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잇꽃 유전자원의 농업적 형질, 총폴리페놀 함량 및 지방산 조성

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Agro-morphological Characters, Total Phenolic Content, and Fatty Acid Compositions of Safflower Genetic Resources

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ABSTRACT

Background: Safflower is an important crop that does not require rich soils. It grows well in dry soils or arid areas with seasonal rain. Exploring the fatty acid profiles and agro-morphological characteristics of diversified collections of safflower provides baseline data for developing improved varieties. In this study, we investigated the variation in agro-morphological characteristics, fatty acid composition and total phenolic content of the seeds, and the relationship between the agro-morphological and biochemical characteristics.
Methods and Results: Agro-morphological characteristics were recorded in the field and laboratory. Total phenolic content was estimated using Folin-Ciocalteu's method and fatty acids were determined using gas chromatography-mass spectrometry. Orange, red, and white petal colors were observed; orange was the dominant pigment. Wide ranges of other agro-morphological characteristics were also recorded. More than 87% of the accessions contained > 50% linoleic acid while approximately 12% of the accessions contained > 50% oleic acid. A strong correlation was observed between palmitic and linoleic acid, and crude fat and oleic acid. A strong negative correlation was observed between crude fat and linoleic acid, palmitic and oleic acid, and oleic and linoleic acid.
Conclusions: Safflower accessions were found to be a poor indicator of essential linolenic acid. The wide variation in agro-morphological and biochemical traits of safflower accessions could potentially help to develop an improved, nutrient-dense safflower cultivar.

Key Words: *Carthamus tinctorius*, Agro-morphological Trait, Fatty Acid Ratios, Linoleic Acid, Total Phenolic Content, Oleic Acid, Unsaturated Fatty Acids

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a draught and salt tolerant underutilized oil seed crop of the Asteraceae family (Pearl and Burke, 2014). It is believed to be originated from southern Asia and have been cultivated in China, Egypt, India, and Iran in the era of human prehistory, and during the Middle Ages in Italy, France, and Spain (Turgumbayeva *et al*., 2018).

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Australia, Ethiopia, India, Mexico and the USA, are the largest safflower producers accounting 85% of the world's production altogether (Liu *et al*., 2016). It is a branching, thistle like herbaceous perennial broad leaf crop (Dajue and Mündel 1996; Park *et al*., 2005).

Studies based on phylogenetic analysis of a combined dataset and unweighted pair group method with arithmetic mean (UPGMA) dendrogram clustering analysis, nuclear DNA assay results, and fluorescent in situ hybridization (FISH) studies showed that *C. tinctorius* L. is most likely domesticated from the wild species, *Carthamus palaestinus* (Chapman and Burke 2007; Sasanuma *et al*., 2008; Agrawal *et al*., 2013; Ambreen *et al*., 2015). The petals, stamens, and pistils of safflower exhibited different colors including, white, yellow, orange, red, and creamy (Kim *et al*., 2020).

Color is an important character that used as an external appearance index for evaluation of the quality of the safflower in assessment of conformity on certain specifications and change in quality due to processing and storage. Safflower crop is also characterized by significant variations in agro-morphological characters including, plant height, leaf length and width, days to flowering, and seed length and weight (Sung *et al*., 2016).

Safflower is an important crop that does not require rich soils and grows well in dry soils or arid areas having seasonal rain. It is a source of both edible and biodegradable oil for technical use, medicinal plant and part of animal feeding mixtures (Golkar, 2014). *Carthamus tinctorius* L. is one of the most studied major *Carthamus* species. Various chemical components from different parts of *Carthamus* species have been reported including phenolic acids, flavonoids, alkaloids, quinochalcones, triterpenes, sterols, volatiles constituents, amino acids, fatty acids, sugars, and others (Akihisa *et al*., 1996; Zhang *et al*., 1997; Takii *et al*., 1999; Kazuma *et al*., 2000; Hotta *et al*., 2002; Mitova *et al*., 2003; Taskova *et al*., 2003; Mikhova *et al*., 2004; Koyama *et al*., 2006; Zhao *et al*., 2010; Zhou *et al*., 2014; Conte *et al*., 2016; Pu *et al*., 2019).

The safflower seed oil is rich in unsaturated fatty acids (Sung *et al*., 2016, 2018). Depending on the variety, growing season, and environment the safflower seed could contain up to 45% oil (Emongor, 2010; Liu *et al*., 2016). Safflower seed is mainly composed of two unsaturated omega-6 (linoleic acid) and omega-9 (oleic acid) fatty acids. Safflower seed oils have been used in various application including preparations of resins for paints and varnishes, edible oils, and cosmetics (Ekin 2005; Emongor 2010; Liu *et al*., 2016).

Linoleic acid is an essential fatty acid that can't be produced by our body in contrast to oleic acid and need to be obtained through diet (Winitchai *et al*., 2011). Linoleic acid is lightweight and thinner that can easily be absorbed by our skin. Hence, in cosmetic industry oils with high content of linoleic acid could be used to control acne while the thicker oleic acid is beneficial for those with dry/aging skin (Downing *et al*., 1986).

