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MORPHOLOGICAL TRAITS BASED GENETIC DIVERSITY IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.)

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Abstract

Safflower (*Carthamus tinctorius* L.) one of the world minor oil crops originated in the Middle East. The breeding potential of the safflower genotypes held in the gene-bank of Plant Genetic Resources Institute (PGRI) has not been exploited to date. Present work was carried out to evaluate 122 genotypes collected from various eco-geographical regions/countries of the world. Observations were recorded for eleven quantitative and five qualitative characters to estimate substantial variation and relationship among the genotypes and identify promising accession(s) for traits of economic significance. A significant level of morphological diversity was noticed for a number of traits. The largest variation was recorded for capsules plant^{-1}, seeds capsule^{-1}, seed yield plant^{-1}, plant height, days to flowering initiation and days to maturity. Relatively low level of variability was distinguished in 100-seed weight, capsule diameter, primary branches plant^{-1}, days to flower completion, time of flowering, flower color, leaf shape and spininess. The correlation analysis indicated that seed diameter, capsules plant^{-1} and seeds capsule^{-1} had highly significant positive contribution to seed yield plant^{-1}. Only one trait, time of flowering showed negative correlation with seed yield plant^{-1}. Principal component (PC) analysis of 122 safflower germplasm displayed significant variation with PC1 having 26.02% of the total variation, 19.97% for PC2, 12.38% for PC3 and PC4 contributed 11.24% of the total variation and revealed that the characters that mainly distinguish the germplasm are: capsule diameter, capsules plant^{-1}, seeds capsule^{-1}, days to maturity, plant height and time of flowering. Cluster analysis recognized five major clusters. Our findings have an important application for safflower germplasm evaluation and preservation.

Introduction

Safflower (*Carthamus tinctorius* L.) belongs to family Asteraceae and is a minor crop which originated in the Middle East and part of Africa, but Mediterranean has the major area of production (Li & Mundel, 1996). It was under cultivation in the Crescent region almost 4000 years ago (Ashri, 1975). Safflower is a diploid (2n=24) annual herbaceous crop which grows well in hot and dry climate (Yuan & Li, 1989). *Carthamus* is derived from Arabic word "quartum or gurtum which refers to the colour of the petals of the flower. This species was grown in India for hundreds of years and is called Kardai in Marathi or Kusum in Hindi. The branching pattern of safflower is secondary and tertiary each terminating in capitulum. It has spiny leaves on branches and stem. The seeds are called achenes which are usually white, shiny and weighs upto 0.1g, having pappus, though sometimes without pappus too (Fernandez-Martinez & Knowles, 1978).

In the wild genus *Carthamus* is reported to have 25 species (Yuan & Li, 1989). Amongst the species of *Carthamus*, only safflower (*Carthamus tinctorius* L.) is grown worldwide containing 12 pairs of chromosomes. Various centers of diversity have been proposed from Far East to Europe (Knowles, 1969). India is the largest producer of safflower flower (68%) in the world with the highest acreage (60%) and production is mainly for the domestic vegetable oil market (Johnson & Marter, 1993). In 1960, safflower was introduced in Pakistan (mainly in Sindh & Baluchistan) as an oilseed crop (Lee *et al.,* 2009).

Fabric painting, food coloring are major beneficiary of using petals of safflower; having vitamin A & iron etc also used as salad (Carvalho *et al.,* 2006; Nimbkar, 2002). Safflower is cultivated on 611436 hectares worldwide yielding 615214 ton (FAO, 2010). Safflower contains flavonoids (Kazuma *et al*., 2000), lignins (Palter *et al.,* 1972), triterpene alcohols (Akihisa *et al.,* 1996) and polysaccharides (Wakabayashi *et al.,* 1997). Safflower is reported to be anticoagulant, antioxidant and neuro-protective (Hiramatsu *et al.,* 1998; Wang *et al.,* 2007).

