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Study on oil content and fatty acid composition in seeds of different genotypes of safflower (*Carthamus tinctorius* L.)

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Abstract
Sixty one safflower (*Carthamus tinctorius* L.) genotypes were evaluated for oil content and fatty acid composition which showed that oil content varied from 23.88 to 42.90 per cent and the highest seed oil content was obtained from EC-6 (42.90%). Among those fatty acids were detected, linoleic acid (13.83-78.66%) was the predominant fatty acid followed by oleic (12.27-80.08%), palmitic (3.65-7.53%) and stearic (1.27-3.28%) acids. The oil content and fatty acid composition of oil among the genotypes were different indicating that synthesis of them is influenced by genotype. Genotypic effects were highly significant (Pr>F at 0.1% level) for seed biochemical traits (oil content and fatty acid composition).

Keywords: Study, oil content, fatty acid composition, different, genotypes, safflower, (*Carthamus tinctorius* L.)

Introduction
Carthamus tinctorius L. (Safflower) belongs to the family Asteraceae originated in the region spanning India, Afghanistan and Ethiopia. Safflower has been used traditionally as a medicinal herb and as a natural dye sources for colouring textile and foods. It is cultivated principally for the oil which is extracted from its seeds and has both food and industrial applications. The seed has an oil content of about 20-45 % (Cosge *et al*., 2007) [8]. Now-a-days, significant attention was generated in consumption and development of safflower seed oil as an excellent health care product (Han *et al*., 2009) [18]. Significant experimental works are required on the genotypes which have not been investigated previously to improve oil content and fatty acid composition in safflower seed. Therefore the objective of the present research was to study the oil content and fatty acid composition of safflower genotypes consisting of elite varieties and checks. These data facilitate for improvement of oil quality and quantity in new genotypes.

Materials and Methodology
Sixty one genotypes including elite germplasm lines and check varieties were used for the study. The pure seeds of all these genotypes were collected from ICRISAT farm of IIOR, Hyderabad. The field experiment was conducted at the research farms of ICAR-IIOR (at Rajendranagar and ICRISAT), Hyderabad during October to February, 2014-15. The experiment was laid out in Augmented Randomized Block Design (Augmented RCB) in three blocks with four checks. The laboratory work was carried out at Biochemistry laboratory of IIOR, Rajendranagar, and Hyderabad.

Estimation of oil content (%)
Estimation of oil content was done through non-destructive method as whole seed by using Nuclear Magnetic Resonance (NMR) analyser. The procedure is described as follows: 1. Sample is kept in the sample tube and weighed, 2. the sample tube is inserted into the instrument; measurement starts automatically. 3. After 30 s, result is reported, and the sample can be removed. NMR analysers detect the signal from receptive atomic nuclei when the sample is placed in a magnetic field. Only those atoms with appropriate nuclear characteristics are detected under the particular conditions used i.e., depending specifically on the magnetic field and radio frequency (RF) used.

Fatty acid profiles (%)
The fatty acid composition of the oil samples was determined by gas chromatography (AOCS Ce 1–62; AOCS 1993) method.

1. The oil sample of each experimental unit (plot) was converted to its Fatty Acid Methyl Esters (FAME). Oil samples (0.2 mL) were dissolved in hexane and transesterified with sodium methylate (0.1 M).
2. Analyses of FAMEs were carried out using an Agilent 6890 model gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a with a split-injection port, flame ionizing detector (FID) and a fused silica capillary column (HP-88, 100 m × 0.25 mm, film thickness = 0.2 µm).
3. The samples (1.0 µL) were injected in split mode (split ratio 1:50). The initial oven temperature was set at 150 °C for 1 min, elevated at a rate of 5 °C $min^{-1}$to 190 °C for 2 min, and then ramped at 5 °C min^{-1} to the final 240 °C for 8 min.
4. The injector temperature was set at 250 °C and the detector temperature was set at 280 °C. Nitrogen with at a flow rate of 1.5 mL min^{-1} was used as the carrier gas.
5. Peak identification was performed by comparing the relative retention times with those of a commercial standard mixture of FAME. The fatty acid content of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) were determined using a computing integrator and showed as the percentage of the oil.
6. The fatty acid content of myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (18:3) were determined. Only the trace amount of myristic and linolenic acids were detected and hence were not further considered.
7. The relative percentage of each fatty acid was determined by integration of each peak in the chromatogram.