Previously, the diversity of safflower germplasm of the National Agrobiodiversity Center (NAC) collected from Asian countries in terms of the fatty acid composition were explored (Shim *et al*., 2004; Sung *et al*., 2016, 2018). Exploring the fatty acid profiles and agro-morphological characters of diversified collections of safflower has a paramount importance to develop new cultivars with improved adaptability and nutritional quality.

Hence, in this study we have investigated: 1) the variation in agro-morphological characters; 2) the fatty acid composition and total phenolic content of the seeds; and 3) the relationship between the agro-morphological and biochemical characters of 237 germplasm collections from 26 countries across the world.

MATERIALS AND METHODS

1. Plant materials and reagents

Hexane, NaOH, 14% boron trifluoride-methanol (BF_3-methanol), gallic acid standard, and Folin–Ciocalteu reagent, and standards of fatty acids were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Seeds of 237 accessions of safflower (*Carthamus tinctorius*) were obtained from the gene bank of the National Agrobiodiversity Center (NAC) of Korea. The accessions were originated from the following countries: Afghanistan (2), Armenia (1) Australia (2), Bangladesh (1), Canada (17), China (30), the former Czechoslovakia (3), Egypt (24), Spain (4), Ethiopia (1), France (2), India (5), Kazakhstan (27), Kyrgyzstan (2), South Korea (4), Morocco (3), Mexico (2), Myanmar (2), Pakistan (1), Russia (1), Sudan (4), Syria (1), Tajikistan (2), Turkey (10), United States of America (72), and Uzbekistan (14). The seeds were sown on March 14, 2018 at the experimental field of NAC in Jeonju (35°49′18″N, 127°08'56"E).

The experimental field is a sandy loam soil with pH 6.0. Each material had 21 seedlings in a plot (row spacing and

plant to plant spacing were 30 ㎝ and 20 ㎝ respectively) in non-replicated design. Rural development Administration's, Jeonju, Korea, recommended agronomic practices for safflower was followed. Plants were drip irrigated and were harvested manually in August, 2018.

2. Agro-morphological characters

Agro-morphological traits such as plant height, leaf length and width, spines, leaf margin, days to flowering and ripening, flower color, seed length and weight were recorded in the field and laboratory following the procedure described by Sung *et al*. (2016).

Plant height (㎝) was measured at full flowering stage from ground level to the main stem's tip. The length and width of the leaf (㎝) were measured starting from the base to the tip. The bract spines were recorded in score of 0 to 2, 0 = spineless, 1 = short spine (< 2 ㎜), and 2 = long spine (> 2 ㎜). Shape of leaf margin were recorded in 0 to 2 score, 0 = smooth, 1 = light split, and 2 = deep split.

Days to flowering were counted as number of days taken where at least three plants showed open flowers starting from the days of sowing. Petal color was described as orange, red, and white. Seed was harvested manually at full maturity. The seed yield was measured using ten plants per accession and reported in gram (g) per plant.

3. Extraction and determination of total phenolic contents

Phenolic compounds were extracted following the procedure described earlier with some modification (Assefa *et al*., 2018). Briefly, seeds of safflower were dried in a VS-1202D drying oven (Vision Scientific, Bucheon, Korea) for 3 days at 40℃. Samples were ground to fine powder and phenolic compounds were extracted from 7 g sample using 75% ethanol (40 ㎖) in an accelerated solvent extractor (ASE) (ASE-200, Dionex, Sunnyvale, CA, USA) under nitrogen gas. The pressure and temperature were set as 1200 psi and 70℃, respectively.

Each extract solution was transferred to a 50 ㎖ conical tube and the solvent was evaporated using a Genevac HT-4X (Genevac Ltd., Ipswich, England) vacuum concentrator at 40℃ for 10 h. Samples were then reconstituted at the appropriate concentration. Each sample was prepared in biological triplicates. Test solutions were filtered using a 0.45 ㎛ syringe filter prior to total phenolic content assay.

Total phenolic content (TPC) was determined based on Folin-Ciocalteu's method (Waterhouse, 2002) after some modifications as described by Assefa *et al*. (2018). Briefly, 100 ㎕ Folin-Ciocalteu reagent was added to a sample solution (100 ㎕) or standard solution and kept at room temperature for 3 min. To this mixture, solution of 2% sodium carbonate (100 ㎕) was added, followed by incubation for 30 min in a dark place. Absorbance of the solutions were recordedusing an Eon Microplate Spectrophotometer (Bio-Tek Inc., Winooski, VT, USA) at 750 ㎚. Distilled water was used as a blank. Results were presented as μg gallic acid equivalent per mg sample (㎍·GAE/㎎). Each sample/standard solution was tested in triplicate.

4. Fatty acid extraction, derivatization, and GC/MS analysis

The total oil content and individual fatty acids were analyzed based on the procedure described by Sung *et al*. (2018).

The total oil was extracted from 1 g of dry pulverized seeds of safflower in hexane using Soxtec™ 2043 (FOSS Tecator AB, Hillerød, Denmark). The solvent was evaporated and the total oil content was determined gravimetrically.