Safflower a multipurpose crop rich in oleic and linoleic acids, is one of the most important oilseed crops (Li & Mundel, 1996). The byproduct after oil extraction has protein that is used as animal feed. Oil production from seed (40%) is like sunflower and olive (Pavlov & Todorov, 1996). Safflower is drought resistant and can be adopted in arid and semiarid areas (Weiss, 2000).

For breeding programme and for conservation of genetic resources, the germplasm is evaluated to use important lines (Mahmood *et al*., 2010). The efficiency of a selection program mainly depends on the degree of genetic variation and heritability of a trait (Falconer & Mackay, 1996). Though, the genetic diversity is measured through applying variety of techniques, yet, the primary source for genetic diversity is through morphological characters (i.e., leaves, stem, branches and flowers etc), mainly its shape, size and variability. In case of safflower, flower color, spininess, is major source of variations (Bradley *et al.,* 1999). As a general rule the achievement in genetic enhancement of the crop and the growth of a species wants the easiness of access of genetic diversity (Mumtaz *et al*., 2011; Jan *et al*., 2012).

Safflower possesses remarkable genetic diversity across different regions of the world (Knowles, 1989). There is only a limited work assessing genetic diversity of safflower based on agro-morphological traits (Ashri 1975; Jaradat & Shahid, 2006). The present investigation encompassing 122 different germplasm of safflower from different agro-climatic zones and various countries of the world using variety of agronomic and morphological data will add valuable information for breeders.

Materials and Methods

In 2012, 122 different safflower genotypes from different eco-geographical were cultivated in National Agriculture Research Centre Islamabad. Average rainfall at Islamabad varies from 500-900 mm with 31% in winter and 69% in summer. The size of plots was 2.5 x 4.7 m^2 with 2 lines per accession, row length 3m, keeping 3.5 m as path between beds, while distance between rows was 1m. Two irrigations were given in addition to pre-sowing irrigation. Hand drill planting and thinning was also carried out. Every thirty days weeds were proscribed. Sixteen morphological characters were chosen for analysis i.e., 11 quantitative and 5 qualitative traits (Table 1).

Quantitative traits were recorded for days to flower initiation, days flower completion, time of flowering, days to maturity, plant height, primary branches $plant^{-1}$, capsules $plant^{-1}$, capsule diameter, seeds per capsule, 100-seed weight and seed yield $plant^{-1}$. International Board of Plant Genetic Resources (IBPGR) descriptors for safflower (*Carthamus tinctorius* L.) were followed as character choice and measurements. Investigation of variance was based on mean values of accessions detected in each block. Kwon & Torrie, (1964) were followed for the correlation coefficient investigations. The level of dissimilarity was assessed through cluster analysis.

Results and Discussion

A significant level of phenotypic variation was noticed among the 122 germplasm accessions for most of the quantitative characters considered (Table 2). Sample of deviation among the genotypes was diverse for different characters. The largest variation was recorded for capsules $plant^{-1}$, seeds $capsule^{-1}$, seed yield $plant^{-1}$, plant height, days to flowering initiation and days to maturity. The variances for the above characters were 933.7, 102.0, 62.2, 52.2, 40.1 and 37.4, respectively. Relatively, a low level of variability was distinguished in 100-seed weight, capsule diameter, primary branches $plant^{-1}$, days to flower completion and time of flowering. The mean values of the safflower genotypes for days to flower initiation, days to flower completion and days to maturity were 175.2, 188.4 and 218.7 with a range of 160 to 188, 177 to 218 and 208 to 229 days, respectively. These characters could be evaluated to know for both early and delayed maturity. The safflower accessions 16320, 16360 and 26732 showed high values (229) in this regard, while a lot of other safflower accessions including 26765, 26787, 26790, 26795, 26796 and 26800 demonstrated earliness (208) in our observations. Both early and delayed maturity are vital for breeding programs trying for variation of plant germplasm to a variety of ecological areas on photoperiod and thermo-sensitivity (Suddihiyam *et al.,* 1992; Rehman *et al.,* 2009). According to Amini *et al*., (2008) to avoid biotic and abiotic stresses, early maturity cultivars are of help as facilitating early harvest. Rest of the traits demonstrated wide genetic deviation and accessions with such a huge level of genetic divergence often used for the identification of best germplasm for varied ecological circumstances.

