*, 2018). Safflower shows high genetic variation across various environments of the world and there is only limited research assessing its genetic variation according to agronomic as well as morphological traits.

Khan et al. (2009) assessed genetic variation for 193 safflower accessions from forty countries and eight geographical regions and showed that cluster analysis as well as and principal component analysis demonstrated wide genetic variation and good potential for crop improvement from various traits. Shinwari et al. (2014) evaluated 122 safflower ascensions from different eco-geographical areas of the world and indicated that the largest variation belonged to the number of capitula per plant, number of seeds per capitulum, single plant yield, plant height, number of days to flowering initiation and days to maturity. They showed that principal component analysis displayed that the traits that differentiate the plant materials were, capitulum diameter, number of capitula per plant, number of seeds per capitulum, number of days to maturity, plant height and time of flowering initiation. Gholami et al. (2018), in sixty-four safflower genotypes investigation based on twenty morphological traits, found a relatively high variation for some traits like the wizened seeds per lateral capitulum, the weight of seeds per lateral capitulum, no of lateral branches, no of capitula per lateral branches, main capitulum diameter, the yield of a single plant, thousand seed weight and seed yield performance. They categorized these safflower genotypes into five clusters but they are not separated based on their geographical origins. This study encompassing 81 various germplasm of safflower from various agro-climatic regions of the world using some of the agronomic and morphologic traits will add good information for knowledge of safflower breeders and aid them in future projects.

2. Materials and methods

2.1. Experimental trial

In the growing season 2021, 81 safflower genotypes from various geographical regions were cultivated in an experimental field in Maragheh (37°23′N; 46°14′E), where the average rainfall is 322 mm with 12.5 °C annual temperature. It is a highland semiarid zone and the soil texture was sandy loam type with 1.6% organic matter. The trial was carried out in a 9×9 lattice with two replicates and the size of each experimental plot was 1.0 × 2.0 m^2 with 4 lines per genotype and a row width of 0.25 m. Hand drill sowing following thinning was carried out and the crop was grown under rainfed circumstances with two irrigations. Number of plants per plot (NPP), plant height (PH), the height of the first lateral branch (HFL), the height of the first lateral capitulum (HFC), stem diameter (SD), number of lateral branches per plant (NLB), number of main branches per plant (NMB), number of capitula per plant (NCP), number of seeds per main capitulum (SMC), and number of seeds per lateral capitulum (SLC) were measured from ten randomly selected plants from each plant based on safflower (*Carthamus tinctorius* L.) Standard Descriptor of IBPGR, FAO. Seed yield (SY) was recorded at maturity and corrected to 12.5% seed moisture and then thousand seed weight (TSW) was recorded using three random sub-samples of the seed yield from each plot.

2.2. Statistical analysis

The dataset was tested for normality by the Anderson and Darling normality test using Minitab version 14.0 (2005) statistical software and phenotypic linear correlation coefficients were calculated for all possible comparisons using the Pearson correlation coefficient to all the measured traits. The genotype by trait (G×T) interaction biplot model (Yan and Frégeau-Reid, 2018) was used to indicate the pattern of G×T interaction two-way interaction data in a graph. The G×T biplot pictures are generated by plotting the symmetric scaled values of the genotypes as well as traits, so that each genotype (entry) or trait (tester) is considered by a marker in each picture. For more information about the G×T biplot procedure and other two-way layouts, see Yan and Frégeau-Reid (2018). The routine statistical analyses were performed via IBM-SPSS version 23.0 (IBM-SPSS, 2015) and G×T interaction biplot pictures were produced by GGEbiplot (Yan, 2001).