To each tube containing the crude fat, 2 ㎖ of 0.5 NaOH was added to transmethylize the fatty acids, vortexed for 5 s, heated for 10 min using a water bath at 80℃. After cooling at room temperature, 2 ㎖ of 14% cold boron trifluoride-methanol solution was added with vortexing for 5 s. Sample solutions were heated again for 10 min at 80℃ in water bath and cooled at room temperature. To this solution, seven ㎖ of n-hexane and 2 ㎖ of H_2O were added, vortexed for 10 s, and centrifuged (temperature, 4℃; speed, 3000 rpm; time, 10 min). The upper layer (hexane part) of the supernatant was filtered using a flower shaped filter paper where anhydrous sodium sulfate powder placed on top of it. Similarly, standards were derivatized. One ㎖ of filtrate was transferred to gas chromatography autosampler vials for fatty acid analysis.

Fatty acid methyl esters (FAMEs) were analyzed by GCMS-QP2010 UltraGas Chromatograph (Shimadzu Co., Kyoto, Japan) equipped with an autosampler using a 19091N-136 INNOWAX column (0.25 ㎜ × 60 m, 0.25 ㎜, Agilent Technologies Inc., Santa Clara, CA, USA). The instrument conditions were described as follows: Column temperature was set initially at 150℃, followed by an increase to 200℃ at a rate of 4℃/min, and set to 220℃ for the last 5 mins; injector port and the detector were set up at 250 and 300℃, respectively. 10 ㎕ of each sample was injected. The carrier gas (N_2) flow rate was set at 0.6 ㎖/min.

The FAMEs in samples were identified by comparison to

their retention time of the authentic standards. The proportion of individual fatty acids in the total fatty acid content was calculated through area-under-the-curve measurements.

5. Statistical analysis

Experiments are conducted in a biological triplicates and results are the average of the replicates. IBM SPSS 25 (IBM Co., Armonk, NY, USA) was used to perform Pearson correlation analysis (PCA). PCA was conducted using Palaeontological Statistics, Version 3.06 (PAST) (Hammer *et al*., 2001). The fatty acid ratios [(oleic acid desaturation ratio (ODR) and linoleic acid desaturation ratio (LDR)] were calculated as follows following a previous report (Velasco *et al*., 1998).

$$ODR \text{ (oleic acid desaturation ratio)} = \frac{\%C18{:}2 + \%C18{:}3}{\%C18{:}1 + \%C18{:}2 + \%C18{:}3}$$

$$LDR \text{ (linoleic acid desaturation ratio)} = \frac{\%C18{:}3}{\%C18{:}2 + \%C18{:}2}$$

RESULTS AND DISCUSSIONS

1. Agro-morphological characters of safflower germplasm

The variation in agro-morphological characters (plant length, leaf length and width, petal color, spine, leaf dentation, seed coat color, and days of flowering, ripening and harvest) of 237 accessions of safflower plant are represented in Fig. 1 and Fig. 2.

All the germplasm collections were branching type and exhibited white colored seed coats except one accession which had a yellow color. The petals were red, orange or white colored. The orange color was the predominant petal color that was observed in 182 accessions followed by red color which was recorded in 49 accessions. The petal color of 47 of the accessions changed with development while 190 accession showed no change of color.

The leaves were ovate to obovate shape, mostly with dentate (21 accessions had moderate and 205 had weak dentations) and few smooth (11) margins. The plant length, leaf length, and leaf width were ranged between 65.7 and 160.8 ㎝, 14.3 and 37.0 ㎝, and 3.3 and 12.1 ㎝, respectively.

The safflower plant started flowering between 63 and 91 days (mean, 81.2 ± 3.7) after sowing, and ripening anywhere between 91 and 108 (mean, 99.2 ± 2.6) days. Plants were ready for harvest in 111 to 132 (mean, 117.4 ± 6.1) days. The days of flowering, plant height, leaf length, and leaf width recorded in this study is in concordance with earlier reports (Shim *et al*., 2004; Sung *et al*., 2016).

