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Biplot analysis of trait relations of some safflower (*Carthamus tinctorius* L.) genotypes in Iran

RAMIN BALJANI, FARIBORZ SHEKARI* AND NASER SABAGHNIA

Department of Agronomy and Plant Breeding
Faculty of Agriculture, University of Maragheh, P.O. Box 55181-83111, Iran
**(e-mail : Shekari_Fb@yahoo.com)*

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ABSTRACT

Safflower (*Carthamus tinctorius* L.) is one of the major oilseed crops worldwide and its genetic improvement for oil yield is very important in breeding of this crop. Effective analysis of obtained dataset from breeding programmes is important at all stages of plant improvement. The genotype by trait (GT) biplot was used for two-way safflower dataset as various genotypes with multiple traits. For this propose, 64 safflower genotypes with specific characteristics were tested. The GT biplot explained 79% of the total variation of the standardized data. The polygon view of GT showed sevenvertex genotypes as 34, 43, 45, 24, 61, 58 and 59. Genotype 34 had the highest values for all of the measured traits except plant height, total biomass and 1000-seed weight. The genotype 58 and the other genotypes of this sector had good amounts of 1000-seed weight, while the vertex genotype 24 and its related genotypes which fall in its sector were good for plant height. Generally, based on vector view, ideal genotype and ideal tester biplots, it was demonstrated that the selection of high seed yield will be performed via number of seeds per capitulum, harvest index, main capitulum diameter, lateral capitulum diameter and number of capitulum per plant. Also, for genetic improvement of oil yield, traits seed yield and harvest index must be considered. These traits should be considered simultaneously as effective selection criteria evolving high seed or oil yielding safflower genotypes because of their large contribution to these traits. The genotypes 34, 42, 43 and 60 could be considered for developing desirable progenies in selection strategy of safflower improvement programmes. Such a similar outcome could be applied in the future to delineate predictive, more rigorous selection strategies as well as to help define breeding concepts for improvement of new safflower cultivars and other crops in the other areas of the world.

Key words : Biplot analysis, *Carthamus tinctorius* L., genotype, safflower, trait

INTRODUCTION

Oilseed crops are the main sources of edible oils and due to increasing demand for edible oils, oilseed crops production needs to be increased via increasing the seed yield potential per unit as well as increasing the cultivation areas. Safflower (*Carthamus tinctorius* L.) as an important oilseed crop which is cultivated mainly for its seeds, which have been used as edible oil and dying or is grown for its flowers, used for colouring and flavouring foods. It was cultivated in 783,000 hectares in the world and 647,000 Mt seed yield was obtained from this area in 2013 (FAOSTAT, 2013). According to Food and Agriculture Organization database, India is the main producer of safflower in the world (25%), but its average yield is relatively low (727 kg/ha) in comparison to world average yield performance (827 kg/ha). Safflower is cultivated in 810 ha in Iran and its average yield is 691 kg/ha (FAOSTAT, 2013).Iranian farmers currently use landraces and local cultivars, which are adapted to local rainfed conditions. The yield performance of these genotypes is very low compared with the highest global yields (1381 kg/ha, produced in United States; FAOSTAT, 2013) and so Iran has started an important safflower breeding programme in recent years.

Increasingthe genetic potential of yield is an importantobjective of safflower breeding programmes in Iran and other areas of the world. The improved safflower genotypes are evaluatedto test their performance and to select the best genotypes in specific traits (Tuncturk and Ciftci, 2004). Some traits including plant

height, first branch height, number of lateral branches, main capitulumdiameter, number of seeds per capitulum, 1000-seed weight andoil content have been reported as the most important traits in genetic improvement of safflower seed yield (Kavani *et al.,* 2001; Bidgoli *et al.,* 2006). The success of safflower breeding will largely depend on the extent of improvement made in both its seed yield and oil content. Yield components not only affect the seed yield, but also influence other yield component traits (Mozaffari and Asadi, 2006; Roopa and Ravikumar, 2008). The ultimate goal of the most breeders is to improve the productivity of crops as measured in terms of the yield performance or oil content (Camas and Esendal, 2006; Ghosh *et al.,* 2010). Seed yield and oil content are very complex traits, and can be dissected into various agronomic component traits. Omidi-Tabrizi (2001) found high association between seed yield and seed number per capitulum, 1000-seed weight and oil content in new spring safflower genotypes and concluded that increasing oil yield was associated with increasing seed yield, which was affected by biomass and number of capitulum per plant.

