MATERNAL AND EMBRYO EFFECTS ON THE OLEIC AND LINOLEIC ACID CONTENTS OF SUNFLOWER OIL.

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ABSTRACT

The oleic and linoleic acid contents of sunflower oil were investigated in selfed and crossed seed of four parental lines differing in levels of these fatty acids. Reciprocal F_1 populations were obtained. Both embryo and maternal genotype control of these acids were found. There were no cytoplasmic effects evident in the reciprocal F_1 populations for any fatty acid in any of the combinations studied. Gene(s) for high linoleic content appeared to be dominant to gene(s) for high oleic content.

INTRODUCTION

The relative effects of the maternal and embryo genotypes on the fatty acid composition of seed oil determines the breeding procedure used in selection for a specific fatty acid. If the percent of a fatty acid depends on the genotype of the embryo, the "half-seed" technique of selection would be very efficient and the first segregating material would be F_2 embryos on F_1 plants. If the fatty acid composition depends mostly on the maternal genotype, selection should be done on F_1 plants using bulk samples of several seeds.

Putt *et al.,* (1969) postulated that, as in other oil crops of the Composite family, the fatty acid composition of sunflower oil is in great part controlled by the genotype of the embryo. However, Miller *et al.,* (1977) reported that, like oil content, oleic and linoleic fatty acid composition seems to be determined primarily by the genotype of the maternal parent.

Different types of control for fatty acid composition have been found in other oil crops. The following studies show that the genotype of the embryo has major control over fatty acid composition: in flax by Yermanos and Knowles (1962); in safflower by Knowles and Mutwakil (1963) and Knowles and Hill (1964); in common rapeseed *(Brassica napus* L.*)* by Downey and Harvey (1963); in turnip rapeseed *(B. campestris)* by Dorrell and Downey (1964). However, in common rapeseed Kondra and Stefansson (1970) and Thomas and Kondra (1972) reported that the genotypes of both the embryo and sporophyte governed fatty acid levels. The genotype of the sporophyte exercised major control of fatty acid composition in soy-beans (Brim *et al.,* 1968) and in corn (Jellum, 1966). Also in corn Poneleit and Bauciman (1970) and De la Roche *et al.,* (1971) reported cytoplasmic effects.

MATERIALS AND METHODS

The sunflower material used in this work was basically of three kinds:

a) Wild *Helianthus annuus* (AO-6): All the wild material came from seed obtained near Ontario, Idaho, in 1971, and accessioned UC71 — 74. Single seeds from this collection were analyzed with other collections for fatty acid composition in 1973, and the plants from seeds with highest levels of oleic acid were crossed (Fernandez-Martinez, 1974). Seed was increased by subcrossing selected plants from seed with highest levels of oleic acid. In Spain that material was identified as AO-6 for "Alto Oleico" No. 6.

b) Wild *Helianthus exilis:* This material came from seed collected near Knoxville, California, and accessioned as UC73 — 80: It was also analyzed in 1973 and identified as having very high levels (about 80%) of linoleic acid (Fernandez-Martinez, 1974).

c) Cultivated sunflower: The self-compatible line, 'P-21', derived from the Russian cultivar 'Peredovik' was used. P-21 consists of equal amounts of male-sterile (msms) and male-fertile (Msms) types (Leclercq, 1966). It is maintained by harvesting seed from *msms* plants that are pollinated by *Msms* plants. The Russian cultivar 'Tchernianka' was also used.

Reciprocal crosses were made between the high oleic source, AO-6, and the high linoleic *H. exilis,* P-21 and Tchernianka, to get F_1 seeds. When the female parent was P-21, male-sterile plants were used. However, where female plants were male-fertile, they were emasculated. Emasculations were made daily on all opening flowers by removing anthers with tweezers and rinsing the flowers with water. F_1 plants from the crosses, male-sterile P-21 x AO-6 and AO-6 x *H. exilis* and its reciprocal, were grown with the parents in greenhouses in Davis and Cordoba. Because all the F_1 plants were self-incompatible they were intercrossed at random to get seed.

The following procedure was followed for fatty acid analyses of parental seed, F_1 seed and seed (F_2) of F_1 plants: Composite samples of 5 — 10 seeds or single seeds were crushed and ground using a glass mortar and pestle with 5 — 10 ml of redistilled petroleum ether (b.p. 35 C). The ground seed mixture was then filtered through a kimwipe tissue in a glass funnel into a 50 ml glass erlenmeyer flask, rinsing with 5 — 10 ml of petroleum ether to remove all the oil from the mortar and funnel. The petroleum ether was evaporated off to near dryness with a rotary evaporator heating the base of the flask in a water bath at 70 — 80 C.