Correlation coefficients of seed yield and yield components are given in Table 3. Data exposed that seed diameter, capsule $plant^{-1}$ and $seeds\ capsule^{-1}$ had highly significant positive contribution with seed yield $plant^{-1}$. Positive significant correlation was observed in days to maturity and primary branch $plant^{-1}$, while positive but not statistically correlation was observed in days to flower initiation, days to flower completion, plant height and 100-seed weight. Only one trait, time of flowering showed negative correlation with seed yield $plant^{-1}$. To enhance yield, number of capitula $plant^{-1}$ is an important trait (Lahane *et al.,* 1999). Following reports of Elfadl *et al.,* 2010; Eslam *et al*., 2010; Safavi, 2011; Ahmadzadeh *et al.,* 2012, we focussed on seed related characters e.g. seeds/plant, 100 kernel weight, and seed yield. For improvement of seed yield and oil content, they recommended to select traits such as seeds/plant and thousand kernel weights.

Table 1. Morphological and seed traits recorded for safflower germplasm.

Trait of interest	Scale	Description of the trait
Quantitative traits:		
Days to flower initiation (DFI)	Days	Number of days from seed sowing until 5% of plants have first flower in each accession
Days to flower completion (DFC)	Days	Numbers of days from seed sowing until 95% flowers completed
Time of flowering (TF)	Days	Number of days taken from flower initiation to completion
Days to maturity (DM)	Days	Number of days from seed sowing until plants reached physiological maturity
Plant height	Inches	Mean height of five random plants from ground level to the apex of the main stem
Primary branches per plant (PB/P)	No.	Total number of branches originating from the core stem which gives rise to other capsule branches from 5 randomly selected plants per accession
Capsules per plant (C/P)	No.	Number of capsules was counted manually from the same 5 plants chosen as sample for every character
Capsules diameter (CD)	mm	Measured by digital vernier caliper
Seeds per capsule (S/C)	No.	Counted number of seeds per capsule obtained from the same 5 capsules used for capsule diameter
100- seed weight (100-SW)	g	Weight of 100 random dried seeds of an average 5 capsules per plant was calculated
Seed yield/plant (SY/P)	g	Average seed weight of five randomly selected plants for each accession was recorded at harvesting
Qualitative traits:		
Leaf shape	-	1 = Ovate; 2 = Oblong; 3 = Lanceolate; 4 = Linear
Leaf margins	-	1 = Entire; 2 = Dentate; 3 = Parted
Angle of branches	-	0 = No branches; 3 = Appressed; 5 = Intermediate; 7 = Spreading; 9 = Drooping
Flower colour	-	1 = Yellow; 2 = Yellow-orange; 3 = Red-orange to red; 4 = White
Spininess	-	1 = No or few spines; 2 = Intermediate spines; 3 = Many spines

Table 2. Variation in quantitative traits of 122 safflower accessions.

Traits	Mean	Minimum	Maximum	SD	CV (%)	Variance
Days to flower initiation (DFI)	175.2	160	188	6.3	3.6	40.1
Days to flower completion (DFC)	188.4	177	218	4.8	2.5	22.6
Time of flowering (TF)	13.2	2	34	5.5	41.8	30.4
Days to maturity (DM)	218.7	208	229	6.1	2.8	37.4
Plant height (PH)	53.7	32.4	66.6	7.2	13.4	52.2
Primary branches plant^{-1} (PB/P)	12.6	4.0	25.7	4.3	34.4	18.6
Capsules plant^{-1} (C/P)	48.2	11.4	198.4	30.6	63.3	933.7
Capsule diameter (CD)	21.6	15.5	30.4	2.8	12.8	7.6
Seeds capsule^{-1} (S/C)	28.2	6.7	54.7	10.1	35.8	102.0
100-seed weight (HSW)	3.4	1.4	13.1	1.2	34.2	1.4
Seed yield plant^{-1} (SY/P)	14.4	3.0	38.1	7.9	54.7	62.2

Table 3. Correlation coefficients among 11 quantitative traits.

