



## Sowing time and irrigation scheduling effects on seed yield and fatty acids profile of sunflower in semi-arid climate

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Received 23 March 2016; Accepted after revision 14 July 2016; Published online 17 January 2017

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### Abstract

A research was conducted in Southern Italy to study the changes in seed yield, oil content and fatty acids composition, in response to irrigation at different stages of growing season (at sowing, Dry; from sowing to beginning of flowering, S-BF; from visible bud to 75% flowering, VB-FF; from beginning of flowering to seed ripening, BF-M; from sowing to seed ripening, Full), in a standard sunflower hybrid sown at normal (April) and late (June) time. High seed yield and oil content combination resulted in the significantly greatest oil yield in 'Full' water regime. MUFAs (mostly oleic acid) were higher in late sowing and they benefit from good soil water availability of 'Full' regime. Contrastingly, PUFAs (*i.e.* linoleic acid content), higher in late season, was depressed by good soil availability during the early growing season (S-BF and S-M regimes). Irrigation at critical stages (*i.e.* flowering) may alleviate the negative impact of water stress upon crop productivity and oil fatty acids composition, resulting in yield and oil quality benefits besides a water saving. Late sowings allow cultivating sunflower as a catch crop, with an increased economic and environmental sustainability of Mediterranean farming systems.

**Keywords:** *Helianthus annuus* L.; Sowing time; Deficit irrigation.

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### Introduction

Sunflower (*Helianthus annuus* L.) is one of the most widespread oilseed crops, which combines high seed yields to great adaptability to a wide range of geographical areas. Its oil represents an important energy source in human diet. The nutritional value of the oil depends on the high concentration of C:18 fatty acids, with oleic and linoleic together accounting for approx. 90% of total fatty acids content. It is well known that a diet rich in mono-unsaturated fatty acids reduces the level of cholesterol associated with low-density lipoproteins (bad cholesterol), as compared to a diet rich in saturated fatty acids, thus reducing the risk of coronary heart diseases (Baldini et al., 2002; Flagella et al., 2002).

Sunflower is a warm-season crop whose cultivation in spring-summer period imposes irrigation in semi-arid Mediterranean areas, where rainfall from May to August is rare. In these conditions, however, the sustainable use of the water resource and the adoption of irrigation strategies that maximise water use efficiency maintaining satisfactory yields, have become a priority (Pereira et al., 2002).

Recently, conventional deficit irrigation (DI) has been proposed to improve water use efficiency. DI is as a water-saving irrigation strategy, which imposes a certain level of water stress to a crop either during a particular period or throughout the whole growing season, with the expectation that any yield reduction is negligible compared to the water benefits gained from the water saving (Eck et al., 1987). However, DI involves an appropriate scheduling of irrigation, because crop sensitivity to water deficit changes with the phenological stages (Istanbulluoglu, 2009).

Although sunflower provides the highest seed yield under full irrigation (Unger, 1983; Flagella et al., 2002; Anastasi et al., 2010), it has been demonstrated that even limited irrigation applied at specific growth stages may have a positive impact on seed yield (Unger, 1983). Indeed, flowering and seed filling stages have been reported as the most critical for water stress in sunflower (Iqbal et al., 2005). Hence, sunflower may become an interesting crop for drought-prone environments. However, the profitability of a sunflower crop primarily depends on the quality of the oil produced in terms of fatty acids composition (Mirshekari et al., 2012).

Raises of oleic acid content in sunflower oil in response to water deficit from flowering to physiological maturity have been reported by Flagella et al. (2002). By contrast, Anastasi et al. (2010) noticed an increase in the percentage of the same fatty acid as water availability increases, whilst Salera and Baldini (1998) observed no effect of water management on this monounsaturated fatty acid. Such contrasting results may be ascribed to the different genotypes, irrigation management and environmental conditions.

Oil composition is greatly affected by the crop agronomic management as well as the environmental conditions, taking into account that the cropping season of sunflower in Southern Italy is spring-summer and that often high temperatures occur during the flowering and seed filling. Therefore, early or delayed sowings, besides irrigation, may exert a major role in determining sunflower seed yield and oil composition. In this regard, some literature reports yield losses in sunflower when sowing date is delayed, both in temperate (Abelardo and Hall, 2002) and subtropical environments (Bange et al., 1998). In turn, increases in oleic acid related to a delay in sowing time have been reported in sunflower in response to increasing temperature, which induces inhibition of oleate desaturase, the enzyme system responsible for the conversion of oleic into linoleic acid (Anastasi et al., 2000, Flagella et al., 2002).

In Italy, sunflower is commonly cultivated as main crop with spring sowing, but the feasibility to grow it as catch crop with summer sowing within cereal-based rotations, has been ascertained in semiarid areas of Southern Italy (Anastasi et al., 2000).

The purpose of this research is to examine the changes in seed yield, oil content and fatty acids composition, in response to irrigation at different stages of growing season, in a standard cultivar of sunflower at two different sowing times (spring and summer), in semi-arid environment.

## **Materials and Methods**

### *Field experiment*

The experiment was conducted in 2009 at Pozzallo (36° 44' N, 14° 51' E, 10 m a.s.l.), in Ragusa province, in South eastern Sicily (South Italy). The soil of the

experimental field (Calcixerollic Xerochrepts, USDA S.T.) had the following characteristics: clay 38.0%, sand 37.0%, silt 25.0%, organic matter 2.6%, pH 8.5, total N 1.6%, available P<sub>2</sub>O<sub>5</sub> 52.3 mg/kg, exchangeable K<sub>2</sub>O 325.0 mg/kg.

The standard hybrid Gloriasol of sunflower was sown at 6 plants/m<sup>2</sup> plant population (70 cm row distance). Before sowing, 100 kg/ha of P<sub>2</sub>O<sub>5</sub> (as superphosphate), 50 kg/ha of K<sub>2</sub>O (as potassium sulphate) and 50 kg/ha of N (as ammonium sulphate) were applied. Further 50 kg/ha of N (as ammonium nitrate) were distributed at floral bud stage.

### *Experimental design*

The experiment consisted of a split-plot experimental design with three replicates, where the following factors were studied: sowing time (April 7 and June 6, indicated as 'Normal' and 'Late' sowing, respectively) and irrigation regime (S or Dry, irrigation at sowing only; S-BF, irrigation from sowing to beginning of flowering; VB-FF, irrigation from visible bud to 75% flowering; BF-M, irrigation from beginning of flowering to seed ripening; S-M or Full, irrigation from sowing to seed ripening). The occurrence of onset of each phenological stage was recorded according to the phenological key proposed by Schneiter and Miller (1981) (Table 1). Water regime was assigned to the main plot and sowing time to the subplot of 16.8 m<sup>2</sup> (2.8 × 6 m). Drip system was used for irrigation.

