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ESTIMATION OF GENETIC DIVERSITY IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) LINES USING MULTIVARIATE ANALYSIS

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ABSTRACT

Thirty-one safflower genotypes along with two checks were grown in Randomized Block Design with three replications at ICRISAT, India during *rabi* 2022-23. The genotypes exhibited significant variability for all the seven quantitative traits under investigation. High PCV and GCV were recorded for number of seeds per capitulum and seed yield per plant; and moderate to high heritability with high GAM were recorded for all the traits, except days to 50% flowering and days to maturity. Seed yield per plant, number of capitula per plant, 100 seed weight, days to 50% flowering and days to maturity had higher vector coefficients in the positive direction and contribute more towards 36.99 per cent of variation of first principal component; 100 seed weight had higher vector coefficients in the positive direction to the 32.87 per cent of variation in the second PC. Based on the K-means clustering method, the genotypes were classified into three groups with Cluster I being the largest, consisting of 14 genotypes followed by Cluster III with 13 and Cluster II with 6 genotypes. The seed yield per plant had higher selection gain percentage (18.2%) followed by number of seeds per capitula (12.8%). The genotype ISF-87-15 is found to have highest oil content of 42.51%. All the studied traits exerted positive genetic gain except seed weight (-1.64%). Five superior genotypes (IIOR Saff-667-19, IIOR Saff-652-19, IIOR Saff-625-19, IIOR Saff-675-19 and IIOR Saff-636-19) were identified based on the MGIDI selection index. These genotypes can be used as superior donor parents in future safflower breeding programmes.

Key words : Oilseed, MGIDI selection index, Principal components.

Introduction

Safflower, a member of the Asteraceae family, (*Carthamus tinctorius* L.) is a traditional oilseed crop. It is cultivated on rich, dark soil with lower soil moisture levels. Safflower has been grown in various parts of the world; spreading from China to the Mediterranean region and all the way down from the Nile Valley to Ethiopia (Weiss, 1971). Farmers cultivate the safflower crop due to its lucrative oil-rich seeds and attractive red flowers. The oil extracted from this crop is highly valued for numerous medicinal properties and is used for human consumption (Dajue and Griffe, 2001; Singh and Nimbkar, 2006). At present, it is commercially grown in India, Kazakhstan, United States of America, Russian Federation, Mexico, China, Ethiopia, Australia, Argentina, Uzbekistan and Pakistan (Sunil *et al*., 2021). Each plant part has several uses *viz.*, as the bird feed, biofuel, flavouring, culinary colouring, and medicinal purposes. India is ranked second in world rankings for both planted area (75,010 ha) and yield (57260 tonnes) with an average productivity of 763 kg/ha (Pushpa *et al*., 2023). The two major safflower cultivazting states in India are Karnataka and Maharashtra, accounting for almost 90% of the total production. Telangana state produces 6910 tonnes of safflower year on 7000 hectares of crop land, with a yield of 987 kg/ha (Indiastat, 2022). In 2022, the world produced over 995,508 tonnes of safflower seeds. This is 15.2% greater than ten years ago and 36.9% more

than the previous year as a whole (Anonymous, 2022). Out of the eighteen main growing countries, Kazakhstan ranked first in the world for safflower seed output in 2022. With 447,457 tonnes produced, Kazakhstan accounted for 44.9% of the world's total production of safflower seeds (Anonymous, 2022). Safflower seeds are mostly used in cooking and contain 24–36% oil. Because the oil has a high concentration of unsaturated fatty acids, which includes linoleic acid (78%) which decreases serum cholesterol, it is highly helpful in lowering blood cholesterol levels (Weiss, 2000). The safflower oil cake is used as cattle feed as it contains about 40-45% protein (Neelima *et al*., 2021). However, because of the unpredictable weather over the past ten years, safflower farming has gradually decreased (Pushpa *et al*., 2023). Understanding and using the genetic diversity across genotypes is essential to developing cultivars that are resilient to climate change, which is the first step towards resolving this problem. Numerous statistical techniques are employed to comprehend the ways in which specific factors impact agricultural yield. Correlation analysis is a vital tool in genotype selection as it facilitates the investigation of the link between component attributes and seed production (Zafarnaderi *et al*., 2013). Principal component analysis (PCA), a multivariate statistical technique, is used to analyse large data sets (Slavkovic *et al*., 2004). The hierarchical agglomerative method known as cluster analysis separates the variables into groups and clusters (Katar, 2013).

