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# Assessment of genetic diversity among safflower germplasm through agro-morphological traits

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## Abstract

The present study was conducted at NARC (National Agricultural Research Centre), Islamabad Pakistan in 2014. The experimental materials comprising of 121 safflower accessions of diverse origin including one check cultivar were evaluated for different agro-morphological traits in order to find out the extent of genetic diversity and relationships among the genotypes and to identify the promising accessions for traits of economic importance. The seed material was provided by the gene bank of PGRI (Plant Genetic Resources Institute). Significant differences were observed for majority of traits. Plant height (cm) followed by days to flowering initiation (days), days to 50% flowering (days), seeds capitulum$^{-1}$, days to maturity (days), capitulum plant$^{-1}$, seed yield plant$^{-1}$ (g), capitulum diameter (mm), leaf length (cm), branches plant$^{-1}$, leaf width (cm) and 100-seed weight (g). Correlation analysis revealed that seed yield plant$^{-1}$ had high significant and positive correlation with branches plant$^{-1}$, capitulum plant$^{-1}$, seeds capitulum$^{-1}$ and 100-seed weight. Similarly days to flowering initiation and days to 50% flowering exhibited significant but negative correlation with seed yield plant$^{-1}$. Principal component analysis showed that 4 out of 12 principal components with an eigenvalue above than 1.0 considered for 74.82% of the total variance. Cluster analysis divided 121 accessions into 5 main clusters (I, II, III, IV and V), each of which having 99, 7, 1, 10 and 4 genotypes. These results could be serving as a useful resource for further characterization and preservation of the safflower germplasm.

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## Introduction

Safflower (*Carthamus tinctorius* L.) is an ancient crop and belongs to the family of Compositae or Asteraceae. It is the only cultivated species of the genus *Carthamus* that contains 2n=24 chromosomes (Singh, 2007). It is a predominately self-pollinating species (Johnson *et al.,* 2007). Depending on insect activity, the out crossing rates average between 15 and 20% (Claassen, 1950). It is believed to have been domesticated somewhere in the fertile Crescent region over 4000 years ago (Knowles, 1969; Ashri, 1975). Knowles, proposed seven diversity centers for safflower germplasm evolution including the Far East, India-Pakistan, the Middle East, Egypt, Sudan, Ethiopia and Europe (Knowles, 1969).

It is a multipurpose crop used for its edible oil as well as its medicinal and industrial applications (Dwivedi *et al.,* 2005). Currently, it is regarded as an important oilseed crop, and is grown commercially for its edible oil rich in polyunsaturated fatty acids (Dwivedi *et al.,* 2005; Singh 2007).Traditionally grown for its brightly colored flowers that are used both as a natural dye and for a number of medicinal purposes (Armah-Agyeman *et al.,* 2002).

In Pakistan, safflower was introduced as an oilseed crop in 1960. It is mainly cultivated in the Sindh and Baluchistan provinces. In 2012-13, its national average yield was 1000 kg/ha. As an oil crop it proved successful in rotation with rice in the northern areas of Sindh and Baluchistan (PARC. 2014).

World production of safflower is lower than other oil seed crops due to lower seed yield and a series of biotic stresses that limit its productivity (Ashri 1971a,b; Knowles 1975; Li and Mündel 1996). These limitations can be overcome by breeding programs providing there is enough genetic variation for the traits in the germplasm.

There are two types of safflower; high linoleic types that have an approximate range of linoleic acid from 3.1 to 88.8%, and high oleic types with an approximate range of oleic acid from 3.9 to 90.6% (Ferna´ndez-Martı´nez *et al.,* 1993). It is one of the prospective oilseed crops, because it yields about 32-40% seed oil and approximately 90% of this oil is composed of unsaturated fatty acids, namely oleic and linoleic acids (Siddiqi *et al.,* 2007; Johnson *et al.,* 1999). Safflower oil, which on average contains 75% linoleic acid, also contains tocopherols, known to have antioxidant effect and high vitamin E content. In recent years, considerable attention has been generated in the consumption and development of safflower seed oil as an excellent health care product and health benefits derived from it including prevention and treatment of hyperlipidemia, atherosclerosis and coronary heart disease (Han *et al.,* 2009).

In development of improved varieties of crops for better production and other attractive traits like disease resistance, insect and pest resistance, shattering resistance, heat and cold tolerance and so many other traits genetic diversity of plant germplasm play an important role and because of using these valuable backup tools of crop plants in the past few years the production and quality has been improved so many times. Since from the initial days (about 10 thousand years ago) of agriculture the genetic diversity has been explored by many plant and animal breeders to gain the required production and quality through bringing the genes for desirable traits and avoiding the others (Narain, 2000).

Characterization and estimation of genetic diversity is so much important step for the competent and successful maintenance and utilization of different crop germplasm (Ghafoor, 1999). And in that respect agro-morphological evaluation is the primary step to be taken for the evaluation and categorization of different crop germplasm (Smith and Smith, 1989). Evaluation and characterization through morphological and phenotypical parameters of different crop germplasm is therefore so much important for all plant breeders (Martins *et al.,* 2006). And that is why it is so much important to make proper strategies for the collection and evaluation of germplasm sources which are locally used in different regions of the world and save them from being vanished (Balkaya and Ergun, 2008).