In 'Full' irrigation treatment, water was supplied replenishing 100% of crop evapotranspiration-ET<sub>c</sub>; for the irrigation, the crop evapotranspiration (ET<sub>c</sub>) method (ET<sub>c</sub> = ET<sub>0</sub> × K<sub>c</sub>), was applied, according to soil-water balance as proposed by Doorenbos and Pruitt (1977). Reference ET (ET<sub>0</sub>) was estimated by means of a class-A evaporation pan and K<sub>c</sub> (crop coefficients) were used according to Doorenbos and Pruitt (1977): 0.4–0.7 from sowing to bud appearance; 0.7–1.1 from bud appearance to end of flowering; 1.1–0.8 from end of flowering to seed filling; 0.8–0.5 from seed filling to seed physiological maturity. The amount of water to supply at irrigation was that required to fill soil up to field capacity in the 0-50 cm soil depth, calculated by the following formula (Doorenbos and Pruitt, 1977):

$$V = 0.66 (FC - WP) \times \Phi \times D \times 10$$

where V is the water amount (mm), 0.66 is the fraction of readily available soil water permitting unrestricted evapotranspiration, FC is soil moisture at field capacity (33.1% of dry soil weight at -0.03 MPa), WP is soil moisture at wilting point (16.6% of dry soil weight at -0.15 MPa),  $\Phi$  is soil bulk density (1.2 g/cm<sup>3</sup>) and D is soil depth at which most roots are expected to develop, equal to 0.5 m (Anastasi et al., 2010) (Table 2).

In the other irrigation treatments, water was supplied using the same method. Besides, the amount of water at the first irrigation was calculated as follows:

$$V = (FC - SWC) \times \Phi \times D \times 10$$

where V is the water amount (mm), FC is soil water at field capacity (33.1% of dry soil weight at -0.03 MPa), SWC is soil water content at the time of the first irrigation (% of dry soil weight),  $\Phi$  is soil bulk density (1.2 g/cm<sup>3</sup>) and D is soil depth, equal to 0.5 m, as above mentioned.

Table 1. Onset of the main phenological stages (date and days after sowing-DAS) in sunflower according to sowing time.

| Phenological stage          | Normal sowing<br>(April 7) |     | Late sowing<br>(June 6) |     |
|-----------------------------|----------------------------|-----|-------------------------|-----|
|                             | Date                       | DAS | Date                    | DAS |
| Visible bud (VB)            | May 28                     | 51  | July 20                 | 44  |
| Beginning of flowering (BF) | June 22                    | 76  | August 5                | 60  |
| 75% flowering (FF)          | July 15                    | 99  | August 26               | 81  |
| Seed ripening (M)           | August 8                   | 123 | September 12            | 98  |

Table 2. Seasonal irrigation volume (mm) in relation to irrigation treatments in the two sowing times.

| Irrigation treatment | Description   | Normal sowing<br>(April 7) | Late sowing<br>(June 6) |
|----------------------|---|----------------------------|-------------------------|
| S (Dry)              | No irrigation following sowing                          | 114                        | 80                      |
| S-BF                 | Irrigation from sowing to beginning of flowering        | 300                        | 201                     |
| VB-FF                | Irrigation from visible bud to 75% flowering            | 391                        | 222                     |
| BF-M                 | Irrigation from beginning of flowering to seed ripening | 367                        | 227                     |
| S-M (Full)           | Irrigation from sowing to seed ripening                 | 599                        | 430                     |

### Field measurements

The following meteorological variables were recorded daily throughout the crop growing season: maximum and minimum air temperature, rainfall, class-A pan evaporation, using a data logger (CR10, Campbell Scientific, Inc. Logan, Utah, USA) located near the experimental field.

During the seed filling period, at three different dates for each sowing time (July 21, July 29 and August 5, for normal sowing; August 27, September 4 and September 12, for late sowing), seed samples from 4 plants per plot were sampled for seed weight measurement (after oven-drying the seeds at 70 °C until constant weight) and laboratory analysis.

Harvest was performed at the beginning of August (normal sowing) and in mid September (late sowing). At harvest, seed yield (t/ha) was evaluated on a test area of 5.0 m<sup>2</sup>. Then, oil yield (t/ha) was calculated from seed yield (t/ha) and seed oil content (%).

Water use efficiency (WUE, kg/m<sup>3</sup>) was also calculated as the ratio between seed yield at final harvest (kg/ha) and water used by the crop (irrigation water + rainfall) (ET, m<sup>3</sup>/ha).

### Seed oil content and fatty acid composition analyses

Representative undehulled seed samples were crushed and oil content was determined on a dry base according to the standard procedure (ISO 659), by extraction in a conventional Soxhlet apparatus using petroleum ether bp 40-60 °C. After the removal of

residual solvent by a rotary evaporator and dehydration over  $\text{Na}_2\text{SO}_4$ , the lipid extracts were filtered.

The fatty acid profile (FAs) of the oil was assessed following the official methodologies (ISO 5508 and 5509) to prepare methyl esters (FAMEs) and to perform gas chromatographic analysis using a HRGC Mega 2 system (Carlo Erba Instruments, Milano, Italy) equipped with flame ionization detector (FID). The stationary phase and operative conditions applied were described in details by Anastasi et al. (2000, 2010). The concentration of each compound was expressed as a percentage of the area under the corresponding peak respect to the total area of picks. Moreover, FAs categories, such as saturated (SFAs), unsaturated (UFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) were calculated and the ratio oleic/linoleic and those between the different FAs categories were derived.

### *Statistical analysis*

Data of seed yield, seed oil content, oil yield and oleic and linoleic acid content, as well as those of WUE were subjected to the Bartlett's test for homoscedasticity and then statistically analyzed by a two-way analysis of variance (ANOVA) using CoStat version 6.003 (CoHort Software, Monterey, CA, USA). The analysis of variance was conducted considering 'sowing time' as random factor and 'irrigation treatment' as fixed factor.

Differences between means were evaluated using the Student-Newman-Keuls (S.N.K.) test, according to Snedecor and Cochran (1989).

Data of seed and oil yields, oil content and FAs concentration pooled across water regimes and sowing times were regressed against ET, as total (whole growing season) or up to start of flowering, using SIGMAPLOT 9.0 (Systat Software Inc., San Jose, California, USA).

## **Results**

### *Weather conditions*

The local climate is typically semi-arid Mediterranean, with mild winters and hot rainless summers. The long-term (twenty years) maximum monthly temperature from April to September ranges between 20.5 and 31.9 °C and the minimum between 10.1 and 20.4 °C; mean rainfall in April is 32.7 mm whilst from May to August it does not exceed 22 mm. Mean rainfall in September is approx. 62 mm (Table 3). Long-term evaporation ranges between 3.2 mm (April) and 5.6 mm (July). The meteorological data recorded during the crop growing season somehow reflected those of long-term period.

