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Survey of safflower (*Carthamus tinctorius* L.) germplasm for variants in fatty acid composition and other seed characters

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Summary

Two hundred safflower accessions, originated in 37 countries, and multiplied in two environments, were evaluated for fatty acid composition of the seed oil and other seed characters. Overall mean values of stearic and palmitic acids were similar in both environments but differed for seed weight and oil content. Oleic and linoleic acids showed also similar overall mean content in both environments but some entries with intermediate contents of these acids displayed significant variation among environments. Oleic and linoleic acids showed a tremendous range of variation, from 3.1 to 90.60 % and from 3.9 to 88.8 %, respectively. The ranges of variation observed for stearic, oleic and linoleic acids indicate that all the reccessive genes, already discovered, controlling high content of these acids, *st*, *ol* and *li*, are present in the collection. Moreover, the upper values of oleic, ten points higher than the published values for the high oleic genotype *olol*, suggest than other genes controlling such levels may be present.

Introduction

The characteristics of vegetable oils, chemically triacyl glycerols, depend, to a large extent, on the nature of their fatty acid constituents, which determine the suitability of oils for edible and industrial uses. Safflower (*Carthamus tinctorius* L.) oil, contains two main unsaturated fatty acids, oleic (18:1) and linoleic (18:2), which together account for about 90% of the total fatty acids. The remaining 10% correspond to the saturated fatty acids palmitic (16:0) and stearic (18:0). Safflower is one of the cultivated oil crops in which a greater number of different types of oil can be developed through the combination of major genes, controlling levels of stearic, oleic and linoleic, and changing environmental conditions (Knowles 1989). The content of oleic relative to linoleic acid is governed by three alleles in one locus (Knowles and Hill 1964). These alleles are *OL* (normal safflower) with linoleic acid content from 75 to 78%, the allele *ol*, found in an introduction from India (India, 57–147) responsible for high levels of oleic acid (71–75%) and low linoleic. Another allele ol_1, found in an introduction from Iran (Iran, 59–779) is responsible for intermediate levels of both oleic and linoleic acids (Knowles and Hill 1964).A genotype with very high levels of linoleic acid (87–89%) and very low oleic (3–7%) was found in an introduction from Portugal (Portugal 253568) (Futehally and Knowles, 1981). High levels of linoleic acid are controlled by a single recessive gene *li* which is in a different locus from *ol* and *st* genes governing levels of oleic and stearic acids. In an introduction from Israel (Israel, 56–

456) a recessive gene *st*, independent of the *ol* and l_i loci was found, which controls higher levels of stearic acid (Ladd and Knowles, 1971). The genotype *stst* has 5–10% of stearic compared with 1–3% in normal oil.

By combination of those loci with different alleles it is possible to develop cultivars with five types of oil (Table 1).

As in other oil crops environmental factors, mainly temperature during seed development, influence the relative proportion of the different fatty acids (Bartolomew, 1971, Knowles, 1972 and 1989) This influence depends on the genotype x environment interaction. High linoleic (*OLOL*) and high oleic (*olol*) genotypes were relatively stable at different temperatures. Variation due to changes of temperature ranged from 75 to 82% for high linoleic and 70–77% for the high oleic genotypes (Bartholomew, 1971). The high stearic genotype showed higher ranges with 4.4% under lower and 10.6% at higher temperatures. Likely, the type having equal amounts of oleic and linoleic acid in the seed (ol_lol_l) show a strong response to temperature. At low temperatures it approached the fatty acid composition of high linoleic safflower, i.e. 75.1% linoleic and 15.0% oleic. At high temperatures the opposite situation prevailed with oleic acid increasing to 53.1% and linoleic decreasing to 38.6% (Knowles, 1972 and Bartholomew, 1971).

