Oil quality of exotic safflower (*Carthamus tinctorius* L.) cultivars in India

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ABSTRACT

Improvement of oil content and quality are important goals in safflower breeding. In this study, a set of 10 high oil exotic safflower cultivars were evaluated at Hyderabad (India) for oil content and fatty acid composition in order to assess their suitability for Indian safflower breeding programmes. Oil content in the exotic varieties ranged from 35.3 per cent (Finch) to 41.4 per cent (Centennial). Overall, the exotic varieties showed about 5 to 10 per cent increase in oil content over the most popular Indian high seed yielding varieties A-1 and Bhima. Furthermore, six varieties showed high oleic acid content ranging from 70.0 per cent to 80.2 per cent. The exotic varietal sources reported in this study would be helpful to expedite breeding high oil-high oleic safflower cultivars in India.

Keywords: *Carthamus tinctorius*, Fatty acid composition, Genetic improvement, Varieties

Safflower (*Carthamus tinctorius* L.) originated in the Fertile Crescent region over 4000 years ago and was domesticated in the Far East, India, Pakistan, the Middle East, Egypt, Sudan, Ethiopia and Europe (Chapman and Burke, 2007). It is a traditional crop of India, primarily grown for extraction of edible oil from seeds. It also has variety of other uses including poultry feed, extraction of natural dye (carthamin) from petals and preparation of industrial and pharmaceutical products. Standard safflower oil is a healthy oil with high amount of polyunsaturated fatty acid (PUFA), linoleic acid (>70%) and high ratio between polyunsaturated and saturated fatty acids among edible oils (Kostik *et al*., 2012). Safflower is also a good source of monounsaturated fatty acid (MUFA), oleic acid (>70%) (monounsaturated, MUFA) (Knowles, 1968), which is more stable and preferred for deep frying applications in the food industry.

Despite the economic importance, safflower cultivation is declining in India, from 10 lakh ha in 1988 to 2.11 lakh ha in 2014 (FAOSTAT, 2014), which is perhaps due to low productivity and profitability. India has a long history of breeding programme in safflower, which has resulted in the release of more than 30 cultivars. Oil content in the popular cultivars remains low (~30%), which is a concern for increasing profitability of safflower cultivation (Nimbkar, 2008). Therefore, improvement of oil content is an important goal of the Indian safflower breeding programmes. Availability of high oil sources is critical for this purpose. The United States Department of Agriculture (USDA) collection of safflower accessions, which includes released varieties has high level of variation for oil content (13% to 46%) and unsaturated fatty acids (oleic or linoleic) (~20% or ~80%) (Johnson *et al*., 1999). Safflower cultivars with high oil have also been reported from Mexico (41.9%) (Montoya Coronado, 2008), Australia (42%) (GRDC, 2010) and Argentina (43.4%) (Baümler *et al*., 2014). Among germplasm sources, exotic varieties with specific traits would be valuable for breeding efforts in different countries (Holland, 2004) because they can be deployed immediately in crossing programmes without much pre-breeding effort. Kadirvel *et al*. (2016) found that a subset of Mexican safflower varieties showed high oil content (~38%) and high oleic acid content (>70%) under Indian conditions, which have already been introduced in All India Coordinated Research Programme (AICRP) on safflower (IIOR, 2015). In this study, our aim was to evaluate a set of 10 exotic safflower varieties at Hyderabad (India) for oil content and quality in order to identify their potential for improvement of oil and oleic acid contents in Indian cultivars.

MATERIALS AND METHODS

Plant material: A set of 10 safflower cultivars (9 American and 1 Canadian) comprised of Centennial, Finch, Oker, Montola 2000, Oleic Leed, Lesaff, S-334, S-518, S-719 and CW-99 was used in this study. Seeds of Centennial, Finch, Oker, Montola-2000, Oleic Leed and Lesaf-496 were obtained from USDA and S-334, S-518, S-719 and CW-99 were obtained from Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP), Mexico. Indian popular varieties namely A-1, Bhima and NARI-57 were used as local checks. Lesaf-496 (PI 603208) is a germplasm line with high oleic acid and high oil contents, developed at the Agriculture and Agri-Food Canada Lethbridge (Alberta) Research Centre (Mündel and Braun, 1999). Details of the safflower varieties are provided in Table 1.

Field trial: Field evaluation of the cultivars was carried out in vertisol at a research farm of Indian Council of Agricultural Research (ICAR)-Indian Institute of Oilseeds Research, Hyderabad (India) during *rabi* season of 2013-14.

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Randomized complete block design (RCBD) with three replications was followed. The trial plot consisted of 5 rows of 5 m length with the spacing of 45 cm (between rows) x 20 cm (between plants). The field was irrigated once by sprinkler after seed sowing to facilitate germination.

Estimation of oil content: The oil content (%) was measured by Nuclear Magnetic Resonance (NMR) Spectroscopy using 30 g of pooled seed samples from each plot as described by Yadav and Murthy (2016).

