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Research Note

Characterization and clustering of safflower germplasm based on seed morphological traits

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Abstract

Safflower (*Carthamus tinctorius* L.) is a minor oilseed crop that originated in the Middle East. Characterization and clustering analysis of genotypes are basic and necessary biometrical tools to assess the variations present in the collection. The present investigation was carried out to study important seed morphological traits *viz.,* area, perimeter, width, length, aspect ratio, radius and circularity by using the camera attached microscope (Euromex Image Focus Plus software) in 60 safflower genotypes obtained from the Indian Institute of Oilseeds Research (IIOR), Hyderabad. The maximum area, perimeter, length and width were documented in CO-1 (36.03 mm^2), CO-1 (25.73 mm), GMU-3438 (9.70 mm) and C0-1 (5.79 mm), respectively. The genotypes GMU-900, GMU-1920 and GMU-4035 exhibited significance for all the traits. In cluster analysis, seven clusters were formed with 7, 6, 6, 8, 16, 1 and 16 genotypes in clusters I, II, III, IV, V, VI and VII respectively. Clusters mean values indicated that, clusters I and II were more diverse and clusters II and VI were close to each other. Based on the aspect ratio cluster VI genotypes contained conical shape seed and cluster I contained the oval shape of seed. Based on circularity, cluster I, IV and III genotypes contains circular bold seeds. The genotypes *viz.,* GMU-900, GMU-4035, GMU-3785, GMU-6944, GMU-1193, GMU-2020 and GMU-2366 present in cluster I recorded larger area and perimeter.

Keywords: safflower, seed morphology, characterization, image analyser, cluster analysis

The safflower (*Carthamus tinctorius* L.) (2n=24) is a member of the Asteraceae family. It ranks seventh among oilseed crops in India *viz.,* groundnut, rapeseed, mustard, soybean, castor, sunflower, linseed, sesame and niger. In ancient Indian literature, it is referred to as kusumba. India, China, Mexico, the United States of America, Ethiopia, Argentina and Australia are the world's largest safflower growers. Globally, safflower was cultivated in 0.70 to 0.98 million ha and the production ranged from 0.53 to 0.83 million tonnes during the last decade (Saisanthosh *et al.,* 2018).

In India, safflower is grown in 0.56 lakh ha with a total production of 0.36 lakh tonnes and 640 kg/ha of productivity (Indiastat agri, 2022). Maharashtra, Karnataka and Andhra Pradesh are the major producers in the country. It is a winter herbaceous crop, branched and also contains spines on the leaves. In India, normally grow in the *rabi* season. The rosette form was present in crop in some period after crop rise, then rapid stem elongation to mature height was observed. It contains globular capitulum with brilliant yellow, orange or red flowers (Dajue and Mündel, 1996). Safflower is one of the world's oldest multi-purpose oilseed crops. Conventionally, it is cultivated for seeds, flowers, fabric dyes, food colouring and medicinal reasons (Li and Mundel, 1996). Carthamin is a natural dye which is produced from bright coloured flower petals. The seeds are used to extract vegetable oil for both domestic consumption and industrial application. The safflower seed oil content varied from 20% to 45% (Liu *et al.,* 2016) and it contains a high level of unsaturated fatty acid content (>75% linoleic or oleic acid), which is used as a cooking oil and has an important function in reducing blood cholesterol levels. In order to address the need for human insulin globally, transgenic safflower has also been developed to produce human insulin from seeds (Boothe *et al.,* 2010).

The conventional techniques for the characterization of seeds are laborious, time-consuming, and fundamentally unreliable. As an alternative, it is possible to differentiate seeds of different varieties from a seed lot using an effective and affordable instrumentation system (Vasanthan *et al.,* 2019). Numerous researchers have worked to find non-destructive techniques for cultivar identification during seed quality control programmes in the last ten years, todetermine the cultivar identity of seed lots and testing of new cultivars for authenticity before registering them as new varieties (Dell' Aquila, 2006). Digital image analysis replaces the manual classification of biological seeds by integrating image acquisition equipment with a computer (Jayas *et al*., 2000)

