

MATERNAL EFFECT ON RESPONSE OF OLEIC ACID CONTENT TO TEMPERATURE IN HIGH OLEIC SUNFLOWER

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SUMMARY

High oleic trait has been rarely studied in reciprocal crosses and in most of these cases the maternal effect on the content of unsaturated fatty acids has not been identified. The aims of this study are: i) to check if any maternal effect on oleic acid content is present in high oleic mutants and (ii) if maternal effect and environment conditions could modify the 90% threshold for oleic acid content. Two high oleic inbred lines with different origins were evaluated: 342mt, a selection derived by Ha 342 USDA (maintainer) and line R978 (male fertility restorer, branched) selected by University of Udine and their reciprocal crosses (R978 × 342mt and 342mt × R978). The two inbred lines respond differently to the same environmental conditions. Reciprocal hybrids showed a different accumulation pattern on the first sowing date, and they showed the same response as their female parent. There is a maternal effect in the early stages of the seed filling phase. Low temperature increased linoleic acid and decreased oleic acid at 13 DAF only in 342mt inbred line and in the 342mt × R978 hybrid. High oleic inbred lines with different genetic backgrounds respond differently to the same environmental conditions. It was determined that there was the importance of female parental line choices in breeding to obtain hybrids insensitive to environmental conditions and with a stable oleic acid content over 90%. For the first time a maternal effect on temperature response was found in high oleic sunflower.

Key words: environment, fatty acids, high oleic genotypes, maternal effect

INTRODUCTION

High oleic sunflower is currently used in the food sector and as raw material for non food applications (biofuels, oleochemical). Non food applications in particular

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require oleic acid content that is stable and higher than 90% (Vannozzi, 2006). In a breeding program targeted to obtain hybrids with an oleic acid content higher than 90% it is needed to understand all phenomena that modify fatty acids accumulation and their ratio in the seed.

Seed fatty acid composition depends on two factors: the genetic background of the embryo and the female plant condition (abiotic and biotic stresses).

High Oleic (HO) mutants were obtained by chemical mutagenesis, with dimethyl sulfate, of normal sunflowers (Soldatov, 1976). High oleic trait is seed-specific and mutation reduced the expression of a $\Delta 12$ -desaturase gene (Lacombe *et al.*, 2004). Its transcript is not accumulated during the grain filling period (Martínez-Rivas *et al.*, 2001). Some linoleic acid is still present in the achenes and it is synthesized by a constitutive desaturase system (Martínez-Rivas *et al.*, 2001; Lagravère *et al.*, 2004).

High oleic trait was controlled by at least three loci: oleHL, supole, and modifier loci (Lacombe *et al.*, 2004). There is variability between HO lines according to the alleles at these different loci. Many genetic approaches have been developed to study the HO mutation and in the literature different conclusions are reported on the number of genes that control the trait and on their dominance.

A small number of studies investigated maternal effect on fatty acids in high oleic sunflower. Only one previous study has reported a maternal effect on high oleic trait (Lacombe and Bervillé, 2000). Reciprocal effects on oleic acid content have been reported by Vares *et al.* (2004). Maternal effect on fatty acids was also found in other oil crops such as canola and soybean (Thomas and Kondra, 1973; Erickson *et al.*, 1988; Gilsinger *et al.*, 2010).

Oil composition in standard sunflower is clearly affected by the environment. Temperature is a major environmental factor that influences fatty acid composition of the seed. High temperature enhances the oleic acid content of normal cultivars (Low Oleic), but conflicting results are reported about temperature effects on oleic acid content of high oleic acid cultivars: no effect (Lagravère *et al.*, 2000) or increase of oleic acid content with temperature (Triboi *et al.*, 2000; Izquierdo and Aguirrezábal, 2008).

The aims of this study are:

1. to check if any maternal effect on oleic acid content is present in high oleic mutants and
2. if maternal effect and environment could modify the 90% threshold for oleic acid content.

