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Quantitative and Qualitative Evaluation of Safflower (*Carthamus tinctorius* L.) Mutants in Comparison with Commercially Released Cultivars in Iran

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ABSTRACT

Considering the adaptability and importance of the safflower for the production of oilseeds in Iran, this research was conducted to evaluate and investigate of main agronomic traits in 12 mutant lines, produced in the National Institute of Genetic Engineering and Biotechnology (NIGEB) along with Sina, Faraman, Omid, Sofeh, Goldasht, Golmehr, Parnian and Padideh cultivars. The project was carried out as a complete randomized block design in three replications under rainfed conditions during the crop season 2021-2022 in the Dryland Agricultural Research Institute-Sararood, Kermanshah, Iran. The results of variance analysis for all measured traits showed significant differences between cultivars and mutants at the probability level of 1%. The highest and lowest weight of 1000 seeds in this research was related to the Faraman cultivar (40.65 g) and Safeh (23.47 g), respectively. The first to third highest seed yield was observed in the Omid and Sina and Mutant No. 8 respectively with an average of 267.3, 242.2 and 233.3 kg/ha. The lowest yield belonged to Padideh and Golmehr cultivars (65.3 and 73.4 kg/ha, respectively).

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1. Introduction

Iran is located in the dry belt of the earth, which has caused a natural shortage of water in this country. For a long time, Iranians have taken measures such as building aqueducts, water dams, and water storage in most areas of the country to cope with this issue. Over time, the increase in population and the expansion of agricultural, industrial and service activities have increased the need to use water. In addition, the unbalanced distribution of activities and population across the country has often turned the natural phenomenon of water shortage into a human phenomenon and the management of the water crisis has resulted in additional pressure on water resources (Mokhtari Hashi and Moradi, 2021).

In addition to the problem of drought, another obstacle of the agricultural sector in Iran is the heavy dependence on oilseed imports. Common oilseeds such as soybeans, sunflowers, and canola have high water requirements and the possibility of developing their cultivation in the country is limited. Therefore, if an oilseed crop with high tolerance to drought stress and the possibility of economic production can be introduced to farmers for dryland cultivation, this will certainly be welcomed. (Rostami Ahmadvandi and Faghihi, 2021)

Safflower (*Carthamus tinctorius* L.) belongs to the Asteraceae family and its seeds are mainly used to prepare edible oils. This crop has been used for many years to use its flowers in coloring and flavoring food and making dyes. The main habitat of this plant is considered to be in the Middle East and an enclosed area between the Mediterranean and the Persian Gulf (Pourdad, 2008; Zhang *et al*., 2019). Safflower

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germplasm all over the world has wide genetic variation in terms of quantitative and qualitative characteristics, compatibility with environmental factors and resistance to biotic stresses. There are more than 25 thousand samples of safflower germplasm that have been stored in 22 institutes from 15 countries (Yang *et al*., 2007).

The study of genetic diversity helps plant breeders in identifying the genetic capacity of traits related to their specific breeding goals, and the study of pattern acceptance and the dependence of genetic diversity on the geographical and climatic diversity of genotypes shows their possible adaptations to different environments (Carvalho *et al*., 2019).

Mutation to create genetic diversity and new genetic materials has been one of the ways to improve different quantitative and qualitative properties in plants. Mutation in the genetic improvement programs of plants can be created in two physical ways by using gamma and X-rays and chemically in the form of using mutagenic substances (Gudin, 2017; Katiyar *et al*., 2022).

Several commercial cultivars have been developed in main crops such as rice, barley, wheat, soy, corn and cotton via mutation through gamma radiation, in order to improve traits such as earliness and resistance to biotic and abiotic stresses (Katiyar *et al*., 2022). An experiment that was conducted as a comparison of the genetic variability capacity of laser and gamma rays on somatic and sexual cells of safflower showed that chromosomal abnormalities (adhesion and multiple aggregations) were caused by gamma rays in the meiosis and mitosis divisions. Therefore, the researchers concluded that gamma rays are more effective in creating genetic diversity in safflower (Dierck *et al*., 2016).

In a study by Rampure et al. (2017) to isolate mutants having suitable traits for safflower improvement, two varieties of safflower, AKS-207 and Bhima were mutagenized by chemical mutagens (ethyl methanesulphonate and sodium azide) and gamma rays. All the mutagens decreased pollen fertility in the first generation with the increase in the dose of mutagen. The screening of the next generation (M2) in this research led to the identification of several putative mutants. The evaluation of these mutants in the M3 generation resulted in some specific traits including earliness, dwarf, highly branched, high seed weight, high oil content and high oleic acid that can be valuable in breeding programs for safflower improvement.

Also, an investigation was carried out for creating mutants in safflower (Okaz *et al*., 2016). Their results showed some promising mutants in the M3 generation. The results also show that the Di methyl Sulfoxide was more effective than the other two treatments (γ-ray & electric shock. The promising mutants were superior to the parental genotypes in the aspect of some traits such as earliness.