To have a variety of better traits of any crop we need first hand information about its genetic diversity (Shinwari *et al.,* 2013). The main objective for studying genetic diversity and associations among germplasm collections is to eventually exploit that information to enhance the development of better performing varieties of the cultivated species (Alawala *et al.,* 2006). For breeding programs to be successful, we have to assess the available germplasm for knowing its diversity (Sultan *et al.,* 2013). Breeding programs depend on the knowledge of key traits, genetic systems controlling their inheritance, and genetic and environmental factors that influence their expression. Safflower genotypes have indicated considerable diversity across different geographical regions of the world (Knowles, 1969). This diversity will be very important to overcome risks posed by different diseases, rapidly changing environmental conditions and most importantly to meet the growing consumption demand. The main goals of the present study were.

To determine genetic diversity in diverse safflower genotypes on the basis of agro-morphological traits
To identify elite genotypes for traits of interest

## Materials and methods

### *Research location*

The present study was conducted at experimental field of PGRI (Plant Genetic Resources Institute), NARC (National Agricultural Research Centre), Islamabad Pakistan during the year 2013-2014. In Islamabad region average annual rainfall is ranging from 500-900 mm in which 30% occurs in winter and 70% in summer. It is located at a height of about 5 hundred and 18 meters from the sea level.

### *Experimental materials*

The experimental materials comprising of 121 safflower accessions of diverse origin including one check cultivar. The seed material was provided by the gene bank of PGRI (Plant Genetic Resources Institute). The experimental materials were planted in the research field of PGRI on 18 October 2013 using augmented design. The field was divided into five beds; while each individual bed was contain 24 accessions. The check was repeated after every 30 accessions. Each accession was grown in a single row of 2 m, while distance between two rows was kept at 45 cm. The crop was raised at optimum growing conditions. The experiment was maintained according to the recommended cultural practices.

### *Traits measurement and statistical analysis*

Quantitative traits were recorded for days to flowering initiation, days to 50% flowering, days to maturity, plant height, leaf length, leaf width, primary branches plant$^{-1}$, Capitulum plant$^{-1}$, seeds capitulum$^{-1}$, capitulum diameter, 100-seed weight and seed yield plant$^{-1}$. All the recorded data was averaged and the means of all the accessions were analyzed for simple statistics (mean, standard deviation, coefficient of variation and variance) among all the genotypes for different parameters. Simple correlation coefficient was calculated through the procedure of steel and Torrie (1980) by means of plot mean values using software statistica 7.0. The agro-morphological traits recorded during the present study were also analyzed by arithmetical taxonomic techniques through multivariate analysis including cluster and principle component analysis (Sneath and Sokal, 1973). Means of all the traits were standardized through Z-scores to avoid the effects of scaling differences before the multivariate analysis. Euclidean distances coefficient were determined for the total pairs of genotypes. Resultant Euclidean distances coefficient matrices were used to assess the association among the safflower genotypes through cluster analysis (NTSYS-pc, version 2.1). Furthermore, PCA (principal component analysis) was performed through the similar data matrix. (Statistica, Version 7.0).

## Results and discussion

During the present investigation, significant amount of genetic variability was found for all the twenty parameters through analysis of variance. Among the studied accessions, significant differences were found for the traits of days to flowering initiation, days to 50% flowering, plant height, leaf length, leaf width, branches plant$^{-1}$, Capitulum plant$^{-1}$, seeds capitulum$^{-1}$, capitulum diameter, 100-seed weight and seed yield plant$^{-1}$. Results of the present investigation are strong agreement with Shinwari *et al.* (2014). They evaluated safflower germplasm and observed high level of genetic diversity for the traits of capsules plant$^{-1}$, seeds capsule$^{-1}$, seed yield plant$^{-1}$, plant height, days to flowering initiation, and days to maturity.

**Table 1.** Basic statistics of agro-morphological traits of 121 safflower ( *Carthamus tinctorius* L.) accessions.

| Traits | Mean | Minimum | Maximum | SD* | CV (%)** | Variance |
|---|---|---|---|---|---|---|
| DFI | 179 | 165 | 201 | 8.1 | 4.5 | 65.5 |
| DFF | 183 | 168 | 205 | 7.8 | 4.3 | 61.5 |
| DM | 205.6 | 194 | 226 | 7.4 | 3.6 | 54.9 |
| LL | 11.1 | 6.1 | 19.2 | 2.5 | 22.1 | 6.0 |
| LW | 3.4 | 2.2 | 5.8 | 0.7 | 19.9 | 0.5 |
| PH | 124.2 | 41.0 | 227.4 | 27.5 | 22.2 | 757.3 |
| PBP | 8.0 | 4.3 | 16.0 | 2.2 | 27.1 | 4.7 |
| CP | 12.4 | 6.0 | 40.0 | 5.1 | 41.5 | 26.3 |
| SC | 25.7 | 15.6 | 65.5 | 7.6 | 29.7 | 58.1 |
| CD | 18.3 | 10.8 | 50.2 | 4.5 | 24.5 | 20.0 |
| HSW | 2.3 | 1.1 | 4.3 | 0.6 | 26.0 | 0.4 |
| SYP | 10.5 | 5.4 | 35.2 | 5.1 | 48.5 | 26.1 |

*Standard deviation **Coefficient of Variation

Whereas DFI= Days to flowering initiation, DFF= Days to 50% flowering, DM= Days to maturity, LL= Leaf length, LW= Leaf width, PH= Plant height, PBP= Primary branches plant$^{-1}$, CP= Capitulum plant$^{-1}$, SC= Seeds capitulum$^{-1}$, CD= Capitulum diameter, HSW= 100-seed weight, and SYP= Seed yield plant$^{-1}$.