MATERIALS AND METHODS

Plant materials

Two high oleic inbred lines with different origin and their reciprocal cross were used. The inbred lines were: 342mt and R978. Line 342mt is a selection derived by Ha 342 USDA, and it is a male sterility maintainer with a single head. Line R978,

selected by University of Udine, is a fully branched type and it is a male fertility restorer. Reciprocal cross (F_1 seeds) was obtained by hand demasculation of female parent and controlled pollination in field conditions at Udine in 2010. All plants were covered with paper bags during the R4 stage (Schneider and Miller, 1981) to prevent cross-fertilization. The reciprocal F_1 seed was planted in the field in 2011 and tested with the parents.

Field experiment

Inbred lines and their reciprocal hybrids (F_1 hybrids plants) were grown in 2011 at the University of Udine, Azienda Agraria Universitaria "A. Servadei" (46° 04' N, 13° 22' E, altitude 109 m). The experiment was designed as a complete randomization scheme with three replications using two dates of sowing. The first date of sowing was 18 April (I) and the second was 31 May 2011 (II). Plants were thinned after seedling emergence from 10 to 7.5 plants m^{-2} . Nitrogen was applied at 100 kg ha^{-1} . Weeds and diseases were controlled, and regular watering throughout the experiment ensured that plants were not subjected to water deficit during the entire growth period. All plants were covered with paper bags at the R4 stage to prevent cross-fertilization.

Sampling

Two types of samples were taken: i) 5 seeds per plant; ii) pieces of sunflower head (see below). Seeds samplings were started at 13 DAF (Days After Flowering). Five seeds per plant (obtained from outer rings of the head) were taken every 7 days from 13 DAF to 35 DAF, in order to determine fatty acid accumulation. Seeds were dried in an oven at 60°C for 24 h. The final harvest was done at physiological maturity (R9; Schneider and Miller, 1981).

Effect of low temperature

To simulate the physiological conditions of seed lipid synthesis in the plant, portions of sunflower head were collected at 13, 20, 27 and 35 DAF. Portions of head were incubated in the air, in Petri dishes containing water, at 10°C for 24 h (García-Díaz *et al.*, 2002). After the cold treatment, pieces of head were dried in an oven at 70°C for 24 h. An index of the ODS (Oleate Desaturase) activity (Green, 1986) was calculated for each sampling using the formula $ODS \text{ activity index} = \%18:2 / (\%18:2 + \%18:1)$, where %18:2 and %18:1 are the percentage of linoleic and oleic fatty acids, respectively. The value of this index is directly proportional to the activity of the enzyme system believed to be responsible for the desaturation of oleic acid (Cherif *et al.*, 1975).

Fatty acids determination

Fatty acid composition was determined by gas chromatography and every fatty acid was expressed as a percentage of the total fatty acids detected in the oil. A gas

chromatograph was fitted with a 60 m HP-88 capillary column (Agilent Technologies, USA). Helium was used as the carrier gas, and the injector, detector and oven temperatures were 230, 250 and 200°C, respectively.

Statistical Analysis

ANOVA (ANALYSIS Of VARIance) and LSD (Least Significant Distance) test with *p*-value adjusted using Bonferroni correction were applied to experimental results to determine the significance of differences among treatments.

RESULTS

Sowing date effects on duration of cycle and F-PM (Flowering to Physiological Maturity) phase in inbred lines and reciprocal F_1 plants are shown in Table 1. Later sowing date resulted in a shorter cycle, likely due to environmental differences during plant development. Within sowing date, reciprocal F_1 plants had equal developmental timing.