To allow the formation of methyl esters, 6 ml of sodium methylate (prepared by dissolving 5 grams of sodium in 1000 ml of methanol) were added and esterification was accomplished by refluxing for 5 minutes using teflon-covered ground glass 50 cm air condensers. After allowing the mixture to cool to room temperature, several drops of phenol red ($C_{19}H_{14}O_5S$) were added and sulfuric methanol was incorporated until the red color disappeared. Once neutralization was accomplished 6 ml of hexane were added and, after heating gently, a saturated solution of sodium chloride was added sufficient to fill up the flask. Two phases were then evident and the floating methyl esters were transferred into a 2 ml vial from which samples were injected into a Hewlett Packard gas chromatograph model 5830A using a flame ionization detector, an electronic integrator, and an automatic sampler model 7671A. The automatic sampler was adjusted to inject 2 — 3 μl of sample.

The stainless column 2 m in length and 3.2 mm in diameter was packed with 10% diethylene glycol succinate on 80/100 W/AW chromosorb. Column temperature was 195 C, injector temperature 250 C and detector temperature 250 C. The nitrogen carrier gas meter reading was 30 ml/min.

Comparisons between means of fatty acid composition of parents, F_1 seed and F_2 seed were made by using Student's "t" tests (Steel and Torrie, 1960). Due to the wide range of percentages observed, fatty acid data were transformed to arc sin percentage.

RESULTS AND DISCUSSION

Table 1 gives the means of the fatty acid composition of the oil from seeds of plants of four parents and F_1 seeds of reciprocal crosses between them. Plants were grown under greenhouse conditions except those of P-21 and Tchernianka which were grown in the field at the same time. Palmitic and stearic acids were combined because values for those fatty acids were similar in all materials. Stearic acid contents were usually in the range 5 to 9% and palmitic acid in the range 2 to 5%.

Table 1. Mean fatty acid composition of seed oil of parents and F_1 seed grown, except where noted, in a greenhouse at Davis, 1974.

Parent or crosses	No. of plants	Fatty acid composition %[1] Palmitic stearic	Oleic	Linoleic
P-21[3]	6	9.8a[2]	22.3a	67.7a
P-21 x AO-6[3]	6	10.4a	21.1a	68.4a
AO-6 x P-21	6	10.3a	53.6b	38.0b
AO-6	6	10.6a	63.2c	26.1c
Tchernianka[3]	2	8.9	22.6	68.3
Tchern. x AO-6[3]	2	10.5	20.4	68.8
AO-6 x Tchern.	2	10.7	55.8a	33.3
AO-6	2	10.3	66.5	23.1
H. exilis	6	7.7a	11.7a	80.4a
H. exilis x AO-6	6	9.2b	14.2a	76.4a
AO-6 x *H. exilis*	6	10.9c	49.3b	39.6b
AO-6	6	10.6c	63.2c	26.1c

(1) Analyses were made on samples of 5 to 10 seeds of each plant.
(2) Means for each fatty acid with the same letters within each group are not significantly different (level of prob. 5%).
(3) P-21 and Tchernianka were grown in the field at the same time as greenhouse material.

Table 2 gives the results of analyses of AO-6, *H. exilis* and F_1 (crossed) seed and F_2 seed (on F_1 plants) under greenhouse conditions at Cordoba. Table 3 gives similar analyses for P-21, AO-6, F_1 seeds and F_2 seeds obtained at Davis under greenhouse conditions.

Table 2. Mean fatty acid composition of the oil of samples of several seeds of individual plants of *H. exilis*, AO-6, crossed (F_1) seeds and F_1 plants (F_2 seed) grown in a greenhouse at Cordoba, 1975-76.

