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High yielding-high oleic non-genetically modified Indian safflower cultivars

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ABSTRACT

High oleic safflower (*Carthamus tinctorius* L.) oil is desired by industry because of its high oxidative stability for broader use in food, fuel, and other products. However, standard safflower oil has only 16–20% oleic acid, and Indian safflower cultivars are non-oleic type. The present investigation was taken up to enhance oleic acid level in Indian safflower. Three non-genetically modified high-oleic lines, ISF-1, ISF-2 and ISF-3 were developed from a cross between low and high oleic genotypes through classical breeding approach. These were tested at 10 locations in India along with two non-oleic high yielding check varieties, A1 and Nari-6 under irrigated and dry growing conditions. ISF-1, ISF-2 and ISF-3 consistently possessed high oleic acid content across locations. The mean oleic acid content in these varieties was 75, 76 and 75%, respectively whereas it was 17 and 14% in non-oleic checks. Oleic acid level in ISF-1, ISF-2 and ISF-3 was relatively low under dry growing conditions (72, 73, 73%) than under irrigated (77, 78, 76%). On an average ISF-1 and ISF-2 gave 15 and 9% higher seed yield and 23 and 27% higher oil yield, respectively than the best check, A1. Oleic acid content was not affected when tested at three dates of sowing with one month interval; however, considerable reduction in seed yield was observed as sowing was delayed. ISF-1 and ISF-2 were licensed to Marico Pvt. Ltd., Mumbai, India for large scale production. These are the first oleic safflower cultivars developed for growing under Indian conditions.

1. Introduction

Safflower (*Carthamus tinctorius* L.) is predominantly an annual herb belonging to family Asteraceae. It is grown as a winter-spring oilseed crop under rainfed and minimal irrigation conditions in wide range of ecological environments including cool and warm climates. Demand for safflower (*C. tinctorius* L.) is increasing world over because of its multi-purpose uses in pharmaceutical, biofuel, cosmetic, food and textile industry, *etc.* (Hiramatsu et al., 2009; Asgarpanah and Kazemivash, 2013; Bae et al., 2002; Meka et al., 2007). Kazakhstan is the largest safflower growing and producing country in the world with 3,10,000 ha area and 1,96,000 tones seed production in 2014; India is the second largest safflower growing country with 1,40,000 ha area and 1,13,000 tones production. Mexico, USA, Argentina, China, Russian Federation, Turkey, Iran, Kyrgyzstan, Tanzania, Australia and Uzbekistan are the other countries growing safflower (www.fao.org/faostat/en/#data/QC). In India, safflower is grown as a winter season crop mainly for its edible seed oil.

Market value of safflower can be improved if cultivars with diversified oil quality are available. Diversification of oil quality is mainly associated with changes in proportion of fatty acids. Safflower is one of the crops with great variation in fatty acid composition. The standard safflower oil has fatty acid composition made up of 6–8% palmitic acid, 2–3% stearic acid, 16–20% oleic acid and 71–75% linoleic acid (Gecgel et al., 2007). High variation ranging from 3.1 to 90.6% in oleic acid content and 3.9 to 88.8% in linoleic acid content was reported in the world safflower germplasm collection (Knowles, 1965; Fernandez-Martinez et al., 1993). Linoleic type safflower oil contains 70–75% polyunsaturated linoleic acid whereas oleic type safflower oil contains 75 to 80% monounsaturated oleic acid, which is similar to olive oil. Linoleic acid is the essential fatty acid, and its high level in safflower oil is associated with reduction of cholesterol level in human blood (Herbel et al., 1998). But linoleic safflower oil polymerizes readily when heated and is less suitable for deep frying. High oleic safflower oil has high oxidative stability which makes it suitable for deep and prolonged frying (Fuller et al., 1967). High single point unsaturation makes oleic safflower oil suitable for a series of chemical reactions. Hence, its demand is increasing in the oleochemical industry to convert into a wide range of chemical products for use in paints, inks, lubricants, biofuels, cosmetics, detergents, bio-based plastics, textile, leather, soaps and detergents, foams, adhesives pharmaceuticals and many other industries (Gunstone, 2001). Safflower germplasm accessions possessing high oleic acid content were successfully incorporated into cultivated varieties (Bergman et al., 2006; Mundel and Bergman, 2009) in USA. An EMS induced safflower mutant containing up to 90% oleic acid was reported by Weiske (1999). In