Table 1: Genotypes, sowing date, cycle duration, duration of Flowering to Physiological Maturation (F-PM) and mean temperatures from F-PM for field experiments

Genotype	Sowing date	Cycle (dd)	F-PM (dd)	Tmin °C	Tmean °C	Tmax °C
342mt	I	110	41	16.2	22.4	28.7
R978	I	113	41	16.1	22.5	29.0
342mt × R978	I	123	53	16.2	23.2	30.5
R978 × 342mt	I	123	53	16.2	23.2	30.5
342mt	II	101	37	16.7	23.9	31.5
R978	II	101	37	16.7	23.9	31.5
342mt × R978	II	108	42	16.7	23.8	31.4
R978 × 342mt	II	109	43	16.7	23.8	31.4

ANOVA results for the main and interaction effects of genotype and sowing date on seed fatty acids composition indicated a significant sowing date by genotype interaction effect. There were some differences between the inbred lines and reciprocal hybrids.

Sowing date did not alter the oleic acid concentration in line R978 and in its R978 × 342mt hybrid. On the other hand, sowing date altered the oleic and linoleic acids concentration in line 342mt and in the 342mt × R978 hybrid (Table 2).

We investigated also the variation in saturated fatty acid concentration through sowing date. Palmitic and stearic fatty acids content did not alter oleic acid concentration (Table 2). Reciprocal hybrids were equal for saturated fatty acids content.

The concentration of oleic acid was affected by the concentration of linoleic acid. This was confirmed by the ODS index. On the first sowing date ODS activity was significantly higher than on the second sowing date only in 342mt and

342mt \times R978. Reciprocal hybrids were not equal for unsaturated fatty acids content through sowing date.

Table 2: Fatty acids composition (%) of seed from self-pollinated inbred lines and hybrids. ODS is an adimensional index of oleato desaturase activity. Results are means \pm SE

Genotype	Sowing date	Fatty acid (%)				ODS
		Palmitic	Stearic	Oleic	Linoleic	
342mt	I	3.54 \pm 0.04c	3.05 \pm 0.22bc	83.99 \pm 0.64c	9.42 \pm 0.38a	0.11 \pm 0.006a
R978	I	3.12 \pm 0.05c	2.76 \pm 0.36bc	91.92 \pm 0.44a	2.19 \pm 0.21cd	0.02 \pm 0.002c
342mt \times R978	I	3.57 \pm 0.12c	3.39 \pm 0.19ab	88.07 \pm 0.44b	4.97 \pm 0.28b	0.05 \pm 0.003b
R978 \times 342mt	I	4.47 \pm 0.13bc	3.31 \pm 0.13ab	90.62 \pm 0.25a	1.60 \pm 0.01cd	0.02 \pm 0.000c
342mt	II	5.21 \pm 0.39a	4.25 \pm 0.09a	87.16 \pm 0.41b	3.39 \pm 0.88bc	0.04 \pm 0.011bc
R978	II	4.87 \pm 0.04a	2.91 \pm 0.07bc	90.81 \pm 0.04a	1.42 \pm 0.04cd	0.02 \pm 0.003c
342mt \times R978	II	3.86 \pm 0.13bc	2.61 \pm 0.10bc	92.35 \pm 0.20a	1.09 \pm 0.12d	0.01 \pm 0.000c
R978 \times 342mt	II	3.76 \pm 0.15bc	2.19 \pm 0.06c	91.56 \pm 0.55a	1.40 \pm 0.23d	0.02 \pm 0.003c
LSD at 5%		0.90	0.96	2.19	1.98	0.02

Values followed by the same letter are not significantly different (LSD at the 5% level, *p*-value adjustment Bonferroni).

The oleic acid accumulation showed some differences between R978, 342mt and hybrids. R978 and R978 \times 342mt showed the same accumulation pattern for both sowing dates. 342mt and 342mt \times R978 showed the same accumulation pattern as the other genotypes within the second sowing date. There were some differences within the first sowing date.

All the genotypes exhibited a synthesis of linoleic acid at early stages (13 DAF), but we reported a difference between the two high oleic inbred lines and among reciprocal hybrids. Line 342mt presented the highest initial linoleic acid content followed by a rapid decrease. Line R978 presented a lower initial linoleic acid content than 342mt followed by a rapid decrease.