In April only, temperatures (both maximum and minimum) were cooler respect those of long-term period. Infact, maximum temperatures during the growing period (April-September) ranged from 17.9 (mid April) to 32.6 °C (late August), those minimum from 6.4 (mid April) to 20.5 °C (early August). After August, both maximum and minimum temperatures shifted down to 27.2 °C ( $T_{\max}$ ) and 18.9 °C ( $T_{\min}$ ) in late September (Table 3). Daily evaporation exceeded 4 mm from late June onwards, peaking at 8.9 mm in late August. Rainfall was scarce during summertime (<35 mm from late May to early September); that at the end of September (approx. 20 mm) was not useful since at that time the crop season was at the end.

The crop intercepted different weather conditions depending on sowing time. In particular, during S-BF period, the plants of normal sowing met average min and max temperatures approx. 6 °C lower than those of late sowing. At FF stage, air temperature was warmer (+6 °C for  $T_{\min}$  and +5 °C for  $T_{\max}$ ) for plants sown in June, but later on, differences in thermal conditions during seed development and filling in plants of the two sowing times became smaller (<1 °C).

Table 3. Mean of ten-day maximum ( $T_{\max}$ ) and minimum ( $T_{\min}$ ) air temperature and reference evapotranspiration ( $ET_0$ ) and sum of ten-day rainfall recorded during the experiment, and long-term (twenty years) monthly values for the same period.

|                                 | Ten-day period     | Month |      |      |      |        |           |
|---------------------------------|--------------------|-------|------|------|------|--------|-----------|
|                                 |                    | April | May  | June | July | August | September |
| $T_{\max}$<br>(°C)              | I                  | 18.2  | 20.8 | 27.3 | 27.1 | 31.9   | 27.5      |
|                                 | II                 | 17.9  | 24.0 | 26.1 | 28.5 | 31.3   | 27.8      |
|                                 | III                | 22.2  | 24.7 | 27.5 | 29.8 | 32.6   | 27.2      |
| $T_{\min}$<br>(°C)              | I                  | 8.5   | 9.4  | 15.7 | 16.6 | 20.5   | 15.9      |
|                                 | II                 | 6.4   | 11.6 | 15.1 | 16.7 | 20.4   | 17.3      |
|                                 | III                | 9.3   | 12.8 | 15.5 | 19.8 | 20.0   | 18.9      |
| $ET_0$<br>(mm d <sup>-1</sup> ) | I                  | 2.1   | 2.4  | 4.3  | 4.2  | 6.9    | 4.6       |
|                                 | II                 | 3.2   | 2.9  | 3.9  | 4.6  | 6.4    | 5.1       |
|                                 | III                | 3.5   | 3.8  | 4.1  | 5.6  | 8.9    | 4.7       |
| Rainfall<br>(mm)                | I                  | 4.0   | 14.0 | 0    | 11.2 | 0      | 0         |
|                                 | II                 | 4.0   | 0    | 0.2  | 0    | 0      | 0         |
|                                 | III                | 0     | 15.4 | 7.8  | 0    | 0      | 18.2      |
| Long term                       |                    |       |      |      |      |        |           |
|                                 | $T_{\max}$<br>(°C) | 20.5  | 24.4 | 28.7 | 31.7 | 31.9   | 28.6      |
| $T_{\min}$<br>(°C)              |                    | 10.1  | 12.8 | 16.4 | 19.4 | 20.4   | 18.5      |
| $ET_0$<br>(mm d <sup>-1</sup> ) |                    | 3.2   | 4.3  | 5.2  | 5.6  | 5.0    | 3.7       |
| Rainfall<br>(mm)                |                    | 32.7  | 9.0  | 3.9  | 2.1  | 6.7    | 61.5      |

### Length of growing season

Growing season was affected by sowing date, being approx. a month longer in normal sowing (Figure 1). The difference in the length of growing season is to ascribe to longer vegetative period in plants sown in April, whose flowering took place 76 days after sowing-DAS (against 60 DAS for plants of late sowing). Differently, sowing time had no relevant effects upon the length of the phenological stages following the onset of flowering.

Water regime, as well, affected crop phenology, but if flowering occurrence did not much differ in relation to irrigation, seeds of well watered plants ripened 15 (in normal sowing) to 9 (in late sowing) days later than those receiving water at sowing only, as a consequence of an extended ripening period. Moreover, an early cut of irrigation at beginning of flowering shortened (9 to 6 days in normal and late sowings, respectively) the growing season as compared to late irrigation regime (from flowering onwards).

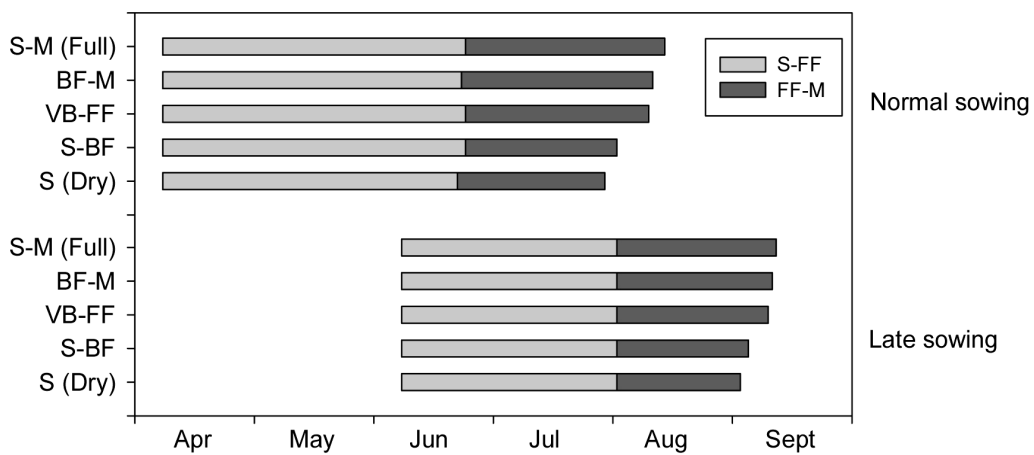


Figure 1. Length of growing season in sunflower in relation to sowing time and irrigation treatment (S= sowing, FF= 75% flowering, M= seed ripening).

### Seed yield

Seed yield was not affected by sowing time. Contrastingly, great yield differences were found in relation to the irrigation (Table 4). Seed yield was maximum under full irrigation for the whole growing season and minimum under dry conditions. Although receiving a similar amount of water, VB-FF and BF-M water regimes produced a different seed yield, which was 37% higher in VB-FF. At the same time, S-BF and VB-FF regimes, although receiving different amounts of water (23% less in S-BF), provided similar yields. These results indicate that in productive terms the crop benefits from irrigation up to flowering more than from flowering onwards. However, ANOVA highlighted a significant interaction *sowing time* × *irrigation treatment*. Indeed, yield differences between the two sowing times were relevant (seed yield more than 40% greater in normal sowing) in S-BF and VB-FF water regimes, indicating that irrigation up to flowering magnified the sowing time effect on seed yield. Contrastingly, no yield differences in relation to sowing time were observed for the dry control.