Seeds of safflower were treated with five doses (5, 10, 15, 20, 25 kR) of gamma rays by Verma and Shrivastava (2014). In their research, some translocation heterozygotes were observed at the meiotic division for 10- and 25-kR doses. The induced mutants showed some variation for example delayed flowering, low flower number, low fertility, and low seed weight as compared to the control. Sometimes, induction of this mutation may bring out favorable morphological variation that can be used in breeding programs.

Therefore, according to the mentioned experiences, it can be concluded that the production of mutants using different methods, such as gamma rays, can be considered in the formulation and development of breeding programs for different crops, including safflower. In this project, 12 mutant lines of safflower plant, produced in the National Research Institute of Genetic Engineering and Biotechnology, along with the safflower commercial cultivars (Sina, Faraman, Omid, Sofeh, Goldasht, Golmehr, Parnian, and Padideh) were investigated under the rainfed conditions.

2. Materials and methods

This research was carried out in the research station farm of the Dryland Agricultural Research Institute (DARI), Kermanshah, Iran during the crop season 2021-2022 which has a moderate climate. The maximum temperature in summer is about 42 centigrade and the minimum in winter is about -12 centigrade. In this project, 12 mutants of safflower, produced in the National Research Institute of Genetic Engineering and Biotechnology (the fourth generation of the mutant resulting from radiation by Gamma rays on the Goldasht cultivar) evaluated in the form of a complete randomized block design with three replications along with Sina, Faraman and Omid cultivars (introduced by Dryland Agricultural Research Institute) and the cultivars Sofeh, Goldasht, Golmehr, Parnian and Padideh (introduced by Seed and Plant Improvement Institute) in terms of seed yield, oil and other agricultural traits under rainfed conditions. Land preparation operations were done in early October without applying fertilizer. The seeds of each mutant and cultivar were planted in an experimental unit with four rows of four meters in November. Planting and weed control were done manually. During the growth period, in addition to the necessary agricultural care (such as regular weed, disease or possible pests control), various traits such as the number of days until the beginning of flowering, the number of days until maturity, the final height of the plant, the number of sub-branches and the number of heads per plant, was recorded and after harvesting, the characteristics of 1000 seeds weight, oil content (using NMR machine made in England) and seed yield in each plot were calculated in kilograms per hectare. Spraying operations against the bollworm (Heliotis) and the red beetle were carried out by spraying with routine pesticides. Variance analysis was done with SAS statistical software (version 9.4). While comparing the mutant lines with different cultivars by LSD method, the superior lines in terms of seed yield, oil content and other suitable traits will be used in the future breeding programs of this crop.

The total rainfall in location was 227.5 mm (90 mm less than the previous crop year). The distribution of rainfall in autumn was 64.3 mm, in winter 109.9 mm and in spring 53.3 mm. In other words, 48.4% of rains occur continuously in winter, 28.2% in autumn and 23.4% in spring. The temperature data show that the average temperature of the last crop year was 13.98 degrees Celsius and the total number of days below zero was 68 days.

3. Results and discussion

Variance analysis for all measured traits at the probability level of 1% (%5 for SB) showed a significant difference between cultivars and mutants in this experiment (Table 1), which indicates the existence of sufficient genetic diversity between cultivars and mutants. The high diversity of introduced cultivars can also be one of the reasons for the significance of all traits in this study. Maximum and minimum values of CV among the measured traits were related to yield (19.48) and phenological traits (0.34), respectively.

The mean comparison results are shown in Table 2. In the following, the mean comparison of each trait is explained separately.

Table 1. Analysis of variance (mean square) of investigated traits in safflower cultivars and mutants under dry conditions.

Source of variation	df	Number of head per plant (HP)	Plant height-cm(PH)	Days to maturity (DM)	Days to flowering (DF)	Oil content % (OC)	Thosand seed weight-g (TSW)	Yeild (kg/ha)	Number of sub-branches (SB)
Replication	2	1.05 ns	2.11 ns	0.35 ns	0.20 ns	0.52 ns	0.11 ns	7.21 ns	0.01 ns
Treat	19	9.36**	145.59**	48.88**	57.35**	6.38**	157.82**	83.69**	1.48*
Error	38	0.76	4.43	0.56	0.32	0.56	3.86	10.24	0.4
CV		14.03	3.21	0.34	0.34	2.66	6.50	19.48	16.71

3.1. Plant height

The highest plant height was related to the Golmehr cultivar (with an average of 83.33 cm and a significant difference with other genotypes) and the lowest plant height was related to mutant number 3 (with an average of 58 cm) (Table 2). It seems that the main reason for the low height of the plants was the low rainfall in the year of the experiment.

