*Indian J. Genet.*, 74(3): 353-361 (2014)
DOI: 10.5958/0975-6906.2014.00853.0

# Genetic variability for morphological and biochemical characters in safflower (*Carthamus tinctorius* L.)

**N. H. Rampure, P. N. Majumdar and R. S. Badere**[*,1]

Department of Botany, Hislop College, Temple Road, Civil Lines, Nagpur 440 001

(Received: August 2013; Revised: May 2014; Accepted: June 2014)

**Abstract**

**Safflower (*Carthamus tinctorius* L.) is cultivated primarily for its oil, which is regarded as 'healthy'. A set of 58 varieties and breeding lines of safflower were analysed for genetic diversity for 23 desirable characters. A great deal of genetic variability was found for the characters like days to bolt and flower, plant height, oil content, test weight of seeds. But for the characters like flower colour, fatty acid profile, the variability was narrow.**

**Key words:** Safflower, fatty acid, oilseeds, protein, correlation

## Introduction

India is a major player in global oilseed production. It has a rich oleaginous plant wealth comprising of groundnut, rapeseed-mustard, soybean, safflower, sesame, niger, castor, linseed etc. [1]. Safflower (*Carthamus tinctorius* L.) is surprisingly overlooked inspite of yielding oil with a rare combination of 'good for taste' and 'good for health'. It has been cultivated for its various purposes such as the dye, yielded by the florets, which is used to colour cotton and silk in religious ceremonies [2, 3] and to colour cheese and flavour sausage in different countries. Safflower has medicinal importance also, as its foliage is used to prevent abortion and infertility in women and the boiled young leaves and thinnings are eaten as vegetable side dish with curry or rice in India, Pakistan and Burma.

The oil of safflower seed is used globally as cooking medium, salad oil and for margarine production having highest polyunsaturated to saturated fatty acid ratio and suitable for use in chilled foods [2]. Safflower is being cultivated since ancient times, but is not popular with farmers and hence is under exploited. The crop is hardy, suitable to tropics but has some drawbacks like spineness, hard hull, low oil content etc. The meal contains toxic substances and high fibre and thus its use as animal feed is limited. The oil with high oleic acid content is considered to be better than that with high linoleic acid content. Thus, modification of fatty acid profile of safflower oil would be a value addition for the crop. A lot of work on analysing associations, genetic diversity for morphological and quality trait in Indian germplasm has been carried out and reported [4, 5]. Yet the work on breeding high yielding genotypes of safflower with quality oil need attention. To popularize the crop as a better alternative to the conventional oilseed crops the priorities such as breeding of high yielding varieties to make it popular among farmers and consumers alongwith the modification of fatty acid composition of seed oil suitable for specific purpose need focus. The prerequisite for breeding is to identify diverse parents for hybridization. Therefore, a study was conducted in some of the varieties and breeding lines of safflower for searching desirable attributes, which could be exploited in breeding programmes.

## Materials and methods

### *Plant material*

Fifty-eight accessions of safflower were procured from Dr. Panjabrao Deshmukh Krushi Vidyapeeth, Akola India. The seed of each accession was obtained from selfed plants to ensure purity. The crop was grown in 10 x 10 feet plot during the year 2008-09 (November

*Corresponding author's e-mail: rsbadere@rediffmail.com
[1]Present address: Dept. of Botany, RTM Nagpur University, M. J. Phuley Educational Campus, Amravati Road, Nagpur 440 03
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

to May) at Kalambha village of Katol tahsil in Nagpur district, ($21^o21'42'N$ and $78^o34'6''E$). The distance between two plants in a row was kept 20cm. and that between two rows 45cm. Observations on various morphological, yield and biochemical parameters were recorded on 20 plants per accession. The characters studied were days to bolt, days to 50% flowering (days needed for appearance of first head in the 50% of plants in the population), flower colour, plant height (measured from soil level to the tip of shoot at maturity), number of primary branches per plant at maturity, spineness, number of spines per leaf, spine density (total number spines per leaf/cm), leaf area (length along the midrib x width at the centre of leaf), heads per plant, head size (widest diameter of the head at maturity), test weight (100 seed weight), seed weight per plant (g), no. of seeds per head, seed length, seed width and hull thickness.

### *Biochemical analyses*

*Oil content*

The oil content was determined by soxhlet method. For this 5g dry seeds were crushed and the meal was dried at $60^oC$ overnight. Next day the meal was weighed and the thimble containing meal was extracted with petroleum ether in soxhlet apparatus for nine hours. The defatted meal was again weighed after drying in a desiccator to calculate the oil content of sample.

*Fatty acid profile*

The fatty acid profile was determined by Gas liquid chromatography essentially using standard method [6]. The seeds were crushed in a vial containing 200µl methylating solution (0.8% methanolic sodium metal). After 10 minutes 800µl of petroleum ether 60-$80^oC$ was added to the vial and the upper layer of petroleum ether containing methyl esters of fatty acids was separated after 5 minutes. 2µl of this solution was injected in the gas chromatograph equipped with capillary BP-20 column and flame ionization detector. Nitrogen at 17psi was used as carrier gas. The oven, injector and detector temperature was set at 160, 180 and $240^oC$, respectively.

