Applied Biological Research 25(2): 160-168; (2023)
DOI: 10.5958/0974-4517.2023.00019.8

GENETIC VARIABILITY AND PRINCIPAL COMPONENT ANALYSIS OF THE SAFFLOWER GERMPLASM MAPPING PANEL (SGMP)

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(Received 27 November, 2022; accepted 26 March, 2023)

ABSTRACT

Worldwide there is focus on the development of safflower (*Carthamus tinctorius* L.) varieties having high seed yield coupled with high oil content. The present study was aimed to evaluate the safflower germplasm mapping panel (SGMP) for major seed yield traits and oil content to improve oil productivity of varieties. SGMP consisting of 200 safflower germplasm accessions and four checks (A-1, PBNS-12, NARI-6, NARI-57) were evaluated for 9 quantitative characters during 2016-17. The distribution of data for phenological characters and seed yield was normal, with the exception for seed size and 100-seed weight. The oil content was positively skewed toward higher values, thus indicating panel homogeneity. The studied traits showed significant variation in the germplasm panel. Seed yield and its related characters had high genotypic and phenotypic coefficients of variation, while oil content showed moderate coefficient of variation. Broad sense heritability and genetic advance over percent mean were high for most traits. The principal component analysis revealed that four components explained 75.3% of total variation. There was a strong correlation between seed yield, capitula plant^{-1}, and number of branches plant^{-1}. Superior accessions for each character were identified over the best check. The safflower germplasm mapping panel had wide range variability for the traits studies, and the best accessions can be used in safflower improvement programme. In terms of seed yield, genotypes A1, EC736504, and GMU6663 performed better. Genotype EC736504 gave higher seed yield and oil content. In terms of seed yield, genotypes A1, EC736504, and GMU6663 performed better; however, genotype EC736504 gave higher seed yield coupled with high oil content. For oil improvement, 26 germplasm lines performed better than the checks.

Keywords: Genetic diversity, principal component analysis, safflower, variability

INTRODUCTION

Safflower is a traditional minor oil seed crop grown in India. There are six species of safflower, but only one species, *Carthamus tinctorius* L., is widely cultivated. Kazakhstan (43.28%), Russian federation (28.5%), USA (6.42%), India (5.46%) and Mexico (3.65%) are the main safflower cultivating regions of the world which together contribute 87% of the world's safflower production (FAOSTAT, 2021). Safflower area has decreased from 0.43 M ha in 2000 to 0.046 M ha in 2021, while safflower production has increased to 0.22 M t in 2007 and then steadily declined to the present level of 0.036 M t in India in comparison to the other main safflower producers globally (FAOSTAT, 2021). The main reasons for India's area decline and low productivity is growing the crop in marginal lands, low oil content, lesser yield, poor crop management and competing high remunerative crops

like sorghum and gram (Nimbkar, 2008). Genetic variability is the most important prerequisite for initiating any genetic improvement programme. Genetic parameters aid in the recognition of gene action as it assists the breeder in selecting the appropriate breeding approach for the experimental material. Heritability and environmental factors are generally influenced by genotypic and phenotypic variations (Bisne *et al.*, 2010). As a result, any attempt at genetic improvement must first comprehend the nature of variability in base germplasm collection. Genetic diversity is required for hybridization in crop development programmes. The use of multiple parents makes it easier to isolate superior recombinants. It is preferable to analyse the structure of yield using breeding approaches.

Several safflower yield contributing traits, such as the number of branches plant^{-1}, number of seeds capitulum^{-1}, number of capitulum plant^{-1}, and 100-seed weight, all have a positive relationship with one another and with seed yield (Sahoo *et al*., 2022). Breeders, on the other hand, may obtain measures for a variety of the observed factors in order to develop a smaller number of variables (principal components) that explain the majority of variance in the observed variables (Jolliffe *et al*., 2016). The principal component analysis (PCA) is a multivariate analysis method that seeks to derive a small number of linear combinations (principal components) of a set of variables while retaining much of the information contained in the original variables. Multivariate techniques could reduce several the phenotypic measurements in large populations into fewer, more interpretable, and easily visualized dimensions (Jombart *et al*., 2010). Although cluster analysis can be used to determine the family relationships, the primary advantage of PCA over cluster analysis is that each genotype can only be assigned to one group (Mohammadi, 2002). Many studies have been conducted on genetic variability and PCA of safflower (Safavi *et al*., 2017; Dhruw *et al.*, 2022; Jabbari *et al*., 2022; Ojaq *et al.*, 2022). The researcher will undoubtedly benefit from understanding the pattern of existing genetic variability, the trend of character association, the identification of promising traits, and the extent of genetic divergence in safflower germplasm. The objective of present study was to use principal component analysis to assess the potential genetic variability and genetic divergence in safflower germplasm mapping panel so as to develop safflower varieties with high oil productivity.