The primary target of breeding programmes is to detect the most favourable genotypes evaluated on the basis of multiple traits and effective interpretation of the obtained data is very important at selection based on traits. An understanding of the causes of genotype (G) × trait (T) interaction (GT) can help identify the most favourable genotypes and their selection. Numerous models have been used in the investigation for a grasp of the causes of interactions, although strategies may differ in overall appropriateness (Flores *et al.,* 1998). Yan *et al.* (2000) combined G main effect and genotype × environment (GE) interaction effect, suggested a GGE biplot, constructed from the first two principal components (PC 1 and PC 2) using a site regression model. The GGE biplot was developed for analyzing multi environment trial data but it can be equally used for all types of two-way data that assume an entry × tester structure (Yen, 2001). The genotypes can be generalized as entries and the traits as testers but, for genotype evaluation, both G and GT must be considered simultaneously. Yan andRajcan (2002) used a genotype × trait (GT) biplot, which is an application of the GGE biplot to investigate the GT data of soybean traits in Canada. Also, Rubio *et al.* (2004) used the same method for analysis of trait relations of white lupin in Spain. The mentioned studies demonstrated that the GT biplot was an excellent graphical tool for visualizing GT data and understanding data structure.

The objective of this research was to explain genotype evaluation on the basis of multiple traits using the GT biplotin order to(i) reveal the interrelationship among safflower traits, (ii) compare among different safflower genotypes on the basis of multiple traits and (iii) recommend possible selection strategies for genetic improvement of safflower.

MATERIALS AND METHODS

Sixty-four safflower genotypes were provided by the 'Genetics and Plant Genetic Resources Research Department" of Iran's Seed and Plant Improvement Institute. Field experiment was established at Experimental Farm of University of Maragheh (37°23'N 46°14'E) in year 2010-11. The experimental design was an 8 × 8 simple lattice design with two replicates. Each plot (2.5 × 4.0 m^2) comprising five rows was 4 m long and 0.5 m apart and distance between plants within rows was 10 cm. Therefore, plant density was 200,000 plants/ha. Plots were over planted and thinned to a distance between plants in the row of 10 cm for an established plant density of 20 plants/m^2. In spring 2011, the trial was irrigated based on 150 mm evaporation from pan class A and total amount of precipitation in agronomic season was 165 mm. The soil type was clay loam with low organic matter (2%) and organic nitrogen content (0.05%). Nitrogen fertilizer hand applied across all plots was applied prior to ploughing at the recommended rate (200 kg/ha ammonium phosphate). Weeds were controlled by hand as needed.

Some traits were examined on 20 plants randomly selected in the mid-rows of plots which were leaf area (LA), dry weight of plant (DWP), plant height (PH), main capitulum diameter (MCD), lateral capitulum diameter (LCD), number of capitulum per plant (NCP) and number of seeds per capitulum (NSC). The 1000-seed weight (TSW) and oil per cent (OP) were measured on a sub-sample of seed harvested from each plot. The area harvested was 4.5 m^2. Only the middle three rows were

harvested and weighed to determine biological yield. Seed yield (SY) was measured at physiological maturity and yield was adjusted to 12.5% seed moisture content. Harvest index (HI) and oil yield (OY) were calculated from the biological yield, oil content and seed yield.

Because the traits of safflower were measured in different units, the biplots were generated using the standardized values of the trait averages using GGE biplot software (Yan, 2001). The biplot analysis was according to Model 2 (Transform=0) within-trait standard deviation standardized (Scale=1), and trait-centred (Centering=2). The polygon views were based on genotype-focused singular value partitioning (SVP=2), while the vector views were based on the trait-focused singular value partitioning and were, therefore, appropriate for visualizing the relationships among traits and genotypes. The genotype by trait (GT) biplot model equation (Yan and Rajcan, 2002) for genotype × trait interaction biplot analysis is presented as follows :

$$\frac{\alpha_{ij} - \beta_j}{\sigma_j} = \sum_{n=1}^{2} \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij} = \sum_{n=1}^{2} \xi^*_{in} \eta^*_{jn} + \varepsilon_{ij}$$

Where, α_{ij} is the mean value of landrace i for trait j, β_j is the mean value of all landraces in trait j, σ_{ij} is the standard deviation of trait j among the landrace means, λ_n is the singular value for principal component n (PCn), ξ_{in} and η_{in} are scores for landrace i and trait j on PCn, respectively, and ε_{ij} is the residual associated with landrace i in trait j. To achieve symmetric scaling between the landrace scores and the trait scores the singular value λ_n has to be absorbed by the singular vector for landraces ξ_{in} and that for traits η_{jn}. That is, $\xi^*_{in}=\xi^*\lambda_n^{0.5}$ and $\eta^*_{jn}=\eta_{jn}\lambda_n^{0.5}$. Because of n=2, only PC 1 and PC 2 are retained in the model and such a model tends to be the best for extracting pattern and rejecting noise from the data.

RESULTS AND DISCUSSION

The GT biplot for wheat dataset, based on Model 2 of Yan and Rajcan's (2002) method, explained 79% of the total variation of the standardized data. This relatively high percentage reflects the complexity of the relationships among the measured traits of safflower. The first two principal components (PC 1 and PC 2) explained 71 and 8%, respectively. In the GT biplot, a vector is drawn from the biplot origin to each marker of the genotype to facilitate visualization of the relationships between and among the traits as well as genotypes. Based on Kroonenberg (1995), Rubio *et al.* (2004) and Sabaghnia and Janmohammadi (2014b), the basic structure among the traits should be captured by the biplots for better interpretations. The polygon view of the GT biplot aids identifies genotypes with the highest values for one or more traits. For this target, the genotypes that are connected with straight lines so that a polygon is formed with all other traits contained within the polygon.