Considering its importance, the characterization of genotypes is vital for the genetic improvement of economically important traits. Generally morphological, biochemical and molecular level characterization has some drawbacks *viz*., time-consuming, destructive, influenced by the environment and cost expensive. The machine vision system of characterization overcomes these problems and has advantages *viz*., quick, simple and non-destructive with great accuracy (Vithu and Moses, 2016). Physical characteristics of grains, such as colour, size, shape, etc., are important. This technique is cost effective and fully computerised quality evaluation system. The morphological characters such as area, perimeter, width, length, aspect ratio, radius and circularity were observed. The characters such as area, width and length were used to find bold seeded genotypes and aspect ratio to find circular genotypes respectively. The minor variation that is difficult to detect by general characterization can be easily screened by non-destructive image analysis system. Artificial intelligence is playing a vital role in every field including agriculture in a more efficient way. Hence the seed morphological traits are assessed by a non-destructive image analysis system used to characterize and clustering the genotypes.

A total of 60 safflower genotypes were collected from the ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad **(Table 1)**. The genetically pure seeds were obtained by selfing of the genotypes during *rabi* season, 2020 at the Breeding experimental farm, Department of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Madurai. Artificial intelligence has an irreplaceable role in the present era of technology and its application in plant science is developing rapidly. The adaption of the technology not only reduces labour burden but also seems to have more advantages like increased accuracy, reduce time, low cost, reliability *etc.,* Digital image analysis is an innovative recent method that is used to measure various morphometric features of the seeds with great accuracy and this technique had been used in different crops (Sau *et al.,* 2018). The seeds were placed on a stage where a camera attached with microscope version 4.0 connected to the computer and seven morphological traits *viz*., seed area, length, width, perimeter, aspect ratio, radius and circularity were recorded for individual seeds and the same was repeated three times. The experimental imagea are presented in **Fig. 1**.

Procedure

(i) Sample preparation

The selfed seeds of each accession were dried and cleaned to remove foreign materials. The seeds were randomly selected for each replication.

(ii) Mounting of camera in a microscope and Instalation of the software

A camera was put into the eyepiece of a microscope using 23.2 mm adapters to fit the typical 23.2 mm tube of a microscope. The driver software ImageFocus 4.0 was installed in the system, in which the images are to be acquired.

(iii)Image acquisition and measurements

The camera settings are adjusted to obtain correct images, are image measurements such as length, breadth, area, perimeter and circularity are done and images are saved as still pictures.

(iv) Calibration

Before measurements of images are made, the calibration table is updated with the proper calibration values for the microscope for all the magnifications and cameras.

The area of object is obtained by multiplication of the object's length which is expressed in mm^2 and the diameter of the smallest circumscribed circle that fits around the object aepressed in mm, or the distance between two points on the object, marked on the screen using the mouse. The perimeter was calculated from multiplication of the object's length, breadth and height in mm. The dimension measured along the horizontal X-axis is calledwidth which is represented in mm. the Aspect ratio is measured from the proportionate relationship between the width and the height of the object (Aggarwal and Mohan, 2010). The circularity is defined as the square root of the ratio of the actual area of the object to