The importance of safflower as an oil crop has decreased in the world, although it is still important in countries such as India and Mexico. The development of cultivars with higher oil content by incorporation of genes which reduce hull content increased the interest of this oil crop (Knowles, 1989). Furthermore, safflower is one of the most important oil crops from the point of view of oil quality. However, the discovery of new types with higher levels of saturated fatty acids, especially high stearic acid stable under different temperatures, would be of interest for oil industries. Likewise, very high levels of oleic acid (87–93%) similar to the high oleic sunflower mutant controlled by dominant genes (Fernández-Martinez *et al.* 1989) are not yet available in safflower.

Therefore, the present study was conducted to survey the variation in fatty acid composition and other seed characters of a collection of safflower to identify new mutants and to study the stability of these characters under different environmental conditions.

Material and methods

The safflower materials of the study were part of a safflower collection provided by the Plant Introduction Service of the U.S. Department of Agriculture. These materials consisted of 200 entries, whose seed was increased by Dr. P.F. Knowles at the University of California, Davis, USA, partly in 1980 and the rest in 1981. Part of this seed was planted several times at the experimental farm of the Agricultural Research Centre, Cordoba, Spain, for evaluation and further multiplication. The remaining part of the original seed received from California was kept under controlled conditions. Both types of seed, the original, multiplied in California and seed multiplied in 1985 under environmental conditions of Cordoba, were used for determination of fatty

Table 1. Fatty acid composition of different safflower mutants

Type of oil	Genotype	Fatty acid composition (%)			
		16:0	18:0	18:1	18:2
Very high linoleic	OLOLliliStSt	3–5	1–2	5–7	87–89
High linoleic	OlOlLiLiStSt	6–8	2–3	16–20	71–75
High oleic	ololLiLiStSt	5–6	1–2	75–80	14–18
Intermediate oleic	ol_lol_lLiLiStSt	5–6	1–2	41–53	39–52
High stearic	OLOLLiListst	5–6	4–11	13–15	69–72

From Knowles (1989)

acid composition and other seed characters, e.g. weight of 100 seeds and oil content. For multiplication in Spain, original seed was sown in unreplicated 5 m long rows with 0.7 m between rows and 20 cm between plants in the row. Plants were furrow irrigated to maintain a maximum plant growth and the experimental area was hand weeded. Planting was carried out in January. At flowering time, several heads of ten plants of each entry were bagged using paper bags. Considering all entries, seed formation and oil synthesis of material multiplied in Cordoba took place between 25 June and 25 July 1985 under high temperature conditions (Table 2). For the original seed increased at Davis this period took place, depending on differences in time to mature between entries and year of multiplication, between 10 June and 31 July 1980 and 1981. Average temperatures at Davis were similar both years but about 5 °C lower than in Cordoba (Table 2).

Oil content, seed weight and fatty acid composition of both, original collection and seed multiplied in Spain, of 200 entries were determined in 1991. The entries originated from the following countries: Afghanistan (7), Algeria (2), Argentina (1), Austria (2), Australia (5), Bangladesh (3), Bulgaria (1), Canada (1), China (1), Ethiopia (9), France (3), Greece (1), Hungary (2), India (31), Iran (28), Irak (3), Israel (9), Italy (2), Japan (4), Jordan (3), Kenya (4), Kuwait (1), Netherland (1), Morroco (3), Pakistan (17), Poland (2), Portugal (7), Romania (2), Spain (6), South Africa (2), Sudan (6), Switzerland (1), Turkey (10), United Arab Republic (10), United Kingdom (1), USA (3) and the former USSR (6). For fatty acid composition a bulk sample of 10 seeds was analysed.

