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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 with 60 safflower genotypes obtained from the Indian Institute of Oilseeds Research (IIOR), Hyderabad. These genotypes were used to measure 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). The maximum area, perimeter, length and width were documented in CO-1 (36.03 mm²), CO-1 (25.73 mm), GMU-3438 (9.70 mm) and CO-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 inferred that, clusters I and II have more diverse and clusters II and VI have close to each other. Based on the aspect ratio cluster VI genotypes contain conical shape seed and cluster I contain 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. Safflower ranks seventh place 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. Determine 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 very important 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 sixty safflower genotypes were collected from the ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad. The list of genotypes were mentioned in **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 recorded seven morphological traits *viz.*, seed area, length, width, perimeter, aspect ratio, radius and circularity were obtained for individual seeds which same was repeated three times. The experimental image was presented in **Fig. 1**.

Table 1. List of safflower genotypes used in the study

S. No.	Accessions	Source/Developed by
1	GMU-184	Indian Institute of Oilseeds Research, Hyderabad, India
2	GMU-704	Indian Institute of Oilseeds Research, Hyderabad, India
3	GMU-855	Indian Institute of Oilseeds Research, Hyderabad, India
4	GMU-900	Indian Institute of Oilseeds Research, Hyderabad, India
5	GMU-1193	Indian Institute of Oilseeds Research, Hyderabad, India
6	GMU-1229	Indian Institute of Oilseeds Research, Hyderabad, India
7	GMU-1303	Indian Institute of Oilseeds Research, Hyderabad, India
8	GMU-1437	Indian Institute of Oilseeds Research, Hyderabad, India
9	GMU-1920	Indian Institute of Oilseeds Research, Hyderabad, India
10	GMU-2020	Indian Institute of Oilseeds Research, Hyderabad, India
11	GMU-2347	Indian Institute of Oilseeds Research, Hyderabad, India
12	GMU-2366	Indian Institute of Oilseeds Research, Hyderabad, India

Table 1. Continued..

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

(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) Mount the camera on a microscope and Install the software

The cameras were put into the eyepiece of a microscope using 23.2 mm adapters to fit the typical 23.2 mm tube of a microscope. Installing the camera drivers and the application software ImageFocus 4.0 is necessary after mounting the camera on the microscope. The image window displays when Image Focus 4.0 launches.

(iii) Image acquisition and measurements

Adjust the camera settings to obtain correct images, do image measurements such as length, breadth, area, perimeter and circularity. Then save still pictures.

(iv) Calibration

Before you can make measurements on an image, the calibration table has to be updated with the proper calibration values for all of the microscope various magnifications and cameras.

The area is referred to a multiplication of the object's length which is expressed in mm² and length means, the diameter of the smallest circumscribed circle that will fit around an object, stated in mm, or the distance between two points marked on the screen using the mouse. The perimeter was calculated from multiplication of the object's length, breadth and height in mm. The length is measured along the horizontal X-axis called width 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 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 discernment among landraces and genotypes was a dynamic effort and a computer-based image analysis

system well determines its role (Geetha *et al.*, 2011 and Kavipriya *et al.*, 2019). 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 were mentioned in Table 2 and seed images displayed at 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). CO - 1 (36.03mm²) had the largest area and GMU 4128 had the smallest area (15.59mm²).

The genotypes GMU-184 (30.75mm²), GMU-704(26.51mm²), GMU-900 (31.92mm²), GMU-1193(29.73mm²), GMU-1437(27.94mm²), GMU-1920(32.88mm²), GMU-2020 (29.74mm²), GMU-2366 (29.96mm²), GMU-3165 (27.43mm²), GMU-3438 (30.16mm²), GMU-3785 (31.46mm²), GMU-4035 (30.50mm²), GMU-4101 (31.32mm²), GMU-4814 (27.71mm²), GMU-5520 (35.37mm²), GMU-6207(27.16mm²), GMU-6944 (29.34mm²), GMU-7107(29.14mm²) and NARI-6 (32.63mm²) were positively significant for seed area indicating that selection within these genotypes is 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 traits which was help to determine that CO -1 was a large seed and GMU 1920 was smaller 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