3. Results and discussion

The genotype by trait (G×T) interaction biplot model indicates that the first principal component effect explained 49%, and the second PC, 24%, of the observed mean squares. The G×T biplot model can evaluate and explore the nature and structure of genotype by trait interaction effect, effectively. The results of the analysis of variance is a perquisite option to decide whether a trait has to proceed for t the G×T biplot model or not (Yan *et al*., 2011), thus in case of significant differences between the interaction of main factors i.e. genotypes and traits, one should go for the G×T biplot model while in case of non-significant interaction use of the G×T biplot model is of no use. Analysis of variance for various traits of safflower (results are not shown) verified to proceed for the G×T biplot model as there were huge variations among genotypes and trait effects for all safflower genotypes. In the vector view of the G×T biplot model (Fig. 1A), two traits are associated positively if the angle between their vectors is less than 90°, associated negatively if the angle is more than 90°, independent if the angle is 90°, whereas, traits with longer vectors are significant and more responsive while traits with shorter ones are less significant and responsive as well as those located at the graph origin are not correlated with the other traits or not-responsive (Yan and Frégeau -Reid, 2018). Fig. 1A shows that the number of capitula per plant (NCP) and the number of main branches per plant (NMB) were positively correlated as shown by the acute angles between vectors. Also, the number of seeds per main capitulum (SMC) and seed yield (SY) were positively correlated as shown by the small acute angles between their vectors. On the other hand, there was a negative association between the height of the first lateral capitulum (HFC) with thousand seeds weight (TSW), and between the number of lateral branches per plant (NLB) with the number of plants per plot (NPP) as shown by the large obtuse angles between their vectors (Fig. 1A). According to Cerrotta et al. (2020), the number of branches per plant, number of capitula per plant and thousand seed weight are the most important traits because their direct association with seed yield has been improved while the positive effect of height of the first lateral capitulum and number of lateral branches per plant have not identified. Also, there was a near zero association between TSW with NPP, between HFC with NPP, and between PH with NLB because of their near perpendicular vectors. Most of the above results are well consistent with those reported by Pearson’s correlation matrix (Table 1), whereas the predictions of the G×T biplot model for traits’ associations can be confirmed from this matrix but some are not consistent because the model described only 73% rather than 100% of the observed mean squares.

Among different options of the G×T biplot model, the polygon view provides detection of the best genotypes in one or more traits and helps to find the best way for the graphic interpretation of the G×T interaction statures among traits and genotypes. For this purpose, the farthest genotypes from the graph origin are lined with straight lines. The vertex entries (genotypes) are the most responsive ones as the best or the poorest in some or all of the measured traits while the perpendicular lines are drawn from the graph origin for categorizing the graph into several sections, each having a special vertex entry. In the G×T biplot model, the polygon-view graph (Fig. 1B) is divided into eleven sectors, and the sector contains genotype G80 on the polygon vertex winner for most traits consisting of the number of lateral branches per plant (NLB), number of main branches per plant (NMB), number of capitula per plant (NCP), number of seeds per main capitulum (SMC), stem diameter (SD), seed yield (SY). While genotype G33 is situated on the vertex of another sector with height-related traits as plant height (PH), the height of the first lateral branch (HFL) and the height of the first lateral capitulum (HFC) (Fig. 1B). Genotype G3 is situated on the vertex of another sector with number of plants per plot (NPP) while genotype G63 is situated on the vertex of another sector with thousand seeds weight (TSW), and genotype G58 is situated on the vertex of another sector with number of seeds per lateral capitulum (SLC). However, it seems that G58 we the best genotype based on the most of measured traits. The identified vertex genotypes in each section gave the highest performance for the traits that fall within that section while the other vertex genotypes did not give the highest performance in any of the traits and were the poorest genotypes in some or all of the traits.