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India, an EMS induced high oleic safflower mutant has been reported recently (Rampure et al., 2015) but it is yet to be stabilized to attain a breeding line status. A genetically modified super high oleic safflower containing 90% oleic acid was developed through gene silencing approach (Wood et al., 2015). High oleic safflower cultivars have not yet been developed in India though India is one of the key producers of safflower. Rising health consciousness among Indian consumers is one of the key factors for increasing preference for high oleic oil. India has big potential in oleochemicals, as it is the main market for oleic safflower oil for the food industry. Domestic production of high oleic safflower is needed to meet its high oleic oil demand. High oleic safflower cultivars would increase commercial value of Indian safflower oil.

Genetically modified high oleic cultivars may not find approval for commercial cultivation in India as India is yet to approve commercial cultivation of genetically modified food crops because of health security of the consumer, biosecurity of agriculture and health, environment protection and the security of national and international trade in farm commodities. The high oleic varieties developed outside India may or may not perform well in India because of varied ecosystems in India. Therefore, the present investigation was undertaken with the objectives: (i) enhancing oleic acid content in Indian safflower through cost effective, environment friendly classical breeding approach, (ii) testing the stability of indigenously developed high oleic safflower lines under dry and irrigated conditions at multilocations, as well at different dates of sowing, and (iii) collaboration of an industry for high seed production and spread of high oleic safflower cultivars in India, and their marketing and creating value through supply chain.

2. Material and methods

At the Indian Council of Agriculture Research-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, India, a cross was made between a high oleic parent, Ole-9-p5-p7 and a non-oleic commercial variety, A1. Oleic acid content in Ole-9-p5-p7 and A1 was 74 and 17%, respectively. A1 is a non-oleic high yielding commercial variety developed in India. Ole-9-p5-p7 was developed in India from the cross (EC-523367-9 × EC-548816-14); EC-523367-9 was a high oleic (61%) selection from an USDA introduction, PI 537695, which segregated for oleic acid content (40–61%). EC-548816-14 was a selection from a low oleic (12% oleic acid) introduction, PI 304474 from Iran.

F_1 of (Ole-9-p5-p7 × A1) cross was advanced up to F_4 in single-plant progeny rows. In F_2 to F_4 generations, seed collected from each progeny in different progeny-rows were assayed for fatty acid composition and those having 70% and above oleic acid level were advanced to F_5 and then in F_5 generation seed from each progeny in a progeny-row having more than 70% oleic acid were bulked to construct F_5 families; these families were advanced to F_7. Finally 95 high oleic F_7 lines were generated; of these high oleic lines, 18 high oleic-high oil lines were evaluated along with check, A1 in a non-replicated trial at Hyderabad during 2014–15 and 2015–16, and three high oleic lines, *viz.*, ISF-1, ISF-2 and ISF-3 were further tested at multilocations and different dates of sowing for understanding their seed yield performance and stability for high oleic acid levels.

2.1. Evaluation of high oleic lines at multilocations

Three high oleic lines, ISF-1, ISF-2 and ISF-3 along with two non-oleic high yielding commercial varieties, A1 and Nari-6, and 10 pre-released test varieties were evaluated at 10 locations during 2015–16 under dry (no irrigation) and irrigated (1–2 irrigations) conditions. Experiment under dry conditions was conducted at four centres namely, Latur (18.4088°N, 76.5604°E), Raichur (16.2120°N, 77.3439°E), Solapur (17.6599°N, 75.9064°E) and Tandur (17.2576°N, 77.5875°E) whereas the experiment under irrigated conditions was taken up at six centres *viz.*, Hyderabad (17.3850°N, 78.4867°E), Nandyal (15.4786°N, 78.4831°E), Indore (22.7196°N, 75.8577°E), Raipur (21.2514°N, 81.6296°E), Sagar (23.8388°N, 78.7378°E) and Sehore (23.2050°N, 77.0851°E). Latur, Raichur, Solapur, Tandur, Hyderbad and Nandyal centres are warmer locations and Indore, Raipur, Sagar and Sehore centres are cooler locations.