Apart from red, orange, or white, petals of safflower exhibit

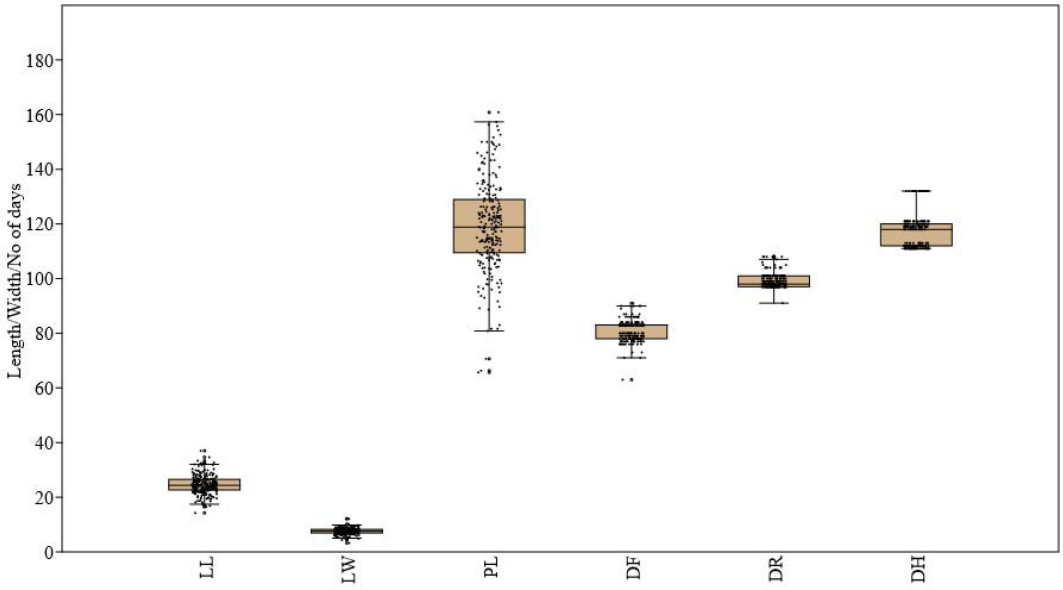

Fig. 1. Box and jitter plot of leaf length (LL, ㎝), Leaf width (LW, ㎝) plant length (PL, ㎝), days of flowering (DF), days of ripening (DR), and days of harvest (DH) of 237 safflower germplasm collections. The box plot with jitter (to avoid dot overlap) shows the summary of the distribution of agromorpholical traits of the germplasm collection. The germplasm collections had the most wide range of distrubution in their plant length while their leaf width showed the narrowest distribution.

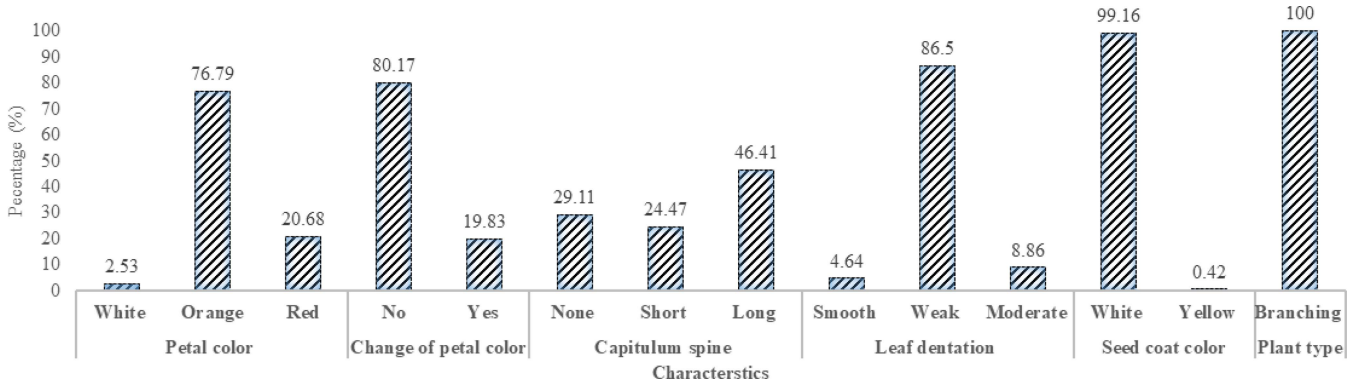

Fig. 2. Distribution of safflower accessions experessed in per cent based on some morphological characters. The figure is generated using microsoft office excel (2016 version). The germplasm collection in this study were branching type and dominantly orange petal color, white seed coat color, and weak leaf dentations. The petal color didn't change with maturity in about 80% of the accessions.

Table 1. Mean and range of seed yield, total phenolic content, crude fat and fatty acid compositions in the seeds of 237 safflower accessions.

Characters	Mean	Range
Total phenol content (㎍·GAE/㎎·DE)	60.36	23.71 - 132.72
Seed yield (g/plant)	83.79	21.20 - 144.90
Crude fat (%)	26.25	14.84 - 41.70
Fatty acid composition (%)		
Palmitic acid (16 : 0)	5.82	4.15 - 7.66
Stearic acid (18 : 0)	2.23	1.49 - 3.25
Oleic acid (18 : 1)	20.08	9.23 - 83.35
Linoleic acid (18 : 2)	71.72	10.46 - 82.62
Linolenic acid (18 : 3)	0.14	< 0.01 - 2.18

various colors such as yellow and cream (Kim *et al*., 2020). The color of safflower plant is an important character that dictate its chemical constituents (Kazuma *et al*., 2000; Golkar *et al*., 2010; Kim *et al*., 2020). The observation about the change in color of the petal with development is supported by Kim *et al*. (2020), Mohammadi and Tavakoli (2015), Flemmer *et al*. (2015), and Kumazawa *et al*. (1994), who indicated that yellow and light-yellow florets of safflower changed to orange, red-orange, dark, or purple at later stages.

The seed yield had shown a wide range of variability (21.2 to 144.9 g/plant) with average value of 83.79 g/plant.