The biplot of Fig. 1a presents data of 64 safflower genotypes in 13 different traits and this following information can be grasped : The vertex genotypes in this investigation are genotypes 34, 43, 45, 24, 61, 58 and 59. These genotypes are the best or the poorest genotypes in some or all of the traits since they had the longest distance from the origin of biplot. Therefore, it seems that 34 had the highest values for all of the measured traits except PH, TB and TSW. The genotype 58 and the other genotypes of this sector had good amounts of TSW. The vertex genotype 24 and its related genotypes which fall in its sector were good for PH. The vertex genotype 45 and its related genotypes which fall in its sector were good for TB. The other vertex genotypes (43, 61 and 59) and related sectors did not have good performance for the measured traits (Fig. 1a). The vertex genotypes and their related genotypes of them which are located in different seven sections of biplot were good candidates for examination heterosis (Yan *et al.,* 2007) for hybrid production in wheat using these genotypes.

Provided that the biplot model described a sufficient amount of the total variation (in this case, 79%), the correlation coefficient between any two traits was approximated by the cosine of the angle between their vectors (Yan and Rajcan, 2002). The correlation coefficients among the traits indicate that the biplot currently indicates relationship among the traits that had relatively large loading on both PC 1 and PC 2. Therefore, the most prominent relations by this Fig. 1b were : a strong positive association amongOY, HI and LA; and among NSC, SY, OP, MCD and NCP; a moderate positive association between PH and TB; between LCD and DWP; and DWP with OY,