**Table 2.** Correlation coefficients among 12 quantitative traits in diverse germplasm of safflower.

| Traits | DFI | DFF | DM | LL | LW | PH | PBP | CP | SC | CD |
|---|---|---|---|---|---|---|---|---|---|---|
| DFF | 0.99** | | | | | | | | | |
| DM | 0.85** | 0.85** | | | | | | | | |
| LL | 0.09 | 0.09 | 0.10 | | | | | | | |
| LW | 0.11 | 0.11 | 0.16 | 0.80** | | | | | | |
| PH | 0.06 | 0.08 | 0.10 | 0.13 | 0.08 | | | | | |
| PBP | -0.04 | -0.03 | -0.01 | -0.02 | 0.05 | 0.05 | | | | |
| CP | -0.04 | -0.02 | -0.01 | 0.00 | 0.12 | -0.06 | 0.54** | | | |
| SC | -0.01 | 0.02 | 0.10 | 0.31** | 0.39** | 0.25** | 0.36** | 0.31** | | |
| CD | 0.04 | 0.06 | 0.08 | 0.07 | 0.15 | 0.13 | 0.05 | 0.04 | 0.16 | |
| HSW | -0.36** | -0.35** | -0.32** | -0.07 | -0.13 | -0.19* | 0.20* | 0.11 | -0.05 | -0.17 |
| SYP | -0.21* | -0.18* | -0.12 | 0.08 | 0.18 | -0.02 | 0.60** | 0.83** | 0.63** | 0.03 |

Where, ** = highly significant, * = significant.

Through analysis of variance highest variance was found for plant height followed by days to flowering initiation, days to 50% flowering, seeds capitulum$^{-1}$, days to maturity, capitulum plant$^{-1}$, seed yield plant$^{-1}$, capitulum diameter. The variances for the above traits were 757.3, 65.5, 61.5, 58.1, 54.9, 26.3, 26.1 and 20.0 respectively (Table 1). Relatively, a low level of variability was distinguished in leaf length (6.0), primary branches plant$^{-1}$ (4.7), leaf width (0.5) and 100-seed weight (0.4) (Table 1). The mean values of the safflower genotypes for days to flowering initiation, days to 50% flowering and days to maturity were 179, 183 and 205.6 with a range of 165 to 201, 168 to 205 and 194 to 226 days, respectively (Table 1).These characters could be evaluated to know for both early and delayed maturity.

The safflower accessions 252042, 253560 and 304499 showed highest values (226, 223 and 223 days, respectively), while a lot of other safflower accessions including 250011, 250601, 262514, 250194, 250010, 250715, 251289 and 251978 demonstrated earliness (194, 195 and 196 days, respectively). 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).

**Table 3.** Principal components of agro-morphological traits in safflower.

| | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Eigenvalue | 3.22 | 2.84 | 1.75 | 1.18 |
| Cumulative Eigenvalue | 3.22 | 6.06 | 7.80 | 8.98 |
| % Total Variance | 26.80 | 23.67 | 14.56 | 9.80 |
| Cumulative % | 26.80 | 50.46 | 65.02 | 74.82 |
| Traits | Eigen Vectors | | | |
| Days to Flower Initiation | 0.885 | 0.252 | 0.314 | 0.120 |
| Days to 50% Flowering | 0.880 | 0.275 | 0.316 | 0.094 |
| Days to Maturity | 0.818 | 0.326 | 0.269 | 0.069 |
| Leaf Length | 0.159 | 0.472 | -0.717 | 0.350 |
| Leaf Width | 0.154 | 0.582 | -0.652 | 0.310 |
| Plant Height | 0.154 | 0.199 | -0.260 | -0.650 |
| Primary Branches Plant$^{-1}$ | -0.334 | 0.602 | 0.376 | -0.109 |
| Capitulum Plant$^{-1}$ | -0.354 | 0.669 | 0.395 | 0.021 |
| Seeds Capitulum$^{-1}$ | -0.134 | 0.754 | -0.168 | -0.208 |
| Capitulum Diameter | 0.111 | 0.215 | -0.200 | -0.557 |
| 100-Seed Weight | -0.586 | -0.006 | 0.192 | 0.372 |
| Seed Yield Plant$^{-1}$ | -0.553 | 0.753 | 0.260 | 0.048 |

According to Amini *et al.,* (2008) to avoid biotic and abiotic stresses, early maturity cultivar facilitates early harvest. Rest of the traits demonstrated wide genetic deviation and accessions with a huge level of genetic divergence often used for the identification of best germplasm for varied climatic conditions.

