

## Maternal effect on response of oleic acid content to temperature in high oleic sunflower

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### ABSTRACT

- High oleic sunflower is currently used in the food sector and as raw material for non food application (biofuels, oleochemical). Non food applications, in particular, require stable oleic acid content and higher than 90%. Seed fatty acid composition depends on two factors that are: the genetic background of the embryo and the female plant condition (abiotic and biotic stresses). High oleic trait has been rarely studied in reciprocal crosses and in most of these cases, maternal effect on the content of unsaturated fatty acids have not been identified (except for soya and rapeseed). 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 x 342mt and 342mt x R978) obtained in 2010 by hand demasculation of female parent and controlled pollination. Field trials were conducted in 2011 on the experimental fields of the University of Udine using a complete randomization scheme using two dates of sowing. Plants were covered with paper bags during the R4 stage to prevent cross-fertilization. Plants were sampled, every seven days, from 13 DAF (Days After Flowering) to 35 DAF. Two types of samples were taken i) 5 seeds per plant; ii) pieces of sunflower head. Head pieces were incubated in a Petri dish with water for 24 hours at 10 °C to evaluate the response to low temperature. The fatty acids were determined by gas chromatography with a 60 m capillary column.
- Key results:
  - i) The two inbred lines respond differently to the same environmental conditions. R978 showed the same oleic acid content through sowing date, while 342mt showed a variation in oleic acid content of 3% between sowing date;
  - ii) Reciprocal hybrids showed a different accumulation pattern in the first sowing date and they showed the same response than their female parental. Reciprocal hybrids were equal in the second sowing date. 342mt x R978 hybrid showed a variation in oleic acid content of 4% from first to second sowing date. In the first sowing date, 342mt x R978 hybrid showed an increase of 1.4% in linoleic acid content from 35 DAF to PM (Physiological Maturity);
  - iii) There is a maternal effect in the early stages of seed filling phase. Low temperature increased linoleic acid and decreased oleic acid at 13 DAF only in 342mt inbred line and in 342mt x R978 hybrid. Low temperature treatment did not cause any variation on oleic acid content in line R978 and R978 x 342mt hybrid.
- High oleic inbred lines with different genetic backgrounds respond differently to the same environmental conditions. There is an activity of a  $\Delta 12$ -desaturase in the early stages of accumulation of reserve lipids in one line and this is inherited from the mother plant.
- It was determined 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 application (biofuels, oleochemical). Non food applications, in particular, require stable oleic acid content and higher than 90% (Vannozzi, 2006). In a breeding program targeted to obtain hybrids with an oleic acid content higher than 90% 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 that are: the genetic background of the embryo and the female plant condition (abiotic and biotic stresses).

High Oleic 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 literature different conclusions are reported on the number of genes that control 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: none 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: i) to check if any maternal effect on oleic acid content is present in high oleic mutants and (ii) 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. Inbred lines used 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 full branched type and it is a fertility restorer. Reciprocal cross (F1 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 (Schneiter and Miller, 1981), to prevent cross-fertilization. The reciprocal F1 seed was planted in field in 2011 and tested with parental.

### Field experiment

Inbred lines and their reciprocal hybrids (F1 hybrids plants) were grown in 2011 at 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<sup>-2</sup>. Nitrogen was applied at 100 kg ha<sup>-1</sup>. 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 sampling 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. Seed were dried in an oven at 60 °C for 24 h. The final harvest was done at physiological maturity (R9; Schneiter 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 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  $ODS \text{ activity index} = \frac{\%18:2}{(\%18:2 + \%18:1)}$ , 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. Gas chromatograph was fitted with a 60 m HP-88 capillary column (Agilent Technologies, USA). Helium was used as 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 F1 plants are showed in Table 1. Later sowing date resulted in a shorter cycle, likely due to environmental differences during plant development. Within sowing date, reciprocal F1 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<br>(dd) | F-PM<br>(dd) | Tmin<br>°C | Tmean<br>°C | Tmax<br>°C |
|--------------|-------------|---------------|--------------|------------|-------------|------------|
| 342mt        | I           | 110           | 41           | 16.2       | 22.4        | 28.7       |
| R978         | I           | 113           | 41           | 16.1       | 22.5        | 29.0       |
| 342mt x R978 | I           | 123           | 53           | 16.2       | 23.2        | 30.5       |
| R978x 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 x R978 | II          | 108           | 42           | 16.7       | 23.8        | 31.4       |
| R978x 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 are some difference between the inbred lines and reciprocal hybrids.

