



Yield Components and Fatty Acids Variation of Canola Cultivars Under Different Irrigation Regimes and Planting Dates

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Abstract

To investigate the effect of different planting dates and irrigation regimes on six canola cultivars, a 2-year (2014–2016) experiment was conducted at the Seed and Plant Improvement Institute of Karaj. The experiments were conducted as the factorial split-plot in a randomized complete block design including six canola cultivars (Gabiella, Brutus, Triangle, Marathon, Danube, and Natali), two irrigation regimes (fully irrigated and irrigation termination at the flowering stage) and two planting dates (October 1 and November 1). The results showed that irrigation termination from the flowering stage prevented the supply of required material for filling the seeds and the metabolism of the seed compounds and reduced the growth period of the seed. Therefore, a reduction was observed in the content of oleic and linoleic acids and proline. Also, water deficit stress caused an increase in the glucosinolate content of the seed. The response of canola cultivars was different in terms of fatty acids, so that the Natali cultivar had higher palmitic, oleic acids, and proline content compared to the other cultivars at both planting dates. Fully irrigated treatment and planting date of October 1 produced the highest amount of seed and oil yield (4659 and 2073 kg ha⁻¹), palmitic acid (5.5%), and proline (22.7 μmol/g) content, and the lowest glucosinolate content (13.2 mg/g). Generally, the Natali cultivar and planting date of October 1 and the fully irrigated regime are highly recommended in the studied area regarding qualitative traits and yield of canola.

Keywords Glucosinolate · Linoleic · Oilseed · Seed yield · Water deficit stress

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Ertragskomponenten und unterschiedlicher Fettsäuregehalt von Rapsorten bei verschiedenen Bewässerungsregimen und Pflanzterminen

Zusammenfassung

Um die Auswirkungen verschiedener Pflanztermine und Bewässerungsregime auf sechs Rapsorten zu untersuchen, wurde ein zweijähriger Versuch (2014–2016) am Seed and Plant Improvement Institute in Karaj, Iran, durchgeführt. Die Experimente wurden als faktorielles Split-Plot-Design in einer randomisierten vollständigen Blockanlage mit sechs Rapsorten (Gabriella, Brutus, Triangle, Marathon, Danube und Natali), zwei Bewässerungsregimen (Vollbewässerung und Beendigung der Bewässerung in der Blütephase) und zwei Pflanzterminen (1. Oktober und 1. November) durchgeführt. Die Ergebnisse zeigten, dass die Beendigung der Bewässerung zum Zeitpunkt der Blüte die Versorgung mit den für die Samenfüllung und den Stoffwechsel der Samenbestandteile erforderlichen Stoffe beeinträchtigt und die Wachstumszeit der Samen verkürzt. So wurde eine Verringerung des Gehalts an Öl- und Linolsäure sowie Prolin beobachtet. Außerdem führte der Wasserdefizitstress zu einem Anstieg des Glucosinolatgehalts in den Samen. Die Rapsorten reagierten unterschiedlich im Hinblick auf die Fettsäuren, so wies die Sorte Natali im Vergleich zu den anderen Sorten bei beiden Pflanzterminen einen höheren Gehalt an Palmitinsäure, Ölsäuren und Prolin auf. Die Vollbewässerung und der Pflanztermin 1. Oktober erbrachten den höchsten Samen- und Ölertrag (4659 und 2073 kg ha⁻¹), den höchsten Gehalt an Palmitinsäure (5,5 %) und Prolin (22,7 µmol/g) und den niedrigsten Glucosinolatgehalt (13,2 mg/g). Generell sind die Sorte Natali und der Pflanztermin 1. Oktober sowie die Vollbewässerung im untersuchten Gebiet hinsichtlich der qualitativen Merkmale und des Ertrags von Raps sehr zu empfehlen.