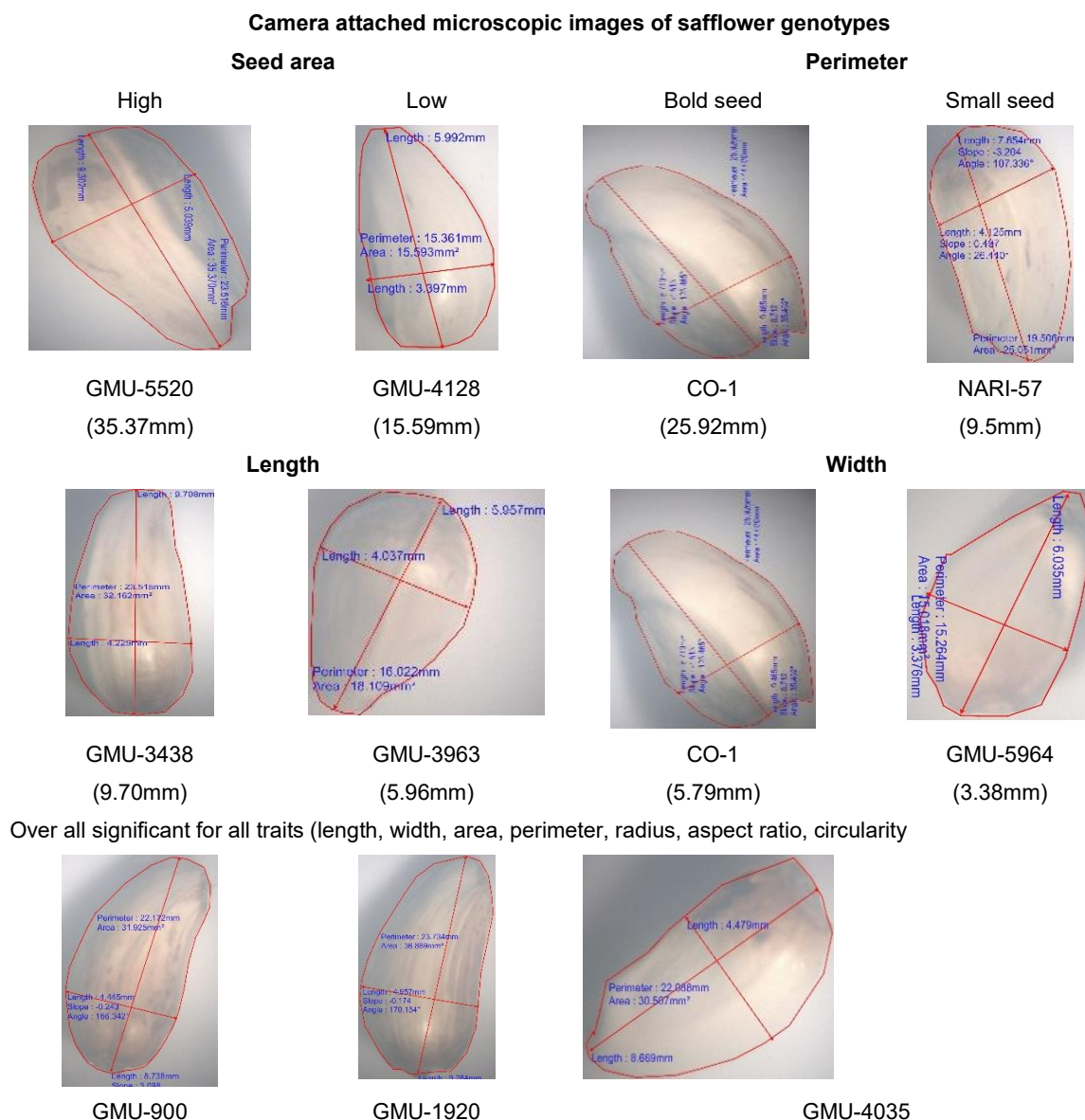


Fig. 1. Camera attached microscopic images of safflower genotypes

(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 Sixty one safflower genotypes, the variety A1 had the larger seed length (9.20 mm), breadth and it was 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.