### Seed oil content

Seed oil content significantly decreased from 50.9 to 45.0%, on average, delaying sowing from normal to late time. Irrigation as well affected seed oil content, which was the highest (>50%) in well-irrigated treatments during seed filling period ('BF-M' and 'Full'), although significant interactions were observed. Indeed, if sowing time had no effect on seed oil content in 'Full' water regime, irrigation interrupted at early flowering (S-BF) led to a 17% seed oil content decrease (from 51.2 to 42.4%) in late crop. According to Johnson and Jellum (1972), this was a probable effect of a warmer course of maximum temperatures (up to 32.6 °C in late August) met by plants of late sowing during seed filling, which combined to a restricted water availability (no irrigation after start of flowering) depressed seed lipogenesis. Differently, continuous water supply to the crop under full irrigation may have compensated to some extent for the negative effects of temperature.

### Oil yield

Oil yield was significantly higher with normal sowing as a result of greater seed oil content (Table 4). High seed yield and high oil content combination also resulted in the significantly greatest oil yield (2.47 t/ha) of crop irrigated for the whole growing season. Nevertheless, the highest seed oil content of BF-M water regime did not compensate for the lowest seed yield and as a result, oil yield was quite low. However, the overall lowest oil yields (<1 t/ha) was found in dry control. Sowing time and water regime significantly interacted on final oil yield. Indeed, wider oil yield differences between sowing times (down to -43% yield decrease in late sowing time) were highlighted in S-BF and VB-FF than in BF-M.

Table 4. Seed yield, seed oil content and oil yield in sunflower as affected by sowing time and irrigation treatment (NS: normal sowing; LS: late sowing). Different letters for the main effects indicate significant differences at  $P \leq 0.05$  by S.N.K.

| Irrigation treatment                 | Seed yield (t/ha) |                   |                   | Oil content (%)   |                   |                    | Oil yield (t/ha)  |                   |                    |
|--------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|--------------------|
|                                      | NS                | LS                | average           | NS                | LS                | average            | NS                | LS                | average            |
| S (Dry)                              | 2.20              | 1.93              | 2.06 <sup>d</sup> | 48.7              | 41.5              | 45.1 <sup>c</sup>  | 1.08              | 0.79              | 0.93 <sup>d</sup>  |
| S-BF                                 | 3.98              | 2.77              | 3.37 <sup>b</sup> | 51.2              | 42.4              | 46.8 <sup>bc</sup> | 2.03              | 1.16              | 1.58 <sup>bc</sup> |
| VB-FF                                | 4.21              | 2.96              | 3.53 <sup>b</sup> | 49.6              | 46.0              | 46.7 <sup>bc</sup> | 2.10              | 1.36              | 1.69 <sup>b</sup>  |
| BF-M                                 | 3.07              | 2.71              | 2.58 <sup>c</sup> | 54.4              | 48.2              | 51.3 <sup>a</sup>  | 1.66              | 1.30              | 1.48 <sup>c</sup>  |
| S-M (Full)                           | 5.42              | 4.49              | 4.95 <sup>a</sup> | 51.0              | 49.1              | 50.0 <sup>ab</sup> | 2.76              | 2.20              | 2.47 <sup>a</sup>  |
| average                              | 3.78 <sup>a</sup> | 2.97 <sup>a</sup> |                   | 50.9 <sup>a</sup> | 45.0 <sup>b</sup> |                    | 1.90 <sup>a</sup> | 1.35 <sup>b</sup> |                    |
| LSD <sub>int</sub> ( $P \leq 0.05$ ) | 0.36              |                   |                   | 4.13              |                   |                    | 0.23              |                   |                    |



### Seed weight accumulation

Seed weight was measured during seed filling period at three dates for each sowing time (29, 37 and 44 days after start of flowering-DAF, for normal sowing and 22, 30 and 38 DAF, for late sowing). Seed was the heaviest under full irrigation (68 and 48 mg for the normal and late sowings, respectively) (Figure 2). Seeds produced with normal sowing had a greater weight than those of the late one. Seed filling course varied with water regime as well. Overall maximum weight accumulation in normal sowing occurred within 30 to 37-day interval after start of flowering and this was more evident in fully irrigated conditions for the whole crop season. Early irrigation from visible bud to full flowering, as well, had beneficial effects on seed weight, whose final value did not significantly differ from that maximum (in S-M regime, 68.1 mg). Differences in final seed weight within water regimes were minimized when sowing was delayed.

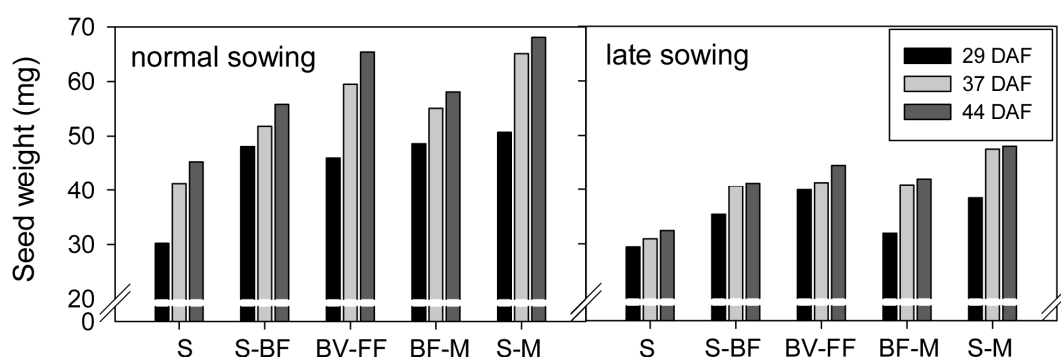


Figure 2. Seed weight at three dates of growing season in sunflower in relation to sowing time and irrigation treatment (DAF= days after start of flowering).

### Fatty acids content

FAs composition was affected by sowing time and water regime. SFAs (mostly palmitic and stearic acids) did not differ with growing season, but were greater under dry and S-BF water regimes (Tables 5 and 6). MUFAs (mostly oleic acid) benefit from irrigation for the whole season. In particular, oleic acid, whose content was 11% higher in seeds of normal sown than those of late sown, exceeded 30% in S-M water regime only, with normal sowing (Table 7). Contrastingly, PUFAs content (*i.e.* linoleic acid content), higher in late sown, was depressed by good water availability during the early stages of growing season (S-BF and S-M regimes). Consequently, an early soil water deficit minimized the MUFAs/PUFAs ratio, whilst good water availability for the whole season maximized the ratio. Water availability also affected the SFAs/UFAs ratio, whose value was higher when an early cut of irrigation (Dry and S-BF regimes) was applied. Oleic and linoleic acids were almost complementary, their sum being rather constant, with both sowing times. However, O/L ratio, greater with normal sowing, shifted to higher or lower values according to the variation in oleic and linoleic acid content with changing water availability, picking in seeds of the fully irrigated crop.

