Effects of different water availability on fatty acid composition of the oil in standard and high oleic sunflower hybrids.

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Summary

During 1998 a trial was carried out, in lysimeters, in order to study the effects of different water regimes on fatty acid composition on high oleic and standard sunflower and the fatty acid accumulation kinetic. The water regimes adopted were: water layer 0.6 m, refunding of 100% of the evotranspiration (ET) and water stress, obtained by withholding the irrigation from flowering to maturity. Two hybrids were cultivated: UD12, characterised by high oleic content and UD87 as standard, both obtained by the Crop Production Department of Udine University. A randomised block design with two replications, was utilised and the main yield characteristics were evaluated at maturity. Achene samples were collected, every 6 days, from last anthesis until maturity in order to study the achene dry weight, oil and fatty acids accumulation. A significant increase of about 5% of oleic acid with respect to the other water regimes, was determined by water stress treatment in the high oleic hybrids. Probably, the water stress determined a reduction of the dry matter and oil accumulation phases, with all the enzyme activities involved, included the Δ-12 desaturase, responsible of the desaturation from oleic to linoleic acid.

Key words: sunflower, water regimes, water stress, high oleic, Δ-12 desaturase.

Introduction

The positive effects on human health from a diet rich in unsaturated fatty acids, and oleic acid in particular, have been well documented by numerous recent studies (Krajcovicova-Kudlakova *et al.*, 1997; Jing *et al.*, 1997; Berry and Rivlin, 1997; Kinter *et al.*, 1996; PoHuang *et al.*, 1996).

Much detailed research has examined the aspects concerning the genetic control of the high concentration of oleic acid in sunflower (Miller *et al.*, 1987; Fernandez-Martinez *et al.*, 1989), the biosynthesis of lipids, both in normal genotypes and high oleic mutants (Ohlrogge *et al.*, 1991; Ohlrogge and Browse, 1995), and the effect of temperature (Harris *et al.*, 1978; Goyne *et al.*, 1979) due to its well-known influence on the enzyme (oleoyl-phosphatidylcholine-desaturase or Δ12 – desaturase) considered responsible for the conversion of oleic acid into linoleic acid (Garces *et al.*, 1989; Garces and Mancha, 1989; 1991). However, little work has been performed to study the possible effects of other agronomic effects on the fatty acid composition, and in particular the effects of different water availability.

For this reason, the main objective of the present work was to study the influence of different water availability on the accumulation kinetics of various fatty acids in high oleic and conventional

sunflower hybrids under a north-east Italian cultivation environment and the effect of various water regimes on the final fatty acid.

Material and Methods

The experiment was performed in 1998 at the Experimental Farm of the University of Udine (46° 02' N, 13° 13' E and 110 m above sea level), utilising 12 rain shelter lysimeters. The main climatic characteristics, divided into the pre and post-flowering phases of the crop, were recorded with an automatic meteorological station located close to the experimental site. Table 1 shows the water regimes adopted.

Two sunflower hybrids were used, one characterised by a high oleic acid content (UD12) and the other a conventional hybrid (UD87), both having the same cultivation cycle and selected by the University of Udine. The crop was sown on 2 May 1998 at a density of 6 plants m^2. At flowering, all the heads of the high oleic hybrid were protected, to avoid pollen contamination.

A randomised block scheme with two repetitions was adopted (2 genotypes x 3 irrigation regimes x 2 replicates).

After harvesting, the achenes were oven dried and used for the following determinations:

- Total evotranspiration (mm);
- Water use efficiency for achene yield (g s.s. l^{-1})
- Production of achenes per unit of surface area (g m^{-2});
- Unit weight of the achenes (mg);
- Number of full achenes per plant (n°);
- Achene oil content (%), with the NMR method (Nuclear Magnetic Resonance).

Starting from 2 days after the end of flowering (F4 stage) and up to physiological maturity (M2 stage), (Merrien; 1986), six samples of 10 achenes were collected from each treatment, every six days. Each sample was taken from four different plants, always from the external part of the head and, once collected, the achenes were immediately dried in an oven and held in a cold store (4°C) until the end of the experiment.

