Isolation of variants with high saturated and high linoleic fatty acids in several wild sunflower species

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Abstract

One objective of sunflower breeding programs has been to search for variability in the fatty acid composition of the seed oil, in order to isolate mutants which could increase the utility of the oil for uses. With this aim, a program was started in 1988 to identify mutants in wild species, with high linoleic acid in the seed oil produced under high temperature conditions or high levels of saturated fatty acids. Fixed mutants with high and stable linoleic content values, under these conditions, higher than 65% as compared with 42% of the cultivated material, were stablished after four generations of selection and selfing or sibcrossing, in the species *H. silphioides* (81%), *H. decapetalus* (80%), *H. divaricatus* (75%) and *H. exilis* (68%). One mutant with very high palmitic acid, 29%, as compared with 6% of the cultivated sunflower, was also isolated in *H. hirsutus*. These characters are being incorporated into cultivated sunflower.

Introduction

The properties of vegetable oils depend on large extent on the fatty acid composition of the lipids stored in the seeds or fruits of oil crop plants. Current cultivated oil crops do not always meet all requirements of the food industry. Therefore, there is an increasing interest in producing modifications in the oil composition of annual oil seed crops. In general, the oil of cultivated sunflower (*Helianthus annuus* L.) is recognized as a high quality edible oil. This oil contain about 10% of saturated fatty acids (palmitic and stearic), the remaining 90% being oleic and linoleic fatty acids (DORRELL, 1978). Oil of current commercial cultivars contains very unstable levels of oleic and linoleic which varies between 15 and 55% and 45 and 75% respectively. These varying levels depend mainly of temperature during seed growth and development (HARRIS et al. 1978).

Modification of the fatty acid composition may be accomplished in two different ways, by natural variability in cultivated and wild species or by mutagenesis. The latter has made possible the identification and isolation of several mutants with altered fatty acid composition in sunflower. The most known is the high oleic mutant with 87 to 90% of this acid and very low levels of linoleic (0,5 to 3%). More recently high stearic (25%) and high palmitic (30%) mutants have been

obtained trough mutagenesis (OSORIO et al. 1995). A high palmitic mutant was also reported by IVANOV et al. (1988). Other type of mutants with high linoleic content (70 to 75%) stable under high temperature conditions during seed formation are most available in sunflower, although variability was reported in cultivated sunflower (DOWNES and TONNET, 1982) and in wild species (SEILER, 1985, DE HARO and FERNANDEZ-MARTINEZ, 1991).

In 1988 we started a program aimed to identify mutants in wild species with high linoleic acid in the oil produced under high temperature conditions. In this paper are reported mutants with high levels of linoleic acid and variants with high palmitic acid, in wild sunflower species.

Materials and methods

Eight species which had showed high linoleic acid content, stable under high temperature conditions, in previous studies (DE HARO and FERNANDEZ-MARTINEZ, 1991) were used for further selection for high levels of this acid. These species are shown in Table 1 together with linoleic acid content and stability.

Progenies of one entry of *H. decapetalus*, not selected in this study but showing very high levels of linoleic (89,8%) under greenhouse conditions, were also included. To study variation for palmitic acid one entry of *H. hirsutus* and one of *H. divaricatus* with levels of this acid higher than 11% in previous studies were evaluated in four environments with differents temperatures during seed formation.

The screening for linoleic acid content was performed by analyzing by gas liquid chromatography (GLC) individual seeds of plants grown under warm temperatures in the field or greenhouse. Combining planting time and the long flowering period of multiheaded wild material it was possible to harvest the seeds under relatively uniform temperature conditions. En each cycle of selection at least 40 seeds for each species were evaluated using the half seed techique. A distal portion of the cotyledons was removed with a scalpel and used for GLC analysis. The rest of the achene was stored and planted for another cycle of selection. Selection of seeds was based on linoleic acid content and linoleic acid stability index (LASI) as defined by DE HARO and FERNANDEZ-MARTINEZ (1991). Selected half seeds of each species were planted and at flowering plant were bagged and sibcrossed starting a new cycle. For the study of variation of palmitic the half seed technique was also used.

For GLC analysis the portion of cotyledon to be analyzed was placed in a vial and lipids were extracted and metylated according to the method described by GARCÉS and MANCHA (1993). Fatty acid composition was determined with a Perkin-Elmer autosystem gas-liquid chromatograph equipped with a flame ionization detector (FID) and a 2 m column packed with 3% SP-2310 SP-2310/2% SP-2300 on chromosorb WAW.

