

7-24-2025

The effects of S-metolachlor on some fatty acids and fatty acids gene expressions in safflower (*Carthamus tinctorius* L.)

GİZEM NARDEMİR

GÜLERAY AĞAR

MAHMUT SİNAN TAŞPINAR

Follow this and additional works at: <https://journals.tubitak.gov.tr/botany>

Recommended Citation

NARDEMİR, G, AĞAR, G, & TAŞPINAR, M (2025). The effects of S-metolachlor on some fatty acids and fatty acids gene expressions in safflower (*Carthamus tinctorius* L.). *Turkish Journal of Botany* 49 (4): 304-313. <https://doi.org/10.55730/1300-008X.2864>



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

This Research Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Botany by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr

The effects of S-metolachlor on some fatty acids and fatty acids gene expressions in safflower (*Carthamus tinctorius* L.)

Gizem NARDEMİR¹ , Güleray AĞAR² , Mahmut Sinan TAŞPINAR^{1,*} 

¹Department of Agricultural Biotechnology, Faculty of Agriculture, Atatürk University, Erzurum, Türkiye

²Department of Biology, Faculty of Science, Atatürk University, Erzurum, Türkiye

Received: 09.12.2024 • Accepted/Published Online: 11.03.2025 • Final Version: 23.07.2025

Abstract: Safflower (*Carthamus tinctorius* L.) is a widely studied plant due to its resistance to abiotic stress, suitability for both summer and winter cultivation, and richness in nutrients and linoleic acid. Thus, it has become more important compared to other oil plants in recent years. Weed infestation affects the growth of this cultivated plant during the greening period. S-metolachlor is a widely used herbicide that prevents the growth of weeds. In this study, three different doses of S-metolachlor (1, 2, and 4 g of active substance per liter) were applied to two safflower genotypes (Dinçer and Remzibey-05), and their effects on oleic and linoleic acid contents were assessed. A decrease in oleic acid content was observed in the Remzibey-05 genotype at all herbicide doses. In contrast, only the 1 g active substance/L caused a decrease in the Dinçer genotype. Linoleic acid content increased in both genotypes. In the early growing period of safflower genotypes, the expression levels of certain fatty acid desaturase genes (*CtFAD2-1*, *CtFAD3*, *CtFAD6*, and *CtFAD7*) were measured to investigate fatty acid proportions and conversions. The expression of *CtFAD2-1* and *CtFAD6* genes, which are responsible for converting oleic acid to linoleic acid, was generally reduced compared to the control. A decrease was observed in the expression of *CtFAD3* and *CtFAD7* genes, which are responsible for converting linoleic acid to linolenic acid.

Key words: Safflower, S-metolachlor, oleic acid, linoleic acid, fatty acid desaturase genes

1. Introduction

Oils are among the main essential nutrients in the human diet. Seeds of sunflower, cotton, soybean, peanut, poppy, sesame, canola, and safflower are harvested globally due to their rich content of minerals, carbohydrates, fats, vitamins, and proteins. The oilseeds of these plants are used in many industrial sectors (Kolsarıcı et al., 2005; Arioğlu, 2016). Among oil plants, safflower (*Carthamus tinctorius*) attracts attention due to its tropical characteristics and ability to adapt to extreme conditions such as cold resistance (Bueno et al., 2020). One of the most important features of safflower is that it can synthesize 78% linoleic acid, which humans and animals cannot produce (Chavan et al., 2011; Eryılmaz et al., 2014). In addition, safflower is an important source of omega-3 and omega-6 essential fatty acids (Kachroo and Kachroo, 2009). Additionally, safflower seeds are notable for their rich content of minerals (zinc, copper, manganese, and iron), vitamins (thiamine B1 and beta-carotene), and α -, β -, and γ -tocopherol (Emongor et al., 2017).

A critical problem faced in safflower cultivation is weed control (Böger et al., 2012). The α -chloroacetamide group of herbicides causes inhibition of very long chain

fatty acid synthesis (VLCFA). The largest family within this group includes S-metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide), one of the three most widely used herbicides in this category, which is commonly applied to control annual grasses and small-seeded broadleaf weeds in crops such as maize, cotton, and soybeans (Rapacz et al., 2024). Moreover, the herbicide S-metolachlor is widely used for weed control in safflower cultivation. Metolachlor-residual, a member of this herbicide family, contains S- and R-isomers, with the S-isomer being more effective than the R-isomer (Jhala et al., 2024). This herbicide has been found to act as a growth inhibitor by suppressing the synthesis of chlorophyll, protein, fatty acids, lipid, isoprenoids (including gibberellins), flavonoids (including anthocyanins), acetyl-CoA, and can selectively kill weeds. The selectivity of S-metolachlor is related to plant metabolic rates. Tolerant plants metabolize S-metolachlor rapidly to prevent its accumulation and persistence at phytotoxic levels (Karam et al., 2003). Plants that are more susceptible to this herbicide absorb the active substance through their roots and translocate it to the growing parts of the plant. The uptake and translocation processes appear to play a

* Correspondence: taspinar@atauni.edu.tr

role in how sensitive a plant is to S-metolachlor (Karam et al., 2003). Following the application of S-metolachlor, the absorption of amino acids (leucine or lysine) and sugars in the plant is affected; oleic acid accumulation increases, and the amounts of linoleic and linolenic acid lipids decrease. In addition to its biochemical effects, the application of S-metolachlor increases the expression of metabolic resistance genes (e.g., glutathione S-transferases) in cultivated plants and makes them resistant to the herbicide (Böger et al., 2012). S-metolachlor has also been reported to cause significant reductions in shoot and root biomass, and the potential for plants to recover from herbicide injury is unknown (Angeles, 2020). S-metolachlor can be applied to sorghum and safflower as a preemergence and postemergence herbicide, but its efficacy is not sufficient to cover the next growing season (Jhala et al., 2024). However, this relatively water-soluble herbicide is absorbed by soil particles and, due to its long persistence in soil and water, can enter surface and groundwater, creating a potential for environmental pollution (Silva et al., 2022).