3.2. The number of sub-branches

In this project, the Sofeh variety showed the highest number of sub-branches (5.33 numbers). this difference was not significant with 5 other genotypes, including some cultivars and mutant number 8. The lowest number of sub-branches was also observed in Golmohr and Padideh cultivars.

3.3. The number of heads

This trait is also one of the important yield-related traits in safflower. In this study, the highest number of heads per plant was related to Sofeh with an average of 10 per plant (no significant difference with Omid and Sina cultivars). The lowest number of heads per plant was found in mutant number 6 with an average of 4.

3.4. Phenological traits

The investigation of the phenological traits in this project showed that the Padideh variety with 225.67

days was the latest and Sina (210 days) was the earliest genotype. According to the comparison table, the average of these two cultivars in flowering were the late flowering and early flowering genotypes with 175.67 and 158.67 days respectively.

3.5. 1000- seed weight

The highest weight of 1000 seeds in this research was related to the Faraman variety (40.65 g) and the lowest value was registered in the Safeh variety (23.47 grams). None of the evaluated mutant lines had superiority or weakness in terms of this trait compared to the mentioned cultivars.

3.6. Yield

The first to third highest yield in this project was related to the varieties Omid and Sina and Mutant No. 8 respectively with an average of 267.3, 242.2 and 233.3 kg per hectare. The lowest yield was found in Padideh and Golmehr cultivars (65.3 and 73.4 kg per hectare, respectively). In other words, these two cultivars showed lower performance than all the studied mutants. Only mutant number 8 shows more and significant performance compared to the Goldasht variety (irradiated variety) in dry conditions.

3.7. Oil content

Based on the results of the variance analysis table, a significant difference was observed between the genotypes studied in this research. The highest percentage of oil content in this research was 31.4% of Sina cultivar (no significant difference with Omid (30.91%)) and the lowest value was related to mutant number 7.

Table 2. Comparison of the average traits of safflower cultivars and mutants under dry conditions.

Var/mutant	Number of heads per plant (HP)	Plant height- cm (PH)	Days to maturity (DM)	Days to flowering (DF)	Oil content % (OC)	Thousand seed weight-g (TSW)	Yield (kg/ha)	Number of sub-branches (SB)
Faraman	7.33	62.67	214.00	164.33	28.63	40.65	157.7	4.33
Goldasht	7.00	64.33	214.33	164.33	28.33	38.69	166.5	4.00
Golmehr	6.00	83.33	224.33	174.67	28.12	24.59	73.4	2.67
Omid	9.67	68.00	212.00	162.67	30.91	26.69	267.3	5.00
Padideh	4.67	73.67	225.67	175.67	27.04	23.67	65.3	2.67
Parnian	7.67	78.00	210.33	160.67	27.99	36.87	153.3	4.33
Sina	9.00	58.33	210.00	158.67	31.40	26.93	242.2	4.33
Sofeh	10.00	74.33	220.33	171.67	30.00	23.47	226.7	5.33
M1	5.33	58.67	213.00	162.33	28.17	34.41	193.9	3.67
M2	5.33	61.67	214.00	164.00	28.98	36.02	123.2	3.67
M3	6.00	58.00	214.67	165.00	26.41	33.84	159.1	3.00
M4	4.33	63.67	213.33	163.00	27.51	36.91	167.6	3.00
M5	4.67	63.00	212.00	161.00	26.78	38.47	121.4	3.33
M6	4.00	58.33	214.00	163.33	27.96	36.63	118.7	3.33
M7	4.33	60.00	214.00	163.00	25.94	35.59	185.1	4.00
M8	5.67	65.33	215.00	164.00	27.91	38.26	233.3	4.33
M9	4.67	65.00	213.33	162.33	26.41	35.20	142.9	3.67
M10	6.67	60.67	215.67	162.67	29.26	32.98	184.2	3.67
M11	6.33	67.33	214.00	164.67	28.96	37.79	177.4	4.00
M12	6.33	65.00	215.00	164.00	26.95	35.43	126.2	3.67
LSD	1.44	3.47	1.23	0.93	1.24	3.44	52.9	1.05

So far, extensive studies have been conducted on the production of plant mutants all over the world. These studies have been done mainly to increase genetic diversity and create some lines with special traits, and even some commercial plant cultivars have been released as a result of these studies. These researches have been conducted in oil crops as well as in other strategic ones. For example, studies in this field have been done in rapeseed (Guo *et al*., 2022), sunflower (Scartazza *et al*., 2020), soybean (Genesio *et al*., 2020), camelina (Neumann *et al*., 2021), sesame (Kouighat *et al*., 2021), olive (Verdura *et al*., 2019) and cottonseed (Zhu *et al*., 2021).

Therefore, the identification of some desirable mutants in crops such as safflower can be well used in breeding programs of this oil crop.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human 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

The main draft of the manuscript was written by Dr. Rostami Ahmadvandi and revised by other authors.

Informed Consent

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

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