MATERIALS AND METHODS

Plant material

A safflower germplasm mapping panel (SGMP) of 200 germplasm accessions, procured from the Germplasm Management Unit of ICAR-Indian Institute of Oilseeds Research, Hyderabad (India), was used in this experiment along with 4 checks (A-1, PBNS-12, NARI-6 and NARI-57).

Experimental design

The experiment was laid out at ICRISAT Farm, Hyderabad, Telangana (India) in 2016-17 in an augmented block design (ABD) in 10 blocks, with four checks replicated in each block. The plot was 5 m long, with plant spacing of 45 x 20 cm. To ensure a healthy crop, the recommended safflower crop cultural, management, and plant protection practices were followed (*Safflower management practices, ICAR-DOR folder https://icar-iior.org.in/sites/default/files/iiorcontent/pops/saf-eng.pdf*).

Observations

The data was collected from five randomly selected plants from each genotype for each germplasm accession, and mean data was used for statistical analysis of phenological traits such as number of branches plant^{-1}, effective capitula plant^{-1}, number of seeds capitulum^{-1}, 100-seed weight (g), seed yield (kg plot^{-1}), seed size (mm), and oil content (%). The traits such as days to 50% flowering, days to maturity, and seed yield were recorded on a plot-by-plot basis. Thirty seeds were chosen at random to determine seed size. At ICAR-IIOR, Hyderabad, the oil content of the sampled seed was estimated

using a bench top NMR-MQC-5 analyser (Oxford. London) supplied with pre-loaded 'easy cal' software as per the calibrated conditions developed by Yadav *et al*. (2016).

Data analysis

Using the R package, randomized complete block design (RCBD) (Aravind *et al*., 2020) available on R-statistics platform (*https://www.r-project.org/*). The data from 9 variables were subjected to augmented analysis of variance (software version 4.0.2). The accessions were compared with controls using a critical difference to identify trait specific accessions (5%). Genetic variability parameters such as phenotypic, genetic variance (PV and GV), broad sense heritability, and genetic advance (GA) were estimated using the standard formula (Burton, 1952). Principal component analysis (PCA) was used to analyze genetic divergence among safflower genotypes using the R package ggplot2.

RESULTS AND DISCUSSION

Phenotypic variability

The data analysis of mapping panel of 200 germplasm accessions and four checks revealed a normal distribution for the majority of characters studied, except seed size and oil content (Fig. 1). The descriptive statistics for 204 germplasm accessions (Table 1) revealed that the days to 50% flowering ranged from 63 (GMU2981) to 106 (EC523374), with a mean of 83 days, and maturity days ranged from 100 (GMU2981) to 147 (GMU5668), with a mean of 122 days. The average number of branches was 10.5 with a range of 34 (GMU1182) to 2 (GMU1182). The mean number of capitula plant^{-1} ranged from 91 (GMU2718) to 6 (EC755683-1) with a mean value of 35.4, while the mean number of seeds capitulam^{-1} ranged from 75 (GMU5335) to 7 (GMU6793) with a mean value of 28.8. The seed size ranged from 9.92 mm (GMU6306) to 1.38 mm (GMU1178), with an average of 7.81 mm. The exotic accession EC736504 gave highest (50 g) seed yield plant^{-1} and GMU7408 showed the lowest (3 g) seed yield plant^{-1}, while the panel's mean seed yield was 20.44 g. The mean test weight was 4.12 g and ranged from 6.2 g (GMU2849) to 2.15 g (EC736521). The mapping panel's mean oil content was 34.47%, with GMU1161 having the lowest oil content of 24.3% and an exotic accession, EC736500-1, had highest oil content (48.4%). Skewness is a metric that describes the asymmetry in the quantitative feature distribution (Fig. 1 and Table 1). The skewness for six characters in the current study was close to "0," indicating normal distribution. The number of branches plant^{-1} and seed size were significantly skewed from the mean, pushing the data to the left (lower values), whereas oil content was positively skewed, pushing the data to the right (higher values). Kurtosis, a statistic that indicates the peakness of a normal distribution curve, was positive and significant for some variables *viz*., flower initiation days, maturity days, number of branches plant^{-1}, number of seeds capitulum^{-1}, seed size, and 100-seed weight. This was a leptokurtic curve, which indicated that the population was heterogeneous. While the remaining four characters' kurtosis values were significant and positive,