Table 1. List of safflower genotypes used in the study

S. No.	Accessions	Source/Developed by	S. No.	Accessions	Source/Developed by
1	GMU-184	ICAR-IIOR, Hyderabad	31	GMU-4009	ICAR-IIOR, Hyderabad
2	GMU-704	ICAR-IIOR, Hyderabad	32	GMU-4035	ICAR-IIOR, Hyderabad
3	GMU-855	ICAR-IIOR, Hyderabad	33	GMU-4093	ICAR-IIOR, Hyderabad
4	GMU-900	ICAR-IIOR, Hyderabad	34	GMU-4101	ICAR-IIOR, Hyderabad
5	GMU-1193	ICAR-IIOR, Hyderabad	35	GMU-4128	ICAR-IIOR, Hyderabad
6	GMU-1229	ICAR-IIOR, Hyderabad	36	GMU-4814	ICAR-IIOR, Hyderabad
7	GMU-1303	ICAR-IIOR, Hyderabad	37	GMU-5146	ICAR-IIOR, Hyderabad
8	GMU-1437	ICAR-IIOR, Hyderabad	38	GMU-5517	ICAR-IIOR, Hyderabad
9	GMU-1920	ICAR-IIOR, Hyderabad	39	GMU-5520	ICAR-IIOR, Hyderabad
10	GMU-2020	ICAR-IIOR, Hyderabad	40	GMU-5571	ICAR-IIOR, Hyderabad
11	GMU-2347	ICAR-IIOR, Hyderabad	41	GMU-5712	ICAR-IIOR, Hyderabad
12	GMU-2366	ICAR-IIOR, Hyderabad	42	GMU-5761	ICAR-IIOR, Hyderabad
13	GMU-2385	ICAR-IIOR, Hyderabad	43	GMU-5815	ICAR-IIOR, Hyderabad
14	GMU-2551	ICAR-IIOR, Hyderabad	44	GMU-5850	ICAR-IIOR, Hyderabad
15	GMU-2758	ICAR-IIOR, Hyderabad	45	GMU-5933	ICAR-IIOR, Hyderabad
16	GMU-2968	ICAR-IIOR, Hyderabad	46	GMU-5964	ICAR-IIOR, Hyderabad
17	GMU-3098	ICAR-IIOR, Hyderabad	47	GMU-5965	ICAR-IIOR, Hyderabad
18	GMU-3165	ICAR-IIOR, Hyderabad	48	GMU-6114	ICAR-IIOR, Hyderabad
19	GMU-3185	ICAR-IIOR, Hyderabad	49	GMU-6207	ICAR-IIOR, Hyderabad
20	GMU-3326	ICAR-IIOR, Hyderabad	50	GMU-6878	ICAR-IIOR, Hyderabad
21	GMU-3438	ICAR-IIOR, Hyderabad	51	GMU-6944	ICAR-IIOR, Hyderabad
22	GMU-3482	ICAR-IIOR, Hyderabad	52	GMU-7107	ICAR-IIOR, Hyderabad
23	GMU-3488	ICAR-IIOR, Hyderabad	53	GMU-7243	ICAR-IIOR, Hyderabad
24	GMU-3708	ICAR-IIOR, Hyderabad	54	GMU-7666	ICAR-IIOR, Hyderabad
25	GMU-3758	ICAR-IIOR, Hyderabad	55	GMU-7688	ICAR-IIOR, Hyderabad
26	GMU-3781	ICAR-IIOR, Hyderabad	56	BHIMA	Dry farming Research Station, Solapur, Mahatma PhuleKrishiVidyapeeth, Rahuri, Maharashtra, India
27	GMU-3785	ICAR-IIOR, Hyderabad	57	JSF-1	AICRP (Safflower) Centre, Indore, RajmataVijayaraje, India& KrishiVishwavidyalaya, Gwalior, Madhya Pradesh, India
28	GMU-3865	ICAR-IIOR, Hyderabad	58	NARI-57	AICRP (Safflower) centre, Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, India
29	GMU-3963	ICAR-IIOR, Hyderabad	59	NARI-6	AICRP (Safflower) centre, Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, India
30	GMU-3965	ICAR-IIOR, Hyderabad	60	CO-1	Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

the area of a circle with the same circumscribed shell and their formula is stated below,

$$C = \sqrt{\frac{A}{AP}}$$

(Abbreviations: A – an actual area of the object, AP – area of a circle with a diameter equal to the circumscribed diameter (or) length of the object)

The collected data were analyzed for the test of significance and the critical difference was determined with a probability of 5% (**Table 2**). Using R studio (R package version 1.0.7) statistical software, the genotypes were clustered based on the seven measured parameters.