Results and discussion.

The linoleic acid content and LASI under warm conditions of nine species and subspecies are shown in Table 2. This index was calculated using linoleic acid values of original material multiplied under cooler conditions. All the species showed variation for linoleic acid content and LASI. This variation was rather wide for some of them as *H. debilis ssp. tardiflorus, H. mollis, H. maximiliani* and *H. rigidus ssp. rigidus* (Table 2). Twenty of the half seeds of each species with higher values (about 10 individuals) were planted and sibcrossed were made at flowering, between selected plants.

In the two following generations the same level of variation, for both linoleic acid content and LASI, was observed for six of the species (data not shown). Therefore, selection was continued only with the other four species (*H. decapetalus, H. divaricatus, H. exilis* and *H. silphioides*). After four cycles of selection the range of linoleic acid and LASI as well as the high mean values (Table 2) suggest that the high values of linoleic acid could be fixed for these four species. Eight of the species used in the present study were reported to be high in linoleic acid under warm conditions (DE HARO and FERNANDEZ-MARTINEZ, 1991). The results obtained indicate intraspecific variation for this character. The pattern of variation and response to selection suggest that the control of high linoleic levels is poligenic. Additional research is needed to study the inheritance of this trait and the possibilities of transfering by interspecific hybridization to cultivated sunflower.

The fatty acid composition of the progenies of two entries of *H. hirsutus* and *H. divaricatus*, selected for high palmitic acid, is presented in Table 3. Both species showed a wide variation for this fatty acid. The higher values in both species (>25%) are similar to the values reported for mutants with high values of this acid (IVANOV et al. 1988, OSORIO et al. 1995). Moreover, *H. hirsutus* showed very high mean of palmitic acid content (24,1%) for all the seeds analyzed and one plant showed an average of 29%. Further studies are needed also in order to fix these mutants and to study the genetics of high palmitic content in these genotypes and their relation with other high palmitic mutants obtained by mutagenesis.

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Table 1.- Linoleic acid content under warm conditions and linoleic acid stability index (LASI) of eight *Helianthus* species used in this study. (From, De Haro and Fernandez-Martinez, 1991).

Species and subspecies	Linoleic acid (%)	LASI
H. debilis ssp. tardiflorus	75,7	0,85
H. divaricatus	75,6	0,93
H. exilis	75,6	0,97
H. gracilentus	74,5	0,89
H. maximiliani	71,4	0,89
H. mollis	77,4	0,95
H. rigidus ssp. rigidus	76,7	0,91
H. silphioides	73,0	0,88
Cultivated check	42,6	0,65

Table 3.- Mean and range fatty acid composition of *H. divaricatus* and *H. hirsutus*.

Species	n° of seeds	Fatty acid composition			
		palmitic	stearic	oleic	linoleic
H. hirsutus	53	24,1 4,0-37,0	5,1 0,1-9,5	31,2 5,4-53,8	38,8 10,0-80,2
H. divaricatus	56	13,2 1,8-30,0	2,40 0,0-9,3	29,9 8,7-57,1	53,6 9,7-83,9
Cultivated Check		5,3	2,4	25,3	60,9

Table 2.- Mean and range of linoleic acid and LASI for cycles 0 and 4 of species of *Helianthus*.

Species and subspecies	Cycle 0				Cycle 4			
	Linoleic acid (%)		LASI		Linoleic acid (%)		LASI	
	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range
H. debilis ssp. tardiflorus	56,2	41,3-69,0	0,63	0,46-0,77				
H. decapetalus	74,3	61,6-88,1	0,83	0,67-0,98	80,1	72,3-85,1	0,89	0,80-0,94
H. divaricatus	69,5	59,0-81,2	0,82	0,70-0,96	75,4	67,1-83,4	0,89	0,79-0,98
H. exilis	69,1	63,1-75,5	0,88	0,81-0,97	68,7	65,5-75,2	0,88	0,84-0,97
H. gracilentus	64,5	53,4-74,0	0,76	0,63-0,88				
H. maximiliani	62,5	39,2-67,0	0,78	0,49-0,84				
H. mollis	57,5	43,4-62,9	0,74	0,55-0,81				
H. rigidus ssp. rigidus	58,9	45,5-72,1	0,69	0,53-0,85				
H. silphioides	75,1	64,9-85,3	0,88	0,76-1,00	81,0	69,7-87,2	0,94	0,82-1,00
Cultivated Check	41,2	35,3-51,7	0,57	0,49-0,72	43,2	41,1-49,4	0,60	0,57-0,69