Statistical analysis

The data were analyzed as per augmented RCB design as implemented in the software Plant Breeding Tools (PB Tools v 1.3) (IRRI, 2013) [19]. Least square means, range and frequency distribution of traits were obtained. Significance of genotypic effect on trait variation was tested using ANOVA estimates.

Results and discussion

Seed biochemical traits

In safflower, relationship between seed biochemical traits between oil content has been analysed to some extent at oil content, fatty acid content and protein content levels. However, deeper understanding on the biochemical mechanisms contributing for high seed oil content in different genotypes would provide more insights on the available germplasm diversity for breeding high oil content. Mean and range values of seed biochemical traits namely oil content and fatty acid components are presented in Table 1.

Oil content

The genotypes set used in this study had an excellent range of variation for oil content (24% to 43%) with the mean of 33% (Table 1). Among checks, the variety Centennial recorded the highest of 41% followed by NARI-57 (35%) and Bhima (30%). Whereas the variety A1 had the lowest oil content (26%). In the present study, the germplasm lines EC-5, EC-6, EC-7, EC-11, EC-20, EC-21 and EC-32 recorded about 40%, which provide good sources for breeding high oil cultivars for Indian conditions. The seed oil content of up to 46% has been reported in safflower germplasm (Johnsson *et al*., 1999, Mukta, 2008) [25].

Genetic variability for oil content in the germplasm collection has been reported across oilseed crops: 13 to 46% in safflower (Johnson *et al*., 1999; Ushakiran *et al*., 2015) [20, 32], 41 to 63% in sesame (Uzun *et al*., 2008; Spandana *et al*., 2013) [33, 30] 27 to 56% in niger (Geleta *et al*., 2011), 21 to 25% in soybean (Aldalin *et al*., 2012) [2], 35 to 43% in rapeseed and mustard (Chauhan *et al*., 2010) [7], 34 to 42% in linseed (Pali and Mehta, 2014), about 40% in sunflower (Mandal *et al*., 2006) [and 40 to 51% in groundnut (Ajay *et al*., 2008) [1]. These studies clearly indicate that the oil content in safflower is comparable to major oilseed crops in India. However, the released safflower cultivars of India possess low oil content (28 to 30%). There is a scope for improving of about 5 to 8% oil content in Indian safflower cultivars (Yadav *et al*., 2012) [38]. Therefore, efforts are underway in this direction by exploiting high oil exotic germplasm sources having about 40% oil content under Indian conditions (Kadirvel *et al*., 2013) [21].

The seed oil content in oilseed crops is affected by the environmental factors. The check variety Centennial recorded the highest oil content (41%) in this study. It has recorded an average of about 40% oil content in a series of field trials over four years in US (Bergman *et al*. 2007) [5] whereas it showed only 29% in a location in Turkey (Arslan 2007) [4]. This observation suggests the role of genotype x environment interaction in determining seed oil content. Therefore, high oil safflower genotypes found in this study are needed to be thoroughly tested across different locations in India before their use in breeding programmes. However, it is encouraging to note that the variety Centennial has maintained high oil content in Indian conditions, which can be immediately used in Indian safflower breeding programmes.

Fatty acid composition

The fatty acid profiles found among genotypes were linoleic acid (14% to 79%), oleic acid (12% to 80%), palmitic acid (4% to 8%) and stearic acid (1% to 3%). The checks had highest linoleic acid (72%-77%) followed by oleic acid (14%-20%), palmitic acid (6%-7%) and Stearic acid (~3%) (Table 1).

Studies have clearly established that linoleic and oleic acid are the major fatty acids, followed by palmitic and stearic acids in the safflower oil (Fernandez-Martinez *et al*., 1993 and Johnsson *et al*., 1999) [11]. Johnsson *et al*. (1999) analyzed a large collection of safflower germplasm of United States Department of Agriculture (USDA) and found that variability for fatty acid profiles were in the range of 11%-83% (linoleic acid), 6% to 82% (oleic acid), 4% to 7% (palmitic acid) and 1% to 5% (stearic acid). The results of the present study correspond well with these findings.