Safflower crop period in warmer locations was September-March while it was November–April in cooler locations. In 2015–16, the maximum temperature recorded during crop period was 31–39 °C at warmer locations and 30–34 °C at cooler locations while the minimum temperature was 11–25 °C and 9–20 °C at warmer and cooler locations, respectively. The relative humidity during crop period in 2015–16 was 51–98% at warmer and 69–98% at cooler locations. The total rainfall received across the locations during crop period in 2015–16 was 1–91 mm. The experiment was conducted in a randomized block design (RBD) with three replications, 45 cm × 20 cm spacing between rows and between plants, and 13.5 sq.m plot size per line per replication. The experiment was sown between last week of September and 2nd week of October, 2015 in warmer locations and between 2nd and 3rd week of November, 2015 in cooler locations. The recommended doses of fertilizers (40N:40P_2O_5:20K_2O kg/ha) were applied to experimental plots; fifty percent of recommended dose of N and full dose of P_2O_5 and K_2O were applied at the time of sowing and the remaining 50% of N was applied 45 days after sowing. Dimethoate 30% E.C. was sprayed at 2 ml/l to protect the crop from safflower aphid (*Uroleucon compositae* T.) damage during peak infestation period, which was between 3rd week of December to first week of January.

Data on seed yield (kg/ha), oil content (%), oleic acid content (%), plant height (cm), days to 50% flowering, days to maturity, 100-seed weight (g), number of effective capitula/plant and number of seeds/capitulum were recorded. The data collected from ISF-1, ISF-2, ISF-3, A1 and Nari-6 was reported, and that of 10 non-oleic pre-released test varieties was not presented.

2.1.1. Evaluation of high oleic lines at different dates of sowing

Three high oleic lines, ISF-1, ISF-2 and ISF-3 were planted along with a non-oleic variety check, A1 at three dates of sowing with one month interval starting from 2nd week of October to 2nd week of December. The experiment was conducted for two years (2014 and 2015) at Hyderabad in a non-replicated trial with a plot size of 56.25 sq.m/line and 45 cm × 20 cm spacing between rows and between plants. The weather data at Hyderabad during the crop period for different dates of sowing in 2014 and 2015 were given in Table 1. The data on seed yield (kg/ha), oil content (%) and oleic acid content (%) were recorded. One irrigation with sprinklers was given after sowing to the experiment in 2014 and 2015.

2.1.2. Evaluation of high oleic lines in large size plots

Three high oleic lines *viz.*, ISF-1, ISF-2 and ISF-3 were evaluated in large size plots (4000 sq.m/line) in a non-replicated trial for two consecutive years (2014–15 and 2015–16) at the research farm of ICAR-IIOR, Hyderabad and the same were evaluated for one year (2015–16) at Agriculture Research Station (ARS), Professor Jayasankar Telangana State Agriculture University, Tandur. The spacing given was 45 cm between rows and 20 cm between plants. The non-oleic high yielding check variety, A1 was included in the experiment conducted at Hyderabad whereas the non-oleic check variety, Manjira was included at Tandur. Sowing was done during 2nd week of October, and one irrigation with sprinklers was given after sowing to the experiment at Hyderabad in both the years and no irrigation was given at Tandur. The soil at both locations was deep black soil. Data on seed yield (kg/ha), oleic acid content (%) and oil content (%) were recorded at both locations.

2.2. Analysis of fatty acid composition

Oil from seed was extracted in hexane on a soxhlet apparatus

Table 1
Weather data during safflower crop period at Hyderabad in 2014–15 and 2015–16.