Schlüsselwörter Glucosinolat · Linolsäure · Ölsaart · Samenertrag · Wassermangelstress

Introduction

The canola (*Brassica napus* L.) plant is a oilseed that has attracted attention in recent years. Canola cultivation has been considered in Iran due to its unique characteristics such as the ability to grow in different regions, the high quantity and quality of oil, and the ability to feed animals (Djaman et al. 2018). Canola is the third most important oil crop globally and is the essential source of vegetable oil and protein for human consumption and animal feed (Friedt et al. 2018). However, there are some factors which restrict plant growth, yield, and oil quality. These include water deficit and high temperature during development and reproductive growth stages, which vary depending on the timing, duration, and intensity of stress factors (Hossein et al. 2015; Tatar et al. 2020). Canola oil contains a lot of unsaturated fatty acids as well as calcium, vitamin A, vitamin C, and carotene (Zhao et al. 2017). The seed oil quality is one of the most important traits for *Brassica* species breeding purposes in arid and semi-arid environments, and the qualitative properties of any oil depend on the composition of its fatty acids (Safavi Fard et al. 2018). Nasiri et al. (2021) reported that the fatty acids composition of canola oil is affected by the agronomic practice; they showed that application of humic acid reduced erusic acid and glucosinolate content.

The watering regime and planting date are among the crucial factors affecting canola plant yield and oil quality (Djaman et al. 2018). The production of the canola in Iran is mainly limited by the water deficit. Rahmani et al. (2019) showed that drought stress in the seed filling stage

has a significant negative effect on canola oil yield and quality. Shahsavari (2019) also reported that seed yield was affected by water deficit in the seed filling stage, and winter cultivars had more seed and oil yield in drought stress conditions.

Planting date is also an essential factor affecting seed yield and seed oil content and composition (Shirani Rad et al. 2017; Geren et al. 2020). By providing the necessary growth rates of rapeseed plants and reducing their vulnerability to frost, the appropriate planting date significantly enhances the seed yield (Moradi Aghdam et al. 2019). In other words, choosing the suitable planting date for different cultivars can help reduce drought stress at the grain filling stage. The results of different studies indicate that applying early or late sowing dates and undesirable temperature regimes over the growth period result in reduced yield and yield components, and the highest seed yield was obtained from the appropriate sowing date (Bashir et al. 2010). Moreover, genetic differences also exist in water deficit tolerance in a wide range of plants, such as winter rapeseed (Kauser et al. 2006). Hence, the selection of cultivars tolerant to water stress in the reproductive stage may provide a basis for the development of the cultivation of this plant in Iran and other regions with similar climatic conditions. Therefore, the present study aimed to investigate the effects of planting dates and different irrigation regimes on oil content and fatty acid composition of different winter canola cultivars at two cropping years.

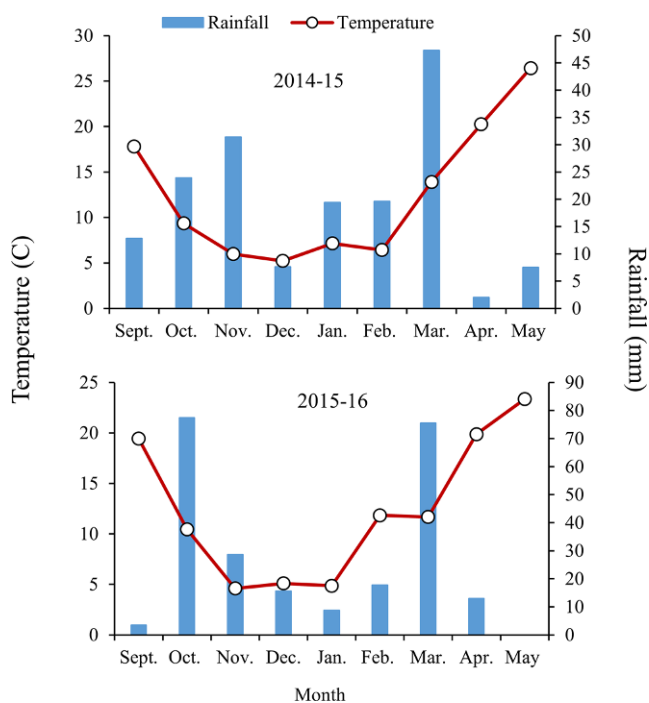


Fig. 1 Mean rainfall and temperature variation of the meteorological station of Karaj region during the growing season (2014–2015 and 2015–2016)