Sowing date did not alter the oleic acid concentration in line R978 and in its R978 x 342mt hybrid. On the other hand, sowing date altered the oleic and linoleic acids concentration in line 342mt and in 342mt x 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 ODS index. In the first sowing date ODS activity was significantly higher than in the second sowing date only in 342mt and 342mt x R978. Reciprocal hybrids were not equal for unsaturated fatty acids content through sowing date.

The oleic acid accumulation showed some differences between R978, 342mt and hybrids. R978 and R978 x 342mt showed the same accumulation pattern for both sowing dates. 342mt and 342mt x R978 showed the same accumulation pattern of the others 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.

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 x 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 x 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 x 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 x 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).

In 342mt and in 342mt x 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 (Fig. 1). 342mt x R978 hybrid showed a continuous increase of linoleic acid accumulation (1.4% from 35 DAF to PM).

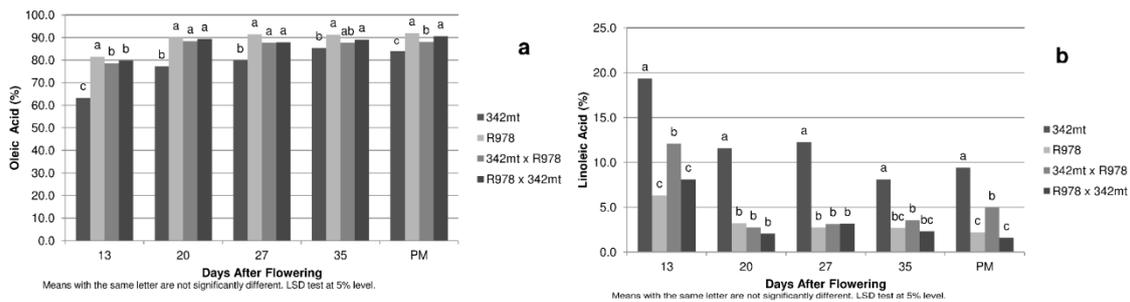


Fig. 1. Oleic (a) and linoleic (b) fatty acids accumulation in lines and hybrid in the first sowing date.

Cold treatment (10 °C for 24h) modified the fatty acid composition only at 13 DAF in line 342mt and 342mt x R978 hybrid (Fig. 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 in the second sowing date. The cold treatment on line R978 and hybrid R978 x 342mt did not modify oleic and linoleic acid content in the seeds incubated at 10 °C (Fig. 2).

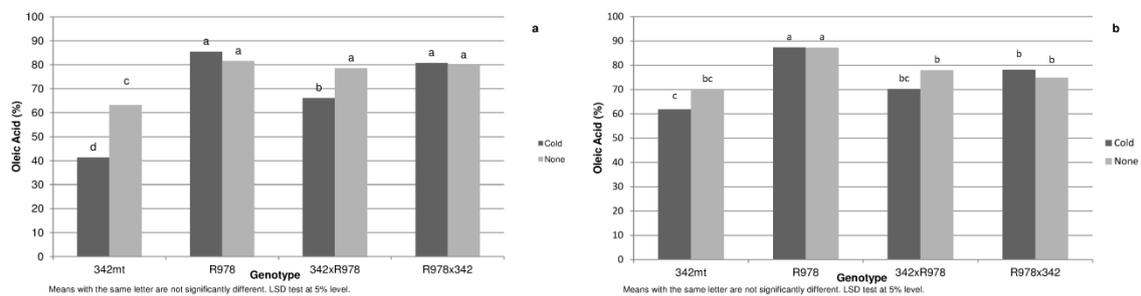


Fig. 2. Low temperature (10 °C) effects on oleic acid content at 13 DAF in the first (a) and in the second sowing date (b).

## DISCUSSION

The composition of fatty acids showed some difference among inbred lines. Line R978 was insensitive to environment and it had the same composition through sowing date. On the other hand, fatty acids

composition in line 342mt varies through sowing date: oleic acid content increases with temperature (Table 1 and Table 2). Temperature was the main environmental difference among sowing date; other factors that may modify fatty acids composition, as water and nitrogen, were kept constants. 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) and other did not show it (Lacombe and Berville, 2000, Lagravère et al., 2000).

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 (Fig. 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 lacked 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 (Fig. 2). The low temperature-induced variations in lipid composition at 13 DAF are different between sowing date in line 342mt and 342mt x R978 hybrid (Fig. 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 this genes show 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 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 suppose to depend by 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 x 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).

In conclusion, high oleic inbred lines with different genetic background are not equal for oleic acid content and they show a different interaction genotype by sowing date. Inbred line 342mt is sensitive to temperature, while inbred line R978 is not sensitive to temperature. Inbred lines show a different fatty acid metabolism. An oleate desaturase is active in early seed filling phase in both lines. 342mt show a greater ODS activity than R978. All this 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 are 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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