Sowing week	Maximum temperature (°C)		Minimum temperature (°C)		Total rainfall (mm)		Relative humidity (%)	
	2014–15	2015–16	2014–15	2015–16	2014–15	2015–16	2014–15	2015–16
2nd week October[a]	20.8–34.2	29.8–34.2	5.2–23.8	9.6–19.7	107.2	12.2	78–94	78–92
2nd week November[b]	25.0–35.4	29.8–36.3	5.2–21.5	9.6–20.9	111.0	2.7	58–98	69–92
2nd week December[c]	25.6–38.2	29.8–39.5	5.2–24.4	9.6–24.1	153.4	4.6	50–98	58–92

Crop period:
[a] 2nd week October–2nd week February.
[b] 2nd week November–2nd week March.
[c] 2nd week December–2nd week April.

(Extraction unit, E-816, Buchi). Methyl esters were obtained by a two-step catalytic process according to the method of Ghadge and Raheman (2005). Extracted oil (100–150 mg) was treated with 2% sulphuric acid in methanol (5 ml) for 2 h at 60 °C. After the reaction, the mixture was allowed to settle for an hour and methanol–water mixture separated at the top was removed. In second step, product at the bottom was trans-esterified using 2 ml of 13% methanolic KOH for 30 min at 55 °C. The organic phase was extracted with hexane and washed with water till neutral pH. The hexane was dried over anhydrous sodium sulphate and concentrated with nitrogen to get methyl esters.

Fatty acid composition was determined using an Agilent 7860A gas chromatograph (GC) equipped with a flame ionization detector (FID) and an auto sampler. Peak separation was performed on a DB-225 capillary column (diameter-250 µm, length-30 m, film thickness-0.25 µm) from Agilent Technologies. The carrier gas was Nitrogen set to a constant gas flow of 1.2 ml/min at 160 °C initial temperature. One microliter of sample was injected at a 20:1 split ratio into the column with the following temperature conditions: 160 °C for 2 min; raised from 150 to 220°C at 6 °C/min. Both inlet and detector were set to 230 °C. Fatty acid composition was determined by identifying and calculating relative peak area per cent by GC post run analysis EZChrom elite compact software. Oil was extracted from seed in hexane on a soxhlet apparatus (Extraction unit, E-816, Buchi). Oil content analysis was done as per the procedure given by Yadav and Murthy (2016).

2.3. Data analysis

Standard error of mean (SE m ±), critical difference (CD) at 5% probability and coefficient of variation (CV %) were analyzed using INDOSTAT statistical software, Indostat services, Hyderabad, India (http://www.indostat.org/agriculture.htm).

3. Results

3.1. Development of high oleic acid lines

Since the genotype of the seed determines the fatty acid composition in oil not the genotype of the plant producing it, the seed analyzed for fatty acid composition was one generation ahead of the generation of the plant producing it. The oil extracted from seeds, which were in F_1, F_3, F_4, F_5, F_6 and F_7 generations of the cross, (Ole-9-p5-p7 × A1), was assayed for fatty acid composition. The oleic parent, Ole-9-p5-p7 had 74% oleic acid content and the non-oleic parent, A1 contained 17% oleic acid content. Cross seed, which was in F_1 generation, had 38% oleic acid content. The seed collected from F_1 plants was not assayed for fatty acid profile because the seed was in F_2 generation and each seed was different in genetic constitution. The F_3 seed collected separately from 50 F_2 plants possessed 13.6–79% oleic acid content. Of the 50 F_2 plants, only nine were advanced to F_3. Two of these F_2 plants were of high oleic type (77.2 and 79% oleic acid), two were of low oleic type (13.6 and 14.7% oleic acid) and the remaining five F_2 plants were of medium oleic type (34.82–45.9% oleic acid).

The two low oleic type F_2 plants produced only low oleic type (10.1–28.2%) F_3 progenies, whereas the two high oleic type F_2 plants produced progenies possessing high oleic acid content (55.04–83.45%) in all filial generation from F_3 to F_7. The five medium oleic type F_2 plants have segregated into high, medium and low oleic type progenies. The high oleic type F_3 progenies of medium oleic type F_2 plants produced only high oleic type progenies (57.73–80.88%) in F_4–F_7 generations while medium type progenies of medium oleic type F_2 plants segregated into low, medium and high oleic types and the low oleic type progenies produced only low oleic type in every generation. The low oleic type progenies appeared in any generation were not selected. Finally, total 95 high oleic lines possessing 70.06–82.17% oleic acid content were developed from the two high oleic and five medium oleic type F_2 plants.