Materials and Methods

The 31 safflower genotypes used in the study were developed by pedigree method using different parental lines. The present investigation was conducted at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad, Telangana, India (17.5111°N latitude, 78.2752° E longitude) in black soil during *rabi* season of 2022-23. The experimental material evaluated in the current study consist of thirty-one safflower genotypes along with two checks (A-1 and PBNS-12) (Table 1) were grown in Randomized Block Design with three replications. Each genotype was raised in five rows with each row 5m length and spacing of 45 cm between the rows and 10 cm between the plants. Recommended agronomic and plant protection practices were followed to ensure a good crop. Data were recorded for seven characters *viz.*, days to 50% flowering, days to maturity, number of capitula per plant, number of seeds per capitulum, 100 seed weight (g), oil content (%), and seed yield per plant (g) (Table 2) in each genotype across all the three replications. The oil analysis from seed was done using the Soxhlet Extraction method.

Table 1 : List of safflower genotypes used in the study.

S. no.	Genotypes	S. no.	Genotypes	S. no.	Genotypes
1	IIOR Saff-604-19	12	IIOR Saff-635-19	23	IIOR Saff-666-19
2	IIOR Saff-615-19	13	IIOR Saff-636-19	24	IIOR Saff-667-19
3	IIOR Saff-616-19	14	IIOR Saff-641-19	25	IIOR Saff-668-19
4	IIOR Saff-617-19	15	IIOR Saff-645-19	26	IIOR Saff-670-19
5	IIOR Saff-618-19	16	IIOR Saff-649-19	27	IIOR Saff-671-19
6	IIOR Saff-623-19	17	IIOR Saff-651-19	28	IIOR Saff-672-19
7	IIOR Saff-625-19	18	IIOR Saff-652-19	29	IIOR Saff-673-19
8	IIOR Saff-626-19	19	IIOR Saff-653-19	30	IIOR Saff-675-19
9	IIOR Saff-627-19	20	IIOR Saff-654-19	31	ISF-87-15
10	IIOR Saff-631-19	21	IIOR Saff-655-19	32	A1 (National check)
11	IIOR Saff-634-19	22	IIOR Saff-660-19	33	PBNS-12 (Varietal check)

Table 2 : Quantitative traits recorded in the study.

S. no.	Traits	Abbreviation	Unit
1.	Days to 50% flowering	DFF	Number
2.	Number of capitula per plant	NCP	Number
3.	Number of seeds per capitula	NSC	Number
4.	Days to maturity	DM	Number
5.	100 seed weight	SW	g
6.	Oil content	OC	%
7.	Seed yield per plant	SYP	g

The safflower oil was obtained by chemical extraction, in which 10 g of decorticated seeds were crushed to extract the oil with hexane solvent for 4 h by Soxhlet type extractor. The oil extract obtained was dried under low pressure at 70°C in a rotary evaporator until the solvent is eliminated (Hosni *et al*., 2022). The weight of the oil was recorded and the oil content was calculated using the formula:

$$\text{Oil content (\%)} = \frac{\text{(Weight of flask with oil – Weight of empty flask)}}{\text{Weight of ground seeds}} \times 100$$

The mean values of the data were subjected to statistical analysis using version 4.3.2 of the R package for Windows. Analysis of variance (ANOVA) was performed for each trait, treating replications as fixed factors and genotypes as random factors following the method described by Panse and Sukhatme (1985) to ascertain the total variances among the safflower genotypes concerning different traits and their significance was tested through 'f' test. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were computed based on Burton's formula (Burton and De Vane, 1953). Heritability and Genetic advance as percentage of mean (GAM) were estimated as suggested by Johnson *et al*. (1955). The contribution of different traits to total variation for all the genotypes were determined by PCA and the first two principal components that had the highest contribution to the variability were used to make biplot. PCA and Cluster analyses were performed by the "factoextra" package in R v1.0.7 (Kassambara and Mundt, 2020). The multi-trait genotype-ideotype distance index (MGIDI) was calculated as per the formula given by