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

S. No.	Genotypes	length	width	area	perimeter	radius	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%

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. Since the majority of safflower seeds are conical and oval. Based on circularity, cluster I, IV and III genotypes contains circular bold seeds.

Except for aspect ratio, all the traits were shown the significant for such 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.

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 conical seed shapes, whereas I and III were oval, based on aspect ratio. In cluster I, the genotypes GMU-900, GMU-4035, GMU-3785, GMU-6944, GMU-1193, GMU-2020 and GMU-2366 contain high area and perimeter. As a result, the breeding programme can obtain a parent from cluster I to generate bold seeded, high yielding safflower varieties. The cluster analysis was carried out on seed morphology trait variations measurement by using grain scanners in different crops such as sorghum land races (Kavipriya *et al.*, 2019) and sesame (Vasanthan *et al.*, 2019). Seed morphological traits are complex traits controlled by polygenes. Quantitative traits showed continuous variation since they are much influenced by environmental conditions. The morphology is very important and also size of the seed has a direct impact on grain yield, germination percentage and nutrient content. Seed morphological traits are observed to be significant

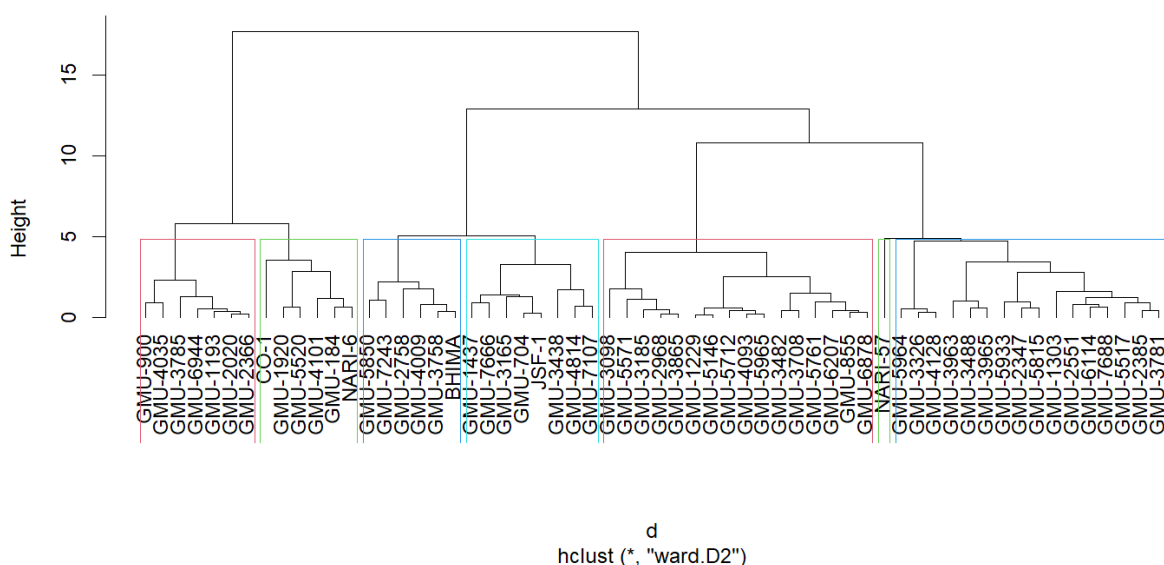


Fig. 2. Dendrogram of 60 safflower genotypes

Table 3. Cluster mean value for seven grain 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

and selection would be effective to develop bold varieties. 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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REFERENCES