Table 1: Descriptive statistics of 204 safflower germplasm accessions

Traits	Mean	SE	Minimum	Maximum	Skewness	Kurtosis	Coefficient of variation (%)
Days to 50% flowering	82.88	0.58	63.0	106.0	0.26ns	2.52ns	1.89
Days to maturity	121.79	0.70	101.0	146.0	0.01ns	2.41*	1.74
Branches plant^{-1} (No.)	10.49	0.31	2.0	34.0	-0.55**	3.81*	52.41
Capitula plant^{-1} (No.)	35.45	1.14	6.0	91.0	0.002ns	3.53ns	32.31
Seeds capitulum^{-1} (No.)	28.85	0.71	7.0	75.3	0.29ns	5.85**	25.50
Seed size (mm)	7.81	0.10	1.3	9.9	-2.39**	9.65**	1.46
Seed yield plant^{-1} (g)	20.44	0.75	2.5	50.5	0.14ns	2.73ns	33.38
100-Seed weight (g)	4.12	0.06	2.1	6.2	0.02ns	2.15**	5.85
Oil content (%)	34.47	0.31	24.7	48.6	0.81**	3.21ns	3.42

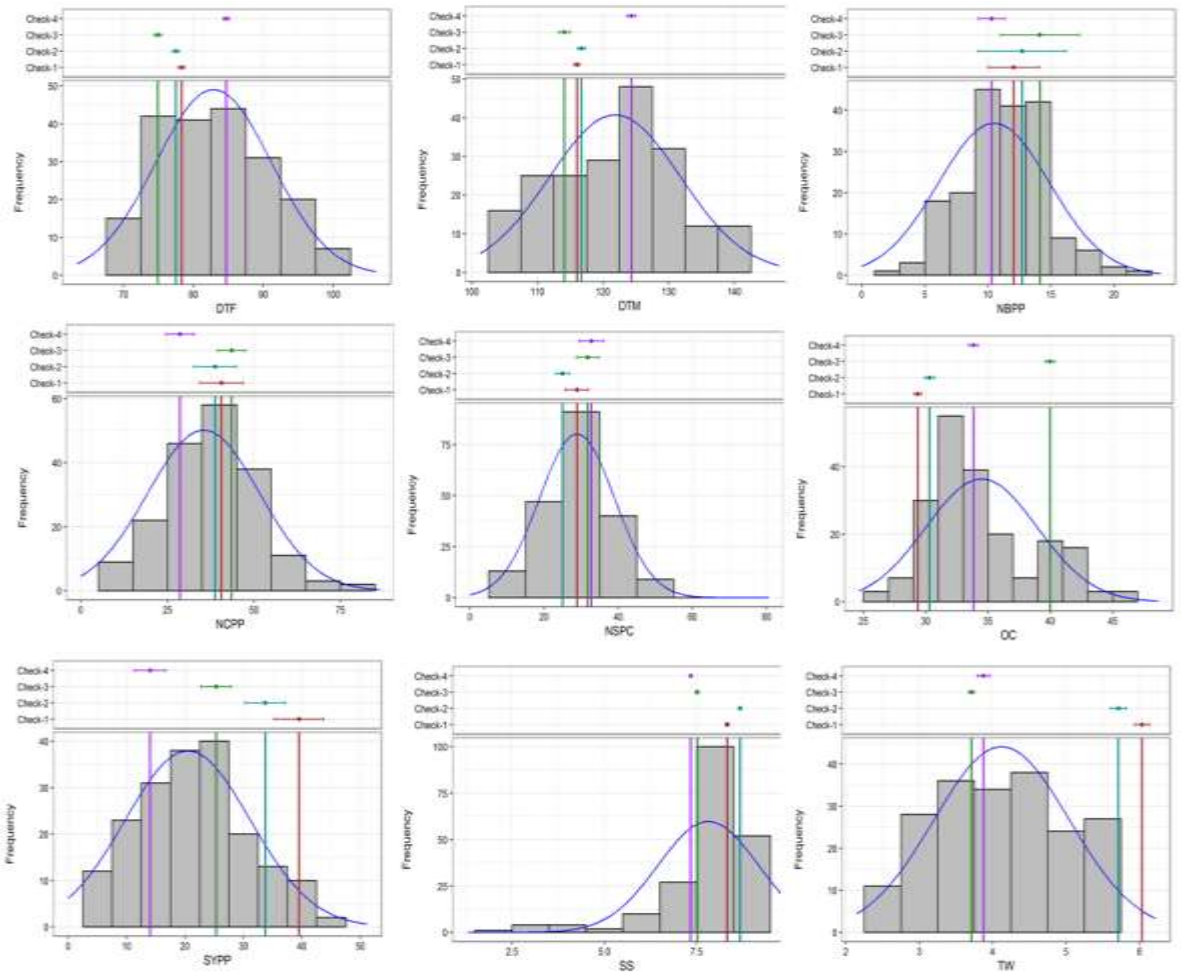

Fig. 1: Frequency distributions for days to flower initiation (DTF), days to maturity (DTM), number of branches plant^{-1} (NBPP), number of capitula plant^{-1} (NCPP), number of seeds capitulum^{-1} (NSPC), oil content (OC), seed yield plant^{-1} (SYPP), seed size (SS) and test weight (TW) in the mapping panel.

indicating a mesokurtic curve (normal distribution). (Jignesh *et al*. (2020) found quantitative trait distribution skewded and leptokutic in F_3 population of groundnut indicating heterogeneity, whereas the distribution in F_5 population was mesokurtic, with little skewness. Because our mapping panel was made up of accessions from various backgrounds, we can expect a leptokurtic curve with a high skewness. The curve for oil content, on the other hand, was positively skewed with no significant kurtosis, indicating that the majority of accessions in the mapping panel were homogeneous for the characteristic.

Analysis of variance