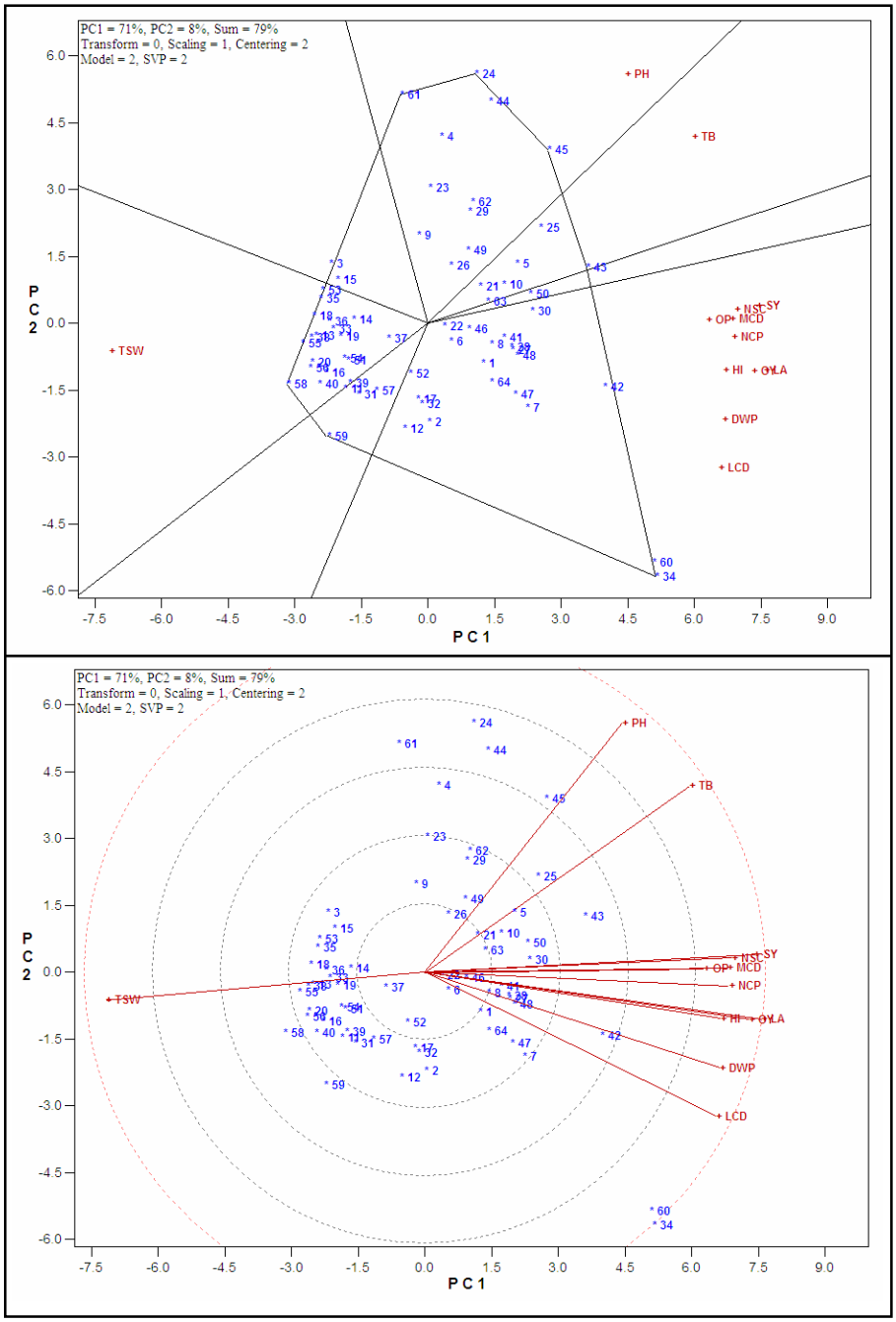


Fig. 1a. (Up) : Polygon view of genotype by trait biplot showing which genotype had the highest values for which traits and 1b. (Down) : Vector view of genotype by trait biplot, showing the interrelationship among all measured traits for 64 safflower genotypes.

HI and LA as indicated by the small obtuse angles between their vectors (r=cos 0=+1). There was a near zero correlation between PH and LCD, between PH and DWP, and between TB and LCD (Fig. 1b) as indicated by the near perpendicular vectors (r=cos90=0). There was a negative correlation between TSW with NSC, SY, OP, MCD and NCP traits (Fig. 1b) as indicated by the near an angle of approximately 180 degrees (r=cos180=-1). Most discrepancies of the biplot predictions and original data (Table 2) were expected because the biplot accounted for <100% of the total variation (79%). The statistical properties of this method have been described in detail by Yan *et al.* (2000) and Yan and Rajcan (2002).

Similar to Fig. 1b, the vector view of biplot was used to estimate the correlation coefficient among traits, the angle between the vectors of safflower genotypes was used to understand the relationships among genotypes (Fig. 2a) and the most prominent relationships of this figure were : a near zero correlation between genotypes 34 and 60 as indicated by the near perpendicular vectors (r=cos90=0). There was a negative correlation between genotypes 43 and 58, as indicated by the near an angle of approximately 180 degrees (r=cos180=-1). Most of the mentioned results can be obtained for the other safflower genotypes and most of them using original correlation coefficients. It is clear that the