Estimation of fatty acid composition: Oil from seed was extracted in hexane on soxhlet apparatus (Extraction unit, E-816, Buchi). Methyl esters were obtained by a two-step catalytic process according to slightly modified method of Ghadge and Raheman (2005). In the first step, the oil (100-150 mg) was treated with 2% sulphuric acid in methanol (5 ml) for 2 hours at 60ºC. After the reaction, the mixture was allowed to settle for an hour and methanol-water mixture that separated at the top was removed. In the second step, product at the bottom was transesterified using 2 ml of 13 per cent methanolic KOH for 30 minutes at 55ºC. The organic phase was extracted with hexane and washed with water till it reaches 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), a split injection port and an auto sampler. Peak separation was performed on a DB-225 fused silica capillary column (diameter-250 μm, length-30 meter, film thickness-0.25 μm) from Agilent Technologies. The samples (0.2 μl) were injected in split mode (split ratio 1:20). The initial oven temperature was set at 160ºC for 2 min, raised to 220ºC (at a rate of 6ºC/min) and held at 220ºC for 10 min. Both inlet and detector were set to 230ºC. The carrier gas was nitrogen set to a constant flow rate of 1.2 ml/min. Peak identification was performed by comparing the relative retention times with those of a commercial standard mixture of FAME (Supelco 37 Component FAME Mix). Fatty acid composition [palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2)] was determined by calculating relative peak areas percent by GC post run analysis EZChrom elite compact software.

Data analysis: Analysis of variance (ANOVA) and mean comparison (LSD at 5% level of significance) of the data were carried out using a statistical analysis package Plant Breeding Tools (PBTools) v 1.3 (IRRI, 2013).

RESULTS AND DISCUSSION

Oil content in the exotic varieties ranged from 35.3 per cent (Finch) to 41.4 per cent (Centennial) whereas Indian check varieties A-1, Bhima and NARI-57 recorded 26.3 per cent, 31.1 per cent and 37.1 per cent, respectively. The most popular Indian variety A-1 recorded the least oil content while Centennial recorded highest oil content. NARI-57, a recently released Indian variety had oil content similar with the exotic varieties. Centennial (77.1%), Finch (75.3%), Oker (76.4%) and S-719 (71.8%) recorded high linoleic acid content. High oleic acid content was recorded in Montola-2000 (80.2%), Oleic Leed (78.6%), Lesaff-496 (70.8%), S-334 (69.6%), S-518 (76.7%) and CW-99 (76.5%). In the Indian check varieties high linoleic content ranging from 72.4 per cent to 74.7 per cent was recorded. Overall, the exotic varieties exhibited 5 per cent to 10 per cent increase in oil content over the most popular Indian high yielding varieties A-1 and Bhima. The results show that exotic varieties could be potential donors for improvement of oil content and oleic acid content of the Indian safflower cultivars.

Comparison of the oil content and fatty acid composition of the varieties, as observed under exotic conditions (Table 1) with the current data collected at Hyderabad (Table 2) indicates reduction in percentage of oil content in most of the varieties (except Finch, Oker and Montola-2000) at Hyderabad. Highest reduction was observed in S-334 (-6.5%) and the lowest reduction was observed in S-518 (-2.1%). The seed oil content is a typical quantitative trait, which is controlled by polygenes (Yermanos *et al*., 1967; Fernandez Martinez *et al*., 1986). Therefore, low heritability and influence of genotype x environment (G x E) interaction are expected. In an evaluation trial in Albania, Montola-2000 recorded 33.4 per cent (by Soxhlet method) (Vorpsi *et al*., 2010). In Turkey, Montola-2000 recorded 35.2 per cent whereas Centennial recorded only 29 per cent (Arslan, 2007). In another experiment under highland conditions in Turkey, Montola-2000 (20.76%), Centennial (27.45%) and Oleic Leed (27.59%) recorded low oil content under non-irrigated conditions (Öztürk *et al*., 2008). Interestingly, these varieties showed higher oil content at Hyderabad. These observations suggest the influence of G x E interaction effects on oil content in safflower in diverse environments. Nevertheless, the oil content of the exotic varieties was substantially higher (5%-10%) than the most popular Indian check varieties A-1 and Bhima at Hyderabad, which is encouraging for their utilization in Indian breeding programmes. Genotypes that show minimal G x E interaction for the target trait would be more desirable for breeding programmes.