Eighteen high oleic-high oil lines evaluated for two years (2014–16) at Hyderabad have recorded 30–34% mean oil content, 80–82% mean oleic content, and 20–127% higher seed yield (902–1591 kg/ha) than non-oleic check variety, A1 (748 kg/ha). The oil and oleic contents in A1 were 25.84 and 17.01%, respectively.

3.2. Performance of high oleic lines at multilocations and different dates of sowing

In the multilocation evaluation trial conducted at 10 locations, oleic acid content of the three high oleic lines, ISF-1, ISF-2 and ISF-3 was much higher than that of non-oleic varieties, A1 and Nari-6 at all locations. Oleic acid content in high oleic lines has not varied when tested in warmer and cooler locations under irrigated conditions, however, it has reduced by about 3–5% in warmer locations under dry conditions (Table 4).

The seed and oil yields of high oleic lines under dry and irrigated conditions were given in Tables 5 and 6. Under irrigated conditions, ISF-1 and ISF-2 gave 22 (2069 kg/ha) and 23% (2092 kg/ha) higher seed yields, respectively, than A1 (1695 kg/ha). ISF-3 recorded seed yield close to A1. Oil content was slightly higher under irrigated conditions than under dry conditions. Oil content of high oleic lines was 28.45–29.6% while it was 26.85% in A1, and oil yields of ISF-1 and ISF-2 were 22 (577 kg/ha) and 27% (599 kg/ha) higher than A1 (473 kg/ha), respectively under irrigated conditions. Oil yield of ISF-3 (471 kg/ha) was at par with A1.

Seed yields of all lines including checks in multilocation trial were much lower under dry conditions than those realized under irrigated conditions. Under dry conditions, ISF-1 could record about 6% higher seed yield (910 kg/ha), though statistically not significant, than the best check variety, A1 (860 kg/ha) while ISF-2 gave slightly lower seed yield (845 kg/ha) and ISF-3 recorded much lower yield (705 kg/ha) than A1 (860 kg/ha). The mean oil content in the high oleic lines under dry conditions was 27.1–28% while it was 26.4% in A1. Oil yields of ISF-1 and ISF-2 were 12 (256 kg/ha) and 13% (258 kg/ha) higher than A1 (229 kg/ha) under dry conditions. Oil yields of ISF-3 were on par with A1 under both dry and irrigated conditions.

Over all the locations including irrigated and dry locations, ISF-1

Table 2
Seed yield, oleic acid content and oil content of oleic lines at different dates of sowing in 2014–15 and 2015–16 at Hyderabad, India.

Year	Entry	Seed yield (kg/ha)			Oleic acid content (%)			Oil content (%)		
		2nd week Oct	2nd week Nov	2nd week Dec	2nd week Oct	2nd week Nov	2nd week Dec	2nd week Oct	2nd week Nov	2nd week Dec
2014–15	ISF-1	2967	2643	1898	78.21	77.98	78.95	29.27	29.01	29.28
	ISF-2	2776	2598	1797	78.74	78.24	79.12	28.03	30.12	30.17
	ISF-3	1737	678	502	76.58	76.96	77.25	27.39	28.47	28.78
	A1 (check)	708	607	577	17	17.2	17.5	25.84	25.69	25.71
	SE m ±	522	571	378	15.2	15.1	15.2	0.7	0.9	0.9
2015–16	ISF-1	2852	2458	1563	78.37	78.98	78.93	29.11	29.25	28.98
	ISF-2	2646	2364	1498	77.94	78.02	78.92	29.00	29.19	30.07
	ISF-3	998	879	571	76.98	76.99	76.55	27.79	27.97	27.79
	A1 (check)	989	878	502	17	17.2	17.5	25.84	25.69	25.86
	SE m ±	509	442	287	15.1	15.2	15.1	0.75	0.83	0.9

Table 3
Seed yield and oleic acid content of high oleic lines under irrigated conditions at Hyderabad in 2014–15 and 2015–16 and under severe drought condition at Tandur in 2015–16.