The following determinations were performed on each of the samples:

- Unit weight of the achenes (mg);
- Oil content, using the method adopted by Champolivier and Merrien (1996), (%);
- Concentration of the main fatty acids, using the esterification method and gas-chromatography as described in detail by Fernandez *et al.*, (1999), (%).

The data obtained were subjected to statistical analysis by ANOVA and, to separate the mean values of the treatments when significant in the F test, Duncan's test was performed at a level of significance of $P \leq 0.05$.

Results and Discussion

Figure 1 represents the dry matter accumulation kinetic for the achenes during the maturation period. At the time of the first sampling, all the values refer to the tissues of the hull, as the embryo had not yet formed. The most significant increase in dry matter was observed between 8 and 14 days from the end of flowering, while between 20 and 28 days the process of accumulation of dry matter in the achene could be considered to have terminated.

Figure 2 shows the rapid increase in the accumulation of oil in the achene between the 8^{th} and 14^{th} day after fertilisation, in correspondence with a significant increase in the achene dry matter (Fig.1). In correspondence with the 20^{th} day from the end of flowering, all the treatments had reached their maximum level of oil accumulation, confirming the results obtained by Champolivier and Merrien (1996).

Figure 3 shows the accumulation kinetics of the main fatty acids in the sunflower oil from a high oleic and a normal hybrid.
The evolution of palmitic acid appeared similar in the two hybrids studied, with a slightly higher final quantity in the normal hybrid (5.5%) than in the high oleic hybrid (3%). The concentration of this acid reduced in the period between the 8^{th} and 14^{th} day after end of flowering (a time in which the level of palmitic acid in the seed appeared to stabilise) by 50% in the normal hybrid and 33% in the high oleic hybrid (Fig.3).
The stearic acid content appeared practically identical in the two hybrids at maturity (4.1 and 4.3 % in the high oleic and normal hybrids, respectively). This acid increased from the 2^{nd} to the 8^{th} day from the end of flowering, probably due to the fact that the embryo had already begun to form in this period, with values which tended to differentiate from those present in the hull.
From the 8^{th} day, with the increase in the biosynthesis of the oil, the enzyme $\Delta 9$ – desaturase started to be active; this enzyme has been proposed as being responsible for the formation of oleic acid (C18:1) by desaturating stearic acid (C18:0). This fact was clearer in the normal hybrid, where in addition to a greater increase in oleic acid there was a simultaneous reduction in the content of linoleic acid (Fig.3). Later, starting from the 14^{th} day, the action of the enzyme $\Delta 12$ –desaturase was clear, with an increase in linoleic acid (C18:2) through the desaturation and respective reduction of oleic acid (Fig.3). In the normal hybrid, the ratio between these two fatty acids stabilised around the 28^{th} day from the end of flowering, with a value very close to unity, as the percentages of the two fatty acids were very similar (44.2 and 45.3%, respectively; Fig.3). These percentages, apparently high for a normal hybrid, were similar to the values obtained in the same environment in previous research (Fernandez *et al.*, 1999) and in experiments performed in controlled environments with similar temperature (Champolivier and Merrien, 1996).
In correspondence with the first samples, the values of oleic and linoleic acid were more similar in the two hybrids than at the end of the experiment (Fig.3). This result can essentially be attributed to two causes: the first is that in sunflower oleic acid is the principal component of the pericarp tissues in both types of hybrid (Graces *et al.*, 1989) and the second is that in high oleic mutants the inhibition of the activity of the enzyme $\Delta 12$ –desaturase, due to a different arrangement of the nucleotide sequence related to the OL locus in the transcription of the gene responsible (Hongtrakul *et al.*, 1998), is not immediate or instantaneously associated with the time of fertilisation. The definitive and constant levels for these two latter fatty acids are, in fact, only reached between the 14^{th} and 20^{th} day after fertilisation (Fig.3).
The presence of a shallow water table (water table) and the complete refunding of evotranspiration (100% ET) treatments gave the best production results for the two hybrids examined. Of the water regimes adopted, at maturity the water table treatment gave significantly higher oil percentages than the other regimes (Tab.1) and the water stress led to the formation of lighter achenes than the other treatments (Tab.1). Of the hybrids, the high oleic had higher evapotranspiration values under conditions of complete refunding of evotranspiration (100% ET) and higher WUE values than the standard hybrid, thus demonstrating itself more efficient in the use of water for the production of achenes. At harvest, the saturated fatty acid (palmitic and strearic) content was unaffected by the water treatments under examination. In contrast, there was a significant positive effect of the water stress on the oleic acid content in the high oleic hybrid (an increase of about 5%; Fig. 4). In contrast, in the standard hybrid, the water stress caused a significant reduction of about 15% of the concentration of oleic acid in comparison with the complete refunding of evotranspiration regime (100% ET; Fig. 4).