Parent or cross	No. of plants	Fatty acid composition %[1] Palmitic+ stearic	Oleic	Linoleic
H. exilis	6	8.8a[1]	19.3a	71.6a
H. exilis x AO-6 (F_1 seed)	6	10.0b	21.9a	67.8a
AO-6 x H. exilis (F_1 seed)	6	9.8b	52.0b	38.0b
H. exilis x AO-6 (F_1 plants)	25	9.7b	26.4c	63.3c
AO-6 x *H. exilis* (F_1 plants)	25	9.6b	28.1c	62.1c
AO-6	6	9.3b	69.0d	21.5d

(1) Means for each fatty acid with the same letters within each group are not significantly different (level of prob. 5%)

The fatty acid compositions of P-21 proved to be very responsive to environmental conditions. In the field at Davis (Table 1) oleic acid content was low (22.3%), whereas in a greenhouse at Davis (table 3) oleic acid content was high (53.8%). AO-6, on the other hand, was consistently high in oleic acid at all locations given in Tables 1, 2 and 3 (the range of averages was 63.2% to 69.1%). It is assumed that the higher temperatures of the greenhouse were responsible for the higher levels of oleic acid of P-21. In this regard its response was similar to a genotype of safflower with intermediate levels of oleic acid (Bartholomew, 1971; Knowles, 1972). Fatty acid composition of Tchernianka was similar to P-21 (Table 1). In contrast to AO-6, *H. exilis* had low levels of oleic acid under greenhouse conditions at both Davis and Cordoba (Tables 1 and 2, respectively).

It is of interest to note the consistent differences in the fatty acid compositions of F_1 seeds of the cross P-21 x AO-6, which were similar to those of P-21, and the F_1 seeds of the reciprocal cross, AO-6 x P-21, where oleic acid levels were consistently higher. Under field conditions (Table 1) P-21 and P-21 x AO-6 F_1 had essentially the same low levels of oleic acid whereas both the reciprocal cross and AO-6 had high levels under greenhouse conditions. Under greenhouse conditions (Table 3), where the differences in the parents were much less, reciprocal crosses were significantly different but similar to the maternal parents. These results suggest that the maternal plant (or sporophyte) exercised a strong influence on fatty acid composition in crosses of both P-21 and Tchernianka with AO-6. Strong maternal effects were also evident in the cross *H. exilis* x AO-6 and its reciprocal (Tables 1 and 2).

Table 3. Mean fatty acid composition of the oil of several seeds of individual plants of P-21, AO-6, crossed (F_1) seed and seeds of F_1 plants (F_2 seed) grown in a greenhouse at Davis 1974-75.

Parent or crosses	No. of plants	Fatty acid composition %[1] Palmitic+ stearic	Oleic	Linoleic
Parents				
P-21	5	9.6a[2]	53.8a	37.5a
AO-6	4	9.3a	68.2c	22.4c
Crossed (F_1)seeds				
P-21 x AO-6	5	9.5a	56.1ab	34.4ab
AO-6 x P-21	4	9.6a	64.5c	25.8c
Seeds on F_1 plants (F_2)				
P-21 x AO-6	27	9.4a	56.2ab	34.3ab
AO-6 x P-21	6	9.3a	57.6b	33.0b

(1) Mean fatty acid contents of each plant were obtained by averaging analyses of single seeds.
(2) Means for each fatty acid with the same letters within each group are not significantly different (level of prob. 5%).

However, sporophytic control was not complete. In most cases F_1 seed showed some influence of the paternal parent. This was true particularly of P-21 (Tables 1 and 3) and *H. exilis* (Tables 1 and 2).

These results are not in agreement with those of Putt *et al.*, (1969) and Zimmerman and Fick (1973) who postulated only gametophytic control similar to safflower and flax and those of Miller *et al.*, (1977) who reported only sporophytic control. It seems evident in the sunflower material used in this study that genetic control of oleic and linoleic acids is partly sporophytic and partly gametophytic. The influence of both the maternal and embryo genotypes has also been found in rapeseed *(Brassica napus)* (Thomas and Kondra, 1972) and in *Lupinus* (Cubero, personal communication).

Results from analyses of F_2 seed on F_1 plants (Tables 2 and 3) indicate no significant differences between reciprocal crosses. This would indicate that there are no significant cytoplasmic effects on fatty acid composition. This is in agreement with the results of Thomas and Kondra (1972) in rapeseed for oleic and linoleic acids. However, in corn Poneleit and Bauciman (1970) and de la Roche *et al.*, (1971) found reciprocal differences in F_1 plants in some combinations.

The data indicate also that the mean values of F_1 plants for oleic and linoleic acids were lower and higher respectively than the mid-parent value, which indicate dominance of the gene(s) for high linoleic (low oleic) content.

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