*Crude protein content*

The protein content of seed meal was determined by micro Kjeldahl method [7]. 300mg of defatted meal (as described above) was digested with 7.5ml concentrated sulphuric acid along with 100mg catalyst ($CuSO_4$, $K_2SO_4$ and $SeO_2$ in 1 : 9 : 0.02 ratio) for 6-12h. Later the digest was diluted to 50ml by distilled water and 5ml of this was introduced in the condenser of Markhom's distillation apparatus. This was followed by addition of 10ml of 40% NaOH to it and the steam was passed through the condenser. The ammonia formed in it was collected in a flask containing 10ml of 2% boric acid containing mixed indicator (0.375% Bromocresol green and 0.250% Methyl red in 95% ethanol). The volume of boric acid was allowed to increase to 20ml and then the distillation was stopped. This solution was subsequently titrated with 0.035N HCl till the appearance of pink colour. The protein content in the meal was calculated from the following formula:

Protein content (mg/g meal) = 104.2 x titre value of 0.035N HCl

All the analyses were carried out in triplicate.

### *Statistical analyses*

Mean, standard error and correlation analysis were performed using MS-Excel. The similarity between accessions for morphological, yield and biochemical parameters was analyzed using Mesquite [8]. This software allows analyzing multistate categorical data and constructing a dendrogram. A character matrix for the accessions was prepared on the basis of class for each character. The character state of each class was designated with a number from 0 to 6. Subsequently, cluster analysis was performed by unweighted pair group method using arithmetic averages (UPGMA) and a dendrogram was constructed by calculating distances (uncorrected distances) between the accessions from the character matrix.

## Results and discussion

In the present investigation, 23 characters of economic importance in safflower were studied. A great deal of variability exists in the Indian germplasm of safflower. These observations are in concurrence with earlier findings [9] which were based on a different set of germplasm. However, in the present study the extent of variability differs with the traits investigated (Table 1). Since safflower is cultivated in India from ancient times, it prompted Vavilov to consider India as one of the centres of origin of safflower. However, Knowles [10] had a counterview to this. However, the results of present investigation showed that while few characters had narrow range of variability, the others were variable to a greater extent. Being an Asteraceous plant, the length of rosette stage affects the flowering time in