Table 2. Minimum, maximum and average temperatures during the period of seed formation of safflower germplasm at Córdoba, Spain and at Davis, California. Córdoba data are for one month from 25 June to 25 July 1985. Davis data are from 10 June to 31 July, 1980 and 1981 (see text)

	Temperature (° C)		
	Maximum	Average	Minimum
Córdoba			
Mean	37.4	28.0	19.4
Range	34.7–43.6	25.6–31.7	16.8–23.4
Davis, 1980			
Mean	31.9	22.9	14.1
Range	24.4–41.1	16.1–28.8	8.9–20.5
Davis, 1981			
Mean	33.1	23.4	14.6
Range	25.0–38.9	16.5–29.2	8.9–22.2

In 1992, 10 plants of each of two entries from Pakistan and Bangladesh PI 280228 and PI 401472 showing higher values of oleic acid were grown for further evaluation under uniform conditions in greenhouse in Córdoba. Two cultivars 'UC-1' and 'Rancho' were used as high and low oleic checks. Plants were analyzed individually and 10 single seed analyses were carried out for each plant using the half seed technique described for safflower (Knowles 1989). This technique, which allows the analysis of a portion of the seed and planting the other portion, was used for further selection of (high oleic) deviating types.

The fatty acid composition for bulk samples of the original seed was determined by gas liquid chromatography using esterification. Fatty acid methyl esters were prepared, after oil extraction with petroleum ether from the seeds, according to the method described for sunflower (Fernández-Martinez and Knowles, 1982). For single seed analyses digestion and transmetylation of lipids from the seeds and extraction of fatty acid methyl esters were carried out in one step according to the following method developed by Garces and Mancha (1992). The samples were heated at 80 °C with a reagent containing methanol: heptane; bencene; 2, 2-dimetoxypropane: H_2SO_4 (37:36:20:5:2). After 120 min two phases were formed, the upper one containing the fatty acid methyl esters ready for GLC analysis. The GLC analyses were performed using a Shimadzu gas chromatograph model GC9A equipped with flame ionization detector and using nitrogen as carrier gas. Oil content was determined by nuclear magnetic resonance.

Results and discussion

Data on seed weight, oil content and fatty acid composition are presented in Table 3 for original seed multiplied in California, US and selfed seed ob-

tained in Cordoba, Spain. Seed weight and oil content showed lower values in the seed from the environment of Cordoba that those for the original seed from California. The mean values of the fatty acids palmitic, stearic, oleic and linoleic were similar at both environments. The ranges for seed weight and oil content were narrower for the seed from the environment of Cordoba. These lower ranges and means could be attributed to the effect of the paper bags used for selfpollination and to the relatively high temperatures during seed formation, end of June to 25 July. The average temperatures in Córdoba in 1985 for this period were 28.0 °C, the minimum and maximum average values were 19.4 and 37.4, respectively, and the absolute maximum values ranged from 35 to 44 °C. These values for the period of seed formation at Davis, in 1980 and 1981, were about 5 °C lower (Table 2).

Entries from Irak, India and Pakistan showed the highest values for oil content. These values were about 5 points lower than those of modern cultivars carrying genes responsible for low hull content (Urie, 1976 and Urie and Zimmer, 1979) that under the same conditions ranged from 43 to 46%. Although the hull content was not determined in the present study, the entries mentioned showing higher oil content, had normal white hull type which can be distinguished visually from low hull genotypes. These entries could be of interest in breeding for high oil content if they are combined with genotypes carrying genes with reduced hull content.

The mean and range of palmitic acid in seeds from both environments were within the normal range reported for this fatty acid (Knowles, 1989). The range observed for stearic acid in both environments was wider than that of the normal cultivated safflower (2–3%). The values higher than 6% (Table 3) correspond to entries from Afganistan and Austria. These entries probably carry the recessive *st* genes found in safflower (Ladd and Knowles, 1971) controlling high stearic values (5–9%).

Oleic and linoleic acids of seed from both origins, U.S. and Spain, showed a tremendous range of variation with extreme values from 3.1 to 84.2% for oleic acid and from 9.1 to 89.2% for linoleic acid with distributions skewed toward high values of these acids. The upper value of linoleic acid, 88.8% and only 3.1% of oleic acid corresponded to an entry from US which may carry the reccesive gene *li* (Futehally and Knowles, 1981), responsible for such levels of linoleic acid, since the normal values for this acid range from 71 to 78% (Knowles 1989). The upper values of oleic acid, 84.7%, were higher than the extreme values for bulk samples for *olol* genotypes with high levels of this acid (Knowles and Hill, 1964 and Knowles, 1989). Due to the interest in this fatty acid it was studied in more detail by analyzing single seeds as shown below. The correlation between oleic and linoleic contents within environments was very high and negative (–0.97), (Table 4). This is in agreement with other studies and other oil crops (Canvin, 1965 and Knowles, 1965).