Other FAs, were found in amounts that, excluding elaidic acid in the dry control, never exceeded 1%.

Table 5. Content of the major FAs (%) in the extracted oil of sunflower as affected by sowing time and irrigation treatment.

| Fatty acid           | Irrigation treatment |       |       |       |            |
|----------------------|----------------------|-------|-------|-------|------------|
|                      | S (Dry)              | S-BF  | VB-FF | BF-M  | S-M (Full) |
| <i>Normal sowing</i> |                      |       |       |       |            |
| Miristic C14:0       | 0.08                 | 0.07  | 0.06  | 0.05  | 0.05       |
| Palmitoleic C16:1    | 0.14                 | 0     | 0.09  | 0.08  | 0          |
| Palmitic C16:0       | 7.40                 | 6.58  | 6.56  | 6.61  | 6.53       |
| Linoleic C18:2       | 65.93                | 58.80 | 59.60 | 60.80 | 57.40      |
| Oleic C18:1          | 21.20                | 28.80 | 28.78 | 27.30 | 31.00      |
| Elaidic C18:1        | 1.08                 | 0.69  | 0.72  | 0.75  | 0.61       |
| Stearic C18:0        | 2.66                 | 2.94  | 2.87  | 2.59  | 2.78       |
| Gadoleic C20:1       | 0.17                 | 0.17  | 0.20  | 0.21  | 0.22       |
| Arachic C20:0        | 0.31                 | 0.44  | 0.25  | 0.32  | 0.28       |
| Behenic C22:0        | 0.76                 | 0.85  | 0.65  | 0.89  | 0.87       |
| Lignoceric C24:0     | 0.27                 | 0.66  | 0.23  | 0.40  | 0.26       |
| <i>Late sowing</i>   |                      |       |       |       |            |
| Miristic C14:0       | 0.06                 | 0.06  | 0.05  | 0.06  | 0.06       |
| Palmitoleic C16:1    | 0.16                 | 0.14  | 0.06  | 0.12  | 0.09       |
| Palmitic C16:0       | 6.97                 | 7.08  | 6.20  | 6.61  | 6.39       |
| Linoleic C18:2       | 66.50                | 61.80 | 66.00 | 63.20 | 60.90      |
| Oleic C18:1          | 20.84                | 25.80 | 23.19 | 25.94 | 27.00      |
| Elaidic C18:1        | 1.16                 | 0.91  | 0.92  | 0.65  | 0.88       |
| Stearic C18:0        | 2.83                 | 2.92  | 2.29  | 2.14  | 2.86       |
| Gadoleic C20:1       | 0.16                 | 0.13  | 0.15  | 0.14  | 0.26       |
| Arachic C20:0        | 0.26                 | 0.35  | 0.25  | 0.25  | 0.33       |
| Behenic C22:0        | 0.74                 | 0.35  | 0.56  | 0.57  | 0.84       |
| Lignoceric C24:0     | 0.32                 | 0.46  | 0.33  | 0.32  | 0.39       |

Table 6. FAs categories, ratios between oleic and linoleic acids and between FAs categories of extracted oil in sunflower (S=saturated; U=unsaturated; M=monounsaturated; P=polyunsaturated).

| Ratio/Index          | Irrigation treatment |       |       |       |            |
|----------------------|----------------------|-------|-------|-------|------------|
|                      | S (Dry)              | S-BF  | VB-FF | BF-M  | S-M (Full) |
| <i>Normal sowing</i> |                      |       |       |       |            |
| SFAs                 | 11.48                | 11.54 | 10.62 | 10.86 | 10.77      |
| UFAs                 | 88.52                | 88.46 | 89.38 | 89.14 | 89.23      |
| MUFAs                | 22.59                | 29.66 | 29.78 | 28.34 | 31.83      |
| PUFAs                | 65.90                | 58.80 | 59.60 | 60.80 | 57.40      |
| SFAs/UFAs            | 0.13                 | 0.13  | 0.12  | 0.13  | 0.12       |
| MUFAs/PUFAs          | 0.34                 | 0.50  | 0.50  | 0.47  | 0.56       |
| Oleic/Linoleic (O/L) | 0.32                 | 0.49  | 0.48  | 0.45  | 0.54       |
| <i>Late sowing</i>   |                      |       |       |       |            |
| SFAs                 | 11.18                | 11.20 | 9.68  | 9.95  | 10.87      |
| UFAs                 | 88.82                | 88.78 | 90.62 | 90.05 | 89.13      |
| MUFAs                | 22.32                | 26.98 | 24.32 | 26.85 | 28.23      |
| PUFAs                | 66.50                | 61.80 | 66.30 | 63.20 | 60.90      |
| SFAs/UFAs            | 0.13                 | 0.13  | 0.11  | 0.11  | 0.12       |
| MUFAs/PUFAs          | 0.34                 | 0.44  | 0.37  | 0.42  | 0.46       |
| Oleic/Linoleic (O/L) | 0.31                 | 0.42  | 0.35  | 0.41  | 0.44       |

Table 7. Oleic and linoleic acid content in sunflower oil as affected by sowing time and irrigation treatment (NS: normal sowing; LS: late sowing). Different letters for the main effects indicate significant differences at  $P \leq 0.05$  by S.N.K.

| Irrigation treatment | Oleic acid (%)    |                   |                    | Linoleic acid (%) |                   |                    |
|----------------------|-------------------|-------------------|--------------------|-------------------|-------------------|--------------------|
|                      | NS                | LS                | average            | NS                | LS                | average            |
| S (Dry)              | 21.2              | 21.3              | 21.2 <sup>c</sup>  | 65.9              | 66.9              | 66.4 <sup>a</sup>  |
| S-BF                 | 28.8              | 25.8              | 27.3 <sup>ab</sup> | 58.8              | 61.8              | 60.3 <sup>bc</sup> |
| VB-FF                | 28.8              | 23.3              | 26.1 <sup>b</sup>  | 59.6              | 66.3              | 62.9 <sup>b</sup>  |
| BF-M                 | 27.3              | 26.0              | 26.6 <sup>ab</sup> | 60.8              | 63.5              | 62.2 <sup>b</sup>  |
| S-M (Full)           | 31.0              | 27.0              | 29.0 <sup>a</sup>  | 57.4              | 60.9              | 59.1 <sup>c</sup>  |
| average              | 27.4 <sup>a</sup> | 24.7 <sup>b</sup> |                    | 60.5 <sup>b</sup> | 63.1 <sup>a</sup> |                    |

'Irrigation x sowing time' interaction not significant.