2. Total phenolic content and fatty acid profiles of seeds of safflower germplasm

The total phenolic content (TPC) of the seeds of safflower germplasm collections was varied widely ranging from 23.7 ± 0.2 to 132.7 ± 0.6 ㎎·GAE/g·of dried extract (DE). Highest average level of total phenols was recorded in accession with red petal color (73.6 ㎎·GAE/g·DE) followed by orange petal colored accessions (57.3 ㎎·GAE/g·DE).

In earlier study, the TPC content in the seeds of 281 safflower germplasm originated from China, Japan, South Korea, and North Korea ranged between 21.0 to 197 ㎎·GAE/g·DE (Sung *et al*., 2018), which is quite in agreement with this study. The TPC of safflower seeds in this study was also found in a similar range with perilla seeds (88.77 - 148.85 mg GAE/g DE) (Kim *et al*., 2019).

The total oil content and fatty acid composition of safflower seeds is summarized in Table 1. The seeds of safflower genetic resources accounted an average crude fat composition of 26.25%.

The total fatty acid was comprised of linoleic, oleic, palmitic, stearic, and linolenic acid in decreasing order. Oleic acid and linoleic acid were recorded to be the major fatty acids in the accessions investigated. The fatty acid composition of safflower seeds has shown a wide variability where the two

unsaturated fatty acids (oleic and linoleic acid) shown the highest range of variation contributing from 9.23 to 83.35% and 10.46 to 82.62%, respectively.

A wide range of variability of the oil composition among samples have also been reported in earlier studies. The total oil content ranged from 12.5% to 34.1%, 15.8% to 32.2%, and 9.8% to 30.3% in seeds of safflower collected from east Asian, south central Asian and south west Asian countries, respectively (Sung *et al*., 2016, 2018). The oil content in seeds of safflower collected from Turkey, Iran, India, Egypt, and USA ranged from 23.1 to 36.51% (Matthaus *et al*., 2015). Another study showed the oil content of safflower from India ranged between 23.8% and 42.9 % (Saisanthosh *et al*., 2018).

The most common fatty acids reported in safflower species include linoleic acid, oleic acid, palmitic, and stearic acid altogether contributing from 96 to 99% of the total fatty acids (Rahamatalla *et al*., 2001; Conte *et al*., 2016; Liu *et al*., 2016; Zhao *et al*., 2019). The oil content and individual fatty acids composition of safflower seed oils reviewed earlier (Liu *et al*., 2016). Linolenic acid, one of the most important unsaturated fatty acid, was found to be a minor constituent (mean, 0.14%) in the accessions investigated. A similar observations was reported in safflower (Cosge *et al*., 2007; Sung *et al*., 2016) and sesame (Mondal *et al*., 2010) seeds, but in perilla seeds linolenic acid was the major fatty acid contributing from 59.19 to 67.28% of the total fatty acid (Kim *et al*., 2019).

The two saturated fatty acids (palmitic and stearic acid) contributed from 4.15 to 7.66% (mean, 5.82%) and 1.49 to 3.25% (mean, 2.23%) of the total fatty acid composition, respectively.

There were two well-defined patterns on the composition of unsaturated fatty acids among safflower accessions: high-oleic-low-linoleic and high-linoleic-low-oleic acid containing accessions. Most accessions (86.5%) had > 70% linoleic acid and < 22.0% oleic acid, while 11.4% of the accessions contained > 66% oleic and < 30% linoleic acid. Only 5 accessions (2.1%) had a relatively balanced composition (30% - 60%) of both linoleic and oleic acid.

This observation could be partly explained by the reports of Knowles and Hill (1964). These authors reported that the composition of fatty acids in safflower is affected by the major gene locus, *ol*. The genotype *olol* causes increased percentage of oleic acid while the genotype *OLOL* favors high linoleic acid. On the other hand, the genotype *ol'ol*' or $OLol_1$ has a balanced proportion (about 45%) of each of the acids but these genotypes are less stable towards changes in temperature resulting slightly higher oleic acid at higher temperature. Later on, a new gene locus (*li*) that controls high levels of linoleic acid in safflower was also reported with high levels of linoleic (87% - 89%) and very low oleic acid (3% - 7%) compositions (Futehally and Knowles, 1981).

The higher mean value of oleic and linoleic acid indicates high quality of the safflower oil for human consumption. A list of top ten safflower accession containing the highest amount of each biochemical trait is presented in Table 2. Oils with high linoleic acid content are considered premium oil. Five accessions (K186176, K186183, K186321, K186374, K248851) with relatively balanced composition of oleic and linoleic acid contributed greater than 91% of the total fatty acids. These accessions could be used to develop new varieties with high content of both fatty acids as suggest earlier for sesame plant (Uzun *et al*., 2008).

Fatty acids are produced in stepwise biosynthetic pathway where oleic acid desaturate to linoleic acid and linoleic acid desaturate to linolenic acid. The average saturated fatty acid (SFA) and unsaturated fatty acid (UFA) compositions were 91.95% and 8.05% in the investigated accessions.