Line	2014–15		2015–16			
	Seed yield (kg/ha)	Oleic acid content (%)	Seed yield (kg/ha)		Oleic acid content (%)	
	Hyderabad[a]		Tandur[b]	Hyderabad[a]	Tandur[b]	Hyderabad[a]
ISF-1	2896	78.21	721	2983	75.45	78.12
ISF-2	2798	79.11	613	2672	75.18	78.24
ISF-3	2067	76.85	502	1808	74.12	75.89
Check[c]	998	16.87	455	801	13.12	16.92
SE m ±	438	15.3	59	489	15.5	15.1

[a] One post-sowing irrigation with sprinklers.
[b] No irrigation.
[c] Manjira was a non-oleic check variety at Tandur and A1 was a non-oleic check variety at Hyderabad.

Table 5
Mean seed yield and oil content of high oleic lines under dry and irrigated conditions in multilocation Initial Varietal Trial-2015–16.

Line	Seed yield (kg/ha)			Oil content (%)		
	Dry[a]	Irrigated[b]	Overall mean[c]	Dry [a]	Irrigated[b]	Overall mean[c]
ISF-1	910 (6)	2069 (22)	1597 (15)	27.1	30.6	29.0
ISF-2	845	2092 (23)	1515 (9)	29.9	33.1	32.0
ISF-3	705	1513	1287	28.0	29.4	28.9
A1 (check)	860	1695	1391	26.4	26.8	27.1
NARI-6 (check)	608	1213	959	29.4	29.7	30.0
$CD_{0.05}$	118	207	–	1.0	1.2	–
CV (%)	17	14	–	2.1	2.0	–

[a] Mean of 3 locations.
[b] Mean of 6 locations.
[c] Mean of 9 locations; figures in parentheses indicate percent increase over the best check variety, A1.

and ISF-2 recorded 15 and 9% higher mean seed yields (1597 and 1515 kg/ha), respectively than the best check, A1 (1391 kg/ha) (Table 5). They also recorded 23 and 27% higher mean oil yields (470 and 485 kg/ha), respectively over A1 (382 kg/ha) (Table 6). The mean oil content in ISF-1, ISF-2, ISF-3 and A1 was 29.1, 32, 28.9 and 27.1%, respectively.

All the lines have reached to 50% flowering in 88–95 days and matured in 128–131 days after sowing. ISF-3 had comparatively short plant height (76 cm) than the other lines (79–86 cm). The 100-seed weight was higher in A1 (5.15 g) that that in other lines (3.62–4.74 g). There was no significant variation among test lines for number of effective capitula/plant while the number of seeds/capitulum has ranged from 24 to 31(Table 7).

Oleic acid and oil contents of ISF-1, ISF-2 and ISF-3 have not changed when planted at different dates of sowing though seed yield has reduced considerably when planted late (November–December) (Table 2). When grown in large size (4000 sq.m/line) plots, ISF-1, ISF-2 and ISF-3 exhibited high oleic acid levels in both test years at Hyderabad under irrigated conditions whereas oleic acid levels in these lines were relatively low when grown under dry conditions at Tandur (Table 3). ISF-1, ISF-2 and ISF-3 recorded much higher seed yields than the check varieties at both locations (Table 3). However, seed yields of

Table 4
Oleic acid content in high oleic lines and non-oleic varieties in warmer and cooler locations under dry and irrigated conditions in 2015–16.