**Table 1.** Mean of important characters in safflower accessions

| Variety/ breeding line | Plant height (cm.) | Seed yield (g) | Oil content (%) | Linoleic acid (%) | Meal protein content (mg/g) | Variety/ breeding line | Plant height (cm.) | Seed yield (g) | Oil content (%) | Linoleic acid (%) | Meal protein content (mg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AKS-207 | 107 ± 2.1 | 19.60 ± 0.64 | 19.0 ± 1.18 | 66.75 ± 0.51 | 28.47 ± 0.75 | AKS 310 | 110 ± 1.1 | 16.89 ± 0.73 | 27.8 ± 0.51 | 72.87 ± 0.55 | 72.57 ± 2.04 |
| Bhima | 121 ± 1.3 | 29.82 ± 1.19 | 22.4 ± 0.97 | 75.40 ± 1.76 | 34.03 ± 0.28 | AKS X 38-1 | 108 ± 1.0 | 32.46 ± 1.18 | 25.2 ± 0.95 | 78.02 ± 0.59 | 51.39 ± 1.58 |
| PBNS 12 | 112 ± 1.7 | 27.72 ± 0.50 | 20.3 ± 1.03 | 79.21 ± 0.61 | 26.74 ± 0.98 | AKS-1 | 94 ± 1.2 | 23.25 ± 1.46 | 25.9 ± 1.14 | 72.70 ± 0.39 | 53.82 ± 1.24 |
| Phule Kusuma | 115 ± 1.9 | 35.40 ± 0.96 | 20.9 ± 0.94 | 75.19 ± 0.93 | 41.67 ± 0.85 | AKS-96 | 103 ± 1.1 | 40.18 ± 1.35 | 24.8 ± 0.59 | 73.37 ± 0.79 | 63.19 ± 1.72 |
| NARI 6 | 129 ± 3.1 | 15.08 ± 1.59 | 26.6 ± 0.16 | 74.59 ± 0.57 | 62.50 ± 1.30 | AKS-113 | 108 ± 1.5 | 35.89 ± 0.83 | 27.3 ± 0.53 | 70.49 ± 1.35 | 75.00 ± 1.30 |
| A-1 | 121 ± 1.6 | 51.68 ± 1.39 | 20.4 ± 0.33 | 73.34 ± 0.57 | 62.85 ± 1.02 | AKS-162 | 97 ± 1.3 | 27.13 ± 1.51 | 25.0 ± 0.60 | 71.39 ± 0.27 | 70.83 ± 1.77 |
| A-2 | 120 ± 1.6 | 43.32 ± 1.31 | 28.9 ± 0.80 | 76.75 ± 1.85 | 54.17 ± 0.49 | AKS-167 | 94 ± 1.4 | 33.14 ± 0.96 | 27.4 ± 0.64 | 75.50 ± 0.46 | 47.22 ± 0.75 |
| N-7 | 93 ± 1.4 | 25.78 ± 0.92 | 23.1 ± 0.71 | 76.54 ± 1.91 | 50.35 ± 0.75 | AKS-192 | 98 ± 0.9 | 32.74 ± 1.17 | 23.3 ± 0.29 | 74.08 ± 0.53 | 53.82 ± 1.24 |
| Manjira | 105 ± 2.4 | 45.73 ± 2.45 | 24.9 ± 1.46 | 73.16 ± 0.11 | 53.47 ± 0.75 | AKS-205 | 103 ± 1.0 | 25.72 ± 1.11 | 23.8 ± 0.34 | 75.09 ± 0.19 | 69.10 ± 1.50 |
| Sharda | 117 ± 1.4 | 45.35 ± 1.27 | 18.3 ± 0.80 | 75.34 ± 0.66 | 60.76 ± 2.04 | PI SPS 21-6 | 104 ± 0.8 | 18.04 ± 0.93 | 25.0 ± 0.19 | 71.13 ± 1.12 | 47.57 ± 1.42 |
| EC-398376 | 92 ± 1.1 | 40.02 ± 1.36 | 23.7 ± 1.14 | 75.46 ± 0.66 | 51.74 ± 4.12 | AKS 70 | 106 ± 0.8 | 24.78 ± 0.91 | 23.2 ± 0.88 | 71.49 ± 1.25 | 68.06 ± 1.02 |
| AKS/GMU 3058 | 98 ± 1.6 | 12.99 ± 1.13 | 25.8 ± 1.40 | 76.29 ± 1.43 | 47.57 ± 1.30 | AKS 104 | 93 ± 0.7 | 21.01 ± 0.88 | 29.6 ± 0.13 | 73.25 ± 1.58 | 71.88 ± 1.77 |
| AKS PI 21-1 | 111 ± 1.2 | 12.84 ± 0.66 | 24.6 ± 0.29 | 77.49 ± 3.28 | 35.42 ± 1.70 | AKS/S-33 | 95 ± 0.7 | 11.65 ± 0.74 | 24.9 ± 0.09 | 73.62 ± 0.12 | 55.56 ± 0.75 |
| AKS-73 | 124 ± 1.3 | 23.27 ± 0.81 | 20.6 ± 0.42 | 71.86 ± 0.81 | 42.71 ± 1.96 | AKS/S-34 | 103 ± 0.9 | 15.69 ± 0.92 | 29.2 ± 0.30 | 73.73 ± 0.89 | 64.24 ± 1.98 |
| AKS-108 | 133 ± 1.7 | 19.08 ± 0.79 | 26.4 ± 0.40 | 67.82 ± 1.62 | 46.18 ± 1.98 | AKS/S-41 | 78 ± 1.0 | 24.13 ± 1.16 | 24.5 ± 0.91 | 71.50 ± 1.70 | 48.26 ± 1.24 |
| AKS-157 | 117 ± 1.3 | 42.38 ± 0.65 | 26.3 ± 0.90 | 76.41 ± 0.49 | 43.75 ± 0.85 | AKS/B-5 | 101 ± 1.0 | 7.63 ± 0.89 | 20.1 ± 0.81 | 80.30 ± 0.65 | 37.85 ± 0.57 |
| AKS-165 | 102 ± 1.6 | 33.74 ± 0.78 | 24.0 ± 0.04 | 76.33 ± 0.88 | 68.40 ± 1.98 | AKS/W-26-1 | 107 ± 1.2 | 10.05 ± 0.86 | 23.5 ± 0.19 | 79.00 ± 0.97 | 45.83 ± 0.49 |
| AKS-191 | 106 ± 1.2 | 26.67 ± 0.86 | 24.3 ± 1.58 | 73.00 ± 0.80 | 64.93 ± 0.28 | AKS/GMU 2724 | 113 ± 0.8 | 17.87 ± 0.85 | 24.4 ± 0.75 | 72.94 ± 0.33 | 75.69 ± 1.86 |
| AKS-200 | 112 ± 0.5 | 27.36 ± 1.25 | 27.3 ± 0.64 | 77.67 ± 0.72 | 87.15 ± 1.13 | AKS/GMU 3293 | 105 ± 1.0 | 18.13 ± 0.81 | 23.5 ± 3.64 | 78.30 ± 0.52 | 56.94 ± 0.28 |
| AKS-217 | 146 ± 0.9 | 27.28 ± 1.51 | 31.4 ± 0.95 | 78.42 ± 0.46 | 78.13 ± 0.98 | AKS/GMU 2924 | 94 ± 0.9 | 24.09 ± 0.85 | 26.3 ± 0.57 | 74.78 ± 0.85 | 48.96 ± 0.85 |
| Tara | 105 ± 1.6 | 26.50 ± 1.14 | 22.1 ± 0.81 | 78.95 ± 0.71 | 83.33 ± 0.85 | AKS 311 | 112 ± 1.0 | 17.85 ± 1.07 | 27.5 ± 0.25 | 75.57 ± 0.85 | 48.61 ± 1.98 |
| Nira | 115 ± 1.8 | 33.98 ± 1.23 | 25.1 ± 0.46 | 78.64 ± 0.14 | 46.53 ± 2.04 | AKS PI 12 | 105 ± 0.9 | 12.96 ± 1.06 | 25.3 ± 0.62 | 72.37 ± 0.23 | 50.69 ± 0.75 |
| JSF-1 | 97 ± 0.6 | 27.17 ± 0.96 | 25.1 ± 0.19 | 78.13 ± 2.21 | 60.42 ± 1.77 | AKS-3 | 104 ± 0.9 | 14.54 ± 0.98 | 26.3 ± 0.93 | 73.15 ± 1.04 | 58.68 ± 1.13 |
| JSI-7 | 105 ± 1.7 | 26.04 ± 0.71 | 25.9 ± 0.47 | 73.36 ± 1.69 | 57.29 ± 2.73 | AKS-103 | 105 ± 1.0 | 14.29 ± 1.08 | 19.6 ± 0.43 | 72.00 ± 1.38 | 59.03 ± 0.75 |
| JSI-73 | 103 ± 1.3 | 31.15 ± 1.39 | 24.0 ± 0.50 | 78.52 ± 0.60 | 68.75 ± 3.07 | AKS-155 | 106 ± 0.9 | 22.06 ± 0.82 | 25.0 ± 0.71 | 74.09 ± 0.57 | 54.51 ± 1.72 |
| JSI-97 | 95 ± 2.0 | 26.81 ± 1.84 | 22.0 ± 0.40 | 76.33 ± 0.43 | 66.32 ± 1.13 | AKS-163 | 113 ± 0.8 | 11.99 ± 0.80 | 20.1 ± 0.68 | 71.84 ± 0.64 | 48.96 ± 0.49 |
| JSI-99 | 44 ± 1.4 | 5.42 ± 0.98 | 23.9 ± 0.19 | 76.32 ± 0.63 | 51.39 ± 1.58 | AKS-170 | 115 ± 0.8 | 23.80 ± 1.12 | 25.3 ± 0.51 | 74.84 ± 0.33 | 47.57 ± 0.75 |
| Sagar Muthiyalu | 100 ± 1.4 | 40.69 ± 1.65 | 28.8 ± 0.61 | 77.42 ± 1.29 | 51.04 ± 2.46 | AKS-198 | 102 ± 0.8 | 23.46 ± 1.04 | 26.6 ± 1.16 | 76.35 ± 1.37 | 48.61 ± 1.24 |
| HUS-305 | 106 ± 1.3 | 11.05 ± 0.79 | 30.3 ± 0.32 | 74.72 ± 0.59 | 55.90 ± 2.21 | AKS-209 | 95 ± 1.4 | 26.85 ± 0.93 | 26.2 ± 0.47 | 76.54 ± 1.21 | 60.76 ± 0.57 |