In plants, fatty acids serve not only as energy stores but also play roles in plant metabolism, membrane structure, and stress response mechanisms. Membrane-dependent fatty acid desaturation (FAD) largely acts as a catalyst to control oil biosynthesis (Grau et al., 1999). While the *FAD* gene products facilitate the formation of double bonds in the hydrocarbon chains of fatty acids, the *FAD2* and *FAD3* gene products are known for their enzymatic activities in fatty acid desaturation in the endoplasmic reticulum (ER) (Shanklin and Cahoon, 1998; Los and Murata, 1998; Dar et al., 2017).

In this study, the effects of S-metolachlor application during the early plant growth period were investigated with respect to fatty acid contents (linoleic and oleic acids). The study also focused on examining the expression levels of genes (*CtFAD2-1*, *CtFAD3*, *CtFAD6*, and *CtFAD7*) responsible for encoding fatty acid desaturation enzymes.

2. Materials and methods

2.1. Plant material

The Dinçer and Remzibey-05 safflower cultivars used as plant material in this study were obtained from the Department of Crops, Faculty of Agriculture, Atatürk University.

2.2. Application of S-metolachlor

Surface sterilization of safflower seeds was performed using 70% ethyl alcohol and 0.1% mercuric chloride (HgCl_2) (Sujatha and Kumar, 2007). Surface-sterilized seeds were placed in Magenta GA-7 vessel boxes (10 seeds per box) (Sigma-Aldrich, St. Louis, MO, USA) containing three different doses of S-metolachlor (Sigma-36163, Sigma-Aldrich, St. Louis, MO, USA) (1, 2, and 4 g of active ingredient per liter) (Table 1) (Grisolia and Ferrari, 1997), and were cultured on nutrient medium [1.2 MS salts (Murashige and Skoog, 1962) and 1% (w/v) phytagel] at 22 ± 1 °C under a photoperiod for 6 days. The study was conducted in three replicates, with each box of Magenta GA-7 as a replicate, according to a completely randomized trial design. At the end of the period, the cotyledons of the plants were harvested and stored at -80 °C.

2.3. Analysis of fatty acid methyl esters (FAMES)

Forty milligrams of plant samples were individually ground in liquid nitrogen and transferred to glass tubes with sterile caps. One milliliter solution I (270 g NaOH, 40 mL methanol, and 450 mL distilled water) was added to the samples. The samples were incubated in a water bath at 100 °C for 5 min. The mixture was then vortexed for 5–10 s and incubated for an additional 25 min. After incubation, 2 mL of solution II (477 mL distilled water, 498 mL HCl [37%], and 450 mL HPLC-grade methanol) was added and vortexed for 5–10 s. The samples were incubated in a water bath at 80 °C for 10 min. After this step, the samples were kept on ice for 2 min. Subsequently, 1.25 mL of solution III (400 mL hexane and 400 mL methyl tert-butyl ether) was added, and the samples were vortexed for 10 min. After vortexing, the samples were kept at room temperature for 10 min, and the upper phase was transferred to new sterile glass tubes. Three milliliters of solution IV (10.8 g NaOH and 900 mL distilled water) were added, and the mixture was vortexed for 5 min. The samples were then incubated at room temperature for 10 min and analyzed using gas chromatography-mass spectrometry (GC-MS) (QP2010 ULTRA, Shimadzu, Kyoto, Japan).

2.4. RNA isolation, cDNA analysis, and qPCR analysis

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used for RNA isolation according to the manufacturer's recommended protocol. RNA concentrations were

Table 1. S-metolachlor doses applied to safflower genotypes.

S-metolachlor (g. active substance /L)	Amount (L ⁻¹)
1.0	1040
2.0	2018
4.0	4160

measured at the A260/280 absorbance ratio using a NanoDrop spectrophotometer (Thermo Fisher Scientific Multiskan GO, Waltham, MA, USA). For complementary DNA (cDNA) synthesis, 0.5 µg of RNA was used for each sample. cDNA synthesis was performed using the RevertAid first strand cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The SYBR Green/Hi-ROX qPCR kit (Thermo Fisher Scientific, Waltham, MA, USA) was used for quantitative PCR (qPCR) analysis (Agar et al., 2019). The forward and reverse primer sequences of target genes and reference genes are given in Table 2.

2.5. Parental data analysis and statistical analysis

Apart from oleic and linoleic acid data, other measurements were analyzed using the SAS 9.3 statistical software (SAS Institute Inc., Cary, NC, USA), and differences between

means were determined at the 5% significance level using Duncan's multiple comparison test. For gene expression analysis, the cycle threshold (Ct) values of the samples were determined. Gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, as described by Livak and Schmittgen (2001).