**Table 4.** Promising accessions of *Carthamus tinctorius* L. identified during present investigation.

| Traits of Interest | Range | Accessions Identified |
|---|---|---|
| Days to maturity | ≤195 days | 250011, 250194, 250601, 251978 and 262514 |
| Primary branches plant$^{-1}$ | ≥ 12 | 250007, 250008, 250528, 250718, 250722, 253569 and 262435 |
| Capitulum plant$^{-1}$ | ≥ 20 | 250353, 250478, 250479, 250720, 251978, 253569, 262435 and 304503 |
| Seeds capitulum$^{-1}$ | ≥ 34 | 239707, 250000, 250006, 250007, 250008, 250009, 250075, 250713, 253569 and 253761 |
| 100-seed weight | ≥ 3.2g | 250079, 250082, 250194, 250473, 250528, 250529, 250605, 251284, 251285, 253898 and 304503 |
| Seed yield plant$^{-1}$ | ≥ 20g | 239707, 250006, 250007, 250353, 250479, 253569 and 262435 |

*Correlation analysis*

The assessment of the extent to which the variables differ together or a measure of a quantity of relationship is called correlation. (Steel and Torrie, 1980). Simple correlation coefficient was carried out among the mean values of 12 quantitative traits of safflower accessions (Table 2).

Correlation analysis revealed that seed yield plant$^{-1}$ had high significant and positive correlation with branches plant$^{-1}$, Capitulum plant$^{-1}$, seeds capitulum$^{-1}$ and 100-seed weight.

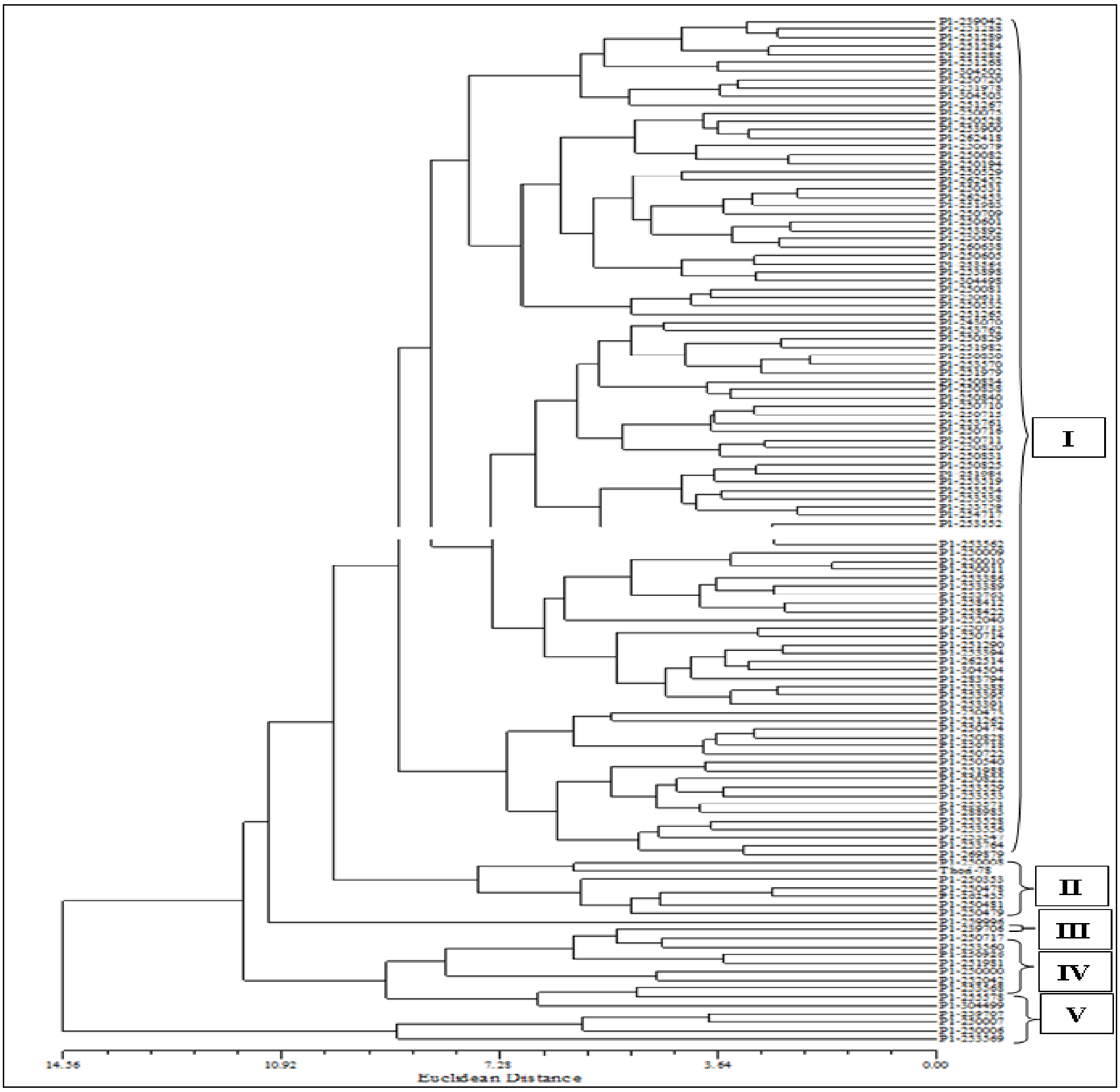

**Fig. 1.** Dendrogram showing relationships between 121 accessions of safflower (*Carthamus tinctorius* L.) based on agro-morphological traits.