In 342mt and in 342mt \times R978, the amount of linoleic acid synthesized is higher than in other genotypes. Reciprocal hybrids were not equal for oleic and linoleic fatty acids content at 35 DAF and at physiological maturity (Figure 1). 342mt \times R978 hybrid showed a continuous increase of linoleic acid accumulation (1.4% from 35 DAF to PM).

The cold treatment (10°C for 24 h) modified the fatty acid composition only at 13 DAF in line 342mt and 342mt \times R978 hybrid (Figure 2). A net increase in linoleic acid content occurred for both sowing dates. The lowest increase was observed in the seeds incubated at 10°C on the second sowing date. The cold treatment on line R978 and hybrid R978 \times 342mt did not modify oleic and linoleic acid content in the seeds incubated at 10°C (Figure 2).

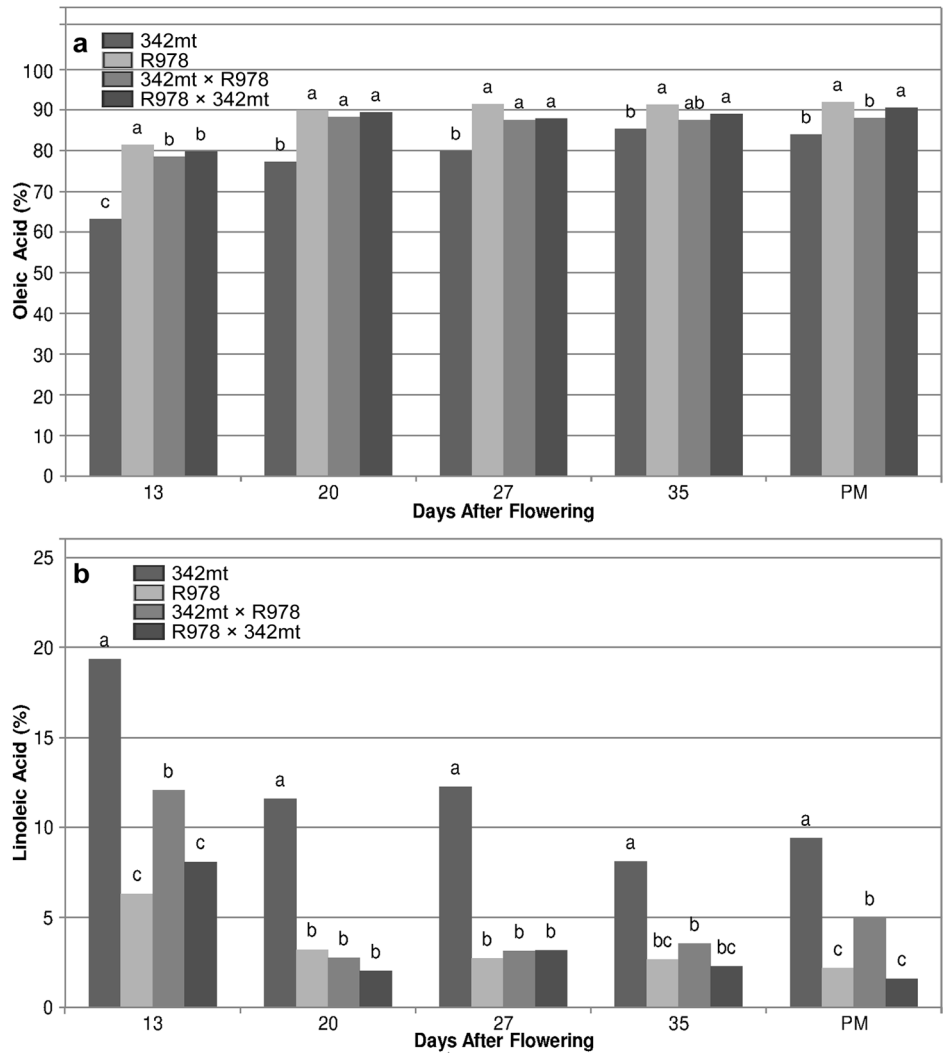


Figure 1: Oleic (a) and linoleic (b) fatty acids accumulation in lines and hybrids on the first sowing date.