safflower. It culminates in bolting during the vegetative growth after which the flowering occurs. The accessions took between one to two months for bolting. However, about two third of the accessions bolted between 33 and 45 days. After bolting, the vegetative growth occurred for more than a month before flowering. Although, the range for days to 50% flowering was from 73 to 119 but more than 90% of accessions flowered not early than 92 days. Plant height, on the other hand, was highly variable ranging from 44.7 to 146.7 cm. Most of the accessions had the average plant height between 89.1 and 119.0 cm. The growth and development of safflower is greatly affected by temperature. Bolting does not occur until the temperature is above 15$^{o}$C. Similarly, the flowering is also affected by the temperature at the time of planting. Ideally when the seeds are sown the temperature should be more than 4.5$^{o}$C. In addtion, for flowering to occur, temperatures higher than that of bolting are needed [11].

In contrast to the above traits, the colour of flower showed very narrow variability with only three shades *viz*., yellow- the most dominant one (35 accessions) followed by white (16 accessions) and orange (7 accessions). Four dominant genes *viz*., *Y, C, O* and *R* have been reported in Indian germplasm [12]. The gene *C* alone or in combination with *O, R* or both produces greyish-white flowers. On the other hand in combination with *Y, C* produces red flowers. The yellowish brown flowers are produced when *Y* and *C* are combined with either *O* or *O* and *R.*

The average number of primary branches per plant were as many as 34.1 but most of the accessions produced not more than 20 branches. Presence of spines is one of the reasons for the crop not being popular amongst farmers. Thus, breeding for non-spiny varieties is one of the challenges for safflower breeders. Out of 58 accessions studied only seven were non-spiny. The number of spines per leaf and spine density varied from 1.2 to 34.8 and 0.2 to 2.3 spines/cm, respectively. The presence of spines, a dominant character over spinelessness, is controlled by four genes *Sa*, *Sb*, *Sc* and *Sd*. *Sa* is the main gene and its combination with any two of the other genes generates variability in spineness through complementary duplicate action [13]. Variability for leaf size (9.4 to 35.2cm$^2$) also existed amongst the accession studied. The leaf area of most of the accessions varied between 13.2 and 21.3 cm$^2$. The large leaves (> 21.4 cm$^2$) and small leaves (< 13.1 cm$^2$) were mere exceptions. Number of heads per plant and head size are the major yield components in safflower and the accessions displayed the variability for both these traits also. The number of heads per plant ranged between 8.3 and 83.8 thus showing a greater variability (Fig. 1). However, in comparison to this the variability for head size was comparatively narrow with diameter of the head varying between 21.5 and 41.4mm. But the head of most of the accessions had the average size between 24.5 and 27.4mm. One of the earlier studies carried out by Ashri *et al*. [14] in 900 lines from 20 countries indicated that the head size varies with regional gene pool.