Unlike oil content, the content of major fatty acids (oleic or linoleic) of most of the test varieties (except S-334) at Hyderabad was comparable with the data from other countries. These observations suggest that oleic or linoleic content of safflower are fairly stable across environments. This is expected because oleic or linoleic content in safflower is qualitatively inherited and highly heritable (Golkar *et al.,* 2011). High stability of oleic or linoleic acid content could possibly be due to high temperature stability of the fatty acid desaturase (FAD2) enzyme in safflower, which is primarily responsible for oleate desaturation in oilseeds (Esteban *et al*., 2004). High oleic acid content in safflower is controlled by a mutation in this gene (ol locus, FAD2-1) (Knowles and Mutwakill, 1963; Hamdan *et al*., 2012), which is recessively inherited. However, range of high oleic acid content (70%-80.2%) indicate the role of modifier genes as postulated by Knowles (1972). Maximum of 87 per cent of oleic acid content has also been reported in safflower lines (Hamdan *et al*., 2009). Due to simple inheritance and larger allelic effect of ol locus, improvement of high oleic acid content in safflower cultivars has been highly successful by conventional breeding efforts (Knowles, 1968).

Table 1 Details of exotic cultivars of safflower used in the study

Cultivar	Seed source	Accession Identity Number	Oil content (%)	Fatty acid composition			Reference
				Oleic acid (%)	Linoleic acid (%)	Palmitic + Stearic (%)	
Centennial	USDA	EC 736516 (PI 538779)	44.1	10.8	79.6	7.7	Bergman et al. (2001)
Finch	USDA	EC 736519 (PI 525458)	37.0	11.0	78.2	8.7	Bergman et al. (1989)
Oker	USDA	EC 736521 (PI 601166)	39.0	-	-	-	Bergman et al. (1985)
Montola-2000	USDA	EC 736515 (PI 538025)	38.3	80.8	12.3	5.6	Bergman et al. (2000); Armah-Agyeman et al. (2002)
Oleic Leed	USDA	EC 736514 (PI 560177)	39.0	76.1	16	5.5	Urie et al. (1979); Fernandez Martinez et al. (1986)
Lesaff-496	USDA	EC 736517 (PI 603208)	42.0	75.0	18.7	6.3	Mündel and Braun (1999)
S-334	INIFAP, Mexico	EC 755660	42.6	81.0	-	-	Silveira Gramont et al. (2009)
S-518	INIFAP, Mexico	EC 755662	40.8	77.0	12.0	-	Montoya Coronado et al. (2008)
S-719	INIFAP, Mexico	EC 755684	40.0	-	-	-	Muñoz-Valenzuela et al. (2007)
CW-99	INIFAP, Mexico	EC 755664	41.0	76.0	-	-	Montoya Coronado (2010)
A-1 (Check)	India	-	26.5	15.6	76.4	8.0	Nagaraj (2001); Kadirvel et al. (2016)
Bhima (Check)	India	-	30.0	16.2	75.5	8.3	DOR (2006); Kadirvel et al. (2016)
NARI-57 (Check)	India	-	37.6	13.4	75.9	10.7	Kadirvel et al. (2016)

Table 2 Oil content and fatty acid composition of exotic safflower cultivars in an evaluation trial at Hyderabad, India

Cultivar	Oil content (%)	Fatty acid composition			
		Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)
Centennial	41.4	7.1	2.3	13.6	77.1
Finch	35.3	6.4	4.1	14.2	75.3
Oker	38.8	7.0	2.2	14.4	76.4
Montola-2000	39.1	4.8	2.0	80.2	13.1
Oleic Leed	36.1	3.6	1.3	78.6	16.5
Lesaff-496	38.4	5.3	2.1	70.8	21. 9
S-334	36.1	5.8	2.0	70.0	22.8
S-518	38.7	4.8	2.4	76.7	16.2
S-719	37.7	7.0	2.7	18.5	71.8
CW-99	37.3	5.0	2.0	76.5	16.5
A-1 (Check)	26.3	6.0	2.6	17.3	74.2
Bhima (Check)	31.1	5.7	2.7	17.1	74.7
NARI-57 (Check)	37.1	7.7	2.5	17.2	72.4
F-value	29.9**	17.7**	12.5**	204.7**	196.2**
LSD0.05	2.0	0.78	0.51	6.12	5.97
CV (%)	3.4	8.2	13.1	8.6	7.6

Increase of oil content is a common breeding goal in safflower. To date, this has been successfully attempted by reducing the hull content (Mundel and Bergman, 2009) as both traits are negatively related (Rao *et al*., 1977). Various hull mutants (reduced hull, partial hull, striped hull and thin hull) with reduced seed coat have been developed in safflower, which have been exploited in breeding of commercial varieties (Mündel and Bergman, 2009). Nutrasaff, a variety with 50 per cent seed oil content has been developed in USA with reduced hull content (Bergman and Flynn, 2008). Rubis (2001) reported a new hull type with over 55 per cent seed oil content. Among the varieties tested in this study, Centennial, Oker, S-334, S-518, S-719 and CW-99 are striped hull types. Genetics of striped hull trait is simple and recessively inherited (Ebert and Knowles, 1966), which can easily be incorporated in the background of improved cultivars through backcrossing.

In conclusion, the most popular safflower cultivars currently grown in India are low oil and high linoleic types. Considering the market demand, new impetus is given to breed high oil yielding varieties with high oleic acid content. The exotic varietal sources reported in this study would be helpful to expedite such breeding efforts in India.

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