Fig. 2A indicates the location of an ideal entry, which is determined by a projection onto the section axis that is equivalent to the largest vector of the entries that had greater performance than average and by a low projection onto the vertical axis while the best genotype is near this location. Thus, genotypes G72 and G30 following to genotypes G58 and G33 were more desirable than other genotypes while the low desirable genotypes were G10 and G21 because they were far away from the position of ideal entry. An ideal tester can detect the genotypes and representative of the main goal trait and regarding the testers or traits are representative items of the main goal trait, the ideal tester should be on the mean traits coordinate axis. The center of the circles shows the ideal tester, which has the largest vector of the traits that had positive projections onto the mean traits coordinate axis (Fig. 2B). A measured trait is preferable if it is located near the ideal tester, therefore, number of seeds per lateral capitulum (SLC) was desirable trait followed by the number of seeds per main capitulum (SMC) and seed yield (SY). In the next step, plant height (PH) and stem diameter (SD) were relatively desirable traits, whereas thousand seeds weight (TSW) and number of plants per plot (NPP), were relatively undesirable traits.

The performance of different safflower genotypes in the seed yield (SY) as an economic target trait is compared in Fig. 3A, where relatively a horizontal line that passes through the graph origin and the selected trait, which is identified as the trait axis and its surrounding oval shows the positive direction of this axis. Thus genotype G58 followed by genotypes G30, G33 and G72, was the most favorable genotype regarding SY while genotypes G10 and G25 were the poorest in the selected trait (Fig. 3A).. For better evaluation of the seed yield (SY), it can be used as a reference for traits evaluation (Fig. 3B), and the most related trait was identified as the number of seeds per main capitulum (SMC). Following SMC, the number of seeds per lateral capitulum (SLC) and stem diameter (SD) was associated with seed yield (SY). In the next step, the number of capitula per plant (NCP), number of main branches per plant (NMB), number of lateral branches per plant (NLB) and plant height (PH) were identified for association with seed yield. However, the most unaltered traits of SY were the height of the first lateral branch (HFL), thousand seeds weight (TSW), and number of plants per plot (NPP) followed by the height of the first lateral capitulum (HFC) (Fig. 3B). For seed yield breeding, it is better to use a number of seeds per main capitulum (SMC), number of seeds per lateral capitulum (SLC) and stem diameter (SD) can be considered.

We found a highly significant association between seed yield and with number of seeds per main and lateral capitula as well as the number of capitula per plant which are in agreement with the report of Gholami et al. (2018), who recorded that number of seeds per capitulum had highly significant positive contribution with seed yield performance. To improve yield, the number of seeds per capitulum and the number of capitula per plant are important traits following to thousand seeds weight based on the report of Pattar and Patil (2020). The higher seed production of safflower was mostly associated with the number of capitula per plant and the number of seeds per capitulum (Koç, 2021). In our study high branching and more capitula or more seeds per capitulum were found as the most influencing traits on yield performance. However, it seems that for improving seed yield performance in safflower, it is better to focus on more number of seeds per capitulum and then try to increase the number of capitula per plant and thousand seeds weight. Also, we found genotype G80 following genotype G58 as the best for most traits especially the number of main and lateral branches, number of capitula per plant, number of seeds per capitulum and seed yield while genotype G63 is a good source for high thousand seeds weight. We found that seed yield, number of seeds per main and lateral capitula and plant height had remarkable discriminant ability as well as good representative potential according to ideal trait biplot information. Thus, the mentioned traits had both characteristics and can be used for the deification of genotypes reliably on a large scale, because they had acceptable representatives of all traits and could discriminate genotypes in a good way (Golkar *et al.*, 2021).

Although the genotype main effect (G) plus interaction effect of genotype by environment (G×E), GGE, biplot methodology was originally used for multi-environment trials interpretation, it applies to two-way type as an entry by tester pattern, such as a genotype by trait (G×T) two-way matrix. The main difference between the two above procedures is that in GT data, various traits have different units, so standardization is needed before analysis. Successful safflower genotypes need to be improved based on effective selection indices in order to ensure their yield performance and economic profitability. The G×T biplot method is an excellent procedure for graphic analysis. Compared with common models, the G×T biplot method has some benefits (Yan and Frégeau-Reid, 2018; Ghaffari *et al.*, 2022), including graphical presentation, increased breeders' ability to understand the data pattern, interpretative potential of the G×T biplot method, facilitating pair-wise genotype comparisons, ranking genotypes based on the target trait, and identification of possible groups of genotypes as well as traits.