$$MGIDI_i = \sqrt{\sum_{j=1}^{f} \left(F_{ij} - F_j\right)^2}$$

where, F_{ij} is ith genotype score for j[th] factor (i = 1, 2, ..., g; j = 1, 2, ..., f), g and f stated as total number of genotypes and their factors. The analysis was done in R studio 4.3.2. using the package of "metan" (Olivoto and Lúcio, 2020) with the 'gamem' and 'mgidi' functions.

Results and Discussion

Genetic variability among the safflower genotypes

Analysis of variance (ANOVA) revealed that the genotypes under the present study significantly different for days to 50% flowering (37.84**), days to maturity (23.11**), number of capitula per plant (212.10**), number of seeds per capitulum (124.11**), 100 seed weight (1.79**), seed yield per plant (170.98**) and oil content (35.23**) (Table 3) indicating that the presence of variability in the experimental material. Similar results were observed by Neelima *et al*. (2021), they also reported that highly significant mean sum of squares for all the above mentioned traits.

Genetic parameters revealed substantial variation for seed yield and its component traits (Table 4). For the tested genotypes, days to 50% flowering ranged from 78-97 with an average of 86 (days). The genotype IIOR-Saff-635-19 exhibited early flowering. The mean values for days to maturity was 133 (days) with range from 123-142 (days). Wide range of variation observed for number capitula per plant, number of seeds per capitulum, 100 seed weight, seed yield per plant and oil content. The number of capitula per plant ranged from 18.4 to 76.40. The highest number of capitula per plant was found in IIOR-Saff-667-19. The mean number of seeds per capitulum was 26.43 and it ranged from 14.60-50.90. The average 100 seed weight was 5.62 g ranging from 3.10-

Table 3 : Analysis of variance of 7 quantitative traits in 31 safflower genotypes.

Source of variation	Df	DFF	DM	NCP	NSC	100 SW	SYP	OC (%)
Replication	2	19.303	2.01	38.69	31.28	0.02	8.46	0.9
Genotype	32	37.84**	23.11**	212.10**	124.11**	1.79**	170.98**	35.23**
Residuals	64	8.37	1.09	45.56	13.81	0.14	9.6	0.32

ns: p>0.05; *: p<0.05; **: p<0.01

Table 4 : Genetic components of variance for seed yield and its components in *rabi* safflower genotypes.

Trait	Mean	Range	GCV(%)	PCV (%)	h^2 (BS)(%)	GAM(%)
DFF	86.06	78-97	3.64	4.95	53.97	5.51
DM	133.59	123-142	2.02	2.17	87.04	3.89
NCP	38.67	18.4-76.40	19.26	26	54.92	29.41
NSC	26.43	14.60-50.90	22.94	26.9	72.68	40.28
SW	5.62	3.10-7.60	13.18	14.83	79.04	24.15
SYP	33.29	15.90-53.10	22.02	23.91	84.85	41.79
OC	30.15	25.48-42.51	11.31	11.46	97.32	22.99

GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, h^2 (BS): Heritability (Broad sense) and GAM: Genetic advance as percentage of mean.

7.60 g; seed yield per plant also showed high variation ranging from 15.90-53.10 g with a mean of 33.29g. The highest seed yield per plant was recorded in the genotype IIOR-Saff-671-19 along with the oil content of 29.57%. The average oil content in the present study was 30.15%, ranging from 25.48-42.51%. The genotype ISF-87-15 was recorded highest oil content of 42.51% along with seed yield of 16.7g/p. The similar wide variability was reported in the study conducted by Mukta *et al*. (2020). Narrow range variation was observed for days to 50% flowering and days to maturity. These findings were in confirmation with the results of Neelima *et al*. (2021), where they noticed that days to 50% flowering and oil content exhibiteda narrow range of variation.