Materials and Methods

This experiment was carried out as a 2-year (2014–2015 and 2015–2016) field experiment within the 400 ha research farm at the Seed and Plant Improvement Institute of Karaj. The study area is located at the latitude of 35° 49' N, 51° 6' E and the elevation is 1321 m above sea level. Mean rainfall and temperature variation of the study area is shown in Fig. 1. Total rainfall at the first and second years of the experiment were 188 and 240 mm, respectively. However, there was a better distribution of rainfall in the first year. Also, no significant difference was observed in the mean temperature of the studied years.

In order to investigate the effect of irrigation regimes on some quality traits of winter canola hybrids, an experiment was conducted as the factorial split-plot in a randomized complete block design with three replications for two cropping years (2014–15 and 2015–16). Experimental treatments consisted of two irrigation methods (conven-

Table 1 The characteristics of canola cultivars used in this experiment

Cultivar	Hybrid/Open Pollination	Winter/Spring	Origin
Marathon	Hybrid	Winter	Germany
Danube	Hybrid	Winter	France
Natali	Hybrid	Winter	France
Gabriella	Open Pollination	Winter	Hungary
Brutus	Hybrid	Winter	Germany
Triangle	Hybrid	Winter	Germany

tional irrigation and irrigation termination from flowering stage to next) and planting dates (at two levels including October 1 [Oct. 1] and November 1 [Nov. 1]) as main plots and the canola cultivars including six cultivars (Gabriella, Brutus, Triangle, Marathon, Danube, Natali) as secondary plots. The properties of cultivars are presented in Table 1.

Irrigation was conducted using the siphon pipes method, and the irrigation intervals were considered based on 80 mm evaporation from class A evaporation pan, and 80% of consumed water at each irrigation was evaporated. The amount of water entering the farm was measured by the water meter. The water required for irrigation was calculated based on 80% of evaporated water, equal to 64 mm water load and/or 640 cubic meters per hectare (Safavi Fard et al. 2018). Accordingly, fully irrigated and early irrigation termination for Oct. 1 plantings received nine and six irrigations, respectively, and for Nov. 1 plantings, they received nine and six irrigations, respectively.

Planting operation was performed manually on plant rows in 1.5 cm depth and we used 4 kg ha⁻¹ seed for sowing the plants. Thinning was conducted to achieve the appropriate plant density. For weed control, Trifluralin herbicide (2.5 L/ha) was applied to the soil. Based on the soil analysis, 150 kg ha⁻¹ of ammonium phosphate, 150 kg ha⁻¹ of potassium sulfate were applied before sowing and 350 kg ha⁻¹ of urea was applied during the growth period (100 kg in the three-leaf stage, 150 kg in stem elongation stage and 100 kg in inflorescence stage). A total of 72 plots (1.8 m width and 6 m length and area of 10.8 m² and plant density of 67 plants per m²) were constructed and there were six planting rows with 30 cm intervals between rows. The properties of the farm soil are shown in Table 2. At the physiological maturity stage in July, to evaluate agronomic and physiological traits, samples were collected from middle lines, and sidelines were removed as marginal effects. Ten samples were randomly selected from approximately 4 m² per plot.

Table 2 Soil physicochemical characteristics in the studied area

Characteristics	Depth (0–30 cm)	Depth (30–60 cm)
Electrical conductivity EC (ds/m)	1.45	1.24
pH	7.9	7.2
Percentage of neutralizing agents	8.56	8.68
The saturated moisture content	36	38
Organic carbon (%)	0.91	0.99
Total nitrogen	0.09	0.07
Available phosphorus (ppm)	14.7	15.8
Available potassium (ppm)	197	155
Clay (%)	28	25
Silt (%)	47	49
Sand (%)	25	26
Soil texture	Loam–Clay	Loam–Clay