Traits	DFI	DFC	TF	DM	PH	PB/P	C/P	CD	S/C	SW	SY/P
DFI	1.00										
DFC	0.54**	1.00									
TF	-0.69	0.25**	1.00								
DM	-0.15	-0.03	0.15	1.00							
PH	0.30**	0.39**	-0.01	-0.18	1.00						
PB/P	0.31**	0.24**	-0.15	0.10	0.31**	1.00					
C/P	0.07	0.14	0.03	0.21	0.04	0.55**	1.00				
CD	0.07	0.25**	0.14	0.24**	0.11	0.07	0.26**	1.00			
S/C	0.18	0.24**	0.01	0.26**	0.06	0.10	0.30**	0.81**	1.00		
SW	-0.32	-0.23	0.16	0.07	-0.26	-0.20	-0.01	0.06	-0.05	1.00	
SY/P	0.08	0.07	-0.02	0.20	0.04	0.19	0.38**	0.44**	0.37**	0.03	1.00

Principal components analysis (PCA) often conducted to build a new set of orthogonal coordinate axes and to find out the relative significance of classification variables. There are no dealings to discover the worth of a coefficient but that is eigenvector (Düzyaman, 2005). According to Sneath & Sokal (1973) top coefficients for some characters designated the relatedness of that trait to relevant PC axes. In our assessments, the first PC contributed 26.02% of the overall variance of the agronomic data, the second 19.97%, the third 12.38% and the fourth 11.24% (Table 4, Fig. 1). PCA revealed that capsule diameter, capsule plant^{-1}, seeds capsule^{-1}, days to maturity, plant height and time of flowering were among the most important descriptors which accounted for more than half of the all phenotypic variation revealed in this 122 safflower germplasm collection. Study of all these traits will help us in recommending best safflower germplasm for Pakistan.

The cluster analysis executed with 11 quantitative and 5 qualitative traits divided 122 accession lines and one check into five different clusters (Fig. 2). Over all most of the variations were observed in the quantitative characters. Cluster I comprised of 60 accessions and was further subdivided into two sub-groups. Sub-group 1 had maximum number of genotypes (46) contributing 37.7%. The results clearly indicated that the accessions in sub-group 1 though tall, having more branches plant^{-1} but low yield potential because of less number of seeds capsule^{-1}. In contrast, the sub-group 2 represented by 14 (11.4%) safflower genotypes, and were late in maturity, taller in height, relatively less number of primary branches plant^{-1}, fewer capsules plant^{-1} but with more seeds capsule^{-1} and hence very low yield potential of plants. Cluster II included 50 accessions, and was also sub-divided into two main sub-groups. Sub-group 1 of cluster II having 41 (33.6% accessions) genotypes, were having late maturity, also short-statured with lowest number of primary branches plant^{-1}. Capsules plant^{-1} were less in number and medium number of seeds capsule^{-1} was found in this sub-group, while the highest as compared to other genotypes was recorded. This group's yield potential was medium. Sub-group 2 in second cluster consisted of nine genotypes (7.3%). These lines were late maturing, with less primary branches & seeds capsule^{-1}. Number of capsules plant^{-1} was less in this cluster, and low yield potential plant^{-1}. While comparing the height, plants of this sub-group were of short stature. Cluster III contained a total of nine genotypes which were also further sub-divided into two sub-groups. Sub-group 1 of cluster III had four genotypes and contributed 3.2% of the accessions.