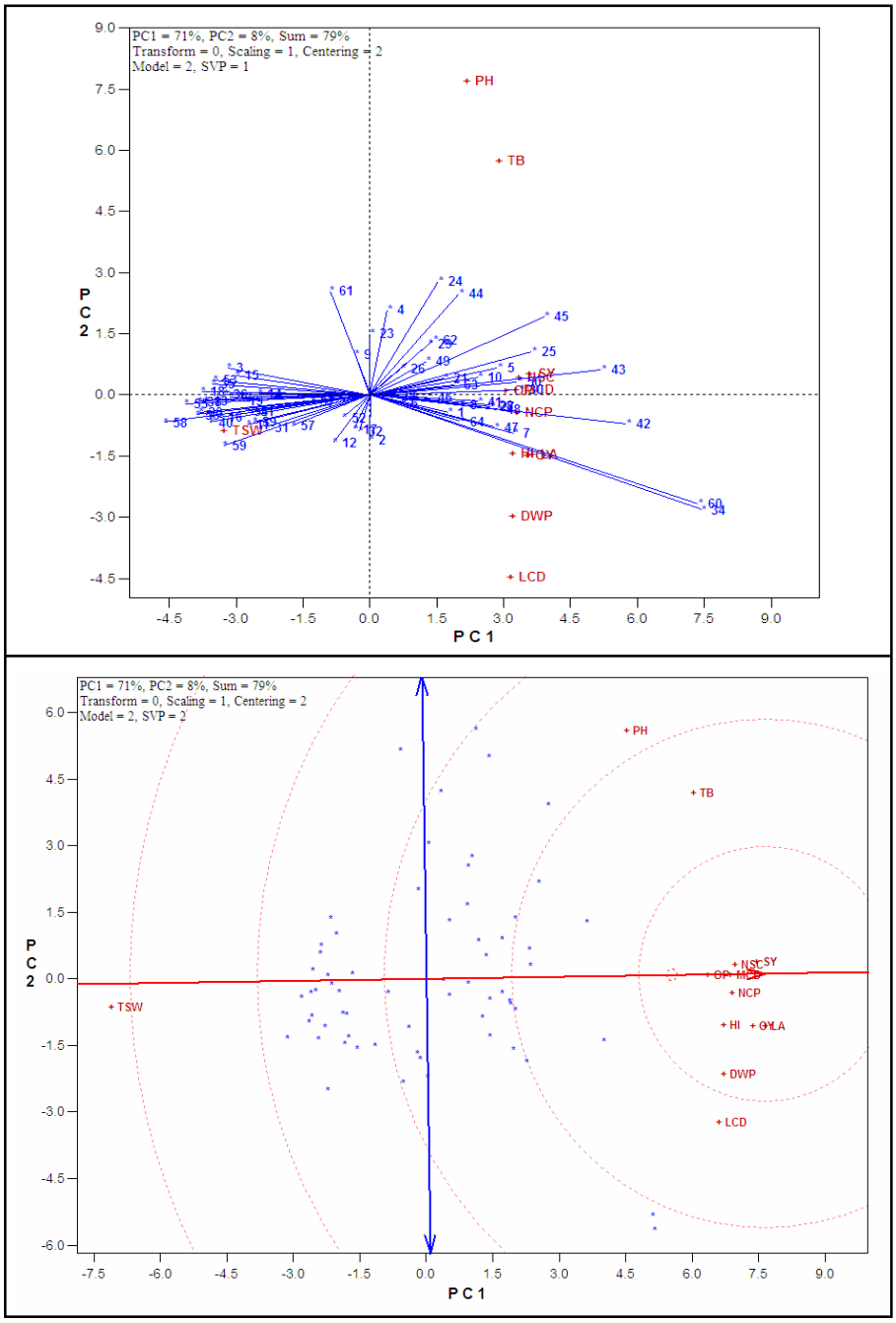


Fig. 2a. (Up): Vector view of genotype by trait biplot, showing the interrelationship among all genotypes traits for 13 measured traits and 2b. (Down) : Ideal tester view of genotype by trait biplot, showing the relationships of traits of 18 measured traits.

Table 1. Name and origin of 64 safflower genotypes

No.	Name	Origin	No.	Name	Origin	No.	Name	Origin	No.	Name	Origin
1	-	-	17	376	-	33	Isfahan-4	Iran	49	PI-506426	China
2	-	-	18	C4130	Iran	34	Isfahan-1	Iran	50	44	-
3	Zarghan	Iran	19	Yimice	Turkey	35	Kordestan-6	Iran	51	62	-
4	12-2-14-32		20	Syrian	Syria	36	C115	Iran	52	17	-
5	Dincer	Turkey	21	Kordestan-4	Iran	37	21	-	53	PI-250537	Egypt
6	-	-	22	508	-	38	324-S6-697	-	54	Kordestan-7	Iran
7	c111	Iran	23	Bregon	Cyprus	39	C441	Iran	55	CW-4440	United States
8	-	-	24	LRV-51-51	Iran	40	60	-	56	168-S6-5841	-
9	27/N	-	25	Kimo-76	Mexico	41	Kordestan-7	Iran	57	Kordestan-1	Iran
10	C444	Iran	26	Gila	United States	42	S-541	United States	58	HARTMAN	United States
11	PI-405985	Iran	27	PI-250190	Pakistan	43	40	-	59		-
12	46	-	28	47	-	44	PI-253384	Palestine	60	Kordestan-8	Iran
13	PI-258417	Portugal	29	PI-9884	France	45	Kordestan-3	Iran	61	Sina	Iran
14	Kordestan-9	Iran	30	Marand	Iran	46	301055	Turkey	62	IRV-55-295	-
15	220	-	31	PI-537636-S	United States	47	38	-	63	ARAK	Iran
16	Kordestan-5	Iran	32	PI-537636-S	United States	48	IL	Iran	64	307-S6-697	-

Table 2. Simple correlation coefficient between measured traits of safflower

	LA+	DWP	PH	MCD	LCD	NCP	NSC	TSW	TB	SY	OP	HI
DWP	0.84*											
PH	0.45	0.27										
MCD	0.81	0.74	0.49									
LCD	0.84	0.79	0.25	0.72								
NCP	0.82	0.81	0.41	0.69	0.75							
NSC	0.84	0.63	0.51	0.69	0.65	0.59						
TSW	-0.83	-0.79	-0.53	-0.74	-0.69	-0.87	-0.72					
TB	0.65	0.55	0.65	0.68	0.41	0.71	0.61	-0.74				
SY	0.86	0.64	0.58	0.75	0.73	0.73	0.87	-0.79	0.67			
OP	0.77	0.77	0.32	0.70	0.58	0.70	0.64	-0.72	0.72	0.61		
HI	0.78	0.55	0.46	0.63	0.72	0.60	0.83	-0.69	0.42	0.95	0.47	
OY	0.88	0.69	0.46	0.73	0.77	0.70	0.88	-0.76	0.53	0.97	0.65	0.97

+For trait abbreviations refer to text.
*Critical values of correlation at P<0.05 and P<0.01 (D. F. 62) are 0.25 and 0.32, respectively.

studied genotypes were completely different from each other and showed remarkable variation due to different geographical origins (Table 1) and thus are good genetic sources for genetic improvement of safflower.