The correlations between the contents of the four fatty acids of seed of the same entries obtained in the Davis and Córdoba environments varied from r=0.34 for palmitic to r=0.61 for oleic acid. These

Table 3. Mean and range of 100 seed weight, oil content and fatty acid composition of 200 safflower accessions multiplied in Davis, California (1980, 1981) and Cordoba, Spain, 1985

Character	Davis		Cordoba	
	Mean	Range	Mean	Range
Seed weight (g)	4.3	2.2–6.6	3.4	2.1–5.4
Oil content (%)	27.7	21.0–40.0	27.1	20.1–37.3
Fatty acid composition (%)				
Palmitic	7.1	4.6–10.0	6.7	4.2–11.9
Stearic	2.9	1.1–7.6	2.3	0.7–6.2
Oleic	20.6	9.5–84.2	20.7	3.1–81.7
Linoleic	68.7	9.1–82.0	70.4	10.4–88.8

values, which indicate an apparent lack of stability of fatty acids of seed grown in different conditions, are illustrated in Fig. 1 for oleic acid. In this figure the oleic acid values of each entry from the original seed (Davis environment) are plotted against the values of the same entry grown in the environment of Cordoba. This figure is not represented for linoleic acid due the high correlation between both acids mentioned above. The blot represented in Fig. 1 enables the entries to be subdivided in four groups taking into account the published mean levels of oleic acid of the already discovered genotypes and their ranges of variation at different temperatures during seed formation (Knowles, 1972 and Bartholomew, 1971).

The first group includes entries with levels of oleic lower than 9%. This group must be formed by individuals with very high frecuency of *lili* genotypes the only genotype showing such levels of oleic acid (Futehally and Knowles, 1981). These very low values were found mostly in the analyses of seeds obtained at Córdoba. Probably the bulk sample of original seed used in the analysis were formed by mixture of genotypes *lili* and *LiLi* and *Lili* and after selfing and multiplication in Spain the frecuency of *lili* genotypes increased.

The second group is formed by entries with low oleic acid content ranging from 9 to 20% in both environments. This group is probably formed mostly by entries with "normal" genotype *(OLOL)* which shows stable levels of oleic, within this range, depending on temperature during oil synthesis (Knowles, 1989).

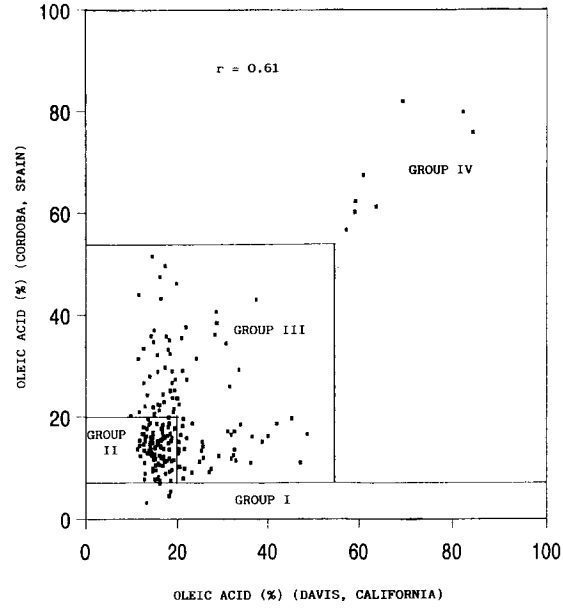

Fig. 1. The relationship between oleid acid content (%) of the seed oil of safflower accessions grown in Córdoba, Spain, 1985 and Davis, California, 1980, 1981.