The phenotypic coefficient of variation (PCV) was higher compared to the genotypic coefficient of variation (GCV) for all the traits, suggesting that the environment plays a significant role in governing the expression of all these traits. High PCV and GCV were recorded for the number of seeds per capitulum (26.9% and 22.94%) and seed yield per plant (23.91% and 22.02%), which indicates a greater variability among the genotypes, thereby implying better chances of selection for improvement of these traits. These results were in agreement with Rathod *et al*. (2021), where they reported that traits like the number of capitula per plant, number of seeds per capitulum and seed yield per plant had high GCV and PCV. In contrast, low GCV and PCV were observed for days to 50% flowering (3.64% and 4.95%) and days to maturity (2.02% and 2.17%). The coefficient of variation does not indicate heritable portion of the trait and only shows the extent of variability present in the traits. Therefore, heritability is calculated, as it is a good index of transmission of characters to offspring (Falconer, 1981). High heritability was observed for traits *viz*., days to maturity (87.04%), number of seeds per capitulum (72.68%), 100 seed weight (79.04%), seed yield per plant (84.85%) and oil content (97.32%). These findings confirmed that a considerable portion of phenotypic variance is due to genotypic factors and effect of environmental factors is negligible indicating that the selection of these traits based on phenotypic selection is rewardable. These results were in agreement with Minnie *et al*. (2018), where they observed high heritability for traits like days to 50% flowering, days to maturity, number of capitula per plant, number of seeds per capitulum, 100 seed weight and seed yield per plant.

Heritability coupled with genetic advance is more reliable in predicting the effect of selection than heritability alone (Johnson *et al.*, 1955). Moderate to high heritability with high GAM was recorded for all the traits except

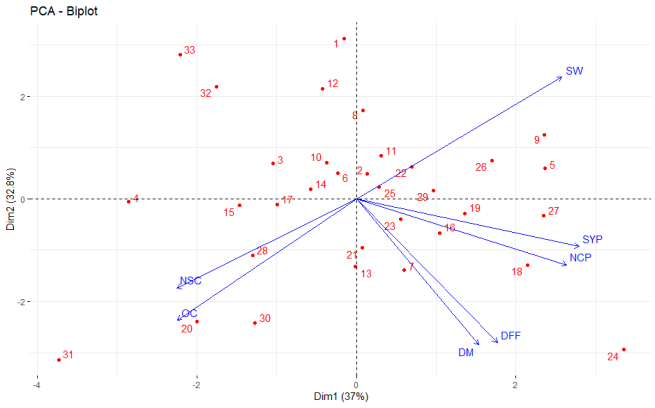

Fig. 1 : PCA Biplots.

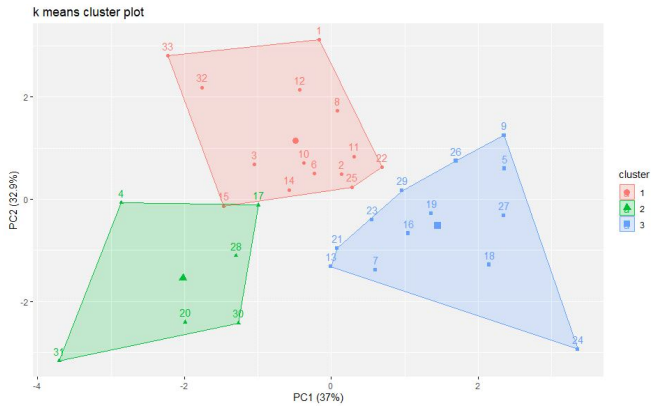

Fig. 2 : Cluster analysis. Three clusters formed to demonstrate the relationship between 31 safflower genotypes.

days to 50% flowering and days to maturity, indicating that traits with high heritability and GAM reflected the influence of additive gene action. Therefore, simple phenotypic selection will be beneficial for improving these traits. Similar results were observed by Pushpavalli (2016) where high heritability coupled with high GAM for 100 seed weight followed seed yield per plant was observed. On the contrary, in the present study, improvement for the traits like days to 50% flowering and days to maturity could be feasible through heterosis breeding as these traits were influenced by non-additive gene action.