In the context of GT biplot analysis, an ideal tester of trait has been defined as the tester that combines several good genotypes in its genetic composition (Dehghani *et al.,* 2008). According to Fig. 2b, the ideal trait was strongly related to NSC, SY, OP, MCD and NCP traits followed by OY, LA, HI and DWP traits. It is interesting that the seed yield existed in these traits. Therefore, these important traits must be regarded in the safflower breeding programmes and determination of selection indices. On the other hand, the studied genotypes were good genetic resources for improving economic and commercial traits (SY, OY and OP). In the ideal entry view of biplot, an ideal genotype has been determined as the genotype that combines several good traits in its genetic composition (Badu-Apraku and Akinwale, 2011). Based on Fig. 3a, the closest genotypes to ideal genotype position were genotypes 42 and 43 followed by 60, 34, 45 and 25. After these genotypes, 50 and 30 could be regarded as the most favourable genotypes based on the ideal genotype (Fig. 3a).

Due to large importance of seed yield in safflower improvement programmes, this trait was compared in the studied genotypes and the relation of seed yield with yield component traits was visually displayed (Fig. 3b). In the context of GT biplot, the SY was evaluated regarding yield component traits (LCD, NCP, MCD, NSC and PH). The most related traits in this context were number of seeds per capitulum (NSC) followed by main capitulum diameter (MCD) and number of capitulum per plant (NCP). The importance of these traits especially number of seeds per capitulum and number of capitulum per plant traits in safflower breeding programmes was reported in the other investigations (Kavani *et al.,* 2001; Bidgoli *et al.,* 2006).

Similarly, due to importance of oil yield in safflower improvement programmes and regarding the role of two other traits (seed yield and oil per cent) on it, this trait was compared in the studied genotypes and the relation of oil yield with some important traits was visually displayed (Fig. 4a). In the context of GT biplot, the SY was evaluated regarding yield component traits (OP, LCD, NCP, MCD, NSC, SY, HI, TSW and PH). The most related traits in this context were number of seeds per capitulum (NSC), seed yield (SY) and harvest index (HI) followed by main capitulum diameter (MCD). In the second step, lateral capitulum diameter (LCD), number of capitulum per plant (NCP), oil per cent (OP) and plant height (PH) can be regarded. Therefore, it seems that increasing of oil yield potential in safflower is most related to seed yield and less related to oil per cent and emphasis of breeders on genetic improvement of seed yield is more responsive in selection efforts.

Due to economic value of oil yield characteristic which is generated by two other important traits (seed yield and oil per cent) in safflower improvement programmes, this trait was compared in the studied genotypes and the relation of grain yield with genotypes was visually displayed (Fig.4b). In the context of GT biplot, the OY was determined and the