In order to determine the percentage of seed oil content, seeds were dried for measuring seed yield in each plot and then 5 g seed was selected and the oil content was determined by NMR (nuclear magnetic resonance) German Broker Brand minispec (Germany) mq20 model based on the International Standard ISO 5511 (Krygsman et al 2004). For this purpose, the device was first calibrated with a refer-

ence sample and at least 3 g of seeds per plot were weighted individually and transferred to the particular cell of the device (Safavi Fard et al. 2018). The number of seeds defined for the device marker was selected as 5 g and in less than a minute, the amount of oil was displayed. The seed oil percentage was obtained by multiplying this amount by the seed yield to calculate the oil yield per hectare. For analy-

Table 3 Analysis of variance of the effect of cropping year, irrigation regime, planting date, cultivar, and their interactions on yield and seed oil and compositions of canola

Sources of changes	Df	Mean Square				
–	–	<i>Oil yield</i>	<i>Seed oil percent</i>	<i>Seed yield</i>	<i>Seed glucosinolate</i>	<i>Proline</i>
Year (Y)	1	1,723,312 **	4.58 **	10,853,181**	75.72 **	262.09 **
Year × rep	4	6023	1.23	19,960	0.34	1.05
Planting date (P-d)	1	35,219,279 **	192.9 **	15,0424,092 **	2377.28 **	1857.82 **
(Y) × (P-d)	1	93,381 ns	7.38 **	229,520 ns	5.04 **	5.34 **
Irrigation (I)	1	10,356.60 **	43.91 **	44,775,057 **	631.66 **	366.82 **
(Y) × (I)	1	3354 ns	2.18 **	81,939 ns	0.22 ns	0.05 ns
(I) × (P-d)	1	437,131*	1.89 **	1,084,895 ns	2.38 **	19.35 **
(Y) × (P-d) × (I)	1	208,620 ns	0.66 ns	1,143,295 ns	0.002 ns	48.87 **
First error	12	33,711	0.49	147,419	0.18	0.14
Cultivar (C)	5	343,621 *	1.65 **	1,452,106 *	21.67 **	16.11 **
(Y) × (C)	5	5084 ns	0.08 ns	25,735 ns	0.02 ns	0.58 ns
(P-d) × (C)	5	83,831 ns	0.34 ns	327,314 ns	5.15 **	4.41 **
(Y) × (P-d) × (C)	5	9959 ns	0.02 ns	49,282 ns	0.11 ns	1.43 **
(I) × (C)	5	1116 ns	0.01 ns	13,061 ns	0.11 ns	0.14 ns
(Y) × (I) × (C)	5	3838 ns	0.005 ns	17,476 ns	0.27 ns	0.07 ns
(P-d) × (I) × (C)	5	498 ns	0.02 ns	1876 ns	0.06 ns	0.07 ns
(Y) × (P-d) × (I) × (C)	5	1092 ns	0.02 ns	5490 ns	0.17 ns	0.05 ns
Error	80	109,425	0.21	550,387	0.24	0.31
CV (%)	–	18.31	1.05	18.08	2.55	3.29
–	–	<i>Erusic acid</i>	<i>Linoleic acid</i>	<i>Linoleic acid</i>	<i>Oleic acid</i>	<i>Palmitic acid</i>
Year (Y)	1	0.014 *	6.38 **	10.5 **	14.4 **	0.14**
Year × rep	4	0.001	0.87	4.65	0.03	0.002
Planting date (P-d)	1	1.44 **	93.02 **	252.007 **	57.04 **	17.5 **
(Y) × (P-d)	1	0.0002 ns	2.90 **	3.27 *	1.13 **	0.0006 ns
Irrigation (I)	1	0.3 **	23.8 **	57.3 **	19.97 **	6.17 **
(Y) × (I)	1	0.0014 ns	0.07 ns	0.05 ns	0.29 **	0.009 **
(I) × (P-d)	1	0.011 *	3.40 **	0.53 ns	0.0006 ns	0.41 **
(Y) × (P-d) × (I)	1	0.0002 ns	0.16 ns	0.24 ns	2.04 **	0.002 ns
First error	12	0.002	0.52	0.12	0.01	0.0007
Cultivar (C)	5	0.012 **	0.67 ns	1.84 *	0.8 **	0.15 **
(Y) × (C)	5	0.00003 ns	0.02 ns	0.01 ns	0.006 ns	0.0006 ns
(P-d) × (C)	5	0.002 ns	0.16 ns	0.48 ns	0.15 **	0.036 **
(Y) × (P-d) × (C)	5	0.00004 ns	0.02 ns	0.04 ns	0.02 ns	0.0004 ns
(I) × (C)	5	0.00004 ns	0.009 ns	0.02 ns	0.03 ns	0.0027 **
(Y) × (I) × (C)	5	0.00009 ns	0.03 ns	0.04 ns	0.08 ns	0.002 *
(P-d) × (I) × (C)	5	0.0003 ns	0.018 ns	0.05 ns	0.05 ns	0.0048 **
(Y) × (P-d) × (I) × (C)	5	0.00006 ns	0.019 ns	0.07 ns	0.009 ns	0.004 **
Error	80	0.0013	0.29	0.60	0.08	0.00066
CV (%)	–	11.50	8.77	4.89	0.32	0.53