The test weight (100 seed weight) also showed wider variability ranging from 3.9 to 9.6g. But most of the accessions had the test weight of < 7.1g (Fig. 2). Similarly, seed weight per plant varied from 5.4 to 51.7g amongst the accessions studied, thus displaying a

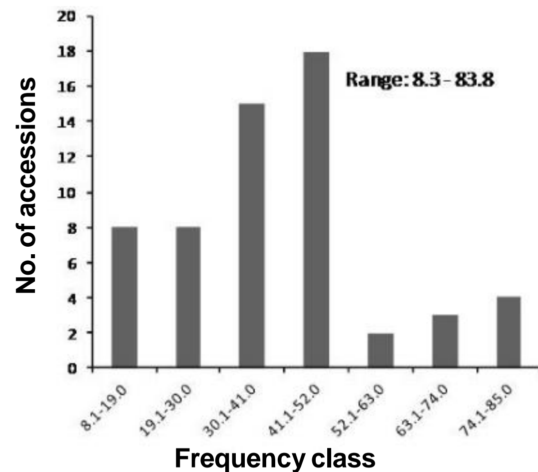


**Fig. 1. The distribution of genotypes for heads/plant in safflower**

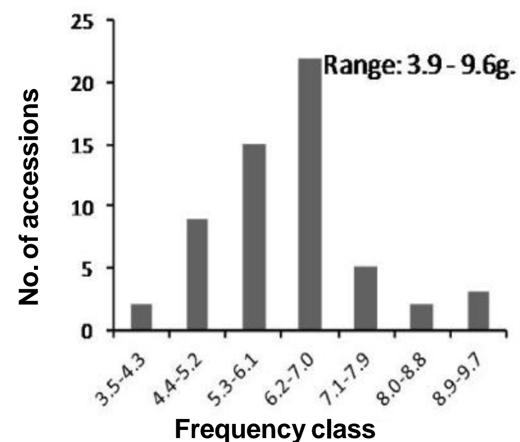


**Fig. 2. The frequency distribution of accessions for test weight in safflower**

broad genetic variability. Moreover, this variability was found to be more or less equally distributed. In contrast to this the variability for number of seeds per head was comparatively narrower (10.5 to 48.0) and most of the accessions formed less than 28.1 seeds per head. The size of seed was estimated in terms of its length and breadth. Both these parameters showed narrow variability. The range for length and breadth varied from 7.6 to 10.9 and 4.2 to 6.1mm, respectively. Hull thickness is another important trait in the breeding of oilseed crops. In the accessions studied, the hull thickness ranged from 0.25 to 0.68mm. However, in almost all the accessions the hull was thinner than 0.52mm. Apart from the normal hull five other hull type variants *viz*., partial hull, thin hull, stripped hull, grey stripped hull and reduced hull have been described in safflower. Moreover, genes determining these types of hull have also been reported. Partial hull (*par par*) has dark seeds due to reduced sclerenchyma and has high oil and protein content. It is recessive to normal hull and is inherited independent of thin hull. The plants with thin hull (*th th*) show structural male sterility and are sensitive to environmental effects. Stripped hull (*stp stp*) is not an preferred character as its oil is pigmented and has undesirable odour. Reduced hull (*rh rh*) is dominant over partilly hull. Similarly, normal hull is dominant or partially dominant over reduced hull [13].

The oil content among the accessions ranged between 8.3 and 31.4%. Moreover, the frequency distribution displayed a perfect bell-shaped pattern. Although, in the present study lower oil content was observed but the accessions with oil content of more than 35% have been reported by Abd-El-Lattief [15]. The fatty acid profile of seed oil showed the presence of four fatty acids *viz*., plamitic, stearic, oleic and linoleic acid; the last one being a major fatty acid followed by oleic acid. The remaining two fatty acids were less than 7.11%. The variability in terms of fatty acid content was also narrow. The palmitic acid varied between 1.18 to 7.11%, while the stearic acid ranged from 1.51 to 5.00%. The major fatty acids i.e. oleic and linoleic acids varied from 10.82 to 22.92% and 66.75 to 80.30%, respectively. Knowles [10] has mentioned five types' of safflower oil and their genotypes according to Futehally. These are very high linoleic (*OlOlliliStSt*), high linoleic (*OlOlLiLiStSt*), high oleic (*ololLiLiStSt*), intermediate oleic (*ol'ol'LiLiStSt*) and high stearic (*OlOlLiListst*). Evidently, this classification is based on the major fatty acid present in the oil. All the accessions analyzed in the present investigation can be designated as high linoleic genotypes. The crude protein content of the seed meal was also estimated in the present investigation which ranged from 26.74 to 87.15mg/g meal.