Conclusions

The different water availability in the soil, during the flowering-maturation period, appeared capable of significantly influencing the concentration of oleic acid at harvest in both genotypes. In particular, it is interesting to highlight how the water stress led to an increase in the concentration of oleic acid in the high oleic hybrid in comparison with the other treatments. In normal sunflower, the

activity of Δ-12 desaturase extends over a long period and can thus be influenced by numerous factors, including variations in temperature, to which it is very sensitive. This is in contrast with the high oleic sunflower hybrid where the same enzyme demonstrates a certain activity only during the very earliest phases of development of the embryo (up to about 10-12 days from the end of flowering), associated with synthesis activity of the lipids, to then diminish rapidly towards negligible values (Garces and Mancha, 1991; Horlogge *et al.*, 1991). Water stress during this period, can cause an anticipation of the embryo development phases and of the accumulation of lipids, with trouble of all the enzymatic activities, including that of the Δ-12 desaturase. This situation is reflected in the final fatty acid composition, in comparison with conditions of normal water availability, which allow a longer grain-filling period. This hypothesis, which could also be widened to any type of environmental stress capable of influencing the accumulation period and which, in any case, would require confirmation with further experimental work, could also explain several variable qualitative results obtained in high oleic hybrids in different years and environments (Monotti *et al.*, 1992; Salera and Baldini, 1998).

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Table 1 – Treatments adopted and analysed characters at harvest on 1998.

Treatments		Analysed characters					
Hybrids	Water regimes (1)	WUE (g s.s. l^{-1})	Total Evotr. (mm)	Seed yield (g m^{-2})	Seed weight (mg)	Seeds per plant (n°)	Seed oil content (%)
High oleic	100% ET	1.88 a	829.0 a	513 a	48.3 a	1326 b	40.9 b
"	Water table	-	-	540 a	46.5 a	1453 a	47.2 a
"	Water stress	1.57 b	579.0 c	304 c	40.6 b	936 c	43.5 ab
Standard	100% ET	1.56 b	717.7 b	493 ab	43.5 ab	1416 a	39.4 b
"	Water table	-	-	432 b	44.6 ab	1210 b	45.9 a
"	Water stress	1.31 c	551.5 c	235 d	39.9 b	737 d	42.8 ab

Mean values followed by different letters are significantly different for $P \leq 0,05$ (Duncan's test).
- missing values
(1) 100 % ET= 100% of evotranspiration refunding; Watertable = watertable at 0,6 m of depth ; water stress= withholding of irrigation from flowering to maturity.