Therefore, these traits could be considered as the most important for yield improvement in safflower. These results were in close agreement with that of Golkar *et al.* (2011) and Tariq *et al.* (2014). Similarly days to flowering initiation and days to 50% flowering exhibited significant but negative correlation with seed yield plant$^{-1}$. High significant and positive correlation was observed between days to flowering initiation, days to 50% flowering and days to maturity. To enhance yield, number of capitulum plant$^{-1}$ and seeds capitulum$^{-1}$ are an important traits (Lahane *et al.*, 1999; Golkar *et al.*, 2011). Hence, seed yield in safflower could be improved indirectly by selecting genotypes producing a greater number of seeds per head and heads per plant.

### *Cluster analysis*

On basis of agronomic and morphologic characters the unweighted. pair group method using, arithmetic averages (UPGMA) is most commonly used (Mohammadi & Prasanna, 2003). Hierarchical cluster analysis based on agro-morphological parameters divided 121 accessions of safflower into 5 main clusters (I, II, III, IV and V, Fig. 1). These main clusters were further sub-divided into 7 sub-clusters. Maximum number of genotypes (99) was present in cluster I, followed by cluster IV (10), cluster II (7), cluster V (4) and cluster III (1) (Fig. 1). Cluster I consisted of 99 accessions, which was further sub-divided into two sub-clusters (I-1 & I-2) comprises of 81 and 18 accessions, respectively.

Cluster II included 7 accessions. There was only one accession in Cluster III. Cluster IV comprised of 10 accessions, which is also sub-divided into two sub-clusters (IV-1 & IV-2). The sub-cluster (IV-1) comprises of 3 accessions while sub-cluster (IV-2) comprises of 7 accessions. Similarly cluster V consisted of 4 accessions. Cluster I comprised of 99 accessions and were medium in maturity, average in plant height, medium in primary branches plant$^{-1}$, minimum in number of capitulum plant$^{-1}$, below average in number of seeds capitulum$^{-1}$ and average in seed yield plant$^{-1}$.