Safflower is one of the best examples of crops with variability for fatty acid composition in seed oil (Bergman, 1997) [5]. Safflower oil contains about 6 to 8% palmitic acid (saturated fatty acid), 2 to 30% stearic acid (saturated fatty acid), 16 to 20% oleic acid (monounsaturated fatty acid) and 71 to 75% linoleic acid (polyunsaturated fatty acid) (Velasco and Fernandez, 2004) [34, 35]. Futehally and Knowles (1981) [12] found safflower genotypes with very high levels of linoleic acid (87-89%) and very low oleic (3-7%). During seed development stage, the linoleic acid predominates every lipid class but the oleic acid decreases with increasing maturation and it becomes almost absent in the fully matured seeds (Nagaraj, 1993) [26]. Furthermore, the fatty acid composition

of safflower oil contains a healthy mixture of all the types of saturated and unsaturated fatty acid. The value of P/S index which is associated to the impact in the human health is also high for safflower (10.55) which makes it one of the most suitable edible oils for mass consumption (Kostik *et al*., 2012) [22]. Interestingly, genotypes having more than 85% oleic levels were also reported in safflower germplasm (Fernandez *et al*., 1993) [11]. This contrasting level of mono-and poly-unsaturated fatty acid profiles makes the safflower oil more attractive for the best commercial uses. High linoleic oil is considered premium quality due to its reported role in reducing blood cholesterol levels (Bergman, 1997) [5]. High oleic oil is more stable because it is less susceptible to oxidative changes during refining, storage and frying hence, it is more preferred by the commercial food industry. High oleic acid is also reported to play a role in reducing blood pressure level (Teres *et al*., 2008). The high oleic edible oils are also available from different plant sources: sunflower (~80%) (Nagarathna *et al*., 2011), soybean (~85%) (Pham *et al*. 2011), groundnut (~80%) (Anderson *et al*. 1998), Ethiopian-mustard (70-80%) (Velasco *et al*., 2003) and olive (70-80%) (Teres *et al*., 2008), which indicate that safflower has comparable levels of oleic acid content with the popular sources. The high level of oleic acid content coupled with very low level of saturated fatty acid content makes safflower is an alternative for high oleic vegetable oil purposes. Furthermore, the high oleic safflower oil is free from linolenic acid, which readily oxidises and imparts rancidity during storage and frying.

Till date, high oleic safflower cultivar has not yet been released in India. In this study, 19 exotic genotypes had high oleic content ranging from 70 to 80%. These genotypes could be the potential sources for breeding high safflower cultivars in India.

Table 1: Mean performance of seed biochemical traits in a set of 57 safflower genotypes compared with four check varieties

Genotype	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Oil Content
Checks					
A1	5.57	2.61	18.79	73.00	25.68
Bhima	5.66	2.48	20.29	71.55	30.17
Centennial	6.75	2.31	14.41	76.51	40.97
NARI-57	7.27	2.63	14.85	75.25	35.16
Genotypes					
HUS-305	6.09	2.31	17.10	74.50	31.05
CO1	6.47	2.61	12.27	78.66	34.19
EC-1	5.66	2.63	24.68	67.03	32.24
EC-2	6.16	2.67	18.07	73.10	28.13
EC-3	6.04	2.60	29.91	61.45	30.00
EC-4	5.48	2.27	16.99	75.26	28.18
EC-5	4.82	2.00	76.72	16.46	40.98
EC-6	6.98	2.73	19.17	71.13	42.90
EC-7	6.90	2.37	16.05	75.50	41.24
EC-8	5.92	2.60	16.69	74.80	39.31
EC-9	3.65	1.27	73.79	21.30	33.13
EC-10	4.47	1.48	78.84	15.21	38.53
EC-11	4.56	1.67	76.77	17.00	41.95
EC-12	6.40	3.28	17.14	73.17	30.38
EC-13	5.08	2.13	75.78	17.01	31.88
EC-14	5.17	2.49	76.33	16.01	35.57
EC-15	6.63	2.71	15.67	74.99	36.62
EC-16	4.93	1.77	79.46	13.83	35.10
EC-17	4.55	1.65	77.04	16.76	34.47
EC-18	5.20	2.60	75.91	16.37	36.68
EC-19	5.93	2.44	14.93	76.78	33.60
EC-20	4.38	2.36	78.16	15.17	40.37
EC-21	4.75	2.31	72.53	20.50	40.12
EC-22	4.91	2.60	75.76	16.82	35.63
EC-23	4.54	1.63	80.08	13.83	33.88
EC-24	4.75	1.82	76.26	17.25	39.01
EC-25	4.86	1.86	75.99	17.37	37.68
EC-26	4.71	1.63	75.62	18.12	36.08
EC-27	5.29	1.67	71.48	21.64	27.79
EC-28	6.17	2.67	20.98	70.27	30.50
EC-29	4.82	2.48	74.23	18.55	36.37
EC-30	5.55	1.87	53.58	39.08	36.05
EC-31	5.97	2.71	39.64	51.76	29.58
EC-32	4.58	1.69	75.50	18.31	40.26
EC-33	6.67	2.96	22.12	68.32	37.06
EC-34	6.15	2.45	14.60	76.89	39.37
EC-35	6.20	2.76	28.56	62.56	29.76
EC-36	6.39	2.38	14.81	76.51	39.47
GMU-1	5.53	2.72	24.07	67.60	32.00
GMU-2	5.47	2.35	16.13	75.96	29.02
GMU-3	5.25	2.32	17.81	74.53	30.02