### Oleic and linoleic acid accumulation rate

Oleic and linoleic acid contents were measured as the seed weight during seed filling. Oleic acid content (as % of oil content) progressively decreased with time irrespective of water regime and sowing time and if its level did not much vary with water regime a month after flowering started (1<sup>st</sup> sample) in normal sowing, two weeks later it was quite lower in unirrigated crop (Figure 3). Differently, in plants of late sowings the initial level of oleic acid slightly differed with water regimes, but at the end of season these differences became negligible.

Linoleic acid progressively increased as oleic acid decreased, thus with normal sowing its initial level was similar among water regimes but at the end of growing season it was the highest under no irrigation. With late sowing, an early water deficit induced by dry regime resulted in the overall highest levels of linoleic acid throughout the whole seed filling period.

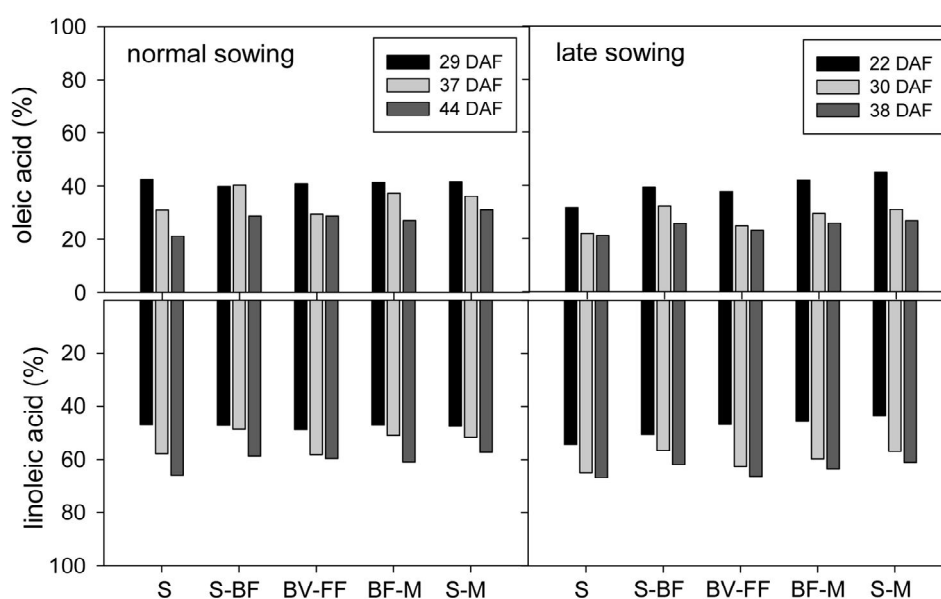


Figure 3. Oleic and linoleic acid content at three dates of growing season in sunflower in relation to sowing time and irrigation treatment (DAF= days after start of flowering).

### Relationships of FAs vs. ET

Seed and oil yield, oil content, FAs and their ratios, were regressed against ET (irrigation + rainfall) (Figure 4). These relationships reveal that seed yield linearly rises with the increase of water availability, especially when ET up to onset of flowering is considered (estimated increase = 8.1 kg/ha/mm). Strict positive relationships there were also for oil yield vs. ET ( $R^2 > 0.93$ ), whilst oil content seemed to be less influenced by water supplied to the crop, either up to flowering or for the whole growing season ( $R^2 < 0.5$ ). Oleic and linoleic acids exhibited opposite relationships when regressed against ET. In both cases, total ET had greater impact on their content and relative ratios (O/L and MUFAs/PUFAs) than water up to flowering. SFAs content did not respond to increasing water availability (not shown).

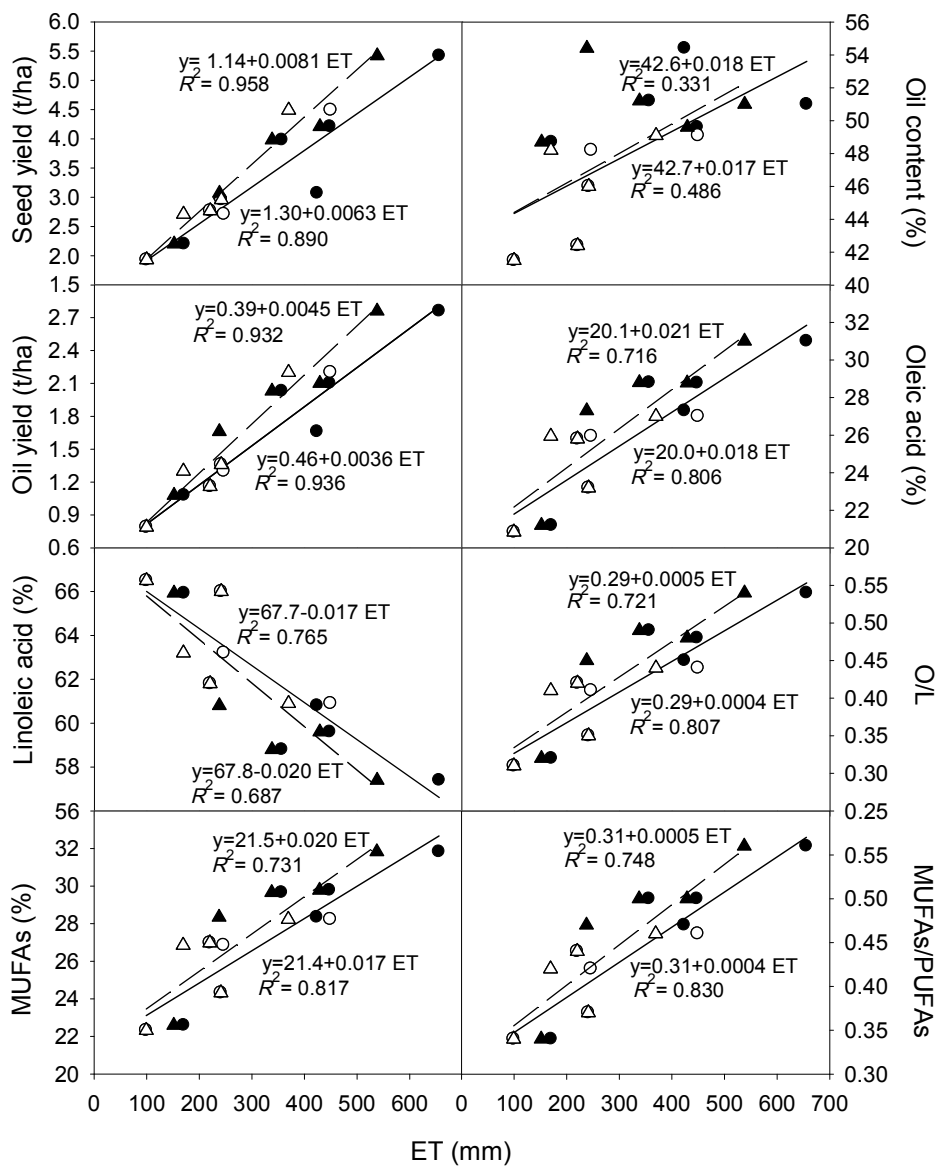


Figure 4. Relationships of seed yield, oil yield, oil content and FAs vs. ET (circle symbols, solid regression line: total ET; triangle symbols, long dash regression line: ET up to start of flowering; closed symbols: normal sowing; open symbols: late sowing).