The higher the UFA, the better is the quality of the oil. The recommendations on the ratio of linoleic to linolenic acid in the diet by The Food and Agriculture Organizations of the United Nations, FAO, is to be between 5 and 10. On the other hand, the ratio of cholesterol-raising fatty acids (SFA) to polyunsaturated fatty acids (PUFA) is recommended to be 1 : 1 and the total intake of each should not exceed 7% of the total energy (Grundy 1997). In this study, the ratio of linoleic to linolenic acid ranged from 34.99 to 11,500 whereas SFA to PUFA ratio was in the range between 0.09 and 0.57. The fatty acid ratios are useful to evaluate the efficiency of the desaturation pathway (Velasco *et al*., 1998).

The ODR and LDR estimate the efficiency of desaturation from oleic to linoleic acid and from linoleic to linolenic acid, respectively. The ODR and LDR values were ranged from 0.11 to 0.90 (mean, 0.78) and 5×10^{-6} to 0.027 (mean 0.002), respectively. The ODR values were quite high compared to the LDR values indicating considerably high amount of linoleic acid and low linolenic acid are produced in safflower seeds. The scatter plots of ODR vs LDR (Fig. 3) shows the efficiency of the desaturation metabolism from oleic to linoleic acid and from linoleic to linolenic acid, respectively.

Table 2. Top ten high seed yield-, fatty acid- and TPC-containing accessions of safflower seeds.

Characters	Range	Accessions			
Total phenol content (㎍·GAE/㎎·DE)	99.52 - 132.72	K186351	K265498	K248852	K265492
		K264676	K18661	K265500	K186617
		K186677	K186722		
Seed yield (g/plant)	131.2 - 144.9	K014637	K186078	K186398	K186394
		K186395	K186399	K186397	K186393
		K248848	K186396		
Crude fat (%)	38.20 - 41.70	K186324	K186588	K186174	K186591
		K186177	K186182	K186168	K186175
		K186387	K186590		
Fatty acid composition (%)					
Palmitic acid (16 : 0)	6.69 - 7.66	909223	K185413	K186390	K186339
		K186168	K186340	K185407	K185957
		K185410	K185404		
Stearic acid (18 : 0)	2.71 - 3.25	K185958	K265504	K014637	K185396
		K265505	K185406	K185401	909223
		K185397	K186156		
Oleic acid (18 : 1)	78.12 - 83.35	K185939	K186079	K186185	K186399
		K186589	K186402	K186336	K186398
		K186388	K186590		
Linoleic acid (18 : 2)	81.68 - 82.62	K263042	K258594	K186184	K263584
		K248850	K248845	K185720	K185411
		K185405	K185400		
Linolenic acid (18 : 3)	0.41 - 2.18	K014636	909220	909223	909221
		K003637	909224	K014635	K003282
		K014637	K014640		

3. Pearson correlation and Principal Component Analysis (PCA)

Agro-morphological traits and fatty acids compositions of safflower showed both negative and positive relations. The Pearson correlation coefficients between agro-morphological traits, TPC, and fatty acids is presented in Table 3.

The total oil content of seeds showed an inverse relationship with days of flowering, ripening, and harvest of safflower germplasm.

Oleic acid showed a negative significant correlation with days of flowering, days of harvesting, and plant length. However, palmitic and linoleic acid showed a positive correlation with the agro-morphological traits (except with leaf width) although insignificant in some cases.

TPC was significantly correlated with the days of flowering, ripening, harvest as well as the plant length. Safflower accessions with longer leaf length were found to contain high levels of linoleic acid, linolenic acid, and TPC, but lower oleic acid compared to the shorter counterparts.

The presence of spines (scaled from 0 to 2; where 0 representing absence of spines and 2 long spines) was associated with high oil content, stearic acid and oleic acid and low levels of other biochemicals investigated. However, safflower seeds with no spines were associated with higher seed (r = -0.2733).

Individual fatty acids showed significant associations each other. There was an inverse relationship between crude fat vs palmitic acid, linoleic acid, linolenic acid, and TPC. Individial fatty acids were uncorrelated with seed yield.

However, a significant correlation (r = 0.5475) between crude fat and oleic acid was recorded. Palmitic acid was negatively correlated with oleic acid (r = -0.7440) and positively correlated with all other fatty acids where greatest correlation was recorded with linoleic acid (r = 0.7290).