3. Results

3.1 Composition of FAMES

The oleic acid content in the Remzibey-05 genotype (24.51%) was higher than that in the Dinçer genotype (10.23%), and the difference between the two genotypes was significant ($p < 0.01$). Since the oleic acid content of the genotypes differed, the genotype $\times$ application interaction was significant (Table 3). When the effect of S-metolachlor doses on the oleic acid content of the

Table 2. Primer sequences and annealing temperatures of the target and reference genes.

Genes	Sequences Forward 5' 3' / Reverse 3' 5'	Bonding temperatures of primers	References
<i>CtFAD2-1</i>	GAGCAACGGAGAGAAGTAACC GAGGGATGATAGAAAGAGGTCC	54 °C 56 °C	Guan et al. (2014)
<i>CtFAD3</i>	AAACACTTCATGGGTTTGGC TCTGAGCAACTTGGTGGGAG	56 °C 54 °C	Guan et al. (2014)
<i>CtFAD6</i>	CCGAATGAAATTGGAAGAGT TGAAAGGTATGTGAGGTGCT	53 °C 54 °C	Guan et al. (2014)
<i>CtFAD7</i>	CTCACTTTCACCCGAATAGC AGGATAAACCCACAAGCAGA	56 °C 55 °C	Guan et al. (2014)
<i>Ubiquitin</i>	GATGGAAGGACTTTGGCTGAC GAACACCACCACCGATACCC	45 °C 45 °C	Liu et al. (2016)
<i>Ct60S</i>	CATCCATTATCCAACAATC AAGAGTAATCAGTCTCCA	45 °C 46 °C	Liu et al. (2016)

Table 3. Oleic acid contents of safflower genotypes under different S-metolachlor dosages (%)^{1,2}.

S-metolachlor (g. active substances/L)	Oleic acid content (%)		
	Dinçer	Remzibey-05	Mean
Control	10.94 ^b	35.56 ^a	23.25 ^a
1.0	17.86 ^a	17.86 ^d	17.86 ^b
2.0	5.77 ^c	20.64 ^c	13.21 ^d
4.0	6.36 ^c	23.97 ^b	15.17 ^c
Mean	10.23 ^B	24.51 ^A	
F-value (Genotype) (G)	-	-	2029.48**
F-value (Treatment) (U)	247.68**	219.43**	189.14**
F-value (GxU)	-	-	267.44**
Coefficient of variation (%)	6.01	3.71	4.46

¹: Means followed by the same letter in the same row or column are not significantly different. F-values marked with ** are significant at the 0.01 probability level. ²: Retention time: 27.50 min.

genotypes was examined, a decrease was observed in the Remzibey-05 genotype at all S-metolachlor doses compared to the control. In the Dinçer genotype, oleic acid content increased at the 1 g active substance/L dose of S-metolachlor compared to the control, and decreased at the other doses (Table 3; Figure 1).

The effect of genotype and S-metolachlor on linoleic acid content was significant ($p < 0.01$) (Table 4). The linoleic acid content of the Dinçer genotype (70.97%) was higher than that of the Remzibey-05 genotype (61.23%), and the difference between them was significant ($p < 0.01$). The linoleic acid content of the genotypes differed

according to the applications, and the genotype $\times$ application interaction was significant (Table 3). While examining the effect of S-metolachlor doses on linoleic acid in the genotypes, it was evident from the data that linoleic acid increased with all doses in both genotypes (Table 3; Figure 2).

3.2. mRNA expression analysis

The expression levels of *CtFAD2-1*, *CtFAD3*, *CtFAD6*, and *CtFAD7* genes in the Remzibey-05 and Dinçer genotypes were evaluated following exposure to S-metolachlor.

The expression levels of the *CtFAD2-1* gene in safflower genotypes under different treatments are

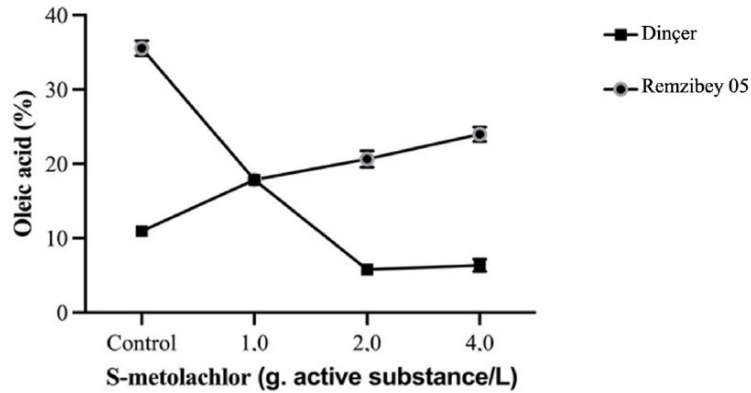


Figure 1. Oleic acid content of genotypes under different S-metolachlor doses (g/active substance)¹.

¹The differences between the means were determined by the Duncan multiple comparison test at a 5% significance level.

Table 4. Linoleic acid contents of safflower genotypes under different S-metolachlor dosages (%)^{1,2}.