** , * significant difference in 1 and 5%, respectively.

sis of fatty acids of samples, the gas chromatography (GC) method was used. The Agilent 6890N GC (Agilent Corporation, Santa Clara, CA, USA), equipped with a flame ionization detector (FID), was used. Chromatography conditions were set according to the procedure AOAC No. 963/22 (AOAC, 2003). The W&G capillary column (60 m, 0.25 mm, 32 μ m) was used (Reiahisamani et al. 2018).

For proline determination, 100 mg of frozen plant material was homogenized in 1.5 mL of 3% sulpho-salicylic acid and the residue was removed by centrifugation. A 100 mL aliquot of the extract was then reacted with 2 mL glacial acetic acid and 2 mL ninhydrin for 1 h at 100 °C and the reaction was then terminated in an ice bath. The reaction mixture was extracted with 1 mL toluene. The chromophore containing toluene was warmed to room temperature and its optical density was measured at 520 nm. The amount of proline was determined from a standard curve in the range of 20–100 mg (Bates et al. 1973). The glucosinolate was measured colorimetrically using Varian Spectrophotometer Cary 100 (Santa Clara, CA, USA) (Makkar et al. 2007).

Data analysis was performed a factorial split-plot design based on randomized complete block design with three replications in 2 years. The main plots were the factorial arrangement of planting dates (Oct. 1, Nov. 1) and irrigation regimes (fully irrigation and water deficit), and the subplots included six canola varieties and hybrids. Data normality were tested using Kolmogorov–Smirnov normality test. Mean comparison analysis was performed using Tukey multiple range test at 5% probability level using SAS v9.1 statistical software v.9.1 (SAS Institute, Cary, NC, USA).

Results

The results showed that the treatments including cropping year, planting date, irrigation ($P < 0.0–1$), and cultivars

($P < 0.05$) had a significant effect on seed yield and the interaction of the studied treatments had no impact on these parameters (Table 3). The highest seed yield was in fully irrigated treatment (4659.7 kg ha⁻¹) and the lowest was in irrigation termination treatment which showed a 23.9% decrease compared to the fully irrigated regime (Table 4). The first planting date (5124.2 kg ha⁻¹) also had a significantly higher seed yield than the delayed planting date (Table 4). Among the studied cultivars, the Natali cultivar had a higher seed yield compared to other cultivars; however there was no significant difference between Natali, Gabriella (4085 kg ha⁻¹) and Marathon (4333 kg ha⁻¹) cultivars. The Danube cultivar had the lowest seed yield with 3748 kg ha⁻¹ (Fig. 2).