The seven seed morphological traits such as length, width, area, perimeter, radius, aspect ratio and circularity were measured in 60 safflower genotypes using camera attached microscope and their mean values are

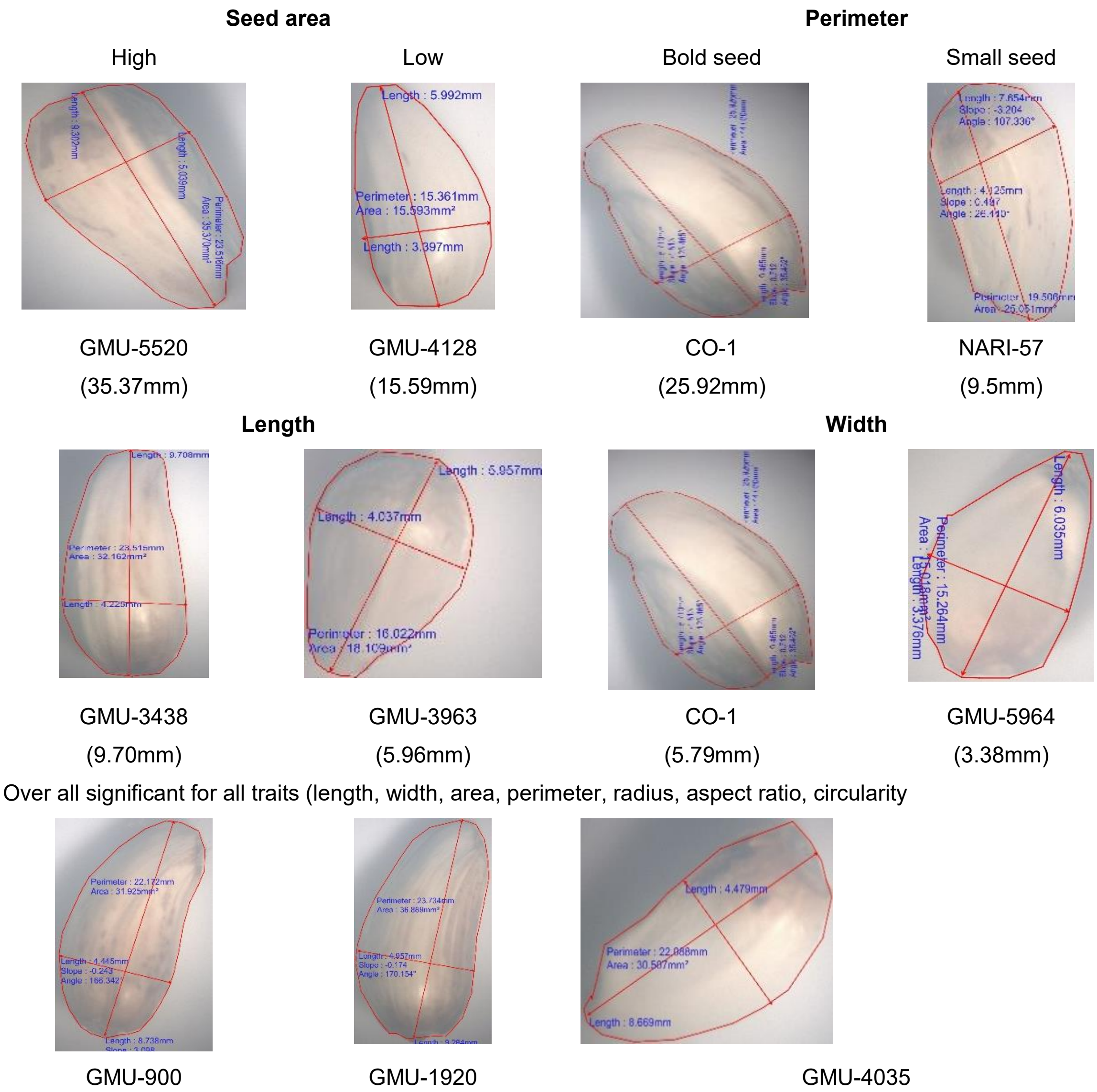

Fig. 1. Camera attached microscopic images of safflower genotypes

presented in **Table 2** and seed images are furnished as **Fig. 1**. The length, breadth and area of the seed affect its size. Seed size is a useful selection parameter since it influences germination rate, field emergence and seed vigour (Hojjat, 2011). Among the genotypes studied, CO - 1 (36.03mm^2) had the largest area and GMU 4128 had the smallest area (15.59mm^2).