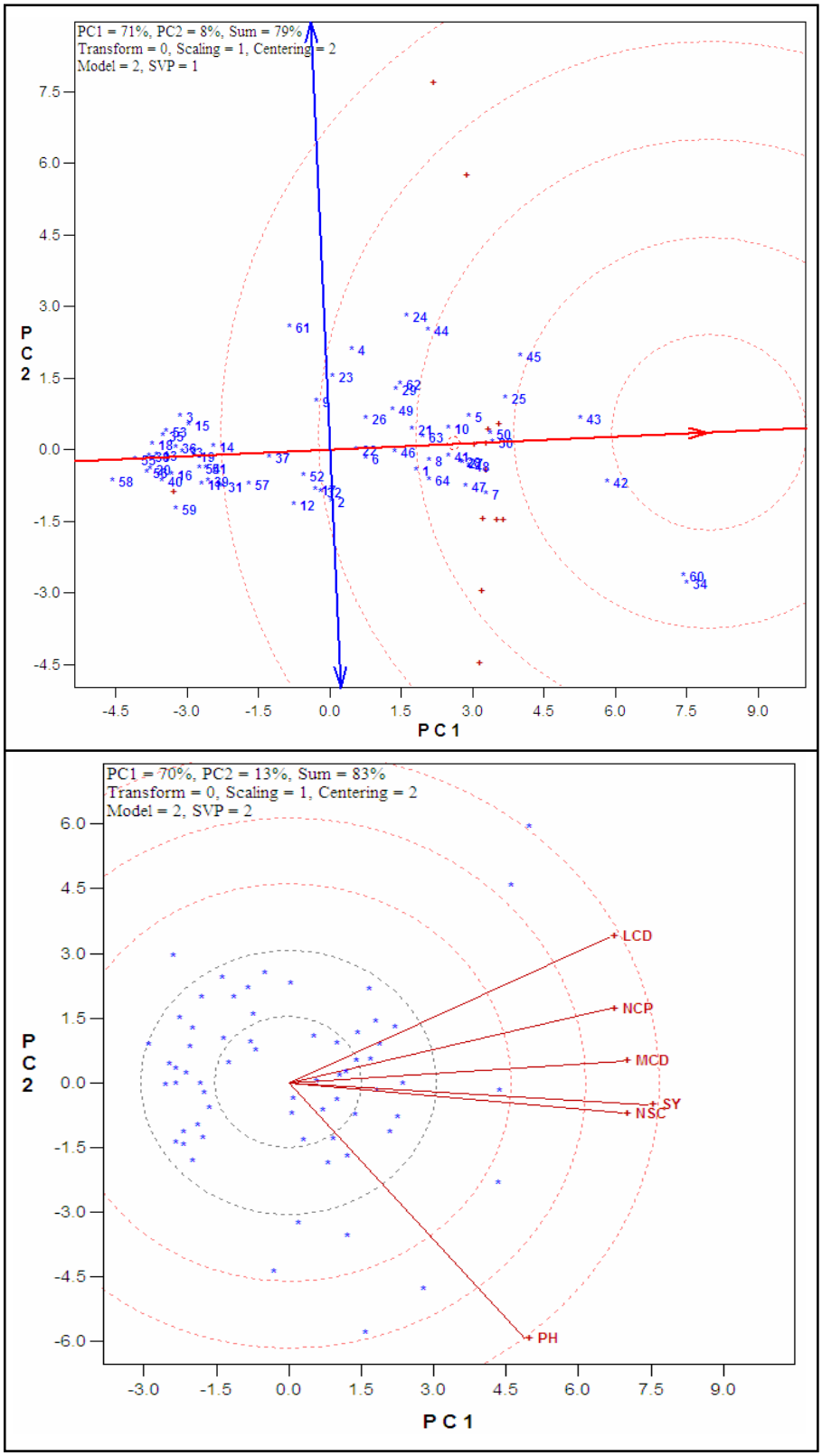


Fig. 3a. (Up): Ideal entry view of genotype by trait biplot, showing the relationships of safflower genotypes with ideal entry and 3b. (Down) : Tester view (seed yield) of genotype by trait biplot, showing the relationships of traits of 13 measured traits regarding SY.