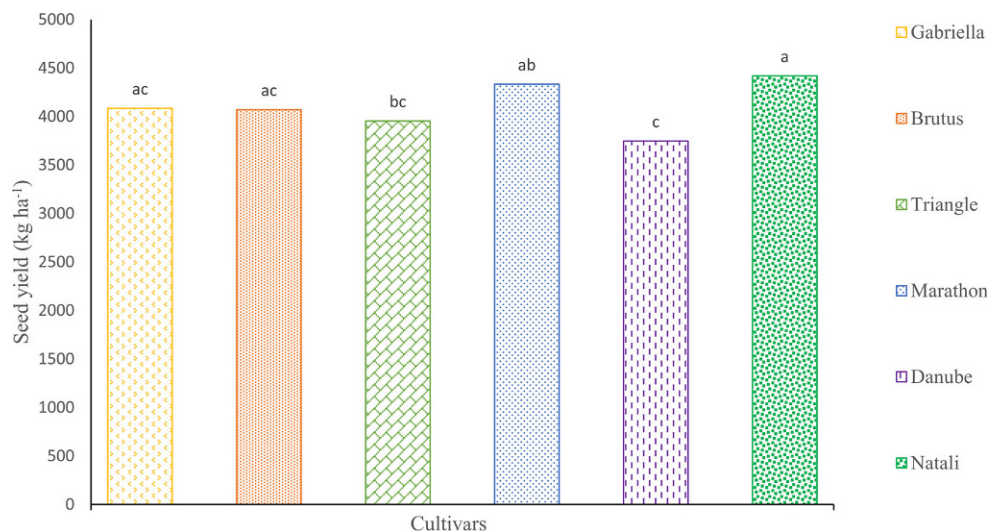
Analysis of variance (ANOVA) showed that a significant effect of cropping year, planting date, irrigation regime, and canola cultivars as well as interaction of irrigation regime and planting date on the seed oil content (Table 3). Results showed that the highest oil percent was obtained from the second year of the experiment (43.8%). The first planting date resulted in a higher oil content than the second planting date. Also, the fully irrigated treatment had the highest amount. The highest percentage of oil was obtained from the Natali cultivar with 44.0% (Table 4). The mean comparison results of the interaction of irrigation regime and planting date are presented in Table 5. The results showed that full irrigation and early planting resulted in an increased seed oil content; however, no significant difference was detected between irrigation regimes in delayed planting (Table 4).

According to the results, the fully irrigated and planting date of Oct. 1 resulted in higher seed oil content compared to other treatments and there were no significant differences between irrigation treatments in delayed planting date (Table 4).

Table 4 Mean comparison results of the main effects of cropping year, planting date, irrigation regimes, and different cultivars on glucosinolate and proline content and seed and oil yield of canola. Means with different letter in a row are statistically different (Tukey, $p \leq 0.05$)

Treatment		Seed glucosinolate (mg/g)	Proline content (μ mol/g)	Oil yield (kg ha ⁻¹)	Seed oil (%)	Seed yield (kg ha ⁻¹)
Year	2014–15	18.6 b	15.8 b	1915 a	43.46 b	4376.7 a
	2015–16	20 a	18.5 a	1696 b	43.82 a	3827.6 b
Planting date	Oct. 1	15.22 b	20.7 a	2300.3 a	44.8 a	5124.2 a
	Nov. 1	23.35 a	13.5 b	1311.2 b	42.5 b	3080.1 b
Irrigation	Regular irrigation	17.19 b	18.7 a	2073.9 a	44.2 a	4659.7 a
	Irrigation termination	21.38 a	15.5 b	1537.6 b	43.1 b	3544.5 b
Cultivar	Gabriella	19.5 c	17.1 b	1802.5 abc	43.6 bc	4085.9 abc
	Brutus	19.3 c	17 b	1785.8 abc	43.56 c	4071.4 abc
	Triangle	19.8 b	16.7 c	1731.7 bc	43.48 cd	3954.9 bc
	Marathon	18.9 d	17.8 a	1917.2 ab	43.88 ab	4333.3 ab
	Danube	20.7 a	15.9 d	1635.6 c	43.3 d	3748.0 c
	Natali	18.1 e	18.1 a	1961.7 a	44.0 a	4419.6 a

Fig. 2 Effect of different varieties on seed yield. Means with different letter in a row are statistically different (Tukey, $p \leq 0.05$)



Canola oil yield was significantly affected by cropping year, planting date, irrigation regime, cultivars, and the interaction of irrigation regimes and planting dates (Table 3). The highest oil yield was obtained in fully irrigated and irrigation cut at flowering stage resulted in decreased oil yield with 26% compared to the fully irrigated crops. Regular planting date also resulted in higher oil yield, compared to the delayed planting date at Nov. 1, with 43%. The Natali cultivar had the highest oil yield ($1961.7 \text{ kg ha}^{-1}$); and the lowest related value was recorded in Danube cultivar, with 1635 kg ha^{-1} .