Members of the sub-group can be distinguished by having fewer primary branches and number of capsules plant^{-1}. They are taller as compared to sub-group 2 and having more seeds capsule^{-1}. They are late maturing lines, and yield potential was higher than any other group. This group is about 4% of the studied germplasm (5 lines). Cluster IV taller in stature, having fewer capsule plant^{-1}, was represented by only 2 lines (1.6%). This group has low yield potential, matures quite late, primary branches are medium in number. The last one line was represented as cluster V, which was also a line of low yield potential and other characteristics similar to cluster IV.

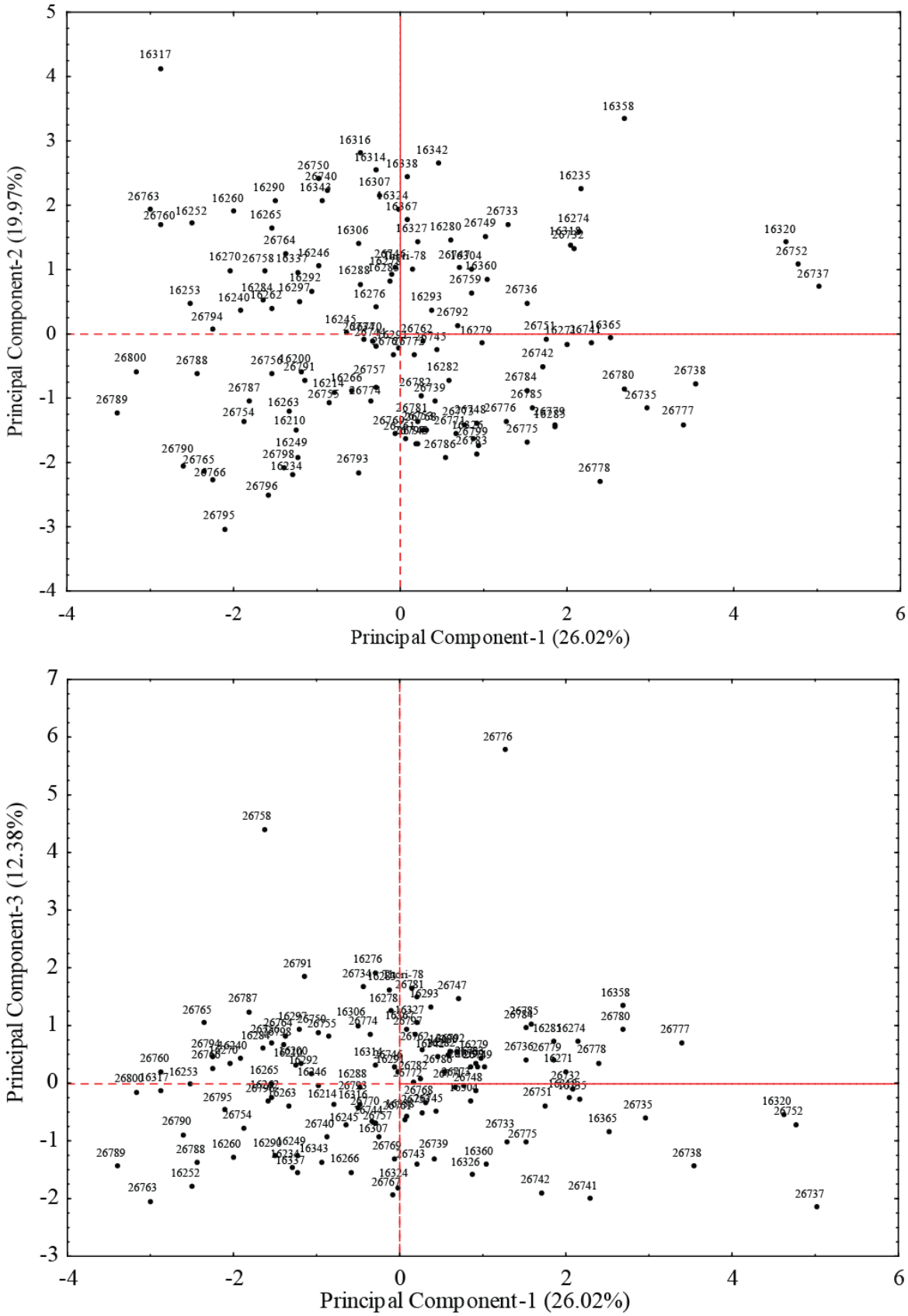

Fig.1. Scatter diagram of 122 accessions of safflower germplasm based on 11 quantitative traits.