Most of the traits showed significant correlation, either positive or negative, with each other. However, the strength of correlation varied with the pair of characters concerned (Table 2). Days to 50% flowering was positively correlated to plant height (r = 0.69) and number of heads per plant (r = 0.34) on one hand, while it was negatively correlated to crude protein content of meal (r = –0.34) on the other. Johnson *et al*. [16] have also mentioned about positive correlation between days to flower and plant height in safflower. While flower colour was negatively correlated to spines per leaf (r = –0.32) and hull thickness (r = –0.29); it showed a positive correlation with head size (r = 0.30). Similarly, head size was also positively correlated to plant height (r = 0.28) and seeds per head (r = 0.66). Such correlations between head size and seeds per head have been reported earlier [14]. Number of primary branches showed correlation with many traits of the plant. In most of the cases like spineness (r = –0.37), spines per leaf (r = –0.37), leaf area (r = –0.41) and head size (r = –0.51) the correlation was negative. Whereas, in case of heads per plant (r = 0.83) and seed weight per plant (r = 0.41) the number of primary branches correlated positively. The above mentioned correlations have been reported earlier [17, 18] but the set of material was different. Such correlation between number of branches and seed weight per plant seems obvious because more branches ensures the synthesis of more assimilates, which results in more accumulation of dry matter after its partitioning in form of seeds [15]. Correlation of spineness with spines per leaf (r = 0.94), spines density (r = 0.93), leaf area (r = 0.37) and seed length (r = 0.43) was highly significant. Similarly, spines per leaf was also positively correlated to spine density (r = 0.89), leaf area (r = 0.55), test weight (r = 0.28) and seed width (r = 0.32), and negatively correlated to palmitic acid content (r = –0.32). Spine density was correlated to seed length (r = 0.43). Similarly, leaf area was positively correlated to head size (r = 0.51), seed width (r = 0.42), oleic acid content (r = 0.33) and linoleic acid content (r = –0.29) in seed oil. Heads per plant was negatively correlated to head size (r = –0.53) and seeds per head (r = –0.29), and positively correlated to seed weight per plant (r = 0.65).

As far as seed characters are concerned oil content was negatively correlated to test weight (r = –0.45), seed length (r = –0.43), seed width (r = –0.40) and hull thickness (r = –0.30), whereas it was positively correlated to crude protein content of the meal (r = 0.33). A comparable report has been made in Iranian breeding lines of safflower by Phalavani [19]. Although, protein content in the seed show negative correlation with seed oil content in flax but such correlation is not necessary in case of protein content of de-fatted seed meal. Simultaneous increase in seed oil and meal protein content in the linseed mutants have been reported [20]. Positive correlation was observed between seed length and seed width (r = 0.37), between hull thickness and test weight (r = 0.41) and between hull thickness and seed width (r = 0.60). Similarly, test weight was also correlated to seed dimensions (r = 0.61). This correlation is reasonable as the larger size of seed would result in more weight of the seed. In the present study it was evident that seed dimensions are positively correlated to hull thickness and negatively correlated to oil content. In turn, as expected, hull thickness was negatively correlated to oil content. Moreover, test weight is also negatively correlated to oil content. Thus, the lower oil yield of the accessions can be explained on the basis of these complex correlations between various components wherein an increase in seed size or test weight would result in thicker hull, which would lower the oil content in the seed. Seeds per head showed negative correlation to heads per plant (r = –0.29), test weight (r = –0.45) and head size (r = –0.53). Such correlations also present a hurdle in developing high yielding varieties. Seed weight per plant was positively correlated to heads per plant and negatively correlated to palmitic acid (r = –0.37). The fatty acid present in seed oil also displayed a correlation for their content in the oil. While stearic acid was negatively correlated to oleic acid (r = –0.40); the oleic acid was negatively correlated to linoleic acid (r = –0.94). During the fatty acid biosynthesis, stearic acid is elongated to oleic acid, which is subsequently desaturated to linoleic acid. Thus, while stearic acid is the precursor of oleic acid and oleic acid itself is the precursor for linoleic acid; the negative correlations observed between stearic acid & oleic acid, and oleic acid & linoleic acid can be understood (Table 2).

The above discussed results clearly indicate that the various yield components like test weight, number of heads per plant, number of branches etc. show various degree of correlations. Such correlations have been mentioned earlier also in Indian germplasm. For example, strong positive correlations have between seed yield per plant and number of heads per plant, number of branches per plant and test weight [4, 5]. Correlations of such magnitude are helpful in devising the breeding strategies for crop improvement. For instance, on the basis of their studies Ranga Rao *et al*. [4] are of view that number of heads per plant, weight of the head and hull percent to be most important components in breeding for higher yield and oil content. Moreover, it has been opined that selection of heads per plant, branches per plant and test weight will indirectly help in selecting the plants or genotypes with higher yield. Further, it has also been suggested to select genotypes with large heads and more number of seeds per head as it has been reported the presence of highly significant positive correlation amongst them has been reported [5] as observed in this case also. The observed correlations may not always indicate that one of the two characters has significant effect on the other. Rather, it is also possible that the gene(s) controlling two characters are linked or the two characters, which exhibit correlation, are under the control (may be partially) of a pleiotropic gene.

The dendrogram constructed (branch lengths are drawn proportional to the distance between accessions) using Mesquite consisted of 10 major clusters (A to J), which means the average of 5.8 accessions per cluster. Out of these only two clusters *viz*., B and C were large enough to include 16 and 13 accessions, respectively. The other clusters contained 1 to 5 accessions within them (Fig. 3). These major clusters can largely be delimited on the basis of spininess and flower colour. Cluster A, B, C, E, F, H and I consisted of accessions which were spiny, while the clusters D and J consisted of non-spiny accessions. There was only 1 cluster i.e. cluster G which contained both, spiny and non-spiny accessions within it. The other morphological feature which was helpful in categorisation of the accessions was the flower colour. The cluster A and H consisted of 4 and 1 accessions, respectively with white flowers. However, the accessions with either yellow or orange flower colour were dispersed in the other clusters with an exception of cluster D and I, which exclusively consisted of the accessions with yellow flower colour. The cluster B had all yellow flowered accessions except the AKS-1, which had white flowers. The cluster C consisted of two sub-clusters with 5 and 8 accessions. Out of these two, the sub-cluster formed by PBNS-12, A-1, Phule Kusuma, Bhima and Sharda had yellow flowers. The

