

# Assessment of Genetic Diversity Based on Agronomic Traits in Safflower International Germplasm under Rainfed Condition of Cold Temperate Region in Iran

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## ABSTRACT

To study the genetic diversity based on agronomic traits and their application in safflower breeding programs, an experiment was conducted in the 2019-2020 crop season at Dryland Agricultural Research Institute (DARI), Sararood, Kermanshah, Iran. A total of 251 safflower genotypes and two cultivars (Faraman and Sina) were planted in a non-repeating observational design. Total rainfall in Sararood station was equal to 518.8 mm, during the 2019-2020 cropping season. Regarding rainfall distribution, the amount of rainfall in autumn, winter and spring was 173.2, 229.1 and 116.5 mm (33.38%, 57.65% and 22.45% of the total annual rainfall), respectively. The results of this research showed genetic diversity in the studied germplasm. Mean seed yield, 1000- seed weight, seed oil content, seed yield, flowering period length, physiological ripening period, plant height, pod diameter and the number of sub-branches per plant for the genotypes were 879 kg/ha, 33 g, 30.4%, 268.1 kg/ha, 170 days, 200 days, 75.5 cm, 13.2 mm and 4.2, respectively. Genotypes PI198009, PI197831, PI271139 and PI305205 produced the highest seed yield (2427, 2483, 2693 and 2965 kg/ha, respectively) and the highest oil yield (675, 798, 804 and 937 kg/ha). The seed oil content of PI306971, PI306954, PI205604, PI306853 and PI343780 genotypes was above 35% and higher than other genotypes. Considerable diversity in the morphological characteristics of the germplasm was observed in this research, which indicates the possibility of using them in breeding programs. Finally, some genotypes were selected that seed yield, 1000- seed weight, seed oil content, plant height, pod diameter and the number of sub-branches per plant were more than the third quarter and the number of days from planting to flowering and their physiological maturity were less than the first quarter. These genotypes were chosen for repeated experiments in the following year.

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## 1. Introduction

The agricultural sector is the largest consumer of groundwater resources. In Iran, due to the use of incorrect methods of irrigation and planting crops with high water requirements, more than 70% of water consumption is wasted in the agricultural sector and less than 30% of it is used (Ghobadi *et al.*, 2021). According to FAO forecasts, until 2030 several western provinces of Iran will suffer from drought, of which Kermanshah province is at the top. In addition, it is predicted that the amount of rainfall in Kermanshah province will decrease by 30% (Miri *et al.*, 2011).

After cereals, oilseeds are the second largest food

reserve in the world. These products have rich fatty acids reserves and are considered a strategic industry in most countries. Vegetable oils are mainly obtained from oilseeds such as soybean, sunflower, cottonseed, peanut and canola. Due to the high water consumption of common oil crops such as rapeseed and soybeans, it is necessary to introduce and replace oil plants with low water consumption. Unfortunately, according to available statistics, more than 90% of the country's oil needs are still supplied from imports (Rostami Ahmadvandi *et al.*, 2021). Common oilseeds such as soybean, sunflower and canola have many advantages but they cannot be cultivated economically in every

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climate and area. For example, soybean is a suit crop in North America and is not well adapted to other lands. Sunflower also needs a lot of fertilizer and is susceptible to a variety of diseases and pests (Robinson, 1987). Therefore, the need for new oil crops with more adaptation and fewer needs is strongly felt (Ghobadi et al., 2021).

In recent years, attention to oilseeds with the ability to cultivate in dryland has increased. Safflower (*Carthamus tinctorius* L.) is a plant belonging to the Asteraceae family. This plant is one of the drought tolerant species that can play an important role in expanding the area under the cultivation of oil crops in dryland conditions. The oil content of safflower seeds varies between 30 and 36% depending on the environmental conditions of the region. Safflower oil is one of the most valuable vegetable oils due to its more than 90% unsaturated fatty acids and high content of linoleic acid (omega-6), oleic acid and high antioxidant activity (Hasani-Javanmard et al., 2021).