The genotypes GMU-184 (30.75mm^2), GMU-704(26.51mm^2), GMU-900 (31.92mm^2), GMU-1193(29.73mm^2), GMU-1437(27.94mm^2), GMU-1920(32.88mm^2), GMU-2020 (29.74mm^2), GMU-2366 (29.96mm^2), GMU-3165 (27.43mm^2), GMU-3438 (30.16mm^2), GMU-3785 (31.46mm^2), GMU-4035 (30.50mm^2), GMU-4101 (31.32mm^2), GMU-4814 (27.71mm^2), GMU-5520 (35.37mm^2), GMU-6207(27.16mm^2), GMU-6944 (29.34mm^2), GMU-7107(29.14mm^2) and NARI-6 (32.63mm^2) were observed to have positively significant seed area, indicating that selection within these genotypes could be fruitful. Similar results were recorded in sorghum landraces (Kavipriya *et al.,* 2019), barnyard millet (Venkatesan and Sujatha, 2018) and sesame (Vasanthan *et al.,* 2019) by using Grain Scanner.

When evaluating grain morphology, the outside border measurement perimeter is also important. The two genotypes CO -1 (25.92mm) and GMU 1920 (23.73mm) had the largest perimeter while NARI-57 had the lowest (9.50mm). The seeds are classified as bold, medium and small based on the area and perimeter and hence CO -1 was classified as a large seed genotype and GMU

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Table 2. Mean performance of 60 safflower genotypes for seed morphological traits