Principal component analysis

Karl Pearson (1901) proposed principal component analysis (PCA) to simplify the large complex datasets into a few components. Eigenvalue of principal components (PCs) that has values greater than one will give more information (Kaiser, 1960). In the present investigation, the first two PCs (Table 5) have contributed to 69.86 per cent of cumulative variance. Jabbari *et al*. (2021), in his study with 122 safflower genotypes observed that the first and second principal components account for 29.5% and 15.9% of the total variation, respectively.

Table 5 : Eigen values, percentage of variance and cumulative percentage of variance of safflower genotypes.

Principal component	Eigen value	Percentage of variance	Cumulative percentage of variance
PC1	2.59	36.99	36.99
PC2	2.30	32.87	69.86
PC3	0.85	12.21	82.07
PC4	0.60	8.59	90.65
PC5	0.31	4.37	95.02
PC6	0.24	3.40	98.42
PC7	0.11	1.58	100.00

Table 6 : Contribution of first two principal components to variation in safflower genotypes.

Trait	PC1	PC2
DFF	0.4712	-0.7419
DM	0.4087	-0.7523
NCP	0.6967	-0.3425
NSC	-0.5940	-0.4633
SW	0.6802	0.6302
SYP	0.7406	-0.2441
OC	-0.5921	-0.6292

The eigen vectors ranged from -1 to +1. The values with higher points in both direction contributes more to the total variance (Anthony, 2014). According to this approach, seed yield per plant (0.740), number of capitula per plant (0.696), 100 seed weight (0.680), days to 50% flowering (0.471) and days to maturity (0.408) had higher vector coefficients in the positive direction (Table 6). Hence, these all variables were significant, contributing more towards the 36.99 per cent variation of the first PC. In the second PC, the 100 seed weight had higher vector coefficients in the positive direction to the 32.87 per cent variation. These results were in agreement with Ali *et al*. (2020), they noticed that the days to flower initiation, days to 50% flowering, days to flower completion, capitula per plant, branches per plant, seeds per capitulum, capitulum diameter and seed yield per plant were the major contributors to the observed genetic variability in the evaluated 96 safflower panel.

The biplot (Fig. 1) provide more information about trait association and clustering of genotypes. The angle between the two variables was less, and it indicates that the two variables are positively correlated. The biplot showed that seed yield per plant had a very high positive relationship with the number of capitula per plant. But the angle between seed yield per plant, oil content and number of seeds per capitula had more, indicating that these traits do not have a positive association. The variables (seed yield plant and number of capitula per plant), which are plotted close to the genotypes indicates that these genotypes (IIOR Saff-653-19, IIOR Saff-649-19, IIOR Saff-652-19, IIOR Saff-673-19, IIOR Saff-671-19, IIOR Saff-667-19 and IIOR Saff-666-19) had higher seed yield compared with others and they are very similar in response. Those genotypes grouped in opposite direction to seed yield per plant indicate had lower seed yield. These results were in agreement with Ebrahimi *et al*. (2023), where they noticed that the genotype G80 was diverse for most of the traits studied.

Table 7 : Genetic gain of different traits based on MGIDI index.

S. no.	Traits	Factors	SG (%)
1	DFF	FA1	3.86
2	DM	FA1	2.09
3	NCP	FA1	5.61
4	SYP	FA1	18.2
5	NSC	FA2	12.8
6	SW	FA2	-1.64
7	OC	FA2	1.68

Cluster analysis

Cluster analysis is a crucial method for data classification as it allows the division of the germplasm into different homogenous groupings according to morpho-genetic features. Through the use of cluster analysis, outliers can be found by maximising variance across groups and minimising variance within them. The 31 genotypes exhibited significant differences for the seven characters studied. Based on the K-means clustering method, genotypes were classified into three groups (Fig. 2). Cluster I was the largest, consisting of 14genotypes, followed by Cluster III, which comprised of 13 and Cluster II had six genotypes. A-1 (national check) and PBNS - 12 (varietal check) were grouped in Cluster I. These results were agreement with Aleem *et al.* (2021), where they observed that 19 safflower genotypes were grouped into three distinct clusters based on thirteen agro morphological traits. Houmanat *et al.* (2021) also found that 45 safflower accessions were grouped into four major clusters based on different physiological traits.