Table 1. Pearson correlation coefficients among safflower traits.

	NPP	PH	HFL	HFC	SD	NLB	NMB	NCP	SMC	SLC	TSW
PH	-0.26										
HFL	0.57	0.26									
HFC	0.09	0.79	0.67								
SD	-0.60	0.74	-0.28	0.31							
NLB	-0.60	0.37	-0.58	-0.08	0.67						
NMB	-0.52	0.44	-0.42	0.08	0.66	0.48					
NCP	-0.60	0.51	-0.47	0.09	0.77	0.70	0.76				
SMC	-0.43	0.65	-0.18	0.31	0.71	0.52	0.68	0.78			
SLC	-0.21	0.64	0.00	0.41	0.58	0.29	0.68	0.54	0.78		
TSW	-0.18	-0.39	-0.38	-0.46	-0.06	0.00	0.03	-0.05	-0.34	-0.36	
SY	-0.08	0.47	-0.13	0.23	0.52	0.32	0.77	0.62	0.73	0.79	-0.03

Critical correlation values P<0.05, degrees of freedom=79 and P<0.01 are 0.22 and 0.28, respectively.

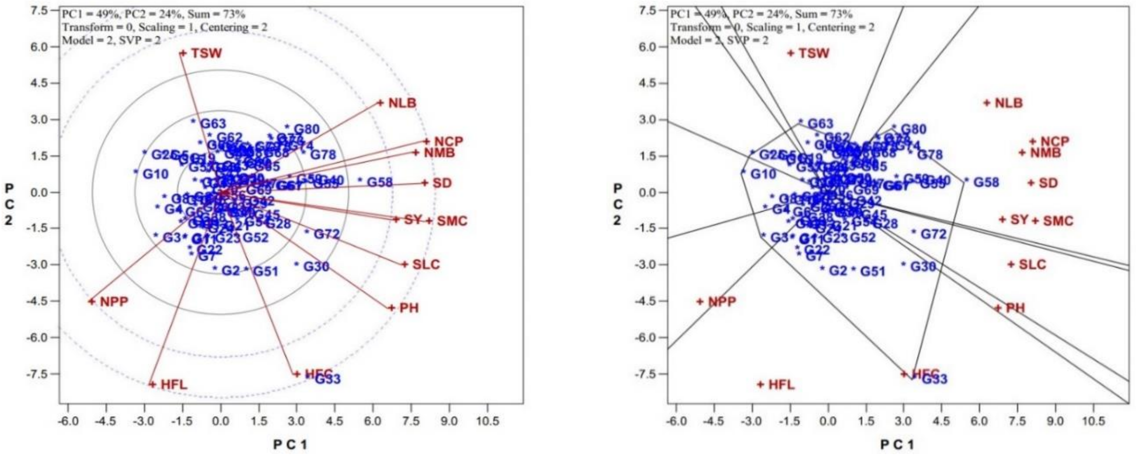

Figure 1. (A) Vector-view of G×T biplot and (B) Polygon-view of G×T biplot indicating the which-won-where pattern for safflower genotypes.