Various safflower genotypes have a wide genetic diversity in terms of quantitative and qualitative characteristics, compatibility with environmental factors and types of resistance to biotic and abiotic stresses. It appears that more than 25,000 samples of safflower germplasm have been stored in gene banks in 15 countries (Yang et al., 2007).

The study of plant genetic diversity helps plant breeders identify crops' genetic capacity and traits related to breeding goals. Also, the follow-up of genetic diversity of genotypes from their geographical and climatic diversity indicates their possible adaptation to different environments (Zebarjadi et al., 2016; Rostami Ahmadvandi et al., 2021).

Considerable variation in safflower genotypes has been reported in the Indian collection. This collection is a rich genetic source for safflower breeding in terms of yield, quality traits and resistance to biotic and abiotic stresses (Dwivedi et al., 2005). In one of the local safflower populations in northwestern Iran, there were plants with a long rosette stage (LRV lines). By selecting the desired genotypes, more than 50 of this local population were introduced with considerable variation in winter cold tolerance (Ghanavati and Knowles, 1977).

30 safflower genotypes were studied based on phenotypic and agronomic traits also, these genotypes were studied with 24 start codon targeted

polymorphism (SCoT) and 10 inter-simple sequence repeat (ISSR) markers at the molecular level reported a wide variety (Rahimi, 2021). In another research, seven quantitative traits of 1618 Iranian and foreign safflower genotypes were evaluated and finally classified into five clusters. This grouping indicated similarities between lines of areas with contrasting ecological conditions (Yazdi-Samadi and Abd-Mishani, 1991). In Brazil, 124 safflower genotypes were compared, which are a part of the State University of Mato Grosso germplasm collection, based on their agronomic characteristics, to provide the initial guidelines for a breeding program. Plant yield, number of chapters per plant, and height contributed the greatest to genetic divergence among the genotypes (Lira et al., 2021).

In a study to evaluate the genetic diversity of 64 safflower genotypes, cluster analysis based on morphological and agronomic traits could not separate genotypes in terms of geographical origin and Iranian genotypes were present in most groups, which indicates there is a great variety of Iranian genotypes (Gholamei et al., 2015). By examining the genetic diversity of 100 safflower genotypes in terms of important agronomic traits, researchers reported a significant difference between genotypes in relation to all traits. The highest and lowest coefficients of genetic variation belonged to oil yield (7.35%) and the number of days to emergence (65.3%), respectively (Omidi et al., 2009).

Researchers also studied the seeds of 12 foreign and six native safflower cultivars and reported a significant difference between seed yield, oil content and fatty acid composition of genotypes. S541, PI537336 genotypes and Arak local population had the highest seed yield, Bacum 92 cultivar had the highest oil content and Rio-70 cultivar had the lowest seed coat (Ghorbanzadeh et al., 2011). Another study evaluated the genetic diversity of 100 safflower genotypes based on agronomic traits research. Factor analysis in this study led to the identification of four factors (seed yield, yield components, phenological and plant height) that accounted for 70% of the total variance. Grouping the genotypes by cluster analysis divided the genotypes into four clusters. The second cluster had the lowest mean seed and oil yields, and plant height and the fourth cluster had the highest mean seed and oil yields (Pourdad and Jamshid Moghadam, 2014).

Due to the importance of safflower in producing oilseeds, this study was conducted to evaluate and

investigate the genetic diversity of some safflower genotypes and the selection of desired genotypes for use in breeding projects.

## 2. Materials and methods

This research was conducted in the research field of Dryland Agricultural Research Sub-Institute (Sararood station), Kermanshah, Iran, during the 2019-20 cropping season. Ecological conditions and soil characteristics of the test site are presented in Table 1.

The total rainfall in Sararood station was 518.8 mm, during the 2019-2020 cropping season. Regarding rainfall distribution, the amount of rainfall in autumn, winter and spring were 173.2, 229.1 and 116.5 mm (33.38%, 57.65% and 22.45% of the total annual

rainfall), respectively. The average temperature was 11.06°C. In 56 days of the mentioned cropping season, the temperature dropped below 0°C.