The third group is formed by genotypes with oleic acid values ranging from 20 to 55% which correspond to the range observed for genotypes ol_1ol_1

Table 4. Correlation coefficients of fatty acid composition of the seed oil of safflower accessions within and between two environments in Davis, California (C) and Córdoba, Spain (S)

	Fatty acid (environment)						
	Palmitic (C)	Stearic (C)	Oleic (C)	Linoleic (C)	Palmitic (S)	Stearic (S)	Oleic (S)
Fatty acid (environment)							
Stearic (C)	0.31**						
Oleic (C)	− 0.40**	− 0.10NS					
Linoleic (C)	0.30**	− 0.04NS	− 0.98**				
Palmitic (S)	0.34**	0.19*	− 0.14NS	0.11NS			
Stearic (S)	− 0.01NS	0.43**	0.12NS	− 0.17NS	0.34**		
Oleic (S)	− 0.23*	− 0.12NS	0.61**	− 0.58**	0.34**	0.44**	
Linoleic (S)	0.17NS	0.06NS	− 0.55**	0.54**	− 0.46**	− 0.50**	− 0.99**

NS = Not significant
* $P \leq 0.5$
** $P \leq 0.01$

and $OLol_1$ (Knowles, 1965 and Bartholomew, 1971). Within this group for some entries differences were observed between the values of oleic acid of the seed obtained in the environments of Davis and Cordoba. These differences were important in some cases reaching levels of oleic acid of 40–50% in one environment and 10–20% in the other. Moreover, both high and low values were observed in both environments depending on the entries considered. This lack of stability suggests that different temperatures regimes were present during seed formation within the same environment for the different entries. It is also possible that in some cases entries were made up by a mixture of types of oil with high frequences of the gene ol_1, genotypes ol_1ol_1, very sensitive to changes of temperature in contrast to genotypes *OlOl* and *olol* which show more stability (Knowles, 1972). In the case of the original seed it was multiplied in two different years at Davis, California, with some differences in temperature during seed formation for the different entries. For the material grown in Spain there were in some cases differences of almost 4 weeks in time to mature between entries with important differences in temperature during the period of oil synthesis, June and July 1985. A significant proportion of the entries showing wider differences in oleic content between Davis and Cordoba environments originated for Iran, the country where genotypes carrying the gene ol_1 were found (Knowles an d Hill, 1964).

The fourth group is formed by individuals showing oleic values higher than 55% (Fig. 1). In this group two different subgroups can be observed, the first (5 entries) with values of about 60% in both environments and the second with values higher than 80%. Due to the interest in higher values of this fatty acid one entry of each group with enough amount of reserve seed was evaluated in Cordoba in 1992 under greenhouse conditions along with high and low oleic checks. For each entry evaluation was performed analyzing single seeds of individual plants.

Results of this evaluation are shown in Table 5. For both entries, two types of oleic acid content,

Table 5. Mean values and ranges of fatty acid composition of two high oleic accessions based on analyses of single seeds of individual plants. Data obtained in greenhouse at Córdoba in 1992