**Table 2.** Correlations between different traits in safflower accessions

| Traits | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 0.69** | | | | | | | | | | | | | | | | | | | | | |
| 5 | | | | -0.37** | | | | | | | | | | | | | | | | | | |
| 6 | | –0.32* | | -0.37** | 0.94** | | | | | | | | | | | | | - 0.32* | | | | |
| 7 | | | | | 0.93** | 0.89** | | | | | | | | | | | | | | | | |
| 8 | | | | -0.41** | 0.37** | 0.55** | | | | | | | | | | | | | | | - 0.29* | |
| 9 | 0.34* | | | 0.83** | | | | | | | | | - 0.29* | | | | | | | | | |
| 10 | | 0.30* | 0.28* | -0.51** | | | | 0.51** | -0.53** | | | | 0.66** | | | | | | | | | |
| 11 | | | | | | 0.28* | | | | | | | - 0.45** | | | | | | | | | |
| 12 | | | | 0.41** | | | | | 0.65** | | | | | | | | | - 0.37** | | | | |
| 14 | | | | | 0.43** | | 0.43** | | | | 0.61** | | | | | | | | | | | |
| 15 | | | | | | 0.32* | | 0.42** | | | 0.61** | | | 0.37** | | | | | | | | |
| 16 | | -0.29* | | | | | | | | | 0.41** | | | | 0.60** | | | | | | | |
| 17 | | | | | | | | | | | -0.45** | | | -0.43** | -0.40** | -0.30* | | | | | | |
| 19 | | | | | | | | | | | | | | | | | | | | | - 0.40** | |
| 20 | | | | | | | | 0.33* | | | | | | | | | | | | | - 0.94** | |
| 22 | -0.34* | | | | | | | | | | | | | | | | 0.33* | | | | | |

* and ** Significant at $p<0.05$ and $p<0.01$, respectively; **Traits:** 1. Days to 50% flowering, 2. Flower colour, 3. Plant height, 4. Primary branches per plant, 5. Spineness, 6. Spines per leaf, 7. Spine density, 8. Leaf area, 9. Heads per plant, 10. Head size, 11. Test weight, 12. Seed weight per plant, 13. Seeds per head, 14. Seed length, 15. Seed width, 16. Hull thickness, 17. Oil content, 18. Palmitic acid content, 19. Stearic acid content, 20. Oleic acid content, 21. Linoleic acid content, 22. Crude protein content

other sub-cluster had all but one accession with white flower. AKS-192 of this sub-cluster had orange flowers. In the same line all the accessions of cluster E had white flowers except AKS/S-41, which had yellow flowers. The cluster G also had two sub-clusters which could be distinguished on the basis of flower colour. The sub-cluster composed of AKS/B-5 and AKS-103 had yellow flowers, while the other sub-cluster of the cluster G had the accessions with orange flowers. However, AKS-3 was an exception in this case with yellow flowers. The utility of qualitative characters like spininess and flower colour in categorising the accessions in broad categories lies in the fact that these were the characters with minimum variation. While there were only two states *viz.*, spiny and non-spiny with respect to spininess but in case of flower colour three states of character was present i.e., yellow, orange and white. The further distribution of the accessions within a cluster depended mostly on the quantitative characters which were in seven different states. This facilitated the arrangement of accessions on different branches of the dendrogram. For example cluster F consisted of two sub-clusters with 2 accessions each. One sub-cluster $F_1$ was formed of AKS-310 and AKS-311, while the other i.e. $F_2$ was formed of AKS-217 and AKS-163. The accessions of $F_1$ bolted after 40 days of sowing and showed 50% flowering after 100 days of sowing. In contrast to this the accession of $F_2$ bolted earlier than them (not later than 35 days of sowing) and showed 50% flower a bit later i.e. around 104 days of sowing. The accessions in these sub-clusters were than segregated on the basis of different characters. Like, in case of $F_1$, AKS-310 and AKS-311 contrasted each other in terms of flower colour

**Fig. 3. A UPGMA dendrogram drawn using Mesquite showing the relationship between the accessions evaluated**

(orange vs. yellow), oleic acid content (12% vs. 16%) and linoleic acid content (72% vs. 75%). On the other hand AKS-217 and AKS-163 were separated on the basis of flower colour (white vs. yellow), plant height (146.7cm. vs. 113.6cm.), oleic acid content (13% vs. 15%) and linoleic acid content (78% vs. 71%) (Fig. 3).

The topology of dendrogram reveals the magnitude of differences in the germplasm, which could be exploited in the breeding programmes. For example, genotypes with characters like spinelessness can be exploited in crossing programmes for introgression of spineless character in the popular varieties. However, the local accessions are best base material to develop new varieties as also stated earlier [21]. Inspite of this there are some important traits like flower colour, fatty acid profile etc. which display a narrow variability. Thus, to broaden the variability in local germplasm introduction of exotic genotype can be resorted to. However, no clear relationship between accessions and geographical diversity was found in earlier studies [22, 23]. An alternative to introduction is induced mutagenesis, which can be particularly employed if novel phenotype has to be conferred to the crop.