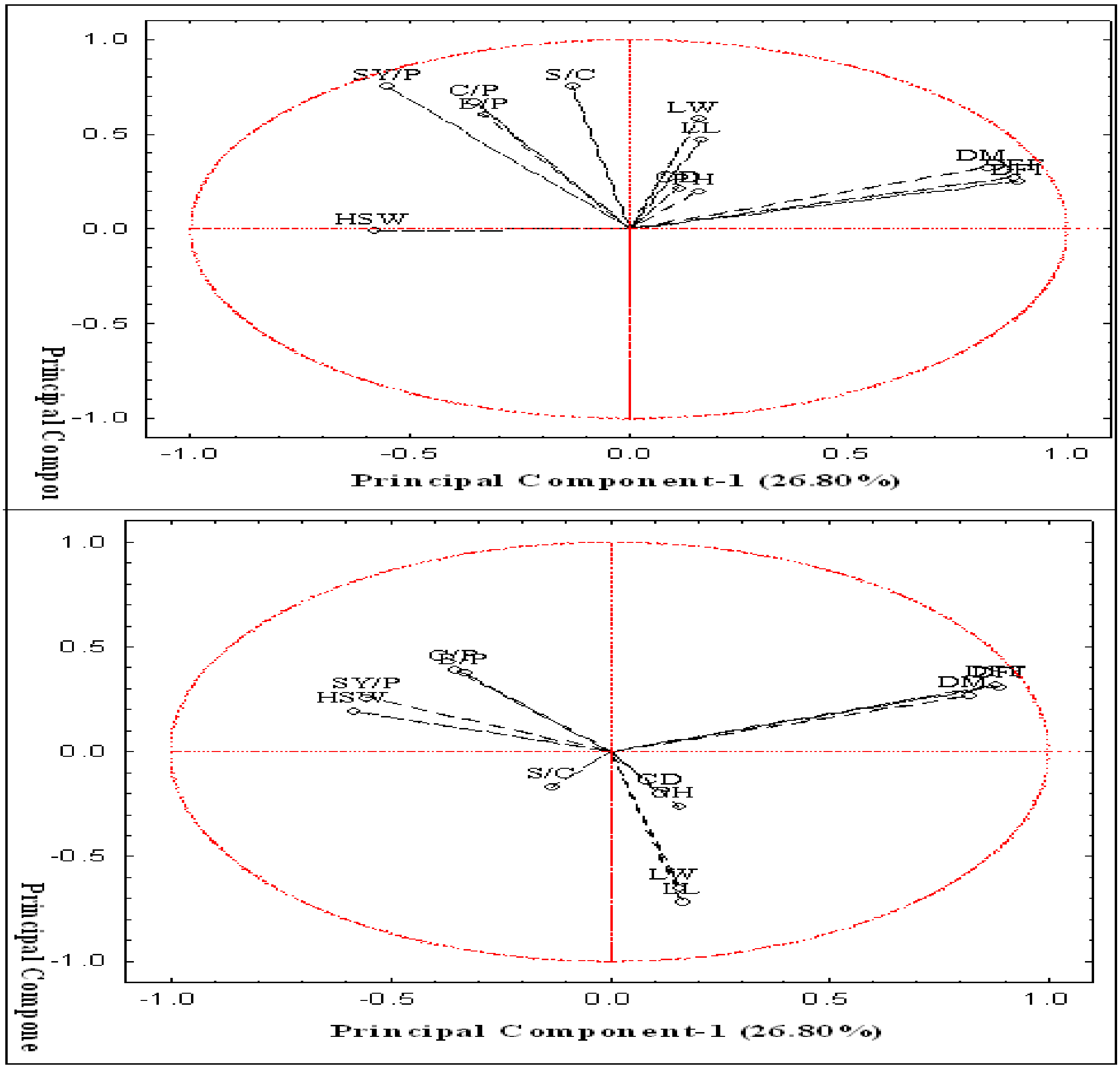

**Fig. 2.** Scatter plots showing the contribution of 12 quantitative traits to the total variation in the first three principal components in 121 accessions of safflower.

Cluster II consisted of total 7 accession which were early maturing, below average in plant height, medium to large in number of branches plant$^{-1}$, medium to high in number of capitulum plant$^{-1}$, above average in number of seeds capitulum$^{-1}$ and above average in seed yield plant$^{-1}$. Cluster III contained only one accession which was early to medium in maturity, shortest in plant height, lowest in number of primary branches plant$^{-1}$, lower to medium in number of capitulum plant$^{-1}$, minimum in number of seeds capitulum$^{-1}$ and minimum in seed yield plant$^{-1}$. Cluster IV consisted of 10 accessions and were Late in maturity, above average in plant height, lower to medium in number of primary branches plant$^{-1}$, medium in capitulum plant$^{-1}$, average in number of seeds capitulum$^{-1}$ and below average in seed yield plant$^{-1}$. Cluster V consisted of total 4 accessions which were medium to late in maturing, tallest in plant height, maximum in number of primary branches plant$^{-1}$ and capitulum plant$^{-1}$, highest in number of seeds capitulum$^{-1}$ and having maximum seed yield plant$^{-1}$.