Multi trait Genotype Ideotype Distance Index (MGIDI)

The MGIDI selection index results figured out that the seven traits were separated into two factors, and the FA1 includes days to 50% flowering, days to maturity, number of capitula per plant and seed yield per plant, and the remaining traits like number of seeds per capitula, seed weight and oil content were included in FA2 (Table 7). The seed yield per plant had a higher selection gain percentage (18.2%), followed by the number of seeds

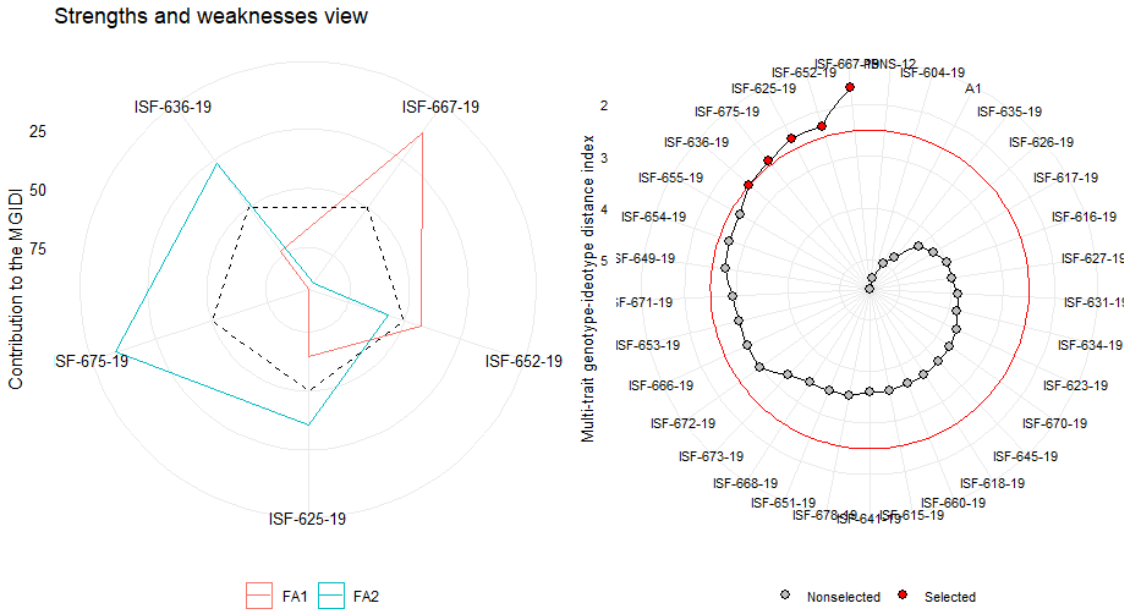

Fig. 3 : Selection of genotypes based on MGIDI selection index.

per capitula (12.8%). All the studied traits exerted positive genetic gain except seed weight (-1.64%). Maranna *et al*. (2021) also observed a negative selection for plant height and days to flowering, which would negatively impact grain yield in soybean. Five superior genotypes (IIOR Saff-667-19, IIOR Saff-652-19, IIOR Saff-625-19, IIOR Saff-675-19 and IIOR Saff-636-19) were identified based on selection pressure (red line that indicates genotypes selected according to the selection pressure) in this study (Fig. 3). The strength and weakness plot revealed the relative contribution of traits on superior genotypes. The traits like days to 50% flowering, days to maturity, number of capitula per plant and seed yield per plant were presented in the first factor (FA1). This FA1 had the least contribution for genotypes IIOR Saff-667-19 and IIOR Saff-652-1 and this indicates that they were the most desirable genotypes for above-mentioned traits. The second factor (FA2) had smallest contribution for genotypes IIOR Saff-675-19, IIOR Saff-636-19 and IIOR Saff-625-19. These genotypes were found to have a high 100 seed weight, number of seeds per capitula and oil content.

The findings of the current investigation can serve as indicator for the selection of the traits associated with high seed yield and oil content in safflower breeding programme. The elite genotypes identified can be used as donor parents for safflower seed yield improvement.

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