INHERITANCE OF OLEIC ACID CONTENT UNDER CONTROLLED ENVIRONMENT

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Summary

An experiment was conducted to study the genetic behavior of oleic acid content in crosses between the lines HA89 (standard fatty acid profile) and HAOL9 (high oleic acid content) under a controlled environment. Plants of both lines were reciprocally crossed in the field in the summer of 1995. F_1 seeds, all of them with high oleic acid content, were sown in pots in 1998 together with seeds of both parents. Reciprocal crosses as well as backcrosses to both parents were done. At the end of flowering the plants were moved to a growth chamber at 25/20 ºC (day/night) with a 16-h day light period. Contrary to the results obtained in the field, F_1 seeds obtained in the growth chamber exhibited segregation for oleic acid content. Taking also into account the observed segregations in the F_2 and BC_1F_1 generations, a genetic hypothesis was elaborated. It was based on the existence of five genes controlling oleic acid content, designated Ol_1, Ol_2, Ol_3, Ol_4 and Ol_5. The genotypes Ol_1Ol_1, $Ol_1ol_1Ol_2Ol_2$ and $Ol_1ol_1Ol_2ol_2$ were high in oleic acid, whereas the genotype ol_1ol_1 had a low oleic phenotype. Conversely, the genotype $Ol_1ol_1ol_2ol_2$ could exhibit low, medium, or high oleic acid content depending on the Ol_3, Ol_4 and Ol_5 genes. This hypothesis is useful to explain the segregations observed in the present study, but it has to be verified with additional experiments.

Introduction

The study of oleic acid inheritance in sunflower is a complex task. A number of studies have been conducted but no general agreement on how oleic acid is inherited has been reached. The first genetic analyses on the high oleic sunflower mutant obtained by Soldatov (1976) concluded that the high oleic acid trait was controlled by a dominant (Urie, 1984) or partially dominant (Fick, 1984) single gene designated *Ol*. In a further study Urie (1985) detected the presence of modifiers as well as an unexplained reversal of the dominance of the *Ol* gene. Miller et al. (1987) identified a second gene, designated *Ml*, affecting high oleic acid content. According to the model proposed by these authors, a high oleic acid concentration was expressed in genotypes having one dominant allele of the *Ol* gene combined with the recessive allele *ml* in homozygous condition. Fernández-Martínez et al. (1989) proposed a model based on the presence of three dominant, complementary genes, designated Ol_1, Ol_2, and Ol_3. According to that model, high oleic acid concentration is only expressed when the three dominant alleles are present. Demurin and Škoric (1996) could not confirm any of the previous hypotheses and concluded that the *Ol* locus exhibited genetically unstable expression. Fernández et al. (1999) postulated a two-gene model, similar to that of Miller et al. (1987), with the main difference that in the new hypothesis the high oleic content was produced by the recessive allele *ol* and the dominant allele *Ml*, both in homozygous condition. Interestingly, Fernandez et al. (1999) suggested that *Ml* might not be a single gene but a gene complex.

Despite the influence of the temperature on the phenotypic expression of oleic acid content is well known (Harris et al., 1978), all the above mentioned genetic studies were carried out under non-controlled environments. The only genetic study on oleic acid inheritance conducted under controlled environment was carried out by Alonso (1988). The author used growth chambers at three temperatures (10ºC, 20ºC and 30ºC) and found a great effect of temperature on oleic acid levels in all generations. In all cases the conclusion was that oleic acid content was controlled by a single gene *Ol*, which depending on the temperature can act either as dominant or partially dominant. No additional genes or modifiers were detected.

The objective of the present research was to study the inheritance of oleic acid content in crosses between the near isogenic lines HA89 (standard fatty acid profile) and HAOL9 (high oleic acid) under a controlled environment.