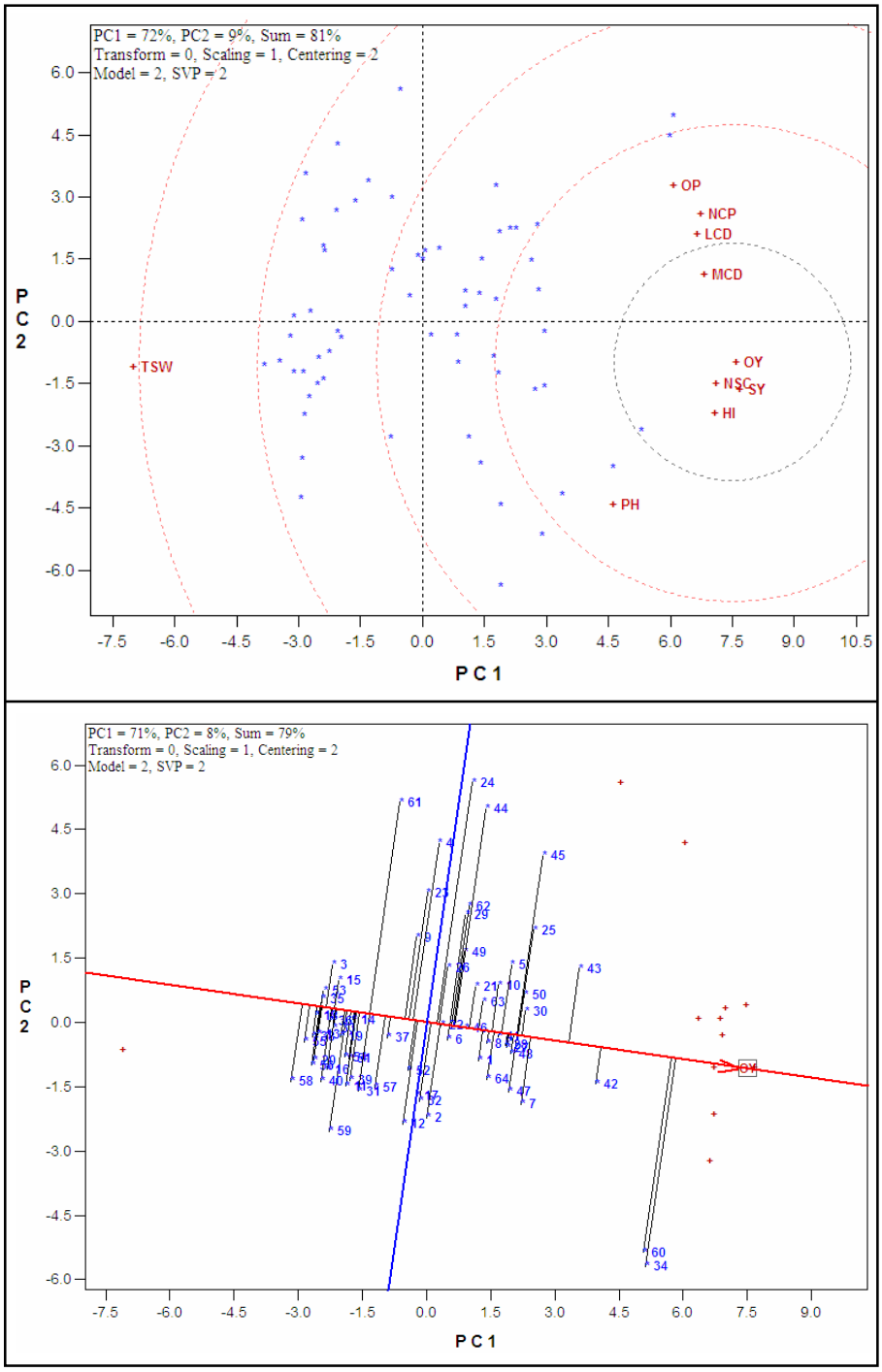


Fig. 4a. (Up) : Tester view (oil yield) of genotype by trait biplot, showing the relationships of traits of 13 measured traits regarding OY and 4b. (Down) : Tester comparison view of genotype by trait biplot showing the relationships of traits with oil yield.

single-arrow line that passes through the biplot origin is referred to as the average-tester axis (ATC) abscissa, and on this line is ranked the genotype in terms of their OY performance. The ATC ordinate divides the ATC abscissa into two at the middle (Yan *et al.*, 2007) and the portion of the ATC towards the right displays the above average genotype and towards the left indicates those genotypes below average. The best genotypes which showed the above average genotype, were genotypes 34 and 60 followed by genotypes 42 and 43 (Fig. 4b). The genotypes

58, 3, 55, 20, 50 and 35 indicated below average performance (Fig. 4b).

The effects of the number of seeds per capitulum (NSC), main capitulum diameter (MCD) and number of capitulum per plant (NCP) were large on seed yield. These results are similar to those obtained by Omidi-Tabrizi (2001) and Ghosh *et al.* (2010). Also the influence of the number of seeds per capitulum (NSC), seed yield (SY) and harvest index (HI) was large on oil yield which showed selection of high yielding genotypes with high HI could be result in high oil yield genotypes. In contrast, 1000-seed weight (TSW) had high negative effect on seedyield followed by oil yield. Selection of the genotypes must be performed carefully keeping in view higher seed yield along with the identified important traits. When the target is to establish associations among the traits that affect seed yield, GT biplot analysis is a more efficient method than the correlation analysis (Rubio *et al.,* 2004; Sabaghnia and Janmohammadi, 2014a). For a possible increase in productivity of safflower production, high yielding genotypes, 34, 42 and 60 must be crossed with each other and significant differences were observed among the 64safflower genotypes. The above genotypes may be used in further breeding programmes and these crosses may yield more transgressive segregants for these traits for grain yield improvement.

Previous investigations on safflower also demonstrated significant differences among genotypes, for seed yield, oil per cent and their related traits (Tuncturk and Ciftci, 2004; Bidgoli *et al.,* 2006). In general, some environmental factors such as rainfall, drought and biotic stresses influence safflower seed yield (Omidi-Tabrizi, 2001; Arslan, 2007), as well as temperature and rainfall at flowering time (Mozaffari and Asadi, 2006; Ghosh *et al.,* 2010). Regarding the production of safflower in arid and semi-arid areas, breeding efforts must consider these environmental factors. It may be concluded from this investigation thatnumber of seeds per capitulum, harvest index, main capitulum diameter, lateral capitulum diameter and number of capitulum per plant appeared to contribute on the seed yield and selection of these traits can produce high yield genotypes. Therefore, indirect selection for higher seed yield may be effective for improving oil yield. Such type of breeding programmes may lead to improve the economic traits in general and farmers living in semi-area in particular.

This study verified that GT biplot was a useful tool for the analysis of safflower dataset and indicated the similarities and differences among traits and genotypes and the similarities among the safflower genotypes from trait aspects could be visualized. The results of analysis by the GT biplot showed that genotypes 34, 42, 43 and 60 over-yielded the mean average of all safflower genotypes tested in this experiment and were identified as the most consistent-performing genotypes. The use of these genotypes by the growers would be ensuring high performance. These genotypes could also be used in a breeding programme to develop new high performing genotypes. Similar conclusions we can address about correlations among traits from GT biplot analysis. Oil yield performance was founded as a key feature for safflower genotypes and was related with the seed yield and harvest index. Therefore, selection based on these features should lead to an increased seed and oil yield. As a benefit GT biplot analysis is the more representative way to show the main feature for each genotype.

CONCLUSION

This investigation demonstrated that the programme for selection of high seed yield instead of oil per cent would be suitable for oil yield improvement of safflower. For genetic improvement of seed yield, traits number of seeds per capitulum, harvest index, main capitulum diameter, lateral capitulum diameter and number of capitulum per plant must be regarded while for genetic improvement of oil yield, traits seed yield and harvest index must be considered. Foursafflower genotypes (34, 42, 43 and 60) were selected with proven high yield potential which could be used as commercial cultivars or after further improvement and selection as pure lines or as parental lines for the development of new safflower cultivars proper for semi-arid areas. The GT biplot analysis was shown to be more informative than conventional methods for distinguishing special features in cultivars. This statistical method was found to be proper for test of safflower cultivars.

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