The accessions in mapping panel showed significant variations for 7 quantitative features, with the exception of number of capitula plant^{-1} and seeds capitulam^{-1} (Table 2). There was a significant variation between checks for 8 quantitative characters that were repeated across 10 blocks, with the exception of the number of seeds capitulum^{-1}. Table 3 displays which accession outperformed the best checks for each character. There were 27 accessions for early maturity and 15 for early flowering during early check. It's worth noting that genotypes with early flowering also showed early maturation. In terms of seed yield, GMU 6663 and EC736504 outperformed the best check A-1, and no accession outperformed the best check in terms of test weight. Further, all the 27 outstanding accessions for high oil content were of exotic origins.

Table 2: Augmented analysis of variance and critical difference for quantitative traits for comparing safflower accessions (Acc.) and their checks

Source	Acc. with checks	Adjusted block	Checks	Acc.	Acc. vs. checks	Residuals	$CD_{0.05}$			
	203	9	3	200	1	27	Acc. vs checks	Checks	Acc. (different block)	Acc. (Same block)
Days to 50% flowering	50.91**	451.22**	173.17**	49.07**	564.44**	2.41	3.73	1.42	5.03	4.50
Days to maturity	65.84**	926.96**	201.29**	63.81**	560.33**	4.42	5.06	1.93	6.82	6.10
Branches plant^{-1} (No.)	15.29ns	238.62**	25.15ns	15.14ns	167.55*	29.89	13.15	5.02	17.74	15.86
Capitula plant^{-1} (No.)	188.04ns	2059.07**	419.73*	184.57ns	219.31ns	133.39	27.79	10.6	37.47	33.51
Seeds capitula^{-1} (No.)	91.05ns	512.32**	122.07ns	90.59ns	17.59ns	54.52	17.77	6.78	23.95	21.43
Seed size (mm)	1.47**	9.01**	4.09**	1.43**	0.77**	0.01	0.27	0.10	0.37	0.33
Seed yield plant^{-1} (g)	112.58**	443.33**	1223.27**	95.92*	2074.79**	51.77	17.31	6.60	23.34	20.88
100-seed weight (g)	0.81**	7.80**	14.65**	0.60**	17.62**	0.06	0.60	0.23	0.80	0.72
Oil content (%)	14.10**	217.74**	230.88**	10.85**	42.97**	1.38	2.82	1.08	3.81	3.40