DISCUSSION

The composition of fatty acids showed some difference among inbred lines. Line R978 was insensitive to environment and had the same composition through sowing dates. On the other hand, fatty acids composition in line 342mt varied through sowing date: oleic acid content increased with temperature (Table 1 and Table 2). Temperature was the main environmental difference among sowing dates;

other factors that may modify fatty acids composition, such as water and nitrogen, were kept constant. Different genetic background and modifier genes could cause differences between high oleic inbred lines (Lacombe *et al.*, 2004). Thus, some high oleic genotypes showed a response to temperature (Izquierdo and Aguirrezábal, 2008), while others did not (Lacombe and Berville, 2000; Lagravère *et al.*, 2000).

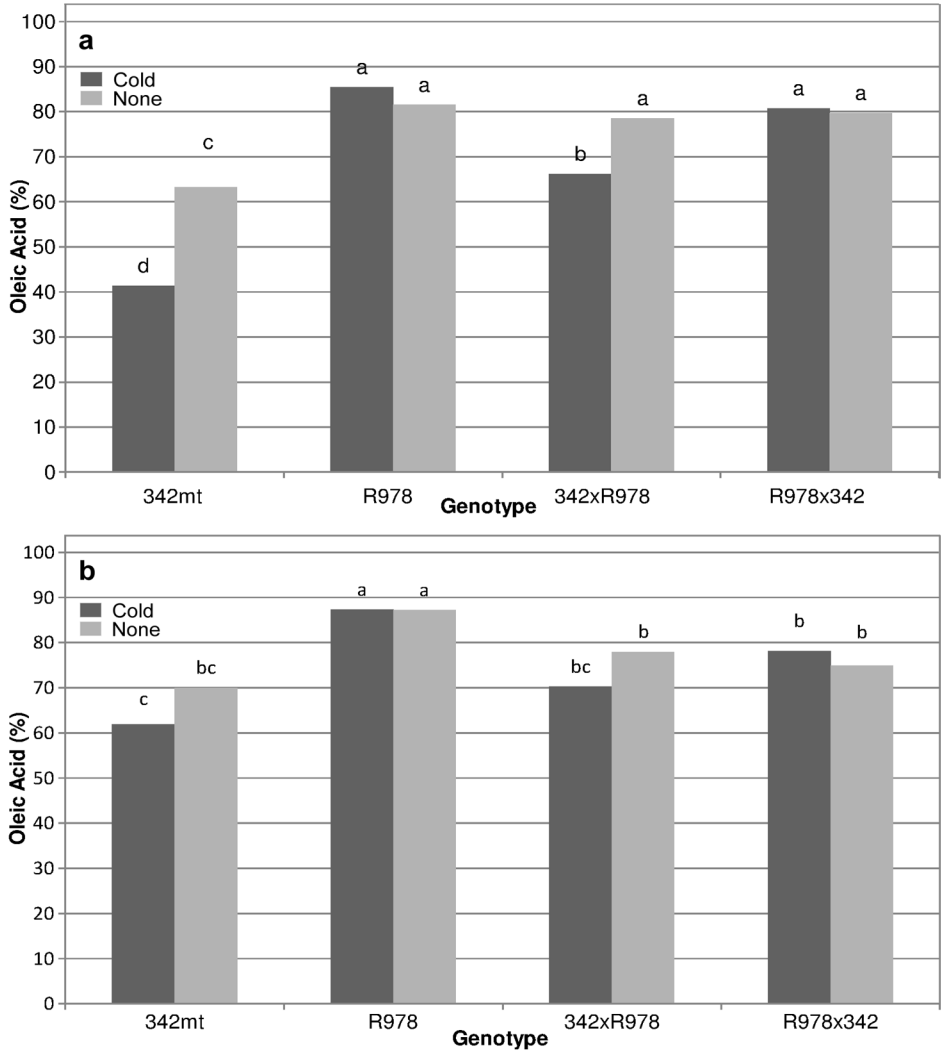


Figure 2: Low temperature (10°C) effects on oleic acid content at 13 DAF on the first (a) and second sowing date (b).