The mean comparison results of the interaction between irrigation and planting dates showed that the planting date Oct. 1 and conventional irrigation had the highest oil yield per hectare, which has increased to 59% based on irrigation termination and planting date of Nov. 1 (Fig. 3).

Analysis of variance of the studied treatments on canola plant leaf proline content showed that the main effect of cropping year, irrigation, planting date, and cultivar and the interaction effect of planting date and cultivar had a significant impact on leaf proline content at 1% probability (Table 3). The mean comparison results showed that the highest proline content was obtained from the plant-

ing date of Oct. 1, which increased to 35% compared to Nov. 1. Fully irrigated treatments had higher levels of proline ($18.7 \mu\text{mol g}^{-1}$ fresh weight) than irrigation termination. Among the studied cultivars, the highest content of leaf proline was found in the Natali cultivar, which was significantly different from the other cultivars, where the Danube cultivars had the lowest proline content with $15.9 \mu\text{mol g}^{-1}$ fresh weight and there was no significant difference between Gabriella and Brutus cultivars (Table 4). The results showed that planting the Natali cultivar at Oct. 1 had the highest amount of proline with $21.97 \mu\text{mol g}^{-1}$ (Fig. 4). Also, fully irrigated at planting date of Oct. 1 had the highest proline content compared to conventional irrigation which showed a 21% increase (Table 5).

According to the results presented in Table 3, cropping year, planting date, irrigation, and cultivar and the interaction effect of irrigation and planting date and planting date and canola cultivar significantly affected seed glucosinolate content ($P < 0.01$; Table 3). The results of mean comparison showed a significant difference in the glucosinolate content in the studied years; and it was 7% higher in the second year compared to the first year. There was also a significant difference between planting date levels and the highest

Table 5 Mean comparison results of the interaction effects of planting date and irrigation regimes on yield and seed oil and compositions of canola. Means with different letter in a row are statistically different (Tukey, $p \leq 0.05$)

Planting date	Irrigation	Seed oil percentage (%)	Proline content ($\mu\text{mol/g}$)	Seed glucosinolate (mg/g)	Erusic acid (%)	Linoleic acid (%)	Oil yield (kg ha^{-1})	Palmitic acid (%)
Oct. 1	Regular irrigation	45.46 a	22.7 a	13.25 d	0.19 d	5.1 d	2623.5 a	5.5 a
	Irrigation termination	44.13 b	18.7 b	17.2 c	0.26 c	5.6 c	1977.0 b	4.9 b
Nov. 1	Regular irrigation	42.92 c	14.7 c	21.12 b	0.37 b	6.4 b	1524.3 c	4.6 c
	Irrigation termination	42.04 c	12.3 d	25.57 a	0.48 a	7.5 a	1098.1 d	4.3 d

Fig. 3 Interaction of irrigation treatments and planting date on oil yield. Means with different letter in a row are statistically different (Tukey, $p \leq 0.05$)