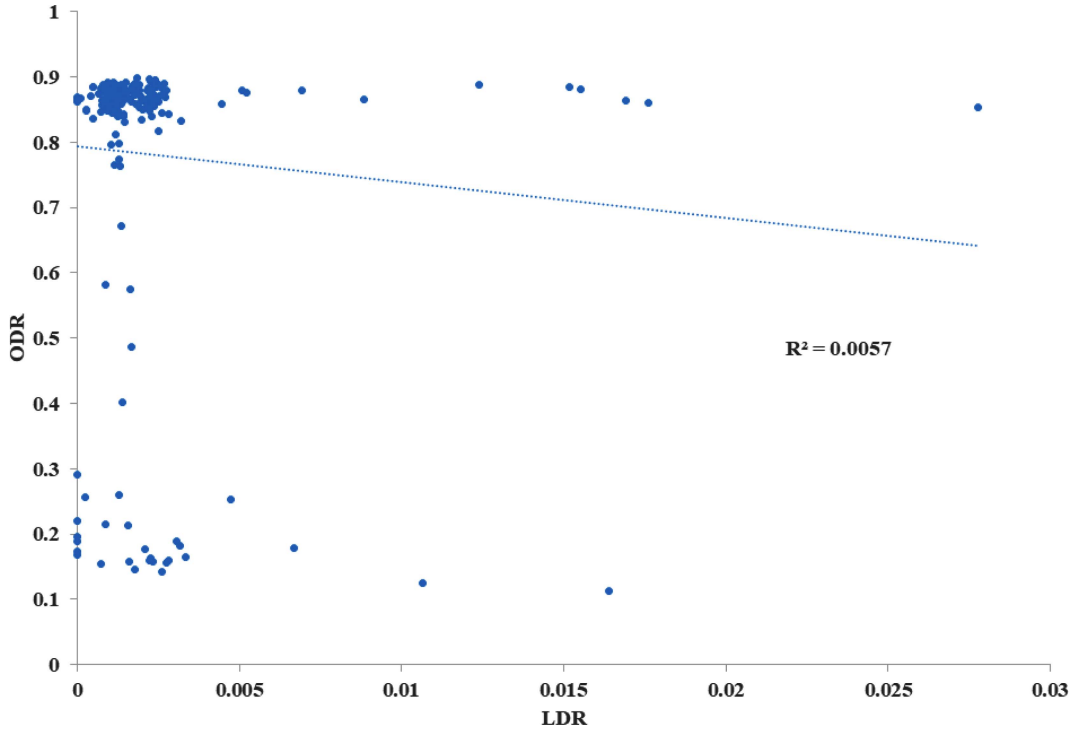

Fig. 3. Scatter plots of oleic desaturation ratio (ODR) vs linoleic desaturation ratio (LDR) in safflower accessions. The scatter plots shows the efficiency of the desaturation systems from C18:1 to C18:2, and from C18:2 to C18:3, respectively. Most accessions showed very high values of ODR low LDR, where values converging towards the vertical axis, indicating considerably high amount of linoleic acid and low linolenic acid are produced in safflower seeds.

Table 3. Pearson's correlation coefficients of some agro-morphological characters, total phenolic content, crude fat, and individual fatty acids in 237 accessions of safflower.

	Spine#	DF[1)]	DR[2)]	DH[3)]	LL[4)]	LW[5)]	PL[6)]	Crude fat	16:0[7)]	18:0[8)]	18:1[9)]	18:2[10)]	18:3[11)]	TPC[12)]
DF	-0.0735													
DR	-0.0923	0.7168*												
DH	-0.0687	0.6202*	0.8122*											
LL	-0.1329*	0.3245*	0.3307*	0.2397*										
LW	0.1406*	0.0484	-0.0239	-0.0547	0.4922*									
PL	-0.2427*	0.5868*	0.4701*	0.4504*	0.3898*	0.1077								
Crude fat	0.3125*	-0.4176*	-0.2035*	-0.236*	-0.1748*	-0.0052	-0.3062*							
C16:0	-0.1393*	0.1832*	0.0548	0.0943	0.1191	-0.0151	0.0885	-0.2699*						
C18:0	0.1795*	-0.0111	-0.0407	-0.0618	-0.1049	0.0699	0.0049	-0.1233	0.3315*					
C18:1	0.2707*	-0.2189*	-0.1013	-0.1473*	-0.1141	0.0711	-0.1538*	0.5475*	-0.7440*	-0.3274*				
C18:2	-0.2736*	0.2184*	0.1020	0.1489*	0.1136	-0.0734	0.1532*	-0.5508*	0.7290*	0.3120*	-0.9996*			
C18:3	-0.2718*	0.1242	0.0937	0.0532	0.1491*	-0.0136	0.1982*	-0.2045*	0.1829*	0.1065	-0.1774*	0.1644*		
TPC	-0.1494*	0.3776*	0.2677*	0.3335*	0.1043	-0.0465	0.3173*	-0.3625*	0.0162	-0.0881	-0.2079*	0.2152*	-0.0706	
SY[13)]	-0.273*	0.1291*	0.0394	-0.0331	0.1284*	0.0171	0.1507*	-0.1308*	0.02401	-0.0792	-0.0042	0.0048	-0.0041	-0.0136

[1)]DF; days to flowering, [2)]DR; days to ripening, [3)]DH; days to harvest, [4)]LL; leaf length (㎝), [5)]LW; leaf width (㎝), [6)]PL; plant length (㎝), [7)]16:0; palmitic acid (%), [8)]18:0; stearic acid (%), [9)]18:1; oleic acid (%), [10)]18:2; linoleic acid (%), [11)]18:3; linolenic acid (%), [12)]TPC; total phenolic content ($\mu g \cdot$GAE/㎎·DE), [13)]SY; seed yield (g/plant). The Pearson correlation table was generated using IBM SPSS 25. The significance of regression coefficients were determined at 95% confidence level ($p < 0.05$). *Values are significant at $p < 0.05$; #The spines of bract were recorded in 0 to 2 score.