Accession or check	Plant no	Fatty acid composition (%)					
		Palmitic + Stearic		Oleic		Linoleic	
		Mean	Range	Mean	Range	Mean	Range
PI280288	1	6.4	5.9–7.0	75.6	72.0–79.1	17.9	14.7–18.6
	2	6.6	6.0–7.3	72.3	68.7–73.4	21.1	18.3–23.2
	3	7.9	6.9–8.3	16.6	15.6–19.4	75.4	73.8–77.1
	4	6.9	6.1–7.4	76.4	75.8–79.1	16.7	14.5–19.0
	5	7.9	7.1–8.2	15.7	14.9–17.6	76.4	74.8–77.9
	6	6.5	5.9–7.1	76.7	74.8–79.1	14.8	13.6–16.5
	7	7.0	6.1–7.9	78.2	77.1–79.3	14.7	13.1–15.7
	8	6.8	6.3–8.0	73.1	71.9–76.2	20.1	18.9–22.1
PI401472	1	5.5	5.3–5.6	89.5	88.7–90.3	4.9	4.1–5.6
	2	5.8	5.6–5.9	89.1	87.4–90.0	5.1	4.4–6.2
	3	6.3	6.0–6.5	88.5	88.1–89.1	5.2	4.5–5.9
	4	5.9	5.7–6.1	88.8	87.7–90.8	5.3	4.3–6.1
	5	5.7	5.4–6.0	87.8	86.9–89.5	6.5	5.1–7.4
	6	5.6	5.4–5.8	88.4	86.6–90.4	6.0	4.0–7.3
	7	8.2	7.6–9.0	15.7	14.9–16.9	76.0	74.1–77.9
	8	6.4	6.1–7.2	88.6	87.6–90.6	4.9	3.9–5.4
	9	5.8	5.7–6.1	88.0	87.9–89.1	6.2	5.9–6.4
CV. 'UC-1' High oleic check		6.8	6.4–7.6	77.9	75.3–79.1	15.3	14.6–17.8
CV. 'Rancho' low oleic check		7.8	6.8–9.0	14.7	13.6–16.4	77.4	75.8–78.5

high and low, were identified in the plants analyzed. Accession number PI-282888 from Pakistan showed 6 plants with oleic acid content ranging from 72.3 to 78.2%, similar to the high oleic check UC-1 with 76.9% of oleic. The other 2 plants showed oleic acid levels from 15.6 to 16.6%, similar to the low oleic check, cultivar 'Rancho' (15.6%). Accession number PI-401472 from Bangladesh showed a plant with low oleic levels and eight with high oleic content. However, the oleic acid levels of the seed of these plants in this accession were extremely high, ranging from 87.8 to 89.5%. The upper content of oleic acid of individual seeds reached up to 90.6%, with only 3.9% of linoleic. These high values have never been reported in safflower before. The maximum values reported in this crop for the high oleic genotype *olol* are around 80% (Knowles, 1972, and Bartholomew, 1971).

The results shown in Table 4 suggest that in the accesion PI-280228, with oleic acid values similar to the cultivar 'UC-1' (genotype *olol*) the alele *ol* described by Knowles and Hill (1964) is present. The original seed received from US was probably a mixture of genotypes, *OLOL* and *olol*, which explains that both, high and low oleic types, appear in the analyses of single plants. High oleic types in germplasm from Pakistan were reported before (Quadrat-i-Khuda *et al.*, 1959 and Knowles 1965).

The analyses of accession number PI-401472 from Bangladesh also shows that there was a mixture of genotypes, with high and low oleic content, in the original seed what explains the lower values of oleic, 80–84%, than the analyses of single plants. However, the extremely high values of oleic acid of this accession, ten points higher than those of the accession from Pakistan and the high oleic check 'UC-1', suggest that other gene/s responsible for higher levels of oleic are present in this material. In fact, although the oleic acid content of the high oleic genotype discovered by Knowles and Hill (1964) is influenced by temperature conditions (Bartholomew, 1971; Knowles, 1972, Knowles, 1989) the range described for this material is only between 70% and 82%.

The research work of Knowles and co-workers in California made it possible to discover different types of oil in series of introductions from different places of the world. In the present study, 200 accessions from 37 countries, part of a bigger collection, have been evaluated for fatty acid composition and oil content. The variation found in these accessions displayed a wider range than that reported before, especially for oleic acid. This range of variation indicates that all the genes previously described, responsable for high values of stearic, *st*, oleic, *ol*, and very high linoleic acid, *li*, are present in the material evaluated.

The very high values of oleic acid (90.6%), never reported before, suggest that other gene/s controlling such high content are involved. It is expected that further studies of the large available safflower world collection reveal additional useful variation for a range of economic characters.

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