## Discussion

Although sunflower is considered as a moderately drought tolerant plant (Stone et al., 2002), it revealed a sensitivity in productive terms to decreasing water availability, when cultivated in Mediterranean semiarid environment as that of the present study. The depressive effects of water deficit upon grain yield were more evident in the crop sown late (June) according to the significant *water regime* × *sowing time* interaction. Anastasi et al. (2010), under the same climatic conditions, found linear positive relationships of seed yield to increasing ET in standard and high oleic sunflower hybrids. However, in the present study, the possibility to save 35 to 48% water (in normal and late sowing time, respectively), by applying irrigation after sowing, solely during the period from bud appearance to full flowering (VB-FF regime), or even more (>50% water saving) stopping irrigation at early flowering (S-BF regime), has been demonstrated. Indeed, the two water regimes resulted in 22% (VB-FF) and 26% (S-BF) yield losses in normal sowing, and 34% (VB-FF) and 38% (S-BF) yield losses in late sowing. Some literature highlighted the importance of irrigation during grain filling in sunflower to attain satisfactory yields (Mirshekari et al., 2012). We observed that plants irrigated from flowering onward only (BF-M regime) were not as yielding as those irrigated earlier from bud appearance to full flowering only (VB-FF regime), although receiving a similar total amount of water, thus revealing how irrigation is beneficial for sunflower when applied during the early stages of growing season up to flowering (WUE 0.94 and 1.23 kg/m<sup>3</sup> for normal and late sowing, Table 8), whereas it has less effects when applied later after flowering (WUE 0.72 and 1.10 kg/m<sup>3</sup>), at least in terms of seed yield. The adverse effect of water deficit from budding to flowering stage on sunflower yield has been reported in literature (Pankovic et al., 1999). Similarly, Roshdi and Rezadoost (2005) observed that soil moisture deficit occurring during flowering decreases head diameter, which in turns leads to a decreased number of filled grains and to reduced seed yield. Anyway, sunflower has an explorative root system able to extract water deeply in the soil profile (Unger, 1984). Therefore, appropriate irrigation up to flowering allows the plant to grow well later on.

Table 8. Water use efficiency (WUE) in sunflower as affected by sowing time and irrigation treatment (NS: normal sowing; LS: late sowing). Different letters for the main effects indicate significant differences at  $P \leq 0.05$  by S.N.K.

| Irrigation treatment        | WUE (kg/m <sup>3</sup> ) |                   |                    |
|-----------------------------|--------------------------|-------------------|--------------------|
|                             | NS                       | LS                | average            |
| S (Dry)                     | 1.29                     | 1.95              | 2.06 <sup>a</sup>  |
| S-BF                        | 1.12                     | 1.26              | 1.62 <sup>b</sup>  |
| VB-FF                       | 0.94                     | 1.23              | 1.19 <sup>c</sup>  |
| BF-M                        | 0.72                     | 1.10              | 1.08 <sup>cd</sup> |
| S-M (Full)                  | 0.83                     | 0.66              | 0.91 <sup>d</sup>  |
| average                     | 1.25 <sup>b</sup>        | 1.49 <sup>a</sup> |                    |
| LSD <sub>int</sub> (P≤0.05) | 0.14                     |                   |                    |

Water plays a major role in regulating seed yield, but sowing time contributes to minimize the negative impact of water deficit, since the depressive effects of water deficit upon crop yield were less evident with early sowing in April (significant interaction *sowing time* × *water regime*). On the other hand, late sowing overall allowed 34% water saving over normal sowing, with 21% yield loss. Decreasing yields following delayed sowings have been reported by Kakani et al. (2002), mostly because of depressive effects of high temperatures upon the reproductive stages of the plant (inhibition of pollen germination and pollen tube growth). However, under the favourable climatic conditions of the experimental site, which is located near the sea, a shortening in growing season following a delayed sowing resulted in a reduced plant growth and dry matter accumulation (Barros et al., 2004). This fact, more than an adverse thermal course, may have accounted for the yield losses. Indeed, temperature during late grain filling was even cooler (approx. -3 °C) than that experienced by the crop in normal sowing.

Extended irrigation was beneficial for sunflower also in terms of oil content. According to the highest seed yield and oil content, oil yield was the highest under full irrigation. These results are consistent with those of literature (Unger, 1983; Mobasser and Tavassoli, 2013).

The oil produced under the thermal conditions of late sowing had a lower concentration of SFAs (stearic + palmitic), thus it is preferred to prevent cardiovascular diseases (Velasco and Fernandez Martinez, 2002). According to Ali et al. (2009), water stress imposed at different stages had no relevant effects on palmitic acid content, whilst it induced a slight rise in stearic acid content when occurring late in growing season (as in S-BF and VB-FF regimes of the present study). Roche et al. (2006) found a reduction of both MUFAs in different sunflower genotypes under irrigation.

Shifting sowing date from April to June resulted in a decreased oleic acid concentration. Indeed,  $\Delta$ -12 desaturase activity, responsible for the oleic vs. linoleic acid conversion (Izquierdo et al., 2006), is inhibited by high temperatures (>30 °C, Garces and Mancha, 1991), as those experienced during the filling stage by plants of normal sowing date. Izquierdo et al. (2006) found that a change in minimum night temperatures of at least +1 °C produced a 15% rise in oleic acid content. Therefore, with proper location and sowing date, a traditional hybrid (as the one of the present experiment) could approach oleic acid concentrations even close to those of typical mid-oleic hybrids.

According to Baldini et al. (2002), oleic acid and linoleic acid exhibited an inverse trend, with the first decreasing and the second increasing with water deficit imposed at different stages. Therefore, although temperature regime remains a major environmental factor in determining fatty acid profile in sunflower oil, soil water availability can play an important role. To this respect, whilst some literature reports a rise in oleic acid content in response to water stress (Flagella et al., 2002), in this experiment this FA was negatively affected by water deficit imposed at the different growth stages. Similarly, Baldini et al. (2002) reported a 15% reduction in oleic acid content under water stress conditions, in a standard hybrid of sunflower, but a 5% increase in a high oleic hybrid under the same soil water conditions. Roche et al. (2006) suggested a different reaction strategy of sunflower genotypes under soil water deficit, which involves an up-regulation or a down-regulation of desaturase in standard (as the one of this experiment) and oleic genotypes, respectively. This fact may account for the highest levels of linoleic acid under water limitation (Anastasi et al., 2010).