## Acknowledgements

Authors are thankful to Dr. S. N. Deshmukh, PKV, Akola for supplying the seeds, Dr. S. E. Pawar, Scientist, BARC, Mumbai for suggestions in conduct of investigation and Dr. R. P. Thakre and Dr. K. M. Borkar, Dept. of Botany, RTM Nagpur University, for allowing to use the GLC facility of their laboratory.

## References

1. **Rao V. R.** 1991. Improved technologies in oil seeds performance and promise under real farm situations.

Directorate of oilseeds Research, Hyderabad, India.

2. **Weiss E. A.** 1971. Castor, Sesame and Safflower. Barnes and Noble Inc., New York, USA.
3. **Weiss E. A.** 1983. Safflower. *In*: Oilseed crops. Longman group Ltd., London, UK: 216-281.
4. **Ranga Rao V., Ramchandran M. and Arunachalam V.** 1977 An analysis of association of components of yield and oil in safflower (*Carthamus tinctorius* L.). Theor. Appl. Genet., **50**: 185-191.
5. **Roopa V. K. and Ravikumar R. L.** 2008 Character association studies on cultivars of safflower (*Carthamus tinctorius* L.). Karnatak J. Agric. Sci. **21**(3): 436-437.
6. **Badere R. S. and Choudhary A. D.** 2004a. Induced low linolenic acid mutants in Indian cultivars of *Linum usitatissimum* L. Physiol. Mol. Biol. Plants, **10**(2): 285-289.
7. **Anonymous**. 1970. Official methods of analytical chemists. Arlington, USA.
8. **Maddison W. P. and Maddison D. R.** 2011. Mesquite: A modular system for evolutionary analysis. (http://mesquiteproject.org)
9. **Narkhede B. N. and Patil A. M.** 1990. Phenotypic stability in safflower. J. Maharashtra Agric. Univ., **15**: 95-96.
10. **Knowels P. F.** 1989. Safflower. *In*: Oil crops of the world. (eds. R. K. Downey *et al.*) McGraw-Hill, New York, USA: 363-374.
11. **Smith J.,** 2005. Safflower oil. *In*: Bailey's Industrial Oil and fat products. (ed. F. Shahidi) John Wiley & Sons, Inc., USA: 491-530.
12. **Narkhede B. N. and Deokar A. B.** 1990. Inheritance of spininess and pericarp types in safflower. J. Maharshtra Agric. Univ., **15:** 279-281.
13. **Dajue L. and Mundel H.** 1996. Safflower *Carthamus tinctorius* L. IPGRI, Rome (Italy) and IPK, Gatersleben Germany).
14. **Ashri A., Urie A. L. and Zimmer D. E.** 1976. Evaluation of the germplasm collection of safflower, *Carthamus tinctorius* L. VII. Variability of capitulum width and outerinvolucral bracts' dimensions. Euphytica, **25:** 225-229.
15. **Abd-El-Lattief E. A.** 2012. Evaluation of 25 safflower genotypes for seed and oil yields under arid environment in upper Egypt. Asian J. Crop Sci., **4**(2): 72-79.
16. **Johnson R. C., Ghorpade P. B. and Bradley V. L.** 2001. Evaluation of the USDA core safflower collection for seven quantitative traits. . *In*: 5th International Safflower Conference: Safflower: A multipurpose species with unexploited potential and world adaptability. (eds. J. W. Bergman and H. H. Mündel) Williston, North Dakota and Sidney, Montana, USA: 149-152.
17. **Camas N., Cirak C. and Esendal E.** 2007. Seed yield, oil content and fatty acid composition of safflower *Carthamus tinctorius* grown in Northern Turkey conditions. J. Fac. Aric.*,* **22:** 98-104.
18. **Eslam B. P., Monirifar H. and Ghassemi M. T.** 2010. Evaluation of late season drought effects on seed and oil yields in spring safflower genotypes. Turk. J. Agric. For., **34:** 373-380.
19. **Pahlavani M. H.** 2005. Some technological and morphological characteristics of safflower (*Carthamus tinctorius* L.) from Iran. Asian J. Pl. Sci., **4**(3): 234-237.
20. **Badere R. S. and Choudhary A. D.** 2004b. Induced mutations in linseed (*Linum usitatissimum* L.). Indian J. Genet., **64**(2): 159-160.
21. **Elfadl E., Reinbrecht C and Claupein W.** 2010 Evaluation of phenotypic variation in a worldwide germplasm collection of safflower (*Carthamus tinctorius* L.) grown under organic farming conditions in Germany. Genet. Resour. Crop Evol. **57**: 155-170.
22. **Mahasi M. J., Pathak R. S., Wachira F. N., Riungu T. C. and Kamundia J. W.** 2005. Development and evaluation of safflower (*Carthamus tinctorius* L.) cultivars for the marginal rainfall areas of Kenya: Morphological characterization, genetic diversity and adaptation studies. Sesame Safflower Newsl., **20**: 68-75.
23. **Elfadl E., Reinbrecht C and Claupein W.** 2010 Evaluation of phenotypic variation in a worldwide germplasm collection of safflower (*Carthamus tinctorius* L.) grown under organic farming conditions in Germany. Genet. Resour. Crop Evol., **57**: 155-170.