S-metolachlor (g. active substance /L)	Linoleic acid contents (%)		
	Dinçer	Remzibey-05	Mean
Control	66.56 ^c	45.61 ^c	56.08 ^d
1.0	74.06 ^a	74.06 ^a	74.06 ^a
2.0	69.51 ^b	62.70 ^b	66.10 ^c
4.0	73.76 ^a	62.56 ^b	68.16 ^b
Mean	70.97 ^A	61.23 ^B	
F-value (Genotype) (G)	-	-	473.31 ^{**}
F-value (Treatment) (U)	37.79 ^{**}	300.14 ^{**}	279.27 ^{**}
F-value (GxU)	-	-	96.13 ^{**}
Coefficient of variation (%)	1.43	1.91	1.66

¹: Means followed by the same letter in the same row or column are not significantly different. F-values marked with ^{**} are significant at the 0.01 probability level. ²: Retention time: 29.30 min.

presented in Figure 3. In the Dinçer genotype, 1 and 4 g active substance/L doses of S-metolachlor decreased the expression of the *CtFAD2-1* gene. At a dose of 2 g active substance/L of S-metolachlor, the expression of the *CtFAD2-1* gene increased to a level close to the control. In the Remzibey-05 genotype, *CtFAD2-1* gene expression decreased at 1 and 2 g active substance/L doses of S-metolachlor, and increased at the 4 g active substance/L dose. *CtFAD3* gene expression decreased at all S-metolachlor doses applied to the Dinçer genotype. The expression of the *CtFAD3* gene in the Remzibey-05 genotype decreased at 1 and 4 g active substance/L doses of S-metolachlor. The expression of the same gene

increased at the 2 g active substance/L dose (Figure 4). The effect of 1, 2, and 4 g active substance/L doses of S-metolachlor on the expression level of the *CtFAD6* gene is shown in Figure 5. *CtFAD6* gene expression decreased in both genotypes. The decrease in the expression of the gene was dose-dependent only in the Dinçer genotype. Therefore, the greatest decrease in gene expression in the Dinçer genotype occurred at the 4 g active substance/L dose of S-metolachlor. When genotypes were evaluated for *CtFAD7* gene expression levels, it was observed that expression decreased in both genotypes regardless of the S-metolachlor dose (Figure 6).

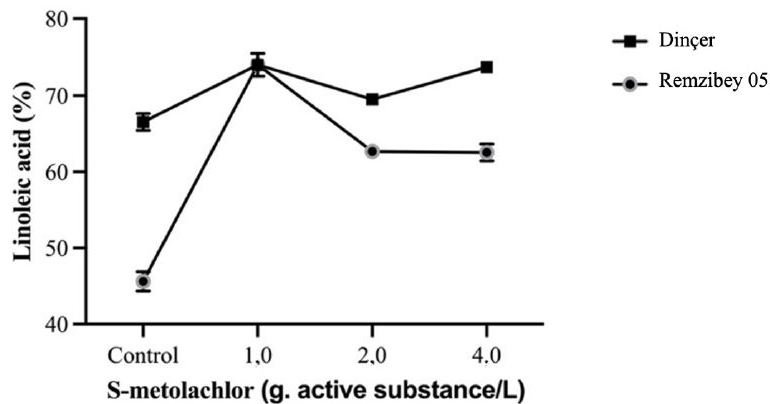


Figure 2. Linoleic acid content of genotypes under different S-metolachlor doses (g/active substance)*.

*The differences between the means were determined by the Duncan multiple comparison test at a 5% significance level.

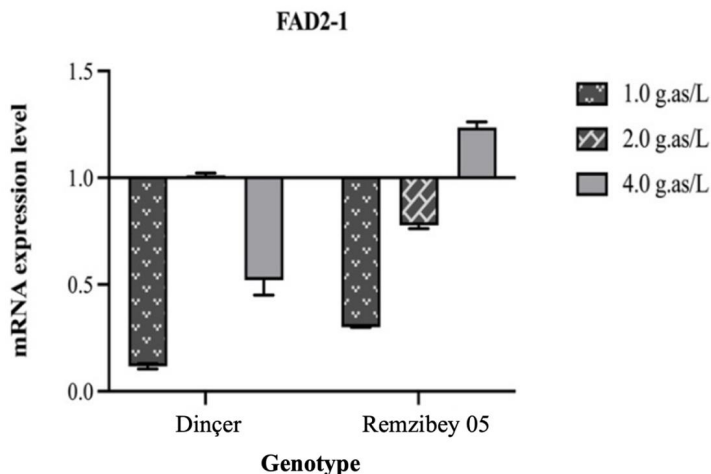


Figure 3. mRNA expression levels of safflower genotypes for *CtFAD2-1* (2^{-ΔΔCt})*.

*Gene expression levels were calculated using the 2^{-ΔΔCt} method, as described by Livak and Schmittgen (2001). as: active substance.

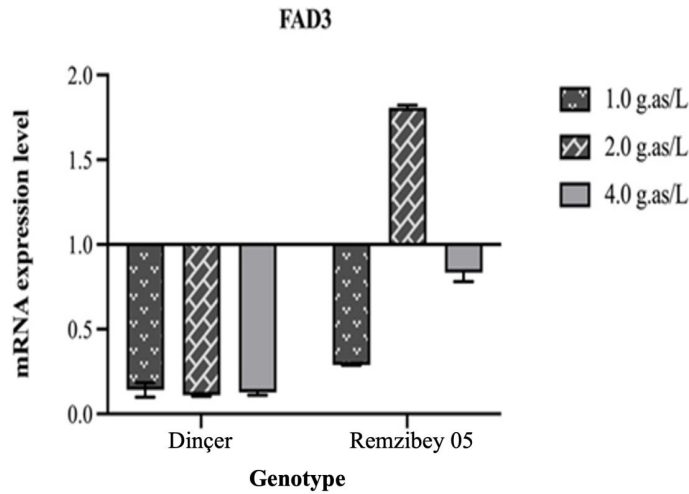


Figure 4. mRNA expression levels of safflower genotypes for *CtFAD3* ($2^{-\Delta\Delta C_t}$)*.

*Gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, as described by Livak and Schmittgen (2001). as: active substance.

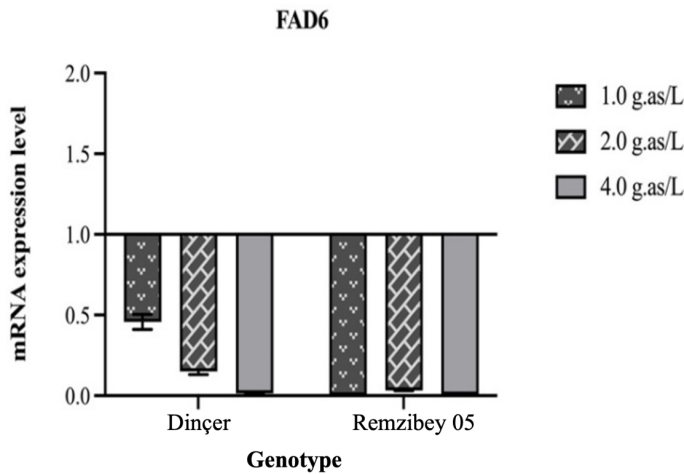


Figure 5. mRNA expression levels of safflower genotypes for *CtFAD6* ($2^{-\Delta\Delta C_t}$)*.

*Gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, as described by Livak and Schmittgen (2001). as: active substance.

4. Discussion

Lipids are essential for maintaining vital metabolic activities (Kolsarıcı et al., 2005). The high linoleic acid content (78%) has led to increased interest in the safflower plant (Chavan et al., 2011; Eryılmaz et al., 2014). S-metolachlor, which is widely used in safflower cultivation, binds to nucleophiles such as glutathione and cysteine in vitro (Leavitt and Penner, 1979). S-metolachlor inhibits the initiation of acetyl-CoA and lipid biosynthesis. In our study, the oleic acid content of the Remzibey-05 genotype decreased

at all S-metolachlor doses compared to the control. In the Dinçer genotype, oleic acid increased only at the 1 g active substance/L dose of S-metolachlor and decreased at the other doses. The linoleic acid content of both genotypes increased at all S-metolachlor doses. Similar to our linoleic acid results, increased linoleic acid content has been observed in maize exposed to S-metolachlor (Canakci-Gulengul et al., 2019). Contrary to our results, although the administration of S-metolachlor affects the uptake of amino acids (leucine or lysine) and sugars, it has

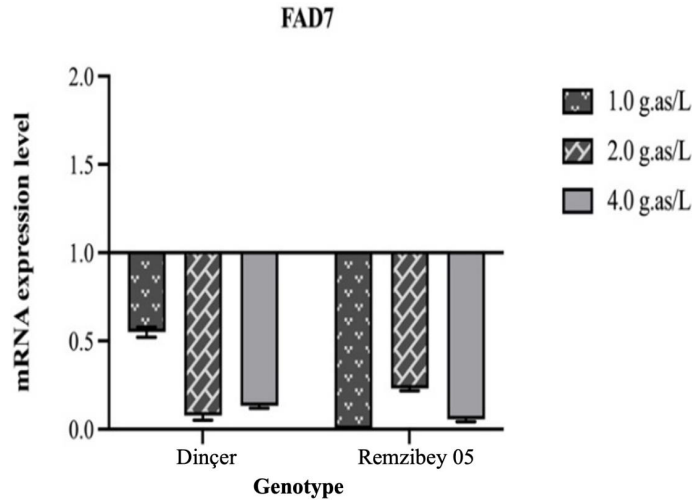


Figure 6. mRNA expression levels of safflower genotypes for *CtFAD7* ($2^{-\Delta\Delta C_t}$).

*Gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, as described by Livak and Schmittgen (2001). as: active substance.

been reported to cause the accumulation of oleic acid and the reduction of various linoleic and linolenic fatty acids (Böger et al., 2012). Furthermore, S-metolachlor treatment was found to increase saturated fatty acids and decrease unsaturated fatty acids (Gutiérrez et al., 2019). Canakci-Gulengul et al., (2019) considered that the decrease in different fatty acids was associated with reduced oxidative stress resistance in maize seedlings treated with atrazine.

Microsomal (*FAD2*) and plastid ω -6 (*FAD6*) are the two enzymes responsible for the conversion of oleic acid to linoleic acid through the eukaryotic and prokaryotic pathways, respectively (Jin et al., 2001; Hernández et al., 2009). In our findings, S-metolachlor doses (1, 2, and 4 g active substance/L) decreased the amount of oleic acid in both genotypes compared to the control. It was found that the amount of linoleic acid increased. In our *FAD2-1* and *FAD6* gene expression results, *FAD2-1* expression decreased at all doses compared to the control, except at 2 g active substance/L in the Dinçer genotype and 4 g active substance/L in the Remzibey-05 genotype. On the other hand, *FAD6* gene expression decreased in both genotypes. Although no correlation was found between these gene expression results and the levels of oleic and linoleic acids, it can be assumed that the *FAD2-2* gene plays a more active role than *FAD2-1* in converting oleic acid to linoleic acid.