## Conclusions

This study indicates that, in semiarid environment, full irrigation maximizes both seed and oil yields and improves oil quality. In turn, water deficit induces remarkable yield losses and modifies oil quality. However, a timely application of irrigation at critical stages (*i.e.* flowering) of growing season may alleviate the impact of water deficit upon seed yield as well as on oil fatty acid composition, resulting in yield and oil quality losses that, compared to a fully irrigated crop, may be considered irrelevant as compared to the benefit in terms of water saving.

Normal sowings in early spring resulted in higher oil yields and lower oil insaturation. In turn, shifted sowing date to early June shortened growing season reduced seed oil content and determined some oil yield losses. However, late sowings allow cultivating sunflower as a catch crop increasing economic and environmental sustainability of farming systems in Mediterranean environment.

## References

- Abelardo, J.V., Hall, A.J., 2002. Effects of planting date, genotype and their interactions on sunflower yield: I. Determinants of oil-corrected grain yield. *Crop Sci.* 42, 1191-1201.
- Ali, Q., Ashraf, M., Anwar, F., 2009. Physico-chemical attributes of seed oil from drought stressed sunflower (*Helianthus annuus* L.) plants. *Grasas y Aceites.* 60, 475-481.
- Anastasi, U., Cammarata, M., Abbate, V., 2000. Yield potential and oil quality of sunflower (oleic and standard) grown between autumn and summer. *Ital. J. Agron.* 4, 23-36.
- Anastasi, U., Santonoceto, C., Giuffrè, A.M., Sortino, O., Gresta, F., Abbate, V., 2010. Yield performance and grain lipid composition of standard and oleic sunflower as affected by water supply. *Field Crop Res.* 19, 145-153.
- Baldini, M., Giovanardi, R., Tahmasebi-Enferadi, S., Vannozzi, G.P., 2002. Effects of water regimes on fatty acid accumulation and final fatty acid composition in the oil of standard and high oleic sunflower hybrids. *Ital. J. Agron.* 6, 119-126.
- Bange, M.P., Hammer, G.L., Richert, K.G., 1998. Temperature and sowing date affect the linear of sunflower harvest index. *Agron. J.* 90, 324-328.
- Barros, J.F.C., de Carvalh, M., Basch, G., 2004. Response of sunflower (*Helianthus annuus* L.) to sowing date and plant density under Mediterranean conditions. *Agron. J.* 21, 347-356.
- Doorenbos, J., Pruitt, W.O., 1977. Guidelines for predicting crop water requirements. In: *Irrigation and Drainage Paper No. 24.* FAO, Rome, 179p.
- Eck, H.V., Mathers, A.C., Musick, J.T., 1987. Plant water stress at various growth stages and growth and yield of soybeans. *Field Crop Res.* 17, 1-16.
- Flagella, Z., Rotunno, T., Tarantino, E., Di Caterina, R., De Caro, A., 2002. Changes in seed and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to sowing date and the water regime. *Eur. J. Agron.* 17, 221-230.
- Garces, R., Mancha, M., 1991. In vitro oleate desaturase in developing sunflower seeds. *Phytochemistry.* 30, 2127-2130.
- Iqbal, N., Ashraf, M.Y., Ashraf, M., 2005. Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage. *Int. J. Environ. Sci. Technol.* 5, 155-160.
- Istanbulluoglu, A., 2009. Effects of irrigation regimes on yield and water productivity of safflower (*Carthamus tinctorius* L.) under Mediterranean climatic conditions. *Agric. Water Manage.* 96, 1792-1798.
- Izquierdo, N.G., Aguirrezábal, L.A.N., Andrade, F.H., Cantarero, M.G., 2006. Modeling the response of fatty acid composition to temperature in a traditional sunflower hybrid. *Agron. J.* 98, 451-461.
- Johnson, B.J., Jellum, M.D., 1972. Effect of planting date on sunflower yield, oil and plant characteristics. *Agron. J.* 64, 747-748.
- Kakani, V.G., Prasad, P.V.V., Craufurd, P.Q., Wheeler, T.R., 2002. Response of in vitro pollen germination and pollen tube growth of Groundnut (*Arachis hypogaea* L.) genotype to temperature. *Plant Cell Environ.* 25, 1651-1661.

- Mirshekari, M., Hosseini, N.M., Amiril, R., Zandvakili, O.R., 2012. Study the effects of planting date and low irrigation stress on quantitative traits of spring sunflower (*Helianthus annuus* L.). Rom. Agric. Res. 29, 189-199.
- Mobasser, H.R., Tavassoli, A., 2013. Effect of Water Stress on Quantitative and Qualitative Characteristics of Yield in Sunflower (*Helianthus annuus* L.). J. Nov. Appl. Sci. 2, 299-302.
- Pankovic, D., Sakac, Z., Kcvrosan, S., Plesnicar, M., 1999. Acclimation to long term water deficit in the leaves of two sunflower hybrids: photosynthesis, electron transport and carbon metabolism. J. Exp. Bot. 50, 127-138.
- Pereira, L.S., Oweis, T., Zairi, A., 2002. Irrigation management under water scarcity. Agric. Water Manage. 57, 175-206.
- Roche, J., Bouniols, A., Mouloungui, Z., Barranco, T., Cerny, M., 2006. Management of environmental crop conditions to produce useful sunflower oil components. Eur. J. Lipid Sci. Technol. 108, 287-297.
- Roshdi, M., Rezadoost, S., 2005. Effect of different levels of irrigation on quantitative and qualitative characteristics of different cultivars of sunflower. Iran. J. Agric. Sci. 36, 1241-1250.
- Salera, E., Baldini, M., 1998. Performance of high and low oleic acid hybrids of sunflower under different environmental conditions. Note II. Helia. 21, 55-68.
- Schneiter, A.A., Miller, F., 1981. Description of sunflower stages. Crop Sci. 21, 901-903.
- Snedecor, G.W., Cochran, W.G., 1989. 'Statistical Methods', 8th ed. Iowa State University Press, Ames, Iowa.
- Stone, L.R., Goodrum, D.E., Schlege, A.J., Jaafar, M.N., Khan, A.H., 2002. Water depletion depth of grain sorghum and sunflower in the central high plains. Agron. J. 94, 936-943.
- Unger, P.W., 1983. Irrigation effect on sunflower growth, development and water use. Field Crop Res. 7, 181-194.
- Unger, P.W., 1984. Tillage and residue effects on wheat, sorghum and sunflower grown in rotation. Soil Sci. Soc. Amer. J. 48, 885-891.
- Velasco, L., Fernandez-Martinez, J.M., 2002. Breeding oilseed crops for improved oil quality. J. Crop Prod. 5, 309-344.