Table 3: Superior accessions over the best checks, identified from the safflower germplasm mapping panel

Characters	Best check	$CD_{0.05}$	Name of the germplasm
Days to flowering	NARI-6	1.42	GMU2981, GMU2855, GMU3475,GMU2895, GMU2860, GMU3607, GMU3858, GMU3652, GMU3525, GMU3508, GMU3379, GMU4862, GMU4907, GMU3822, GMU3907
Days to maturity	NARI-6	1.93	GMU2981,GMU3475,GMU3858,GMU2860,GMU95,GMU2895,GMU2855,GMU3875,GMU3607,GMU3525,GMU40,GMU3652, GMU3508, GMU3907,GMU3605, GMU638, GMU593, GMU3923, GMU3379, GMU4862, GMU3886, GMU2976,GMU1015, GMU1085, GMU391, GMU2912, GMU4558
Branches plant^{-1} (No.)	NARI-6	5.02	GMU1182,GMU1153
Capitula plant^{-1} (No.)	NARI-6	10.6	GMU2718,GMU1182,EC736495,GMU1695,EC755663
Seeds capitula^{-1} (No.)	NARI-6	6.78	GMU5335, EC755660, EC523373, EC736521, GMU1153, EC755661, EC736494, EC736498, GMU6869, EC736529-1
Seed size (mm)	PBNS-12	6.60	GMU6306,GMU3924,GMU3907,GMU2860,GMU1153,EC736496,GMU6851,GMU3281,GMU2849,GMU2749,GMU2895, GMU3531,GMU2328, GMU2759,GMU4178,GMU2616,EC736485,GMU2855,GMU4223,GMU3607,GMU3966, EC755679-1,GMU3719, GMU4201, GMU6878, GMU4558, GMU3964, EC755673, GMU3875, EC755676, EC755659, EC755663, GMU3992,GMU2444
Seed yield plant^{-1} (g)	A-1	0.23	EC736504, GMU6663
100-seed weight (g)	A-1	1.08	Nil
Oil content (%)	NARI-6	0.10	EC736500-1,EC736501-1, EC736495,EC736499, EC736498, EC736497, EC736496, EC755670-2, EC755673, EC736506, EC755683-1, EC755671, EC736511, EC755684, EC736517, EC755666, EC736492, EC736504, EC736494, EC736516, EC736485, EC755665, EC755664, EC755688, EC755687, EC755672, EC736521

Table 4: Estimation of genetic parameters for quantitative traits of safflower in mapping panel

Variables	Mean	PV	GV	EV	GCV	PCV	ECV	h^2BS	GA	GAM
Days to 50% flowering	82.88	66.78	64.37	2.41	9.68	9.86	1.87	96.39	16.25	19.61
Days to maturity	121.79	103.05	98.63	4.42	8.15	8.34	1.73	95.71	20.04	16.46
Branches plant^{-1} (No.)	10.49	17.02	-12.87	29.89	-	39.35	52.14	-	-	-
Capitula plant^{-1} (No.)	35.45	246.41	113.02	133.39	29.99	44.27	32.58	45.87	14.85	41.89
Seeds capitula^{-1} (No.)	28.85	106.67	52.15	54.52	25.03	35.79	25.59	48.89	10.42	36.10
Seed size (mm)	7.81	1.84	1.83	0.01	17.32	17.38	1.46	99.29	2.78	35.60
Seed yield plant^{-1} (g)	20.44	93.59	41.82	51.77	31.64	47.32	35.20	44.69	8.92	43.63
100-seed weight (g)	4.12	0.86	0.80	0.06	21.76	22.57	6.01	92.92	1.78	43.27
Oil content (%)	34.47	20.49	19.11	1.38	12.68	13.13	3.40	93.28	8.71	25.27

GV = Genotypic variance, PV = Phenotypic variance, GCV = Genotypic coefficients of variation, PCV = Phenotypic coefficients of variation, hBS = broad-sense heritability (%), GA = Genetic advance at 5% selection intensity (%), GAM = Genetic advance as the percentage of the mean at 5% selection intensity (%)