Contrary to our findings, *FAD2* gene expression was reported to increase in avocado fruits and cotton cobs under cold stress (Wang et al., 2004; Kargiotidou et al., 2008; Teixeira et al., 2009). On the other hand, no significant change in *FAD2* and *FAD6* gene expression

was reported at low temperatures in *Arabidopsis* and soybean (Okuley et al., 1994; Heppard et al., 1996). It has been reported that the expression of *ALA10* (a P4-type ATPase) in *Arabidopsis* leaves decreases *FAD2* gene expression and affects lipid sequestration (Botella et al., 2016). Furthermore, in the same study, changes in *ALA10* expression were found to affect fatty acyl desaturation of phosphatidylcholine by disrupting the balance between *FAD2* and *FAD3*. Changes in this protein may be one of the main reasons for our findings regarding fatty acids.

Environmental stresses affect the physiological activities of living organisms. It has been reported that the *FAD3* and *FAD7* genes examined in this study encode omega-3 desaturase, and that these two genes originated from a common ancestor (Iba et al., 1993). Additionally, the active role of these genes in fatty acid metabolism is to convert linoleic acid to linolenic acid (Iba et al., 1993). The lack of linolenic acid formation observed in our oil analysis results can be explained by decreased *FAD7* gene expression in both genotypes at all doses; and decreased *FAD3* expression in the Dinçer genotype at all S-metolachlor doses and in the Remzibey-05 genotype at 1 and 4 g active substance/L S-metolachlor doses. It has been reported that *FAD3a* and *FAD3b*, which belong to the *FAD3* gene family, are duplicate genes. In addition, it has been reported that *FAD3a* adds extra amino acids at the N-terminus of *FAD3* (You et al., 2014). This may explain the upregulation at 2 g active substance/L in the Remzibey-05 genotype. It has also been stated that the *FAD3* gene is not only associated with abiotic stress but also positively affects linolenic acid levels during growth

and development (Aron del et al., 1992). Compared to the control group, reduced development under stress conditions supports this conclusion.

On the other hand, under stress conditions, the production of reactive oxygen species (ROS) occurs in plants (Akram et al., 2017). Polyunsaturated fatty acids (PUFAs) are common lipid components and are easily oxidized by unstable ROS (Alché, 2019). This situation may explain the formation of fatty acids in our research independent of gene expression. Rapid metabolism of herbicidally active S-metolachlor into polar compounds (including putative glutathione, dipeptide, and cysteine conjugates) has been reported (Jhala et al., 2024). Hwang et al. (2023) stated that the evolution of S-metolachlor resistance observed in the S-metolachlor-resistant *A. palmeri* biotype can probably be attributed to the enhanced activity of glutathione S-transferases. Besides the contribution of GSTs to the metabolism of S-metolachlor in Palmer amaranth, recent studies suggest that cytochrome P450 enzyme (CYPs) activity also contributes to S-metolachlor resistance in this plant (Concepcion et al., 2023).

5. Conclusions and recommendations

In our study, all applications of S-metolachlor were found to decrease the amount of oleic acid and increase the amount of linoleic acid in both safflower genotypes. In addition to fatty acid analysis, it has been reported that the expression of genes involved in lipid metabolism varies depending on genotype and herbicide application. It may be recommended to investigate new methods that positively affect S-metolachlor-induced fatty acid changes in safflower genotypes and clarify the mechanisms responsible for differences in gene expression. In addition, during the evaluation of the parameters in our study, it was visually observed that root development in S-metolachlor-treated plants was negatively affected. It can be suggested

that further studies may provide new insights into the negative effects of S-metolachlor on root development in safflower.

Author contributions

All authors contributed to the conceptualization and design of the study. Laboratory work and study organization were carried out by Gizem NARDEMİR and Mahmut Sinan TAŞPINAR. The first draft of the article was written by Güleray AĞAR, and all authors commented on previous versions of the manuscript. All authors have read and approved the final version.

Funding

This study was supported by the Atatürk University Scientific Research Projects Coordination Unit (Project no: FYL-2019-7076).

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors would like to express their gratitude to the Atatürk University Scientific Research Projects (BAP) Coordination Unit for its support.

References

- Agar G, Taspinar MS, Yildirim E, Aydin M, Yuce M (2019). Effects of ascorbic acid and copper treatments on metallothionein gene expression and antioxidant enzyme activities in *Helianthus annuus* L. exposed to chromium stress. *Journal of Plant Growth Regulation* 39 (2): 897-904. <https://doi.org/10.1007/s00344-019-10031-0>
- Akram NA, Shafiq F, Ashraf M (2017). Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Frontiers in Plant Science* 8: 613. <https://doi.org/10.3389/fpls.2017.00613>
- Alché JDD (2019). A concise appraisal of lipid oxidation and lipoxidation in higher plants. *Redox Biology* 23: 101136. <https://doi.org/10.1016/j.redox.2019.101136>
- Angeles J (2020). Early season response of tomatoes to differential soil residue levels of preplant herbicide. MSc, California State University, Fresno, CA, USA.
- Arioğlu H (2016). Oilseed and crude oil production in Turkey, problems and solution suggestions. *Journal of the Central Research Institute of Field Crops* 25(SPECIAL ISSUE-2). 357-368.