In this experiment, 251 safflower accessions (prepared from the available germplasm at USDA) along with the introduced cultivars in Iran for dryland areas (Faraman and Sina) were examined. Land preparation operations including plowing, disc plow and fertilizing were carried out in late September 2019. Seeds were sown manually in four rows with a distance of 30 cm and a length of 2 m. During the growing season, the necessary crop care was performed, including regular weeding and controlling diseases or possible pests.

**Table 1. Geographical, ecological conditions and soil properties of the soil at the experimental site.**

|                            |   |
|----------------------------|---|
| Longitude                  | 47°, 20'                                    |
| Latitude                   | 34°, 20'                                    |
| Altitude                   | 1351.6 m                                    |
| Climate                    | moderate and cold                           |
| Average long-term rainfall | 441 mm                                      |
| Soil texture               | clay- loam (clay: 44%; silt: 47%; sand: 9%) |
| Organic carbon             | 0.9%  |
| Nitrate                    | 23 ppm                                      |
| Ammonium                   | 20 ppm                                      |
| av. p                      | 8-15 ppm                                    |
| av. k                      | 420 ppm                                     |

Out of 251 genotypes, the seeds of 43 genotypes did not germinate and finally, 208 genotypes remained and were evaluated. During the growing season, the appearance of the plants was recorded in terms of spine status (Spine and Spineless) and color of flowers (Red, Orange, White and Yellow). Flowering dates and physiological maturity were recorded for each plot. The first rainfall occurred on November 29. Fertilizers, herbicides and pesticides were not used in this experiment and weed control was done mechanically. At the end of the growing season, in each plot plant height (cm) and head diameter (mm) were measured and the number of sub-branches per plant was counted. After harvest, 1000- seed weight (g) and seed yield (kg/ha) was measured. The seed oil content of each genotype was also measured by an NMR device (University of Oxford, England).

In order to evaluate the genetic diversity between the studied genotypes, statistical parameters including mean, range and standard deviation were calculated for

each of the traits. Finally, genotypes that seed yield, 100-seed weight, seed oil content (by NMR method), oil yield, plant height, head diameter and the number of sub-branches per plant were more than the third quarter and the number of days from planting to flowering and their physiological maturity were less than the first quarter, were selected as superior genotypes.

## 3. Results

Regarding the qualitative traits, 146 genotypes had the spine and 62 genotypes were spineless out of 208 genotypes. Florets of 71 genotypes were red, 68 genotypes were orange, 13 genotypes were white, 11 genotypes were yellow and 45 genotypes were multicolored.

The origins of 106, 59 and 13 genotypes were India, Iran and Turkey, respectively. Other genotypes originated in countries such as China, Spain, Afghanistan, the United States, Egypt, Palestine,

Ethiopia, Pakistan, Morocco, Australia, and Sudan. The origin of the two genotypes was unknown (Fig. 1).

The safflower germplasm in the Dryland Agricultural Research Institute (DARI) is one of the richest gene reserves of this crop in Iran and even in the Middle East. Therefore, the evaluation of this germplasm is done annually and documented with two

main goals: 1) initial evaluation for agricultural traits and 2) revitalization of this germplasm. Seed propagation is another positive result of this kind of experiment. This research showed a considerable diversity among the selected ecotypes that can be used in breeding programs.