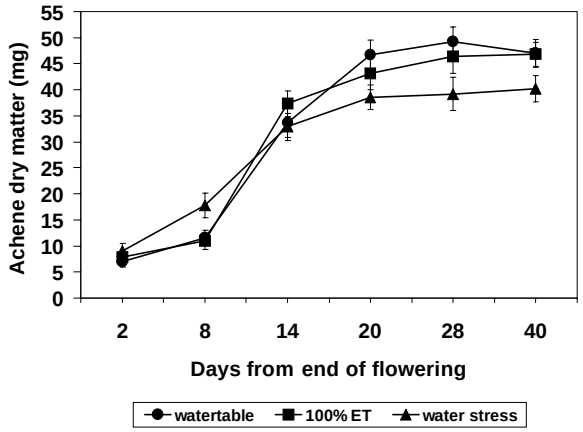

Figure 1 – Time course of dry matter accumulation in achenes of plants submitted to a shallow water table (water table), to a complete refunding of evotranspiration (100% ET) e to a flowering – maturity period of water stress (water stress). The values reported referring to the mean of the two hybrids. The vertical bars represent the standard error of the mean.

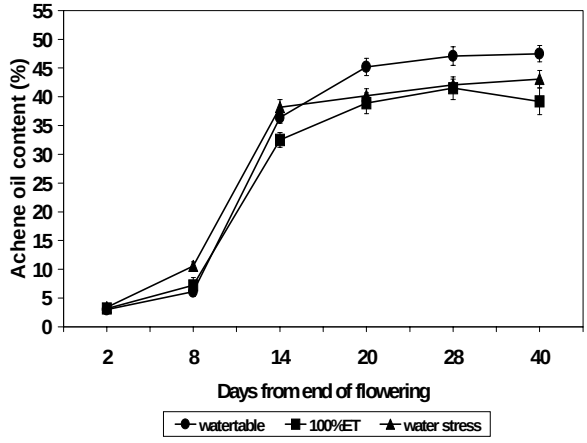

Figure 2 – Time course of oil accumulation in achenes of plants submitted to a shallow watertable (watertable), to a complete refunding of evotranspiration (100% ET) e to a flowering – maturity period of water stress (water stress). The values reported referring to the mean of the two hybrids. The vertical bars represent the standard error of the mean.