- Arondel V, Lemieux B, Hwang I, Gibson S, Goodman HM et al. (1992). Map-based cloning of a gene controlling omega-3 fatty acid desaturation in *Arabidopsis*. *Science* 258 (5086): 1353-1355. <https://doi.org/10.1126/science.1455229>
- Böger P, Wakabayashi K, Hirai K (editors.) (2012). *Herbicide Classes in Development: Mode of Action, Targets, Genetic Engineering, Chemistry*. 1st ed. Heidelberg, Berlin, Germany: Springer Verlag.
- Botella C, Sautron E, Boudiere L, Michaud M, Dubots E et al. (2016). ALA10, a phospholipid flippase, controls FAD2/FAD3 desaturation of phosphatidylcholine in the ER and affects chloroplast lipid composition in *Arabidopsis thaliana*. *Plant Physiology* 170 (3): 1300-1314. <https://doi.org/10.1104/pp.15.01557>
- Bueno PDL, Santos RF, Bassegio D, Lewandoski CF, Maziere CL et al. (2020). Safflower genotypes affected by nitrogen fertilization in subtropical conditions. *Australian Journal of Crop Science* 14 (9): 1420-1426. <https://doi.org/10.21475/ajcs.20.14.09.p2420>
- Canakci-Gulengul S, Kirecci OA, Karabulut F (2019). Changes based on oxidative stress in metolachlor and atrazine treated maize seedlings. *Pakistan Journal of Botany* 51 (2):421-426. [https://doi.org/10.30848/pjb2019-2\(5\)](https://doi.org/10.30848/pjb2019-2(5))
- Chavan SP, Lokhande VH, Nitnaware KM, Nikam TD (2011). Influence of growth regulators and elicitors on cell growth and α -tocopherol and pigment productions in cell cultures of *Carthamus tinctorius* L. *Applied Microbiology and Biotechnology* 89 (6): 1701-1707. <https://doi.org/10.1007/s00253-010-3014-4>
- Concepcion JCT, Brown NK, Morris JA, Hutchings S, Kaundun SS et al. (2023). Comparative analysis of S-metolachlor metabolism among Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*A. tuberculatus*) populations from the U.S. Abstract 397 in *Proceedings of the Weed Science Society of America Annual Meeting*, Arlington, Virginia, January 30–February 2.
- Dar AA, Choudhury AR, Kancharla PK, Arumugam N (2017). The FAD2 gene in plants: occurrence, regulation, and role. *Frontiers in Plant Science* 8:1789 <https://doi.org/10.3389/fpls.2017.01789>
- Emongor V, Oagile O, Phuduhudu D, Oarabile P (2017). Safflower production. In *Botswana: The Regional Universities Forum for Capacity Building in Agriculture–RUFORUM*
- Eryılmaz T, Cesur C, Yesilyurt MK, Aydın E (2014). Safflower (*Carthamus tinctorius* L.), Remzibey-05 seed oil methyl ester: fuel properties for potential diesel engine application. *Turkish Journal of Agricultural and Natural Sciences* 1 (1): 85-90.
- Grau LP, Kinney AJ, Pierce Jr JW, Wierzbicki AM, Yadav NS (1999). U.S. Patent No. 5,952,544. Washington, DC: U.S. Patent and Trademark Office.
- Grisolia CK, Ferrari I (1997). In vitro and in vivo studies demonstrate non-mutagenicity of the herbicide metolachlor. *Brazilian Journal of Genetics* 20 (3): 411-414. <https://doi.org/10.1590/s0100-84551997000300009>
- Guan LL, Wu W, Hu B, Li D, Chen JW et al. (2014). Developmental and growth temperature regulation of omega-3 fatty acid desaturase genes in safflower (*Carthamus tinctorius* L.). *Genetics and Molecular Research* 13 (3): 6623-6637. <https://doi.org/10.4238/2014.august.28.7>
- Gutiérrez IB, Mesquita AF, Nunes C, Coimbra MA, Gonçalves FJ et al. (2019). Impacts of S-metolachlor and terbuthylazine in fatty acid and carbohydrate composition of the benthic clam *Scrobicularia plana*. *Ecotoxicology and Environmental Safety* 173: 293-304. <https://doi.org/10.1016/j.ecoenv.2019.02.034>
- Heppard EP, Kinney AJ, Stecca KL, Miao GH (1996). Developmental and growth temperature regulation of two different microsomal [omega]-6 desaturase genes in soybeans. *Plant Physiology* 110 (1): 311-319. <https://doi.org/10.1104/pp.110.1.311>
- Herrández ML, Padilla MN, Mancha M, Martinez-Rivas JM (2009). Expression analysis identifies FAD2-2 as the olive oleate desaturase gene mainly responsible for the linoleic acid content in virgin olive oil. *Journal of Agricultural and Food Chemistry* 57 (14): 6199-6206. <https://doi.org/10.1021/jf900678z>
- Hwang JI, Norsworthy JK, Carvalho-Moore P, Barber LT, Butts TR et al. (2023). Exploratory analysis on herbicide metabolism and very-long-chain fatty acid production in metolachlor-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.). *Journal of Agricultural and Food Chemistry* 71 (15): 6014-6022. <https://doi.org/10.1021/acs.jafc.3c00196>
- Iba K, Gibson S, Nishiuchi T, Fuse T, Nishimura M et al. (1993). A gene encoding a chloroplast omega-3 fatty acid desaturase complements alterations in fatty acid desaturation and chloroplast copy number of the fad7 mutant of *Arabidopsis thaliana*. *Journal of Biological Chemistry* 268 (32): 24099-24105. [https://doi.org/10.1016/s0021-9258\(20\)80498-0](https://doi.org/10.1016/s0021-9258(20)80498-0)
- Jhala AJ, Singh M, Shergill L, Singh R, Jugulam M et al. (2024). Very long chain fatty acid–inhibiting herbicides: Current uses, site of action, herbicide-resistant weeds, and future. *Weed Technology* 38: e1. <https://doi.org/10.1017/wet.2023.90>
- Jin UH, Lee JW, Chung YS, Lee JH, Yi YB et al. (2001). Characterization and temporal expression of a ω -6 fatty acid desaturase cDNA from sesame (*Sesamum indicum* L.) seeds. *Plant Science* 161 (5): 935-941. [https://doi.org/10.1016/s0168-9452\(01\)00489-7](https://doi.org/10.1016/s0168-9452(01)00489-7)
- Kachroo A, Kachroo P (2009). Fatty acid–derived signals in plant defense. *Annual review of phytopathology* 47: 153-176. <https://doi.org/10.1146/annurev-phyto-080508-081820>
- Karam D, Lara FR, Cruz MB, Pereira Filho IA, Pereira FTF (2003). Características do herbicida S-Metolachlor nas culturas de milho e sorgo. *Embrapa Milho e Sorgo. Circular Técnica* 36 (in Portuguese).
- Kargiotidou A, Deli D, Galanopoulou D, Tsafaris A, Farmaki T (2008). Low temperature and light regulate delta 12 fatty acid desaturases (FAD2) at a transcriptional level in cotton (*Gossypium hirsutum*). *Journal of Experimental Botany* 59 (8):2043-2056. <https://doi.org/10.1093/jxb/ern065>
- Kolsarıcı Ö, Gür A, Başalma D, Kaya MD, İşler N (2005). Yağlı Tohumlu Bitkiler Üretimi). Yağlı tohumlu bitkiler üretimi. *Türkiye Ziraat Mühendisliği VI. Teknik Kongresi; Türkiye*. p. 409-429.