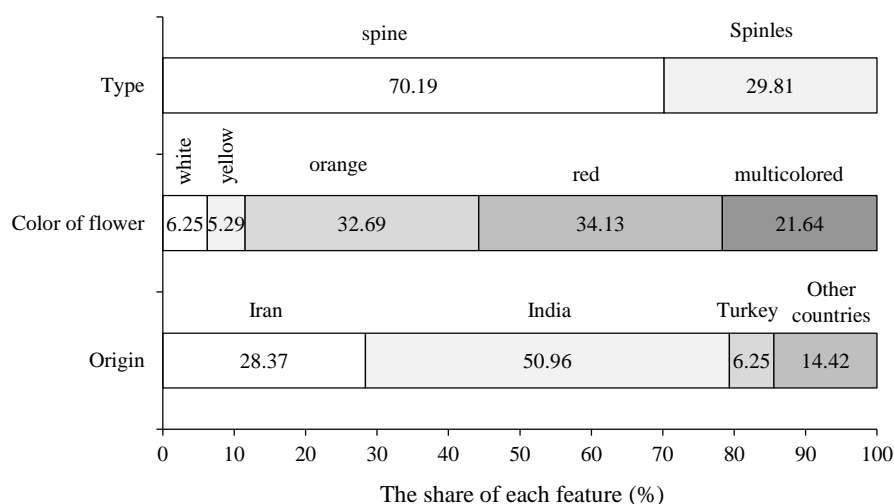


Figure 1. The share of each qualitative trait (%).

The seed yield of PI198009, PI197831, PI271139 and PI305205 genotypes (2427, 2483, 2483, 2693 and 2965 kg/ha, respectively) were higher than the seed yield of Sina and Faraman cultivars as controls of the research (1950 and 2116 kg/ha, respectively).

There was a significant variation between seed and oil yields of the studied genotypes so the range of changes for seed yield varied from 85 to 2966 kg/ha and for oil yield from 22.9 to 937.2 kg/ha. The mean seed and oil yields of the genotypes were 879 and 268.1 kg/ha, respectively (Fig. 2).

The mean of 1000- seed weight genotypes was 33 g and the range of variation was between 19.6 to 49.4 g.

The seed oil content of genotypes with a mean of 30.4% ranged from 22.9 to 36.3%.

The studied genotypes reached flowering and physiological maturity on a mean of 170 and 200 days after planting, respectively. The range of their changes was 17 days for flowering and also 17 days for physiological maturity. In the study of Saeedi et al. (2004) native safflower cultivars had the highest diversity in seed yield and the lowest diversity in the number of days to flowering and physiological maturity, which is in accordance with the results of the present experiment.

Table 2. Statistical indicators of the studied traits in Safflower germplasm.

| Characters            | Min   | Max    | Range  | Mean   | Std. deviation |
|-----------------------|-------|--------|--------|--------|----------------|
| Seed yield (kg/ha)    | 85.5  | 2965.7 | 2880.2 | 877.99 | 488.69         |
| 1000- seed weight (g) | 19.6  | 49.4   | 29.8   | 33.05  | 6.64           |
| Oil content (%)       | 22.95 | 36.27  | 13.32  | 30.37  | 2.63           |
| Oil yield (kg/ha)     | 22.86 | 937.16 | 914.3  | 268.15 | 153.3          |
| Day to flowering      | 164   | 181    | 17     | 169.60 | 3.50           |
| Day to maturity       | 194   | 211    | 17     | 199.89 | 3.33           |
| Plant height (cm)     | 41    | 121    | 107    | 75.52  | 15.05          |
| Head diameter (mm)    | 5     | 28     | 23     | 13.25  | 3.87           |
| Sub-branches in plant | 2     | 12     | 10     | 4.22   | 1.72           |

The genotypes were very diverse in terms of plant height so the plant height of different genotypes with a mean of 75.5 cm was between 41 and 121 cm. The range of genotype head diameter changes was between 5 to 28 mm and its mean was 13.2 mm. The number of sub-branches in plants of different genotypes was between 2 to 12 and the mean was 4.2 (Table 2). The highest and lowest standard deviation was related to seed yield (48.9) and the number of sub-branches (1.7), respectively (Table 2).