Genetic variability parameters

The results pertaining genetic variability parameters *viz*., phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h^2 BS) and genetic advance as a percentage of the mean (GAM) for all the nine characters are given in Table 4. The highest magnitude of both GCV and PCV were observed for seed yield plant^{-1} (31.64 and 47.32%, respectively), followed by the number of capitula plant^{-1} (29.99 and 44.27%) and the number of seeds capitula^{-1} (25.03 and 35.79%) suggesting that these characters were under the influence of genetic control whereas 100-seed weight, seed size and oil content had medium GCV and PCV, probably due to the significant environmental effect. Moderate PCV and GCV in oil content have been reported by Neelima *et al*. (2021) while similar highest magnitude of both GCV and PCV for capitula plant^{-1} and seeds capitulum^{-1} were observed by Kavani *et al*. (2000) and Reddy *et al*. (2004). The PCV values were higher than GCV for capitula plant^{-1}, seeds capitula^{-1} and seed yield plant^{-1}, which implied the effect of environment on the variability in these traits (Neelima *et al*., 2021).

Higher broad sense variability was found associated with the characters *viz*., days to 50% flowering (96.39%), days to maturity (95.71%), seed size (99.29%), 100-seed weight (92.92%) and oil content (93.28%). Rathod *et al*. (2021) reported similar results for days to 50% flowering and 100-seed weight. Higher heritability was observed for 100-seed weight by Dhutmal *et al*. (2006). Pushpavalli *et al*. (2017) reported low to moderate heritability and genetic advance as percentage of mean for seed yield plant^{-1} and test weight. This suggested greater effectiveness of selection and improvement to be expected for these characters in future breeding programme as the genetic variance was mostly due to the additive gene action. Higher genetic advance as percent of mean was observed for the number of capitula plant^{-1}, number of seed capitula^{-1}, seed yield plant^{-1}, seed size and 100-seed weight (Table 4). Similarly, high genetic advance was observed for the number of seed capitula^{-1} and seed yield plant^{-1} (Dambal *et al*., 2016; Valli *et al*., 2016; Rathod *et al*., 2021). The 100-seed weight recorded high heritability coupled with high expected genetic advance, indicating the presence of additive gene action and effectiveness of phenotypic selection.

Principal component analysis (PCA)

Multivariate analysis was used to study the patterns and relationships between variables. SGMP was subjected to principal component analysis for nine safflower features. The first principal component (PC1) contributed maximum towards variability (26.84%), followed by PC2 (22.37%), PC3 (14.61%), and PC4 (11.51%), respectively. Bahmankar *et al*. (2017) studied PCA in 20 safflower cultivars, and found that 80% of total variance in data was explained by three principal components, where the contribution of the first, second and third components was 34, 32 and 14%, respectively. Similarly, PCA results showed that the three components accounted for 29.5, 15.9 and 11.1% of the

Table 5: Eigen values, proportion of total variance and factor loading of nine quantitative traits represented by four principal components (PC)

Characters	PC1	PC2	PC3	PC4
Eigen value	2.420	2.010	1.310	1.040
Variation	26.840	22.370	14.610	11.510
Cumulative Percent variation	26.840	49.210	63.820	75.330
Days to 50% flowering	0.506	-0.165	-0.305	0.266
Days to maturity	0.429	-0.207	-0.418	0.365
Branches plant^{-1} (No.)	0.172	0.530	-0.219	-0.128
Capitulum plant^{-1} (No.)	0.174	0.589	-0.037	0.104
Seeds capitula^{-1} (No.)	0.333	0.073	0.205	-0.043
Oil content (%)	0.380	-0.063	0.530	-0.203
Seed size (mm)	-0.075	-0.164	0.467	0.683
Seed yield plant^{-1} (g)	0.088	0.487	0.280	0.350
100-seed weight (g)	-0.478	0.159	-0.248	0.366

total variation with 48 genotypes in the findings of Ojaq *et al*. (2020). Phenological characters such as days to 50% flowering (0.506) and days to maturity (0.429) and oil content (0.38) had major positive association in PC1, seed yield (0.487) and its attributing characters like the number of branches plant^{-1} (0.530), number of capitula plant^{-1} (0.589) were major traits with positive association in PC2, oil content (0.530) and seed size (0.467) was the main factor in PC3 and seed size (0.683), 100-seed-weight (0.366) and seed yield plant^{-1} (0.350) was represented in PC4 (Table 5). The biplot of germplasm by quantitative characters was created for PC1 and PC2 (Fig. 2). The number of branches plant^{-1} was significantly and positively correlated with the number of seeds plant^{-1}, number of capitula plant^{-1} and number of seeds capitulum^{-1} because they were plotted together in biplot; however, oil content, days to maturity, and days to flowering initiation were not significantly correlated because they were plotted on different axes. Since they were on the opposite sides of an axis, the seed yield was negatively correlated to the seed size and oil content to test weight. The checks were divided into axes, with genotypes A-1 and PBNS-12 on the same axis, NARI-6 on different axis, and