Materials and methods

Plants of the inbred line HA89, with standard seed oil fatty acid profile, and its near isogenic high oleic HAOL9 were grown in the field under a high-temperature environment (spring-summer 1995) and reciprocally crossed. Fatty acid analyses of half-seeds revealed that oleic acid ranged from 83.5% to 90.3% for F_1 seeds of the cross HA89 X HAOL9, and from 80.5% to 89.5% for F_1 seeds of the cross HAOL9 X HA89. Seeds of both parents together with F_1 seeds from the reciprocal crosses were sown in pots and grown in the greenhouse in 1998. F_1 plants were self-pollinated and backcrossed to both parents to obtain the F_2 and BC_1F_1 seed, respectively. Reciprocal crosses between HA89 and HAOL9 plants were repeated to obtain F_1 seed under the same conditions. Immediately after the end of flowering the plants were moved to a growth chamber at 25/20 ºC (day/night) with a 16-h day light period and a photon flux

density of 300 μE m^{-2} s^{-1}, where they were kept till the seeds reached maturity.

The fatty acid composition of the seed oil of the parents, F_1, F_2, and BC_1F_1 generations was analysed on half seeds by gas-liquid chromatography of fatty acid methyl esters (Garcés and Mancha, 1993). Since all generations were grown at the same time in a growth chamber with controlled temperature, discrete classes for oleic acid content in segregating generations were established on the basis of the ranges of variation of the parents.

Results and discussion

Oleic acid content ranged from 18.6% to 52.4% in HA89 and from 85.7% to 92.0% in HAOL9. In contrast with the results under summer field conditions, F_1 seeds of reciprocal crosses between both lines exhibited wide variation for oleic acid content: from 29.9% to 92.8% and from 26.3% to 91.8%, respectively in two F_1 populations from the cross HA89 X HAOL9; from 35.3% to 92.5% and from 25.5% to 92.4%, respectively in two F_1 populations from the cross HAOL9 X HA89. Such wide variation was not unexpected, since previous studies have reported deviation of F_1 seeds from the expected high oleic acid phenotypes (Miller and Zimmerman, 1983; Urie, 1984; Urie, 1985; Fernández-Martínez et al., 1989; Demurin and Škoric, 1996; Fernández et al., 1999). Such deviation of F_1 seeds is not explained with any of the hypotheses for oleic acid inheritance reported in the literature. For example, Urie (1985) and Fernández-Martínez et al. (1989) interpreted the presence of deviating F_1 seeds on the basis of the action of modifiers on the effect of the major genes.

Segregation patterns for oleic acid content in the F_1, F_2 and BC_1F_1 generations are shown in Table 1. All these segregations could be explained with a genetic hypothesis based on the existence of five genes controlling oleic acid levels. We have designated them Ol_1, Ol_2, Ol_3, Ol_4 and Ol_5. According to this hypothesis, the homozygotes ol_1ol_1 (HA89) and Ol_1Ol_1 (HAOL9) produce low and high oleic acid levels, respectively regardless of the genetic configuration for the other four genes. Conversely, the phenotypic expression of the heterozygote Ol_1ol_1 is influenced by the levels of other genes. It produces high oleic acid levels when combined with either Ol_2Ol_2 or Ol_2ol_2, whereas the genotype $Ol_1ol_1ol_2ol_2$ will yield high, medium or low oleic acid content depending on the genetic configuration for the other three genes, Ol_3, Ol_4 and Ol_5 (Table 2).

The genetic hypothesis presented in Table 2 allowed us to explain all the phenotypic segregations observed in the different generations evaluated in this experiment, which could not be explained with any of the existing hypotheses on oleic acid inheritancve in sunflower. It is worth noting that segregation for oleic acid content in the F_1 generation can be also interpreted assuming that the parent lines did not breed true for the Ol_2, Ol_3, Ol_4 and Ol_5 genes. This is possible because they do not have phenotypic effect when the Ol_1 gene is in homozygosis and, therefore, there has not been selection pressure on these genes. Furthermore, this hypothesis does not contradict previous hypotheses. For example, Fernández-Martínez et al. (1989) described the presence of three major genes, Ol_1, Ol_2 and Ol_3 and, additionally, some modifiers, which could be the Ol_4 and Ol_5 genes suggested in the present study. Similarly, Fernández et al. (1999) hypothesized the genetic control of oleic acid content by a major gene, *Ol*, and a second gene *Ml* that the authors considered as a possible gene complex. Moreover, the existence of three genes affecting the expression of the genotype $Ol_1ol_1ol_2ol_2$ can explain to a certain extent the unstable expression of the *Ol* gene (which

might be assumed as the Ol_1 gene of our hypothesis) discussed by Demurin and Škoric (1996).