S. No.	Genotypes	Length (mm)	Width (mm)	Area (mm^2)	Perimeter (mm)	Radius (mm)	Aspect ratio	Circularity
1	GMU-184	8.43*	5.35*	30.75*	22.54*	2.68*	1.58	2.03*
2	GMU-704	8.45*	4.15	26.51*	20.69*	2.08	2.04*	1.77
3	GMU-855	7.60	4.45*	26.32	19.62	2.23*	1.71	1.86*
4	GMU-900	8.73*	4.44*	31.92*	22.17*	2.22*	1.97*	1.91*
5	GMU-1193	8.02*	4.67*	29.73*	20.76*	2.34*	1.72	1.93*
6	GMU-1229	6.81	4.35	23.06	17.99	2.18	1.57	1.84
7	GMU-1303	6.96	3.61	18.59	17.06	1.81	1.93*	1.63
8	GMU-1437	8.07*	4.21	27.94*	20.47*	2.11	1.92*	1.86*
9	GMU-1920	9.28*	4.95*	32.88*	23.73*	2.48*	1.88*	1.99*
10	GMU-2020	8.15*	4.79*	29.74*	20.92*	2.40*	1.70	1.91*
11	GMU-2347	7.65	3.90	22.35	18.81	1.95	1.96*	1.71
12	GMU-2366	8.11*	4.74*	29.96*	21.21*	2.37*	1.71	1.92*
13	GMU-2385	6.99	3.99	20.16	17.47	2.00	1.75	1.70
14	GMU-2551	6.43	3.66	17.82	16.51	1.83	1.76	1.67
15	GMU-2758	8.75*	3.59	25.06	21.02*	1.80	2.44*	1.69
16	GMU-2968	6.79	4.63*	23.77	18.41	2.32*	1.46	1.87*
17	GMU-3098	6.27	4.96*	21.88	17.44	2.48*	1.27	1.87*
18	GMU-3165	8.77*	4.46*	27.43*	21.67*	2.23*	1.97*	1.77
19	GMU-3185	6.62	4.67*	22.98	18.00	2.34*	1.42	1.86*
20	GMU-3326	6.03	3.51	15.65	15.41	1.76	1.72	1.61
21	GMU-3438	9.70*	4.23	30.16*	23.52*	2.11	2.30*	1.82
22	GMU-3482	7.35	4.14	23.97	18.78	2.07	1.78	1.81
23	GMU-3488	6.29	4.03	19.89	16.72	2.01	1.56	1.78
24	GMU-3708	7.49	4.28	24.29	19.19	2.14	1.75	1.80
25	GMU-3758	8.20*	3.78	24.71	20.40*	1.89	2.17*	1.74
26	GMU-3781	6.75	3.87	19.84	17.16	1.93	1.75	1.71
27	GMU-3785	8.69*	4.99*	31.46*	21.68*	2.49*	1.74	1.90*
28	GMU-3865	6.72	4.59*	24.07	18.42	2.29*	1.47	1.89*
29	GMU-3963	5.96	4.04	18.11	16.02	2.02	1.48	1.74
30	GMU-3965	6.61	4.15	20.28	17.25	2.08	1.59	1.75
31	GMU-4009	8.49*	3.93	25.43	20.75*	1.96	2.16*	1.73
32	GMU-4035	8.66*	4.48*	30.50*	20.09*	2.24*	1.94*	1.88*
33	GMU-4093	6.99	4.46*	22.95	18.03	2.23*	1.57	1.81
34	GMU-4101	7.95*	5.22*	31.32*	21.69*	2.61*	1.52	1.98*
35	GMU-4128	5.99	3.40	15.59	15.36	1.70	1.76	1.61
36	GMU-4814	8.72*	3.87	27.71*	21.61*	1.94	2.25*	1.78
37	GMU-5146	6.87	4.34	23.12	18.28	2.17	1.58	1.83
38	GMU-5517	6.60	3.89	20.91	17.45	1.95	1.70	1.78
39	GMU-5520	9.30*	5.04*	35.37*	23.51*	2.52*	1.85	1.95*
40	GMU-5571	7.16	4.74*	26.17	19.16	2.37*	1.51	1.91*
41	GMU-5712	7.22	4.42*	23.51	18.49	2.21*	1.63	1.81
42	GMU-5761	7.17	4.34	25.18	19.26	2.17	1.65	1.87*
43	GMU-5815	7.36	3.90	20.85	18.43	1.95	1.89*	1.68
44	GMU-5850	7.54	3.55	20.68	18.40	1.78	2.12*	1.66
45	GMU-5933	7.54	3.98	23.75	18.97	1.99	1.89*	1.77
46	GMU-5964	6.04	3.38	17.02	15.26	1.69	1.79	1.58
47	GMU-5965	6.93	4.44*	23.04	18.56	2.22*	1.56	1.82
48	GMU-6114	6.82	3.72	19.84	17.58	1.86	1.83	1.71
49	GMU-6207	7.82	4.47*	27.16*	20.07*	2.24*	1.75	1.86*
50	GMU-6878	7.71	4.46*	25.70	19.75	2.23*	1.73	1.83
51	GMU-6944	8.26*	4.71*	29.34*	20.79*	2.36*	1.76	1.88*
52	GMU-7107	8.83*	4.05	29.14*	21.50*	2.03	2.18*	1.82
53	GMU-7243	8.14*	3.56	22.72	19.25	1.78	2.29*	1.67
54	GMU-7666	7.89*	4.27	25.21	20.17*	2.14	1.85	1.79
55	GMU-7688	6.49	3.76	19.42	16.73	1.88	1.73	1.73
56	BHIMA	8.08*	3.70	25.02	19.77	1.85	2.18*	1.76
57	JSF-1	8.30*	4.09	26.05	20.25*	2.05	2.03*	1.77
58	NARI-57	7.65	4.12	25.05	9.50	2.06	1.86	1.81
59	NARI-6	8.83*	5.33*	32.63*	22.75*	2.67*	1.66	2.01*
60	CO-1	9.46*	5.79*	36.03*	25.92*	2.90*	1.63	2.16*
	MEAN	7.62	4.28	24.96	19.31	2.14	1.80	1.81
	Sed	0.12	0.07	0.72	0.33	0.03	0.03	0.01
	CD(0.05)	0.25	0.13	1.44	0.66	0.07	0.06	0.03

*Positively significant at 5%

1920 was small seeded among the observed genotypes. The same results were observed in sorghum landraces (Kavipriya *et al.,* 2019 and Chinnasamy *et al.,* 2021), barnyard millet (Venkatesan and Sujatha, 2018) and sesame (Vasanthan *et al.,* 2019) by using Grain Scanner.

The size and shape of the seed were influenced by the length and width of the seed. The genotype GMU 3438(9.70mm) had maximum length and GMU 3963 (5.96mm) had minimum length since it has the lowest area. The genotype CO-1 had the highest width (5.79mm) while GMU 5964 had the smallest (3.38 mm). CO-1 had the largest seed area because it had the highest width among genotypes observed.