Entry	Oleic acid content (%)													
	Warmer locations								Cooler locations					Overall mean
	Dry					Irrigated			Irrigated					
	Raichur	Annigeri	Tandur	Solapur	Mean	Nandyal	Hyderabad	Mean	Raipur	Indore	Sehore	Sagar	Mean	
ISF-1	70.07	73.38	71.7	71.46	72	76.16	78.28	77	75.8	79.2	75.6	77.6	77	75
ISF-2	74.22	75.13	70.31	74.22	73	76.5	78.81	78	78.3	78.7	77.64	78.62	78	76
ISF-3	76.86	71.48	70.01	74.02	73	74.91	76.28	76	75.0	80.4	79.2	77.12	76	75
A1 (C)	16.05	14.41	17.64	22.25	15	18.12	14.45	16	13.9	22.3	14.94	16.41	18	17
Nari-6 (C)	16.81	11.61	15.18	12.12	15	15.87	14.25	16	12.0	13.7	11.53	12.66	12	14
$CD_{0.05}$	0.60	0.53	0.74	0.60	–	0.51	0.68	–	0.61	0.71	0.56	0.71	–	–
CV (%)	1.1	1.0	1.4	1.1	–	0.9	1.2	–	1.1	1.2	1.0	1.3	–	–

C: non-oleic check variety.

Table 6
Mean oil yield of high oleic lines under dry and irrigated conditions.

Line	Oil yield (kg/ha)		
	Dry[a]	Irrigated[b]	Mean[c]
ISF-1	256 (12)	577 (22)	470 (23)
ISF-2	258 (13)	599(27)	485 (27)
ISF-3	204	471	382
A1 (check)	229	473	382
NARI-6 (check)	185	367	297
$CD_{0.05}$	25	33	–
CV (%)	17	14	–

[a] Mean of 3 locations.
[b] Mean of 6 locations.
[c] Mean of 9 locations; figures in parentheses indicate percent increase over the best check, A1.

all lines were very low at Tandur; this may be because of severe drought condition prevailed during crop period in 2015–16. The total rainfall during crop period in 2015–16 was 37 mm at Tandur and 12.2 mm at Hyderabad, and the relative humidity was 75–94% at Tandur and 77–92% at Hyderabad.

4. Discussion

Knowles and Hill (1964) reported that the levels of oleic and linoleic acid contents in safflower is controlled by three alleles (*OL, ol*^{1}*, ol*) of a single locus, and the combination of *olol* gives rise to high oleic content (64–83%), *OLOL* gives low oleic content (10–15%), *ol*^{1}*ol*^{1} confers medium oleic content (35–50%) and various combinations of these genes give intermediate levels. Knowles (1972) assumed that the existence of modifying genes intensify or diminish expressions of the major gene *ol* in safflower. Knowles (1989) also reported that oleic acid content of *olol* genotype was usually 71–75% of total fatty acids. Hamdan et al. (2012) showed the dominant role of the FAD2-1 gene and the involvement of at least one modifying gene with positive effect further increases oleic acid content in safflower. However, the complex role of modifying genes is still a matter of investigation. The *ol* allele was found to be a defective microsomal oleate desaturase *FAD2-1* with a single nucleotide deletion in the coding region that leads to premature termination of translation and subsequent nonsense-mediated mRNA decay of *FAD2-1*, a process that typically degrades transcripts containing a premature termination codon (Guan et al., 2012; Liu et al., 2013). Hamdan et al. (2012) described a SSR-based molecular marker for the *OL* locus.

In our study, it was observed in all generations from F_3 to F_5 (F_4–F_6 seed) that the plants having less than 30% oleic acid content produced only low oleic (< 30%) type progenies and those having 30 to 55% oleic acid produced low, medium and high oleic type progenies and those possessing 60% and above oleic acid content produced only high oleic acid type progenies (60.4–83.55%), however, the number of progenies possessing 60–69% oleic acid content were less than those containing 70% and above oleic content.

In our experiment, the high oleic type and low oleic type plants bred true while the intermediate type produced low, medium and high oleic type progenies though the number of low and high oleic type progenies were lower than that of medium oleic type progenies in all filial generations studied. Nonetheless data on oleic content in F_2 (seed generation) was not available, based on oleic level of F_1 and the segregation pattern of high, low and medium oleic type F_2 plants in different generations, we can suggest *olol* genotype for high oleic, *OLOL* for low oleic and *OLol* for medium oleic type plants. This also suggests recessive gene control of high oleic acid content in our material.