Differences in segregation patterns for oleic acid content between the present study and previous ones might have been caused by differences in temperature during seed maturation. Temperature is a major factor affecting the expression of oleic acid content (Harris et al., 1978). Therefore we necessarily assume that the behavior of the *Ol* genes might be different under different conditions. Probably some of the genes have no phenotypic expression at higher temperatures, as indicated by lack of segregation for oleic acid content of the F_1 generation of reciprocal crosses between HA89 and HAOL9 obtained in 1995 under summer field conditions (see above).

In conclusion, crosses between HA89 and HAOL9 produced segregations in the F_1, F_2 and BC_1F_1 generations that were interpreted considering a genetic hypothesis with five genes controlling oleic acid content. The hypothesis is useful to explain the segregations observed in the present study, but it has to be verified with additional experiments. We speculate that the phenotypic expression of some of these genes is dependent on the temperature during seed maturation and therefore the genetic hypothesis might not be useful to understand segregations for oleic acid under different conditions. Therefore further experiments under controlled environments with different temperatures are needed to get a better understanding on the genetic system controlling oleic acid levels and on how it is influenced by the temperature.

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Table 1. Number of seeds having a different oleic acid content and chi-square analyses in the F_1, F_2 and BC_1F_1 seeds from reciprocal crosses between HA89 and HAOL9.