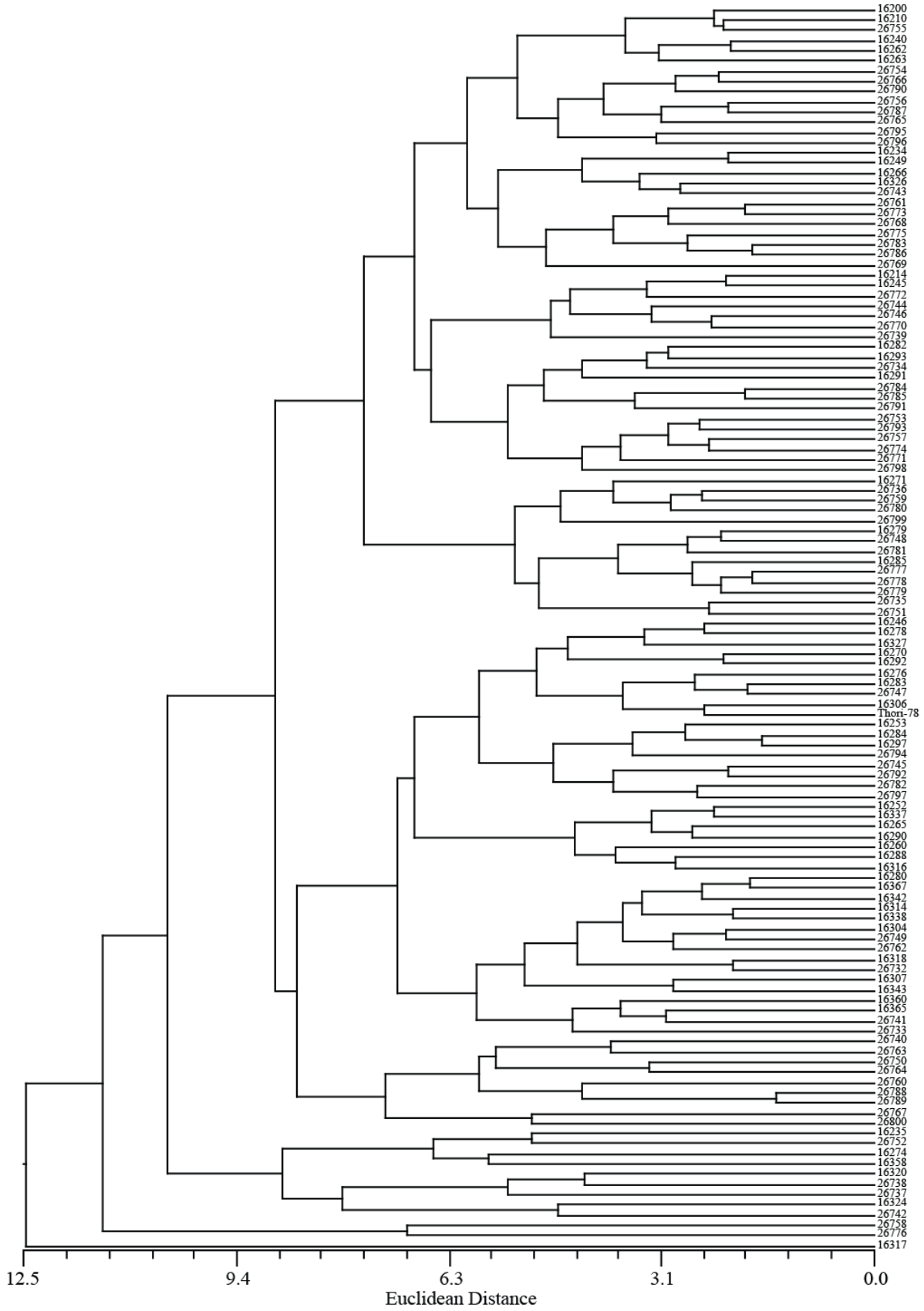

Fig. 2. Cluster analysis showing the relationships among 122 accessions of safflower germplasm based on quantitative and qualitative traits.

Table 4. Principal components of safflower germplasm.

Traits	PC1	PC2	PC3	PC4
Eigenvalue	2.86	2.20	1.36	1.24
Cumulative eigenvalue	2.86	5.06	6.42	7.66
%cent of variance	26.02	19.97	12.38	11.24
Cumulative variance	26.02	45.99	58.37	69.61
	Eigenvectors			
Days to flower initiation (DFI)	0.540	-0.692	-0.190	-0.288
Days to flower completion (DFC)	0.578	-0.261	0.594	-0.018
Time of flowering (TF)	-0.122	0.570	0.731	0.316
Days to maturity (DM)	0.228	0.525	-0.177	0.157
Plant height (PH)	0.408	-0.433	0.450	0.125
Primary branches plant^{-1} (PB/P)	0.577	-0.241	-0.190	0.616
Capsules plant^{-1} (C/P)	0.584	0.219	-0.270	0.546
Capsule diameter (CD)	0.671	0.489	0.115	-0.396
Seeds capsule^{-1} (S/C)	0.706	0.394	0.004	-0.420
100-seed weight (HSW)	-0.279	0.490	-0.124	-0.044
Seed yield plant^{-1} (SY/P)	0.551	0.349	-0.259	-0.011

Table 5. Promising accessions of safflower identified on the basis of traits of interest for future use.

Trait of interest	Range	Accessions identified
Days of maturity	< 212 days	16200, 16210, 16271, 16274, 16276, 26753, 26754, 26764, 26765, 26766, 26769, 26770, 26787, 26788,26789, 26790, 26795, 26796 and 26800
Branches plant^{-1}	≥ 18	16274, 16320, 16326, 26733, 26737, 26738,2 6743, 26752, 26761, 26772, 2677 and 26784
Capsules plant^{-1}	≥ 100	16274, 16318, 16324, 26732, 26737, 26738 and 26742