The genotypes such as GMU-184 (8.43mm), GMU-704 (8.45mm), GMU-900 (8.73mm), GMU-1193 (8.02mm), GMU-1437 (8.07mm), GMU-1920 (9.28mm), GMU-2020 (8.15mm), GMU-2366 (8.11mm), GMU-2758 (8.75mm), GMU-3165 (8.77mm), GMU-3758 (8.20mm), GMU-3785 (8.69mm), GMU-4009 (8.49mm), GMU-4035 (8.66mm), GMU-4101 (7.95mm), GMU-4814 (8.72mm), GMU-5520 (9.30mm), GMU-6944 (8.26mm), GMU-7107 (8.83mm), GMU-7243 (8.14mm), GMU-7666 (7.89mm), BHIMA (8.08mm), JSF-1 (8.30mm), NARI-6 (8.83mm) and CO-1 (9.46mm) were significant for length and they offer an effective selection platform for this trait.

The genotypes GMU-184 (5.35mm), GMU-855 (4.45mm), GMU-900 (4.44mm), GMU-1193 (4.67mm), GMU-1920 (4.95mm), GMU-2020 (4.79mm), GMU-2366 (4.74mm), GMU-2968 (4.63mm), GMU-3098 (4.96mm), GMU-3165 (4.46mm), GMU-3185 (4.67mm), GMU-3785 (4.99mm), GMU-3865 (4.59mm), GMU-4035 (4.48mm), GMU-4093 (4.46mm), GMU-4101 (5.22mm), GMU-5520 (5.04mm), GMU-5571 (4.74mm), GMU-5712 (4.42mm), GMU-5965 (4.44mm), GMU-6207 (4.47mm), GMU-6878 (4.46mm), GMU-6944 (4.71mm) and NARI-6 (5.33mm) were significant for width.

The genotypes GMU-184, GMU-900, GMU-1193, GMU-1920, GMU-2020, GMU-2366, GMU-3165, GMU-3785, GMU-4035, GMU-4101, GMU-5520, GMU-6944, NARI-6 and CO-1 were significant for three traits such as length, width and area. As a result, they can be deployed as a parental line in a breeding programme to develop bold seeded high yielding varieties. Among the 60 safflower genotypes, A1 had the largest seed length (9.20 mm) and breadth when measured by using the digital grain vernier meter (Saisanthosh *et al.,* 2018). The same work was carried out in sorghum landraces (Kavipriya *et al.,* 2019), barnyard millet (Venkatesan and Sujatha, 2018) and sesame (Vasanthan *et al.,* 2019) by using Grain Scanner.

The aspect ratio is the ratio of width and height of the grain (ratio of the major axis to the minor axis). The shape of the safflower seed such as oval, conical and crescent was observed. Majority of safflower seeds are conical and oval. Based on circularity, cluster I, IV and III genotypes contained circular bold seeds.

Except for aspect ratio, all the traits were shown to be significant for genotypes *viz.,* CO-1, NARI-6, GMU-6944, GMU-5520, GMU-4101, GMU-3785, GMU-2366, GMU-2020, GMU-1193 and GMU-184. The genotypes GMU-900, GMU-1920 and GMU-4035 were positively significant for area, perimeter, length, width, circularity, radius and aspect ratio. The observed genotypes showed a significant variation in traits and hence they can be used in the breeding programmes.