GMU-4	5.18	2.57	17.92	74.23	29.87
GMU-5	6.23	2.40	17.76	73.52	31.86
GMU-6	5.46	2.28	16.52	75.64	28.50
GMU-7	5.55	2.24	14.24	77.89	29.00
GMU-8	5.58	2.52	15.50	76.31	26.77
GMU-9	5.39	2.09	23.23	69.20	27.51
GMU-10	5.74	2.71	17.04	74.43	28.74
GMU-11	5.77	2.62	23.76	67.75	28.44
GMU-12	5.54	2.95	20.19	71.23	28.16
GMU-13	5.86	2.11	14.49	77.45	31.67
GMU-14	6.55	2.31	17.83	73.22	27.47
GMU-15	5.76	2.12	16.54	75.49	23.88
GMU-16	5.72	3.67	16.80	73.72	36.04
GMU-17	6.43	2.58	15.05	75.85	25.37
GMU-18	5.31	2.06	15.16	77.38	28.96
GMU-19	6.09	2.48	13.98	77.37	29.65
Mean	5.68	2.37	34.76	57.20	33.25
Range	3.65-7.53	1.27-3.28	12.27-80.08	13.83-78.66	23.88-42.90
SD	0.79	0.42	26.38	25.51	5.02

Frequency distribution

The oil content showed continuous distribution in the present study suggesting quantitative nature of trait variation among genotypes. Similarly, quantitative differences were found for saturated fatty acids, palmitic acid and stearic acid. However, distributions of linoleic acid and oleic acid were discrete and two clear cut groups (low and high) were emerged suggesting qualitative nature of variation.

Yermanos *et al*. (1967) [39] reported that variation for oil content in safflower was quantitative, which resulted from complex inheritance involving many genes. In contrast, variation for the major fatty acids, linoleic acid and oleic acid, was qualitative, which resulted from simple inheritance with the involvement of a single locus, which was recessively inherited. Genetic basis of oil content in safflower has not yet been elucidated. Experience in other oilseed crops suggests that the seed oil content is genetically complex and involves many quantitative trait loci (Delourne *et al*., 2006). However, qualitative nature of inheritance of major fatty acids, oleic and linoleic content in safflower has been fairly explained. Hamdan *et al*. (2009) [17] reported that genotype with >75% oleic acid had the involvement of a single recessive gene (*ol*) whereas the genotype with >85% oleic acid had the involvement of two loci such as the major gene (*ol*) and a modifier gene. Presence of dominant gene, *Ol* leads to high linoleic acid content in safflower. In contrast, Miller *et al*. (1986) [24] reported that two alleles, dominant allele *Ol* and a recessive allele *ml* were required to obtain high oleic acid content (>82%) in sunflower. The distribution of the alleles, *Ol* or *ol* of a single gene and the modifiers across genotypes contributes for variations in the linoleic or oleic acid content in germplasm collection.

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