- Aggarwal, A. K. and Mohan, R. 2010. Aspect ratio analysis using image processing for rice grain quality. *International Journal of Food Engineering*, **6** (5):1-14. [Cross Ref]
- Boothe, J., Nykiforuk, C., Shen, Y., Zaplachinski, S., Szarka, S., Kuhlman, P., Murray, E., Morck, D. and Moloney, M.M. 2010. Seed-based expression systems for plant molecular farming. *Plant Biotechnology Journal*, **8**(5): 588-606. [Cross Ref]
- Chinnasamy, K., Arumugam, Y., Jegadeesan, R. and Chockalingam, V. 2021. Linear discriminant analysis in red sorghum using artificial intelligence. *The Nucleus*, **64**(1): 103-113. [Cross Ref]
- Dajue, L. and Mündel, H.H. 1996. Safflower, (*Carthamus tinctorius* L.) *Biodiversity International*, **7**.
- Dell'Aquila, A. 2006. Red-Green-Blue (RGB) colour density as a non-destructive marker in sorting deteriorated lentil (*Lens culinaris* Medik.) seeds. *Seed Science and Technology*, **34**(3): 609-619. [Cross Ref]
- Geetha, V.V., Balamurugan, P. and Bhaskaran, M. 2011. Characterization of mustard genotypes through image analysis. *Research Journal of Seed Science*, **4**(4): 192-198. [Cross Ref]
- Hojjat, S. S. 2011. Effects of seed size on germination and seedling growth of some Lentil genotypes (*Lens culinaris* Medik.). *International Journal of Agriculture and Crop Sciences*, **3** (1): 1-5.
- Indiastat agri. 2022. Ministry of Agriculture and Farmers welfare, Government of India. (<https://www.indiastat.com/>).
- Jayas, D.S., Paliwal, J. and Visen, N.S. 2000. Multi-layer neural networks for image analysis of agricultural products. *Journal of Agricultural Engineering Research*, **77**(2): 119-128. [Cross Ref]
- Kavipriya, C., Yuvaraja, A., Vanniarajan, C., Ramalingam, J. and Subramani, A. 2019. An image analyser: A rapid and non-destructive method for characterization and diversity assessment of sorghum landraces. *Electronic Journal of Plant Breeding*, **10**(3):1176-1184. [Cross Ref]
- Li, D. and Mundel, H.H. 1996. Safflower *Carthamus tinctorius* L. Promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, Rome, Italy.
- Liu, L., Guan, L.L. and Yang, Y.X. 2016. A review of fatty acids and genetic characterization of safflower (*Carthamus tinctorius* L.) seed oil. *World Journal of Traditional Chinese Medicine*, **2** (2): 48. [Cross Ref]
- Saisanthosh, K., Keshavulu, K., Joesph Raju, T., Palchamy, K., Mukta, N. and Sultana, R. 2018. Variability studies for seed morphological traits in safflower genotypes. *International Journal of Current Microbiology and Applied Sciences*, **7** (10):2204-2216. [Cross Ref]
- Sau, S., Uchhesu, M., Dondini, L., De Franceschi, P., D'hallewin, G. and Bacchetta, G. 2018. Seed morphometry is suitable for apple-germplasm diversity-analyses. *Computers and Electronics in Agriculture*, **151**: 118-125. [Cross Ref]

- Vasanthan, V., Geetha, R., Menaka, C., Vakeswaran, V. and Chidambaram, K. 2019. Characterization of sesame varieties through image analysis. *Electronic Journal of Plant Breeding*, **10**(2): 785-790. [[Cross Ref](#)]
- Venkatesan, S. and Sujatha, K. 2018. Characterization of barnyard millet cultivars using seed image analysis. *Seed Research*, **45**(2): 1-3.
- Vithu, P. and Moses, J.A. 2016. Machine vision system for food grain quality evaluation: A review. *Trends in food science and technology*, **56**: 13- 20. [[Cross Ref](#)]