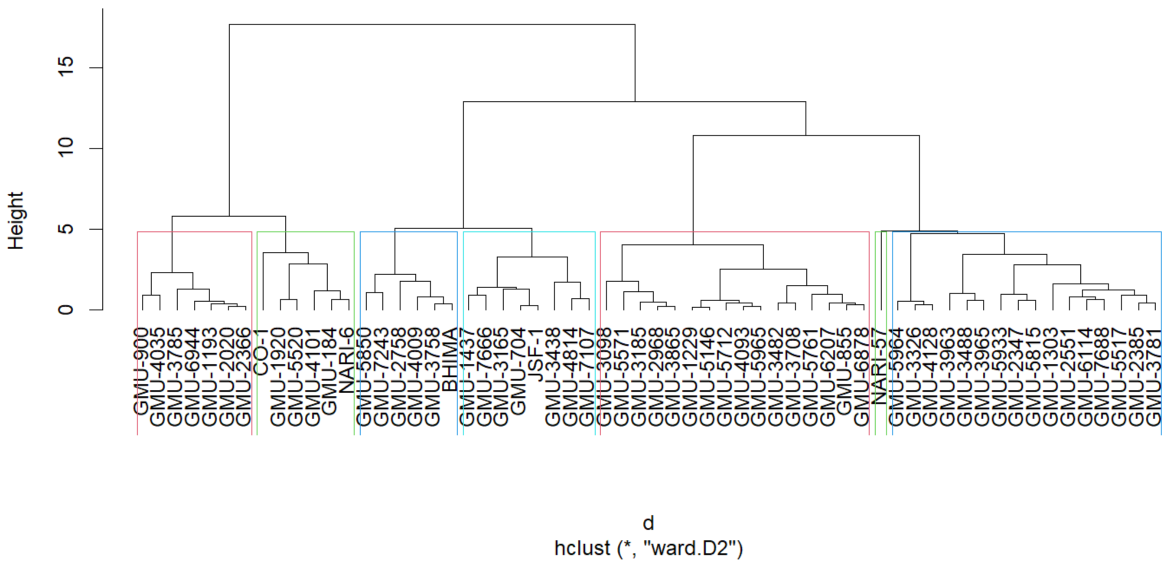

Fig. 2. Dendrogramof 60 safflower genotypes

Table 3. Cluster mean value for seven seed parameters

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
length	8.88	8.60	7.10	8.38	6.66	8.21	7.65
Width	5.28	4.17	4.48	4.69	3.80	3.69	4.12
Area	33.16	27.52	24.20	30.38	19.38	23.94	25.05
Perimeter	23.36	21.24	18.72	21.09	17.01	19.93	9.50
Radius	2.64	2.09	2.24	2.35	1.90	1.84	2.06
Aspect ratio	1.69	2.07	1.59	1.79	1.76	2.23	1.86
circularity	2.02	1.80	1.85	1.90	1.70	1.71	1.81

Table 4. Intra and inter-cluster distance

Cluster	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	**1.99**	4.43	4.37	2.59	6.77	6.22	6.95
Cluster 2		**1.54**	3.07	2.44	3.79	2.27	4.84
Cluster 3			**1.31**	2.57	2.98	3.94	4.04
Cluster 4				**1.09**	4.63	4.07	5.06
Cluster 5					**1.71**	3.17	3.80
Cluster 6						**1.34**	4.68
Cluster 7							**0**

Based on the morphological traits observed, the genotypes were grouped into seven clusters in cluster analysis. In each cluster, the number of genotypes was 7, 6, 6, 8, 16, 1 and 16 in clusters I, II, III, IV, V, VI and VII respectively. The dendrogram was drawn using R studio (**Fig. 2**).

Cluster mean values for different traits such as area, perimeter, width, length, radius and circularity are noted in **Table 3** and their intra and inter-cluster distance details are mentioned in **Table 4.**

In cluster analysis, clusters VI and II were composed of conical seeded genotypes, whereas I and III were oval. In cluster I, the genotypes GMU-900, GMU-4035, GMU-3785, GMU-6944, GMU-1193, GMU-2020 and GMU-2366 contained high area and perimeter. As a result, for breeding programme to generate bold seeded genotypes with high yield, accessions in cluster I could be exploited as parents. Seed morphological features are complex traits controlled by polygenes. The size of the seed has a direct impact on grain yield, germination percentage and nutrient content. Hence, seed morphological traits could be effective index for development of bold varieties. Similar studies of seed morphology using grain scanners and grouing them based on seed features have been done in sorghum land races (Kavipriya *et al.,* 2019) and sesame (Vasanthan *et al.,* 2019). The boldness of the seed is highly influenced by the length, breadth, area, perimeter, aspect ratio and circularity of the seed. The screened genotypes that possessed better grain morphology could be effectively used in a future breeding programme related to develop a better variety. Overall, the camera-attached microscope is a clear, simple and non-destructive instrument for genotyping individuals based on grain morphology.

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