- Leavitt JRC, Penner D (1979). In vitro conjugation of glutathione and other thiols with acetanilide herbicides and EPTC sulfoxide and the action of the herbicide antidote R-25788. *Journal of Agricultural and Food Chemistry* 27 (3): 533-536. <https://doi.org/10.1021/jf60223a004>
- Liu F, Guo DD, Tu YH, Xue YR, Gao Y et al. (2016). Identification of reference genes for gene expression normalization in safflower (*Carthamus tinctorius*). *Revista Brasileira de Farmacognosia* 26: 564-570. <https://doi.org/10.1016/j.bjp.2016.05.006>
- Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25 (4): 402-408. <https://doi.org/10.1006/meth.2001.1262>
- Los DA, Murata N (1998). Structure and expression of fatty acid desaturases. *Biochimica Biophysica Acta (BBA) - Lipids and Lipid Metabolism* 1394: 3-15. [https://doi.org/10.1016/s0005-2760\(98\)00091-5](https://doi.org/10.1016/s0005-2760(98)00091-5)
- Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* 15 (3): 473-497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Okuley J, Lightner J, Feldmann K, Yadav N, Lark E (1994). Arabidopsis FAD2 gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. *The Plant Cell* 6 (1): 147-158. <https://doi.org/10.1105/tpc.6.1.147>
- Rapacz D, Smolinska-Kempisty K, Wolska J (2024). Molecularly imprinted polymers toward herbicides – progress in development and the current state of the art depending on the polymerization environment –short review. *Journal of Environmental Chemical Engineering* 12 (2): 112159. <https://doi.org/10.1016/j.jece.2024.112159>
- Shanklin J, Cahoon EB (1998). Desaturation and related modifications of fatty acids. *Annual Review of Plant Physiology and Plant Molecular Biology* 49: 611-641. <https://doi.org/10.1146/annurev.arplant.49.1.611>
- Silva QM, Palmieri MJ, Andrade-Vieira LF (2022). Effects of a S-metolachlor based herbicide on two plant models: *Zea mays* L. and *Lactuca sativa* L. <https://doi.org/10.20944/preprints202209.0351.v1>
- Sujatha M, Kumar D (2007). In vitro bud regeneration of *Carthamus tinctorius* and wild *Carthamus* species from leaf explants and axillary buds. *Biologia Plantarum* 51 (4): 782-786. <https://doi.org/10.1007/s10535-007-0160-3>
- Teixeira MC, Coelho N, Olsson ME, Brodelius PE, Carvalho ISD et al. (2009). Molecular cloning and expression analysis of three omega-6 desaturase genes from purslane (*Portulaca oleracea* L.). *Biotechnology letters* 31 (7): 1089-1101. <https://doi.org/10.1007/s10529-009-9956-x>
- Wang X, Beno-Moualem D, Kobiler I, Leikin-Frenkel A, Lichter A et al. (2004). Expression of Δ^{12} fatty acid desaturase during the induced accumulation of the antifungal diene in avocado fruits. *Molecular Plant Pathology* 5 (6): 575-585. <https://doi.org/10.1111/j.1364-3703.2004.00249.x>
- You FM, Li P, Kumar S, Ragupathy R, Li Z et al. (2014). Genome-wide identification and characterization of the gene families controlling fatty acid biosynthesis in flax (*Linum usitatissimum* L.). *J Proteomics & Bioinformatics* 7: 310-326. <https://doi.org/10.4172/jpb.1000334>