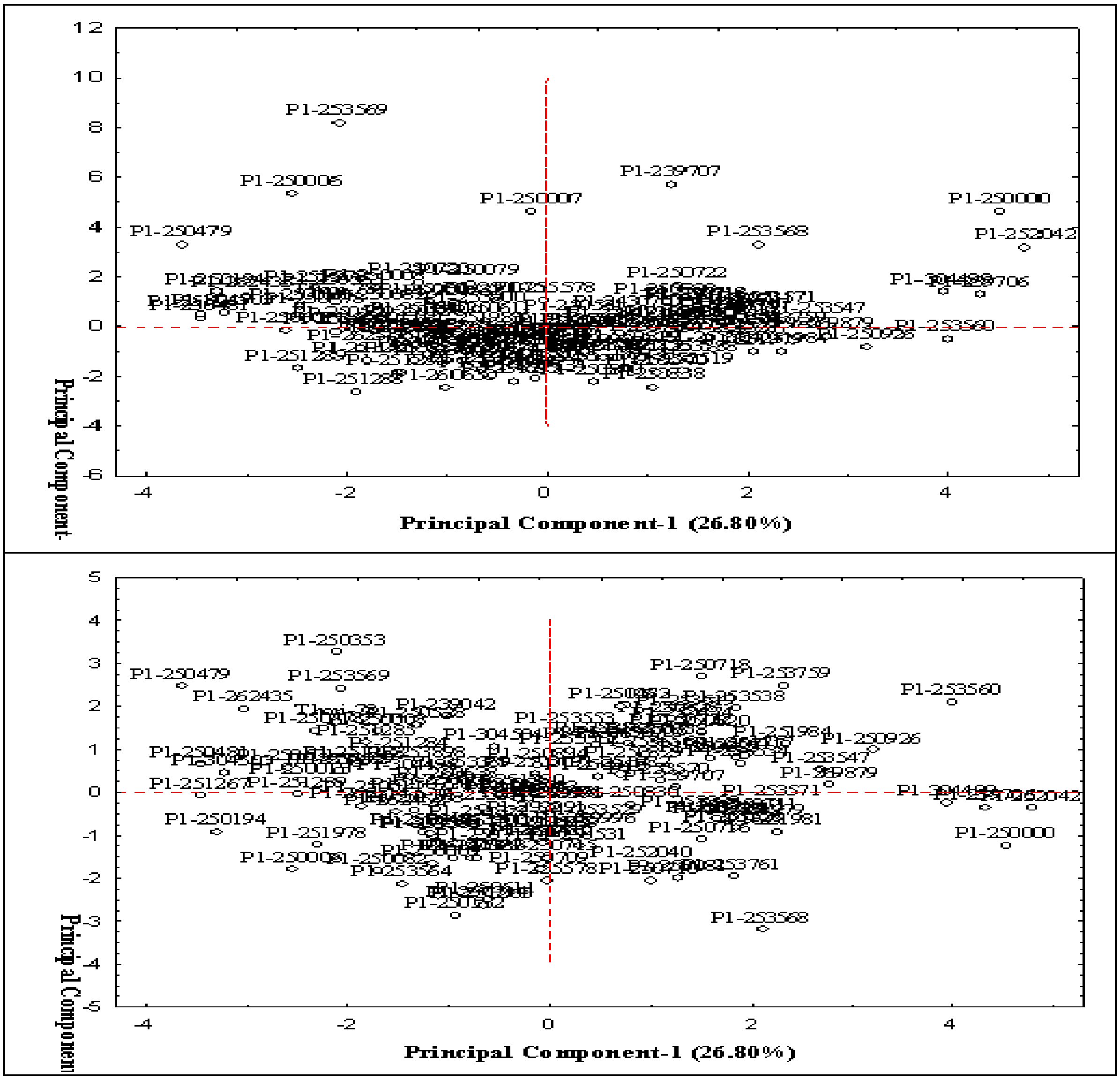

**Fig. 3.** Scatter plots showing the genetic relationships among 121 accessions of safflower as revealed by first three principal components.

The results of cluster analysis indicated that there is considerable variation between the accessions for the studied agro-morphological traits and it also indicated that the studied accessions were distinctly divided into its particular groups. Grouping of safflower accessions into different clusters was not associated with the geographic distribution instead accessions were mainly grouped was due to their agro-morphological difference. These result is in agreement with that of shinwari *et al.,* (2014).

The present study explored notable range of genetic variability in *Carthamus tinctorius* L. accessions for some of the traits of economic importance providing a resource for development of improved verities of safflower in future breeding programme.

*Principal component analysis*

Principal, component analysis based on 12 agro-morphological traits showed that 4 principal components with an eigenvalue above than 1.0 accounted for 74.82% of the total variation among 121 accessions of Safflower.

PCI which is most important explained 26.80%, PCII contributed 23.67%, PCIII contributed 14.56% and PCIV accounted for 9.80% of total morphological variability (Table 4, Fig. 2). The coefficients describing four principal components are shown in the Table 3. PCA revealed that days to flowering initiation, days to 50% flowering, days to maturity, primary branches plant$^{-1}$, capitulum plant$^{-1}$ and seed yield plant$^{-1}$ were among the most important descriptors which accounted for more than half of the all phenotypic variation in the evaluated safflower germplasm. Further, study of all these traits will help us in recommending best safflower germplasm in future breeding programme. The findings of the present investigations were supported by the findings of Shinvari *et al.* (2014). They were observed that division of accessions into different clusters through principal component analysis was only due to their morphological alteration instead of their topographical circulation.

## Conclusion

Significant genetic variability was observed between the evaluated genotypes for different parameters including days to maturity, plant height, primary branches plant$^{-1}$, capitulum plant$^{-1}$, seeds capitulum$^{-1}$, 100-seed weight and seed yield plant$^{-1}$. Most of parameters of studied genotypes were found to correlate significantly.

This shows that there is a great potential for improvement in these genotypes for the said traits. Furthermore, these traits can be used in future for selecting the most promising genotypes.

The present research study will be useful to provide morphological based genetic diversity knowledge of safflower to various researchers, academia and genetic resources managers dealing with the crop.

## References

**Alawala S, Suman A, Arro JA, Veremis JC, Kimbeng CA.** 2006. Target region amplification polymorphism (TRAP) for assessing genetic diversity in sugarcane germplasm collections. Crop Science **46,** 448-455.

**Amini F, Saeidi G, Arzani A.** 2008. Study of genetic diversity in safflower genotypes using agro-morphological traits and RAPD markers. Euphytica **163,** 21-30.

**Armah-Agyeman G, Loiland J, Karow R, Hang AN.** 2002. Safflower. Oregon State University Extension Service. EM 8792.

**Ashri A.** 1971a. Evaluation of the world collection of safflower, *Carthamus tinctorius* L. II. Resistance to the safflower fly, Acanthiophilushelianthi R. Euphytica **20,** 410-415.

**Ashri A.** 1975. Evaluation of the germplasm collection of safflower (*Carthamus tinctorius* L.) V. Distribution and regional divergence for morphological characters. Euphytica **24,** 651-659.

**Balkaya A, Ergun A.** 2008. Diversity and use of pinto bean (*Phaseolus vulgaris*) populations from Samsun, Turkey. N. Z. J. Crop Horticulture Science **36,** 189-197.

**Claassen CE.** 1950. Natural and controlled crossing in safflower (*Carthamus tinctorius* L.). Journal of Agronomy **42,** 381-384.

**Dwivedi SL, Upadhyaya HD, Hegde DM.** 2005. Development of core collection in safflower (*Carthamus tinctorius* L.) germplasm. Genetic Resources and Crop Evolution **52,** 821-830. http://dx.doi.org/10.1007/s10722-003-6111-8.