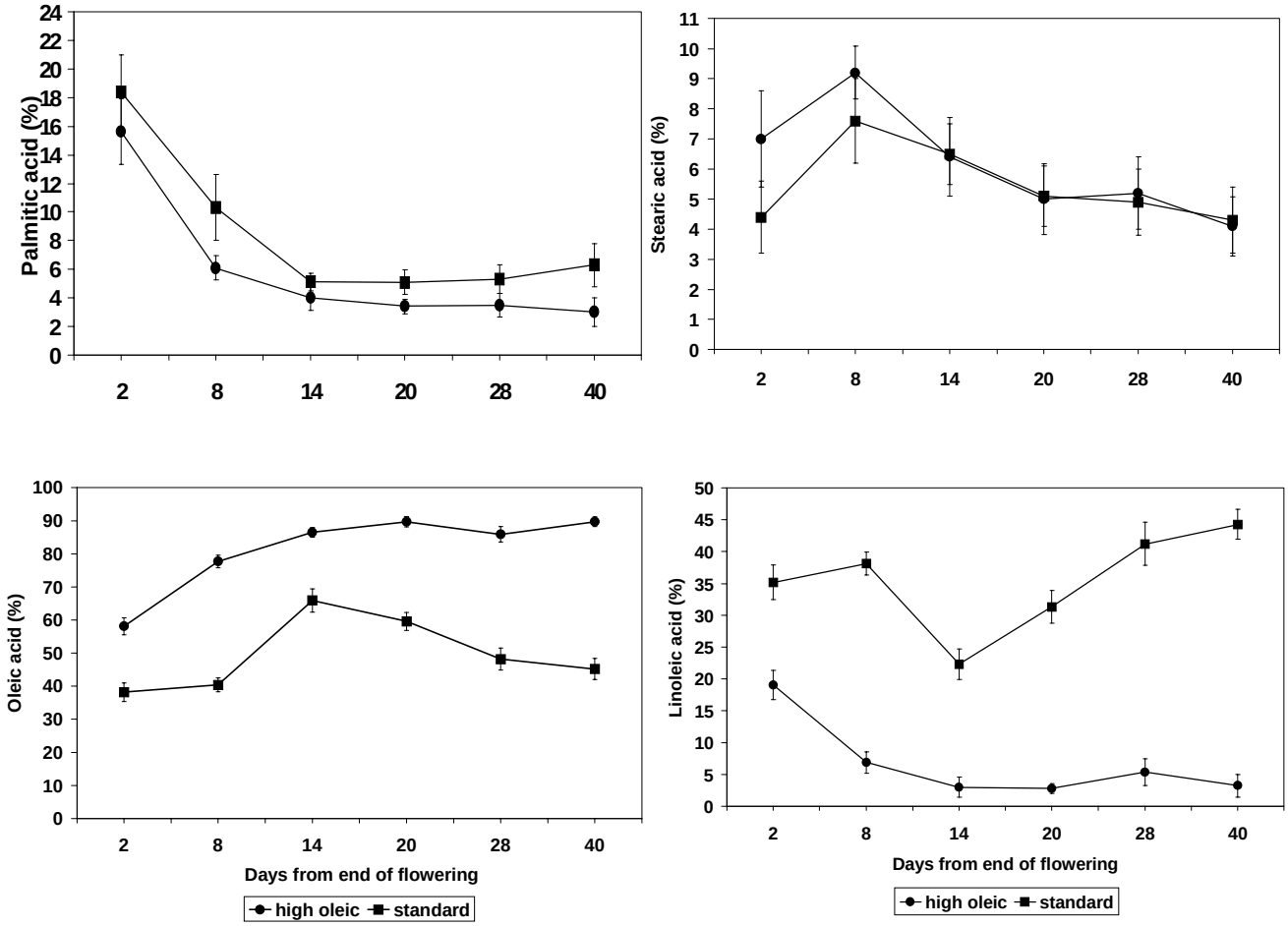

Figure 3 – 1998. Time course of fatty acids accumulation in the high oleic and standard sunflower hybrids. The value reported referring to the mean of the treatments. The vertical bars represent the standard error of the mean.

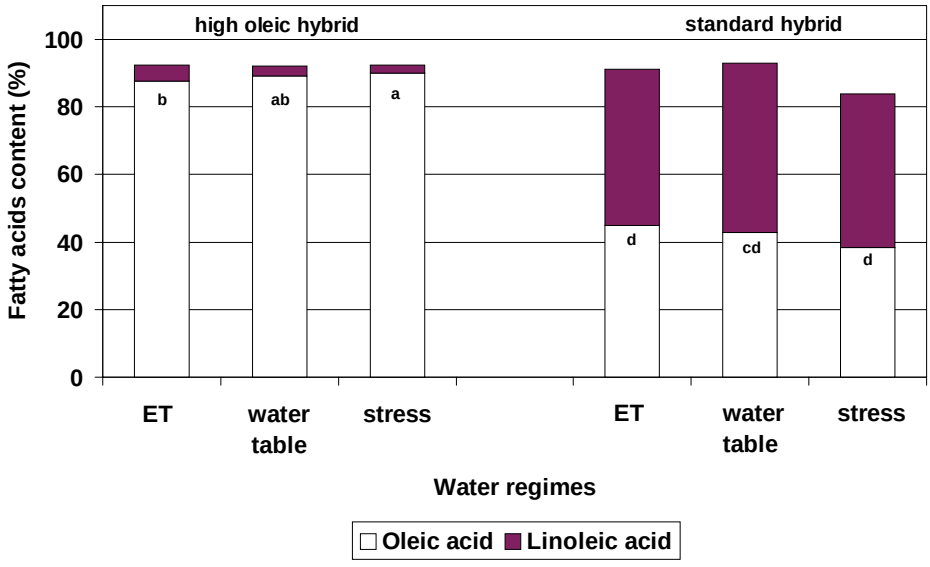

Figure 4 – 1998. Effect of water regimes on oleic acid and linoleic acid content in the achenes at harvest time.