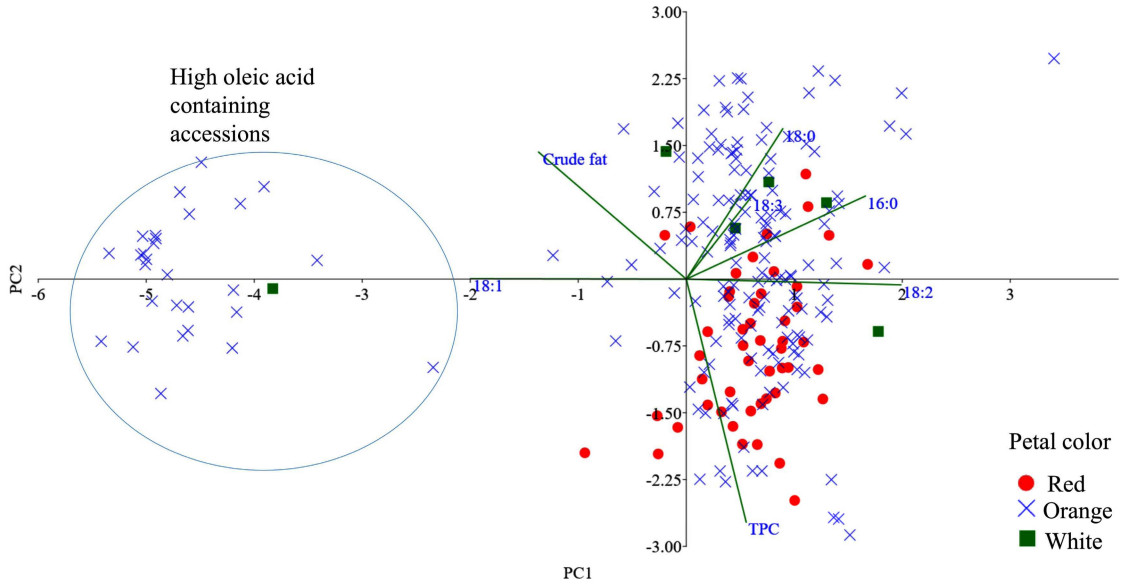

Fig. 4. PCA-biplot of 237 red-, white-, red-petal colored safflower accessions based on the biochemical traits (total phenolic content, crude fat, and individual fatty acid compositions) of their seeds. 16:0; palmitic acid, 18:0; stearic acid, 18:1; oleic acid, 18:1; linoleic acid, and 18:3; linolenic acid. Two clear groups of germplasm collections were formed at the negative and positive side of the PC1 due to high 18:1 and high 18:2 compositions, respectively. Red colored safflower accessions were more converged to the right bottom quadrant of the axis, due to their higher level of total phenolic content.

Table 4. Loadings, eigenvalues, and the variances of the principal component analysis (for the first five PC's) of total phenol content, crude fat, and individual fatty acid compositions of 237 safflower seed samples.

	PC 1	PC 2	PC 3	PC 4	PC 5
Total phenol content	0.14817	-0.72822	-0.016	0.32248	0.58495
Crude fat	-0.36519	0.38075	-0.26502	-0.2027	0.69344
Palmitic acid (16:0)	0.44288	0.24902	-0.15954	-0.30903	0.31895
Stearic acid (18:0)	0.23864	0.45063	-0.21215	0.83277	0.036178
Oleic acid (18:1)	-0.53368	0.00099	0.10839	0.1751	0.018002
Linoleic acid (18:2)	0.53057	-0.01712	-0.11404	-0.18285	-0.03123
Linolenic acid (18:3)	0.15899	0.24362	0.9134	0.042672	0.26952
Eigenvalue	3.24391	1.26737	0.960924	0.741088	0.523217
% Variance	46.342	18.105	13.727	10.587	7.4745

A similar observation was reported in safflower (Sung *et al*., 2016). However, quite the opposite is true in sesame (Uzun *et al*., 2008; Mondal *et al*., 2010) which could indicate a difference in activities of various biosynthetic pathways of fatty acid production in different plants. The greatest significant and negative relationship was found between the two major fatty acids, oleic and linoleic acids (r = -0.9996). The inverse relationship between oleic and linoleic acid is also reported in safflower and other oilseed crops (Baydar and Erbaş 2005; Mondal *et al*., 2010; Sung *et al*., 2016).

The PCA bi-plot representing scatter plots of the safflower samples and loading plots of crude fat, individual fatty acids, and TPC is represented in Fig. 4 and Table 4.

PCA showed that PC1 and PC2 had eigenvalues greater than 1 and contributed 46.3% and 18.1% of the variations, respectively. Seeds of safflower with red petal color contained high levels of TPC as shown in the PCA. The PCA plot distinguished the genetic resources which contained high percentage of oleic acid from other resources and located at the negative side (left side) of the PC1.

Genetic resources located to the positive side of the PC1 were mainly high linoleic acid- and palmitic acid-containing accessions. Seeds of accessions with red pigment petal contained low percentage of oleic acid. The main contributions for first principal component were from oleic acid, linoleic acid and palmitic acid, where the former contributed negatively. TPC had the highest negative contribution to the PC2, whereas stearic acid had the greatest positive contribution.

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