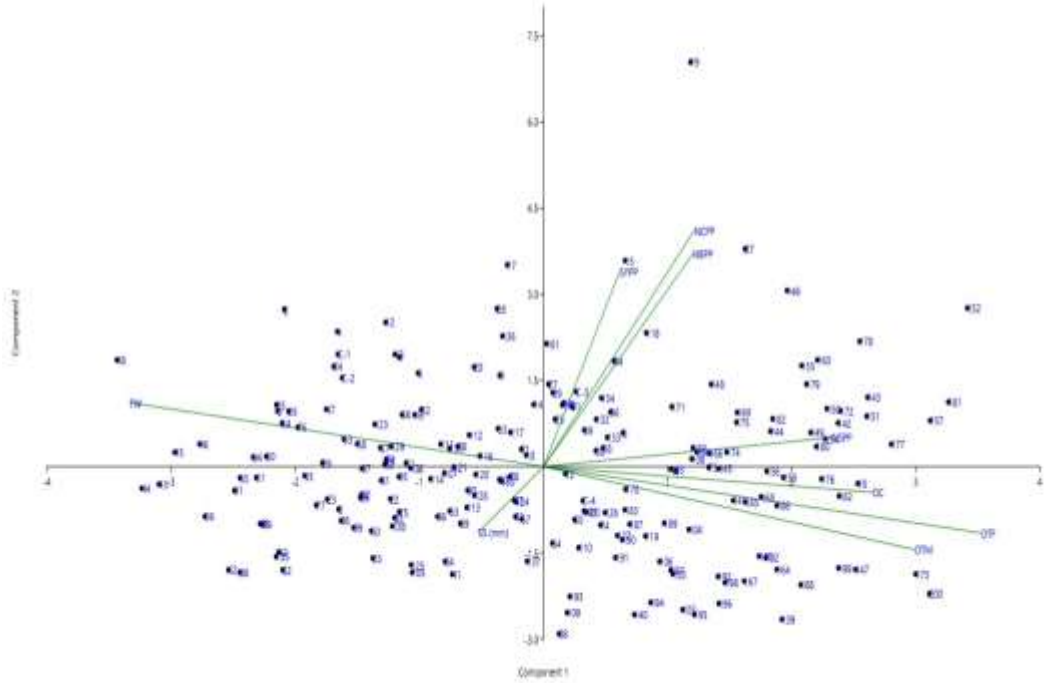

Fig. 2: Biplot for germplasm accessions and quantitative traits of the safflower germplasm mapping panel

NARI-57 on the axis opposite to A-1. Throughout breeding operations significant genetic gain is expected from qualities with high variability (Gana, 2013). The PCA for agro-morphological features has previously been conducted on safflower by Golkar *et al*. (2011), Shinwari *et al*. (2014), Arif *et al*. (2016), Safavi *et al*. (2017) and Jabbari *et al*. (2022). Germplasm accessions from various sources were randomly distributed across all components, and PCA revealed no strong correlation between diversity pattern and geographic origin.

The majority of quantitative features are represented by a consistent, homogeneous mapping panel with significant variance. In this large mapping panel population, the majority of characteristics had high broad sense heritability and genetic progress. The use of this mapping panel accessions to improve a specific quantitative attribute can result in significant genetic gain. Each characteristic phenotypic value quantifies the importance and contribution of each component to overall variation. The main contributors to genetic divergence in safflower genotypes were plant height, seed yield plant^{-1}, and number of seeds plant^{-1}, which accounted for the majority of phenological variables. Thus, when using these traits in breeding programme, the prominent traits that come together in different principal components contribute to explain variability and have a tendency to remain together so may be taken into account.

Acknowledgments: The authors gratefully acknowledge the financial support given by ICAR-Indian Institute of Oilseeds Research, Hyderabad, India.

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