Seeds capsule^{-1}	≥ 40	16235, 16271, 16274, 16279, 16280, 16320, 16358, 26735, 26736, 26737, 26749, 26751, 26752, 26777, 26780 and 26759
100-seed weight	≥ 4.5g	16290, 16307, 16314, 16316, 16317, 26733 and 26800
Seed yield plant^{-1}	≥ 30g	16235, 16320, 26733, 26735, 26737, 26741, 26752 and 26769

Clustering of accessions into various groups was not related to topographical circulation instead accessions were primarily congregated owing to their morphological alteration. These results are not concordant to Gupta *et al.,* (2001). We may argue that this may be because of change in assembling positions. Secondly, different environmental conditions may also be reason affecting gene flow etc. According to Baydar & Gurel (1999) a few environmental factors could also induce the gene flow among populations from various geographical sources. We could observe genetic divergence based on morphological characters. The scientists working on safflower will interpret these results for their benefit. Elite safflower germplasm selection was carried out on the basis of important traits of economic interest such as days of maturity, primary branches plant^{-1}, capsules plant^{-1}, seeds capsule^{-1}, 100-seed weight and seed yield plant^{-1} (Table 5). Our results indicated that the genetic material studied had a considerable level of variability that could be exploited in future breeding programs. Further research on these selected accessions will save a lot of time for the breeder in future.

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