We were successful in producing high oleic type lines (70–82%) by substituting the gene *OLOL* with the recessive *olol* through simple crossing and intense selection for high oleic type progenies. Besides exhibiting high levels of oleic acid, ISF-1 and ISF-2 recorded increased seed and oil yields over the best non-oleic check variety, A1. So they would be competitive to low oleic high yielding varieties with additional advantage of high oleic acid levels when grown commercially in India. The present study denotes that simultaneous improvement of oleic content, oil content and seed yield was possible through simple classical breeding approach.

Differences in fatty acid composition due to year, location and genotype have been reported in safflower (Camas et al., 2007; Oz, 2016). Temperature and moisture were the major factors affecting linoleic acid and oleic acid contents. Knowles (1972) reported that commercially grown high linoleic and high oleic types were temperature stable. Canvin (1965) also reported that temperature had not affected fatty acid composition of oil and oil content of safflower. The three high oleic lines, ISF-1, ISF-2 and ISF-3 possessed high levels of oleic acid at all locations under varying environments indicating high stability of these lines for oleic acid content. Knowles (1989) reported that *OLOL* and *olol* were more stable with regard to temperature change. There was no difference in oleic acid content between cooler locations and warmer locations when irrigation was provided. However, there was 3–5% reduction in oleic acid content when high oleic lines were grown in warmer locations under dry conditions. Ashrafi and Razmjoo (2010) reported 14% reduction oleic acid content in safflower cultivars when grown under drought conditions. Reduction in oleic acid contents under drought stress was also reported in canola (Pritchard, 2007) and sunflower (Petcu et al., 2001). The high oleic lines, ISF-1, ISF-2 and ISF-3 have remained stable with regard to oleic acid and oil contents when planted at different dates of sowing which varied in maximum and minimum temperatures. High oleic oils are dominating the edible oil market in recent years. India has imported around 853,660 MT high oleic oil during April 2014 to May 2016, out of which, 37,171 MT of oil was high oleic safflower oil worth of US $ 94,358,838 imported from Mexico, Argentina, Australia and USA (www.eximpulse.com). The high oleic safflower cultivars developed under the present investigation would definitely improve production of high oleic safflower oil in India and may reduce to some extent the burden of import of oleic oil. The high oleic safflower lines, ISF-1 and ISF-2 have already been licensed for three years to Marico Pvt. Ltd., Mumbai, India through entering into a Memorandum of Understanding (MoU) between ICAR-IIOR and Marico Pvt., Ltd. for commercial

Table 7
Mean values of yield traits, plant height, and phenological traits.

Entry	100-seed weight (g)[a]	Number of effective capitula/plant[a]	Number of seeds/capitulum[a]	Plant height (cm)[a]	Days to 50% flowering[a]	Days to maturity[a]
ISF-1	4.74	24	31	87.3	90	131
ISF-2	4.25	25	29	86.6	89	128
ISF-3	4.35	27	27	76.7	91	131
A1 (check)	5.15	24	27	83.2	89	128
NARI-6 (check)	3.62	24	24	79	95	130
$CD_{0.05}$	0.31	NS	2.6	8.2	1.2	2.2
CV (%)	6.5	–	13	3.4	1.7	1.2

[a] Mean over all 9 locations including dry and irrigated locations.

production. Marico is India's leading consumer goods company and one of the key players of edible oils (NPCS, 2016). The large scale production of these cultivars during winter season of 2016 has already been initiated by Marico Pvt. Ltd. Once the production is increased, the marketing and creating value through supply chain would be taken up by Marico Pvt. Ltd.

Currently, GM food crops are not in cultivation in India as these are yet to be approved for commercial cultivation. The only genetically modified cash crop under commercial cultivation in India is cotton, which is not a food crop. The high oleic-high yielding non-GM safflower varieties need not to face the regulations and hurdles that were faced by genetically modified crops; and testing, seed production and supply of non-GM high oleic safflower cultivars would be easy and hassle free. ISF-1 and ISF-2 are more suitable to Indian growing conditions as they were indigenously developed.

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