It seems that difference in oleic acid content at maturity is due to a diverse pattern of fatty acid accumulation and ODS activity and so to different metabolism in high oleic mutants (Figure 1). Different fatty acid metabolism in high oleic hybrids

was already found by Lagravère *et al.* (2004). At 13 DAF, line R978 showed an intermediate linoleic content among those reported by Lagravère *et al.* (2004). According to Martínez-Rivas *et al.* (2001) and Lagravère *et al.* (2004), two desaturase systems are involved in the biosynthesis of unsaturated fatty acids. The first one is a constitutive system present in the whole plant, and the second is especially devoted to storage metabolism in the achenes. During the first part of the seed filling period, the constitutive desaturase could be responsible for a low synthesis of linoleic acid in all kinds of hybrids. The specific desaturase is then involved in the accumulation of high quantities of linoleic acid in the standard hybrids, but its activity is lacking in high oleic mutants. We can suppose that 342mt and R978 are different for constitutive system. In 342mt there is a constitutive system more active or active for more days than in R978. This is confirmed by low temperature effect. At 13 DAF, R978 did not show any variation in oleic-linoleic ratio, while 342mt showed a strong response to low temperature (Figure 2). The low temperature-induced variations in lipid composition at 13 DAF are different between sowing date in line 342mt and 342mt \times R978 hybrid (Figure 2). It could be related to the environmental temperature that modifies the activity of the enzyme (Garcés *et al.*, 1992) and to a “memory effect” of early temperature regime on the fatty acid desaturation mechanism (Izquierdo *et al.*, 2002). Furthermore, Schlueter *et al.* (2007) found many oleate desaturase genes in soybean and they reported that some of these genes showed temperature-dependent changes in transcript accumulation in developing pod. A mechanism comparable with soybean may be proposed in sunflower.

Absence of temperature sensitivity from 20 DAF is due to the seeds' age (Garcés *et al.*, 1992).

It seems that temperature response during seed filling phase is related to a maternal effect. The maternal effect, small but significant, on oleic and linoleic acid contents, was detected in reciprocal hybrids plants. The difference was found on response to low temperature at 13 DAF that we assume depends on the constitutive desaturase. Within the second sowing date, environment could mask maternal effect and so reciprocal hybrids are equal. When parents displaying small differences in fatty acid composition are crossed, environment could easily mask any maternal effects (Gilsinger *et al.*, 2010). Different response magnitude between 342mt and its 342mt \times R978 hybrid could be related to a different duration of F to PM. It is important to note that the magnitude of the maternal effect could be the result of some number of morphological or physiological processes, all of which could be controlled by genes in the nucleus or cytoplasm (Gilsinger *et al.*, 2010).

CONCLUSION

High oleic inbred lines with different genetic background are not equal for oleic acid content and they show a different genotype by sowing date interaction. Inbred

line 342mt is sensitive to temperature, while inbred line R978 is not sensitive to temperature. Inbred lines showed a different fatty acid metabolism. An oleate desaturase is active in early seed filling phase in both lines. 342mt showed a greater ODS activity than R978. All these differences depend on different nuclear modifier genes and on maternal effect. For the first time a maternal effect on temperature response was found in high oleic sunflower. Maternal effect could modify oleic acid content and so the 90% threshold, a goal in a breeding program to develop high oleic hybrids for industrial use.

Further analysis is needed to understand the type of maternal effect and its magnitude in different genetic background and in different environments. We are going to study maternal effects and cytoplasmic inheritance using reciprocal backcrosses. We are going to study reciprocal segregants to obtain information about the persistence of reciprocal differences in later generations.

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