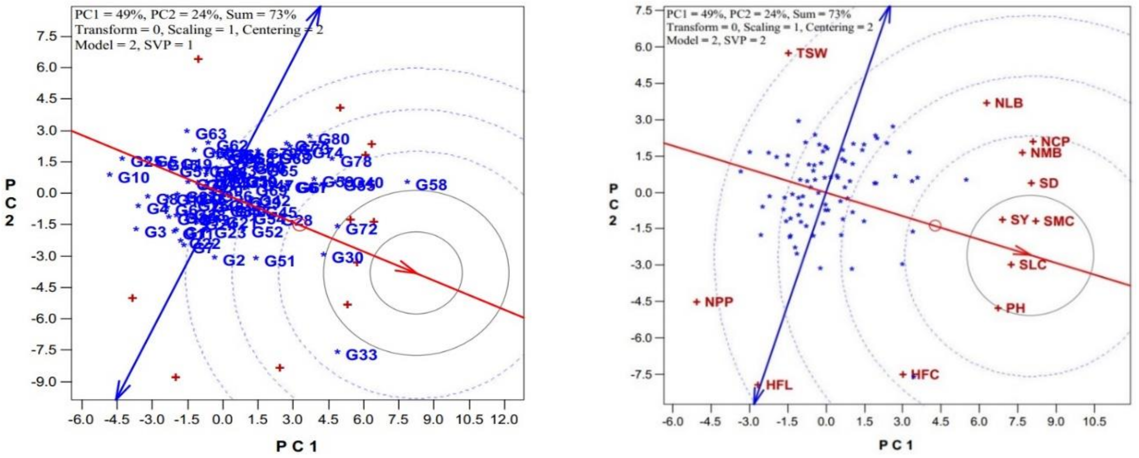

Figure 2. (A) Ideal entry of G×T biplot and (B) Ideal tester of G×T biplot for safflower genotypes.

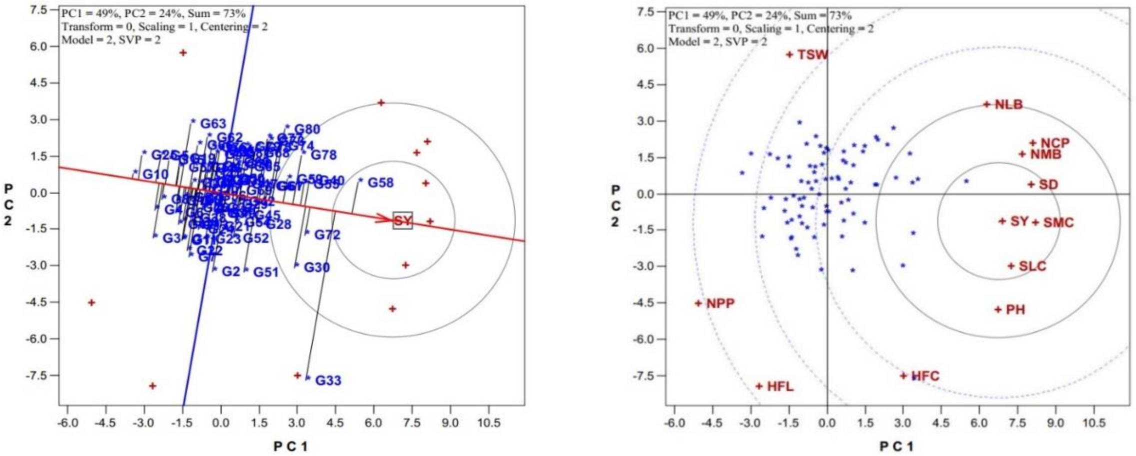

Figure 3. (A) Ranking of genotypes based on seed yield (SY) and (B) ranking of traits based on seed yield (SY) in G×T biplot.

Abbreviation

G×T, genotype by trait; NPP, number of plants per plot; PH, plant height; HFL, height of the first lateral branch; HFC, height of the first lateral capitulum; SD, stem diameter; NLB, number of lateral branches per plant; NMB, number of main branches per plant; NCP, number of capitula per plant; SMC, number of seeds per main capitulum; SLC, number of seeds per lateral capitulum; SY, seed yield; and TSW, thousand seed weight.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

Hossein Ebrahimi performed the experiment and measured traits, Naser Sabaghnia designed, analyzed and wrote the manuscript, Abdollah Javanmard and Amin Abbasi read and checked manuscript.

Informed Consent

The authors declare not to use any patients in this research.

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