**Ferna´ndez-Martı´nez J, Rio MD, Haro AD.** 1993. Survey of safflower (*Carthamus tinctorius* L.) germplasm for variants in fatty acid composition and other seed characters. Euphytica **69,** 115-122.

**Ghafoor A**. 1999. “Genetic diversity and gene-action in *Vigna mungo* based on morphological and biochemical markers”. (PhD thesis, Quaid-i-Azam University, Islamabad, 1999), 192.

**Golkar P, Arzani A, Rezaei AM.** 2011. Determining relationships among seed yield, yield components and morpho-phenological traits using multivariate analyses in safflower (*Carthamus tinctorious* L.). Annals of Biological Research **2(3),** 162-169.

**Han X, Cheng L, Zhang R, Bi J.** 2009. Extraction of safflower seed oil by supercritical $CO_2$. Journal of Food Engineering **92,** 370-376.

**Johnson RC, Bergman JW, Flynn CR.** 1999. Oil and meal characteristics of core and non-core safflower accessions from the USDA collection. Genetic Resources and Crop Evolution **46,** 611-618.

**Johnson RC, Kisha TJ, Evans MA.** 2007. Characterizing safflower germplasm with AFLP molecular markers. Crop Science **47,** 1728-1736.

**Knowles PF.** 1969. Centers of plant diversity and conservation of crop germplasm: Safflower. Economic Botany **23,** 324-329.

**Lahane PS, Mukewar AM, Zope JS, Kalpande HV, Kalpande VV.** 1999. Genetic variability for different traits in safflower (*Carthamus tinctorius* L.). Journal of Soils and Crops **9,** 130-132.

**Li D, Mündel HH.** 1996. Safflower: *Carthamus tinctorius* L. promoting the conservation and use of underutilized and neglected crops 7. Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, International Plant Genetic Resources Institute (IPGRI), Rome, Italy.

**Martins SR, Vences FJ, Miera LE, Barrosa MR, Carnide V.** 2006. RAPD analysis of genetic diversity among and within Portuguese landraces of common white bean (*Phaseolus vulgaris* L.). Science Horticulture **108,** 133-142.

**Mohammadi SA, Prasanna BM.** 2003. Analysis of genetic diversity in crop plants - salient statistical tools and considerations. Crop Science **43,** 1235-1248.

**Narain P.** 2000. Genetic diversity conservation and assessment. Current Science **79(2)**, 170-175.

**PARC.** 2014. Pakistan agricultural research council, status paper, oilseed crops of Pakistan.

**Rehman AU, Habib I, Ahmad N, Hussain M, Khan MA, Farooq J, Ali MA.** 2009. Screening wheat germplasm for heat tolerance at terminal growth stage. Plant Omics Journal **2,** 9-19.

**Shinwari S, Akbar F, Rabbani MA, Mumtaz AS, Shinwari ZK.** 2013. Evaluation of genetic diversity in different genotypes of *Eruca sativa* from Pakistan by SDS-PAGE Analysis. Pakistan Journal of Botany **45,** 1235-1240.

**Shinwari ZK, Rehman H, Rabbani MA.** 2014. SDS-PAGE based genetic divergence in safflower (*Carthamus tinctorius* L.). Pakistan Journal of Botany **46(3),** 811-815.

**Siddiqi EH, Ashraf M, Akram NA.** 2007. Variation in seed germination and seedling growth in some diverse lines of safflower (Carthamus tinctorius L.) Under Salt Stress. Pakistan Journal of Botany **39,** 1937-1944.

**Singh RJ.** 2007. Genetic resources, chromosome engineering and crop improvement. CRC Press Inc. Boca Raton, Florida, USA.

**Smith JSC, Smith OS.** 1989. The description and assessment of distances between inbred lines of maize: The utility of morphological, biochemical and genetic descriptors and a scheme for testing of distinctiveness between inbred lines. Maydica **34,** 151-161.

**Sneath PH, Sokal RR.** 1973. Numerical Taxonomy; The principles and practice of numerical lassification. W.F. Freeman & Co., San Francisco, USA. 573p.

**Steele RG, Torrie JH.** 1980. Principles and Procedures of Statistics. A Biochemical Approach, McGrow-Hill, New Yark, pp. 187-188.

**Suddihiyam P, Steer BT, Turner DW.** 1992. The flowering of sesame (*Sesamum indicum* L.) in response to temperature and photoperiod. Australian Journal of Agricultural Research **43,** 101-116.

**Sultan M, Zakir N, Rabbani MA, Shinwari ZK, Masood MS.** 2013. Genetic diversity of guar (*Cyamopsis tetragonoloba* L.) landraces from Pakistan based on RAPD markers. Pakistan Journal of Botany **45,** 865-870.

**Tariq M, Tariq MA, Shah MKN, Ijaz M, Hassan MF, Aftab M, Aadal NK, Hussain T.** 2014. Genetics and interrelationship of yield and yield related attributes in some genotypes of safflower (*Carthamus tinctorius* l.) under rainfed conditions. Journal of Biology, Agriculture and Healthcare **4(3).**