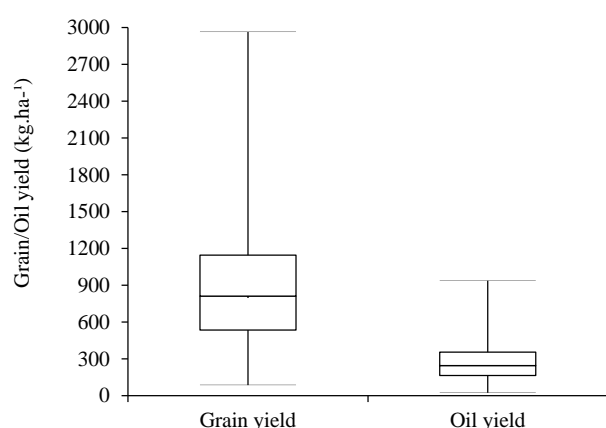


Figure 2. Box plot of seed and oil yields- The horizontal line within the box represents the median (second quartile, Q2). The lower and upper limit of the box represents the first and third quartile (Q1 and Q3), respectively. The bottom and top horizontal lines outside each box represent the minimum and maximum data, respectively.

#### 4. Discussion

Genetic diversity was the basis of plant breeding and knowledge of this diversity can make breeders much more successful in carrying out breeding programs. Rafiei and Saeedi (2005) studied the genetic diversity of 68 selected genotypes from native safflower populations in different regions of Iran along with 13 foreign genotypes. The seed yield of the genotypes ranged from 1285 to 3524 kg/ha and the oil content varied between 24.7 to 37.5%. Also, in terms of flowering and physiological maturity periods, plant height and resistance to powdery mildew, the differences between lines and genotypes were significant. In addition, the genetic diversity of Iranian lines did not follow their geographical diversity.

In the study of Ma'ali Amiri et al. (2001), some Iranian genotypes were placed in the group of foreign cultivars by cluster analysis based on quantitative and qualitative traits, which shows their closeness with foreign genotypes. In both methods, the mismatch

between genetic and geographical diversity was confirmed.

Genetic diversity in safflower ecotypes has been proven in different studies using various markers, including morphological markers (such as the present experiment) and molecular markers (Ali et al., 2019; Hassani et al., 2020; Golkar and Mokhtari, 2018; Atole et al., 2018). The existence of this diversity can give hope to researchers to carry out programs.

In most recent research breeding, in order to identify high-quality safflower germplasm and provide theoretical reference for breeding programs, 482 safflower germplasm were evaluated based on their genetic diversity, correlation, principal component by some agronomic traits including plant height, branch height, the height of the top branch, number of primary branches, number of secondary branches, seedling survival rate, initial flowering, flower color, with or without spines on leaf margin, with or without spines on bract, leaf margin, and thousand-grain weight from 30 counties. Results of this experiment showed considerable genetic diversity among these recourses. (Qin et al., 2022).

#### 5. Conclusion

The safflower collection created by the deputy director of the Dryland Agricultural Research Institute, Sararood is the largest safflower collection in the country and even in the Middle East.

Every year, a part of this collection is evaluated, revived and purified. In this study, 208 safflower genotypes were evaluated. The results showed considerable genetic diversity for most of the traits in the studied germplasm. Mean seed yield, oil yield and physiological ripening period for the genotypes were 879 kg/ha, 268 kg/ha and 200 days, respectively. The variation range of these traits was 288 kg/ha, 914 kg/ha and 17 days, respectively.

The seed oil content of PI306971, PI306954, PI205604, PI306853 and PI343780 genotypes was above 35% and higher than other genotypes. The duration of planting to physiological maturity of PI198009, PI250601 and PI283750 genotypes was 194 days and shorter than other genotypes, so they were known as the earliest genotypes. Finally, superior genotypes were selected and along with the control cultivars will be evaluated in repeated experiments in the following year.



## Conflict of Interests

All authors declare no conflict of interest.

## Ethics approval and consent to participate

No humans or animals were used in the present research.

## Consent for publications

All authors read and approved the final manuscript for publication.

## Availability of data and material

All the data are embedded in the manuscript.

## Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

## Informed Consent

The authors declare not to use any patients in this research.

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