Generation	No. of seeds with oleic acid content			Ratio tested	χ^2	*P*	Proposed genotype(s)[b]
	Low	Medium	High[a]				
F_1 (HA89XHAOL9)	11	31	56	1:2:5	2.30	0.32	$ol_1ol_1Ol_2ol_2Ol_3ol_3Ol_4Ol_4ol_5ol_5$ (HA89) X $Ol_1Ol_1ol_2ol_2Ol_3ol_3Ol_4Ol_4ol_5ol_5$ (HAOL9)
F_1 (HA89XHAOL9)	16	8	72	3:1:12	0.89	0.64	$ol_1ol_1Ol_2ol_2Ol_3ol_3ol_4ol_4ol_5ol_5$ (HA89) X $Ol_1Ol_1Ol_2ol_2Ol_3ol_3ol_4ol_4ol_5ol_5$ (HAOL9)
F_1 (HAOL9XHA89)	3	7	86	2:2:28	1.71	0.42	$Ol_1Ol_1Ol_2ol_2Ol_3ol_3Ol_4ol_4Ol_5Ol_5$ (HAOL9) X $ol_1ol_1Ol_2ol_2Ol_3ol_3Ol_4Ol_4Ol_5Ol_5$ (HA89)
F_1 (HAOL9XHA89)	5	8	83	2:2:28	0.84	0.66	$Ol_1Ol_1Ol_2ol_2Ol_3ol_3Ol_4ol_4Ol_5Ol_5$ (HAOL9) X $ol_1ol_1Ol_2ol_2Ol_3ol_3Ol_4Ol_4Ol_5Ol_5$ (HA89)
F_2 (HA89XHAOL9)	45	6	90	312:36:676	0.42	0.81	$Ol_1ol_1Ol_2ol_2Ol_3ol_3Ol_4ol_4Ol_5ol_5$ (F_1)
F_2 (HA89XHAOL9)	62	17	65	120:36:100	2.33	0.31	$Ol_1ol_1ol_2ol_2Ol_3ol_3Ol_4ol_4Ol_5ol_5$ (F_1)
F_2 (HA89XHAOL9)	66	8	70	24:4:36	4.27	0.12	$Ol_1ol_1ol_2ol_2Ol_3ol_3Ol_4Ol_4Ol_5ol_5$ (F_1)
F_2 (HAOL9XHA89)	50	8	84	24:4:36	0.50	0.78	$Ol_1ol_1ol_2ol_2Ol_3ol_3Ol_4Ol_4Ol_5ol_5$ (F_1)
F_2 (HAOL9XHA89)	46	1	94	72:4:180	1.93	0.38	$Ol_1ol_1Ol_2ol_2Ol_3ol_3Ol_4Ol_4Ol_5ol_5$ (F_1)
F_2 (HAOL9XHA89)	31	0	111	1:3	0.76	0.38	$Ol_1ol_1Ol_2Ol_2ol_3ol_3ol_4ol_4ol_5ol_5$ (F_1)
BC_1F_1 (HA89)	48	0	46	1:1	0.04	0.84	$ol_1ol_1Ol_2Ol_2ol_3ol_3ol_4ol_4ol_5ol_5$ (HA89) X $Ol_1ol_1Ol_2Ol_2ol_3ol_3ol_4ol_4ol_5ol_5$ (F_1)
BC_1F_1 (HA89)	54	2	38	36:1:27	0.28	0.87	$ol_1ol_1Ol_2ol_2ol_3ol_3Ol_4Ol_4Ol_5ol_5$ (HA89) X $Ol_1ol_1Ol_2ol_2Ol_3ol_3Ol_4Ol_4Ol_5ol_5$ (F_1)
BC_1F_1 (HAOL9)	0	2	94	1:31	0.34	0.56	$Ol_1Ol_1Ol_2ol_2Ol_3Ol_3Ol_4ol_4Ol_5Ol_5$ (HAOL9) X $Ol_1ol_1Ol_2ol_2Ol_3Ol_3Ol_4ol_4Ol_5Ol_5$ (F_1)
BC_1F_1 (HAOL9)	0	2	92	1:31	0.31	0.58	$Ol_1Ol_1Ol_2ol_2Ol_3Ol_3Ol_4ol_4Ol_5Ol_5$ (HAOL9) X $Ol_1ol_1Ol_2ol_2Ol_3Ol_3Ol_4ol_4Ol_5Ol_5$ (F_1)

[a]Phenotypic classes were determined according to the ranges for oleic acid content of the parents HA89 (18.6% to 52.4%) and HAOL9 (85.7% to 92.0%) grown under the same conditions.

[b]The proposed genotypes are given as an example of several possible allelic combinations.

Table 2. Hypothesis on phenotypic expression for oleic acid content of the different allelic combinations in the *Ol*$_1$, *Ol*$_2$, *Ol*$_3$, *Ol*$_4$ and *Ol*$_5$ loci. HO=high oleic acid content, MO=medium oleic acid content, LO=low oleic acid content.

$\underline{Ol_1Ol_1}$					**HO**
Ol$_1$*ol*$_1$	$\underline{Ol_2Ol_2}$				**HO**
	$\underline{Ol_2ol_2}$				**HO**
	ol$_2$*ol*$_2$	*Ol*$_3$*Ol*$_3$	$\underline{Ol_4Ol_4}$		**HO**
			$\underline{Ol_4ol_4}$		**HO**
			$\underline{ol_4ol_4}$		**MO**
		Ol$_3$*ol*$_3$	*Ol*$_4$*Ol*$_4$	$\underline{Ol_5Ol_5}$	**HO**
				$\underline{Ol_5ol_5}$	**HO**
				$\underline{ol_5ol_5}$	**MO**
			Ol$_4$*ol*$_4$	$\underline{Ol_5Ol_5}$	**MO**
				$\underline{Ol_5ol_5}$	**MO**
				$\underline{ol_5ol_5}$	**LO**
			$\underline{ol_4ol_4}$		**LO**
		ol$_3$*ol*$_3$			**LO**
$\underline{ol_1ol_1}$					**LO**