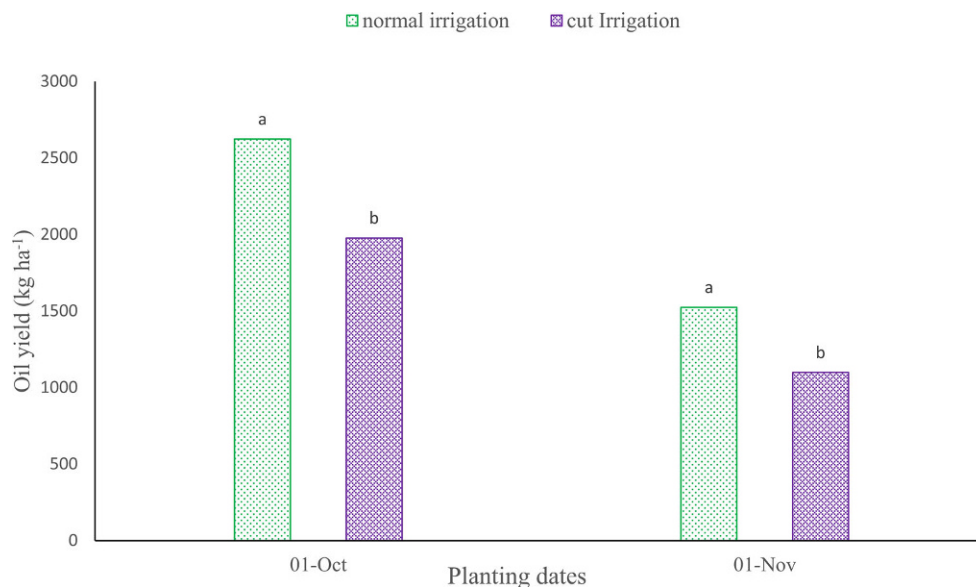
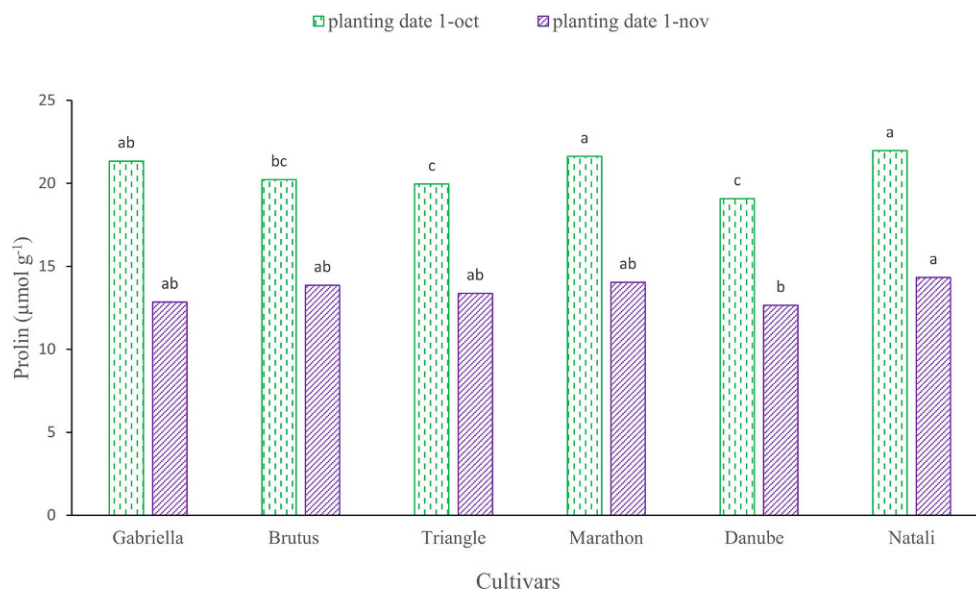


Fig. 4 Interaction of planting date and cultivars tested on the amount of proline. Means with different letter in a row are statistically different (Tukey, $p \leq 0.05$)



glucosinolate content was obtained from a planting date of Nov. 1, which was 35% higher than the first planting date on Oct. 1. Among the irrigation treatments, irrigation cut at the flowering stage resulted to the highest glucosinolate content which recorded 19.6% increase compared to the fully irrigated regime (Table 4). The mean comparison results of the interaction of planting date and irrigation regimes showed that the highest glucosinolate content (25.27 mg/g) obtained in delayed planting and irrigation cut at the flowering stage, which had significant difference with other treatments. Cultivation of canola seeds at regular planting date (Oct. 1) and irrigation termination at flowering stage resulted to the lowest content (17.2 mg/g; Table 5). Also, according to Table 6, at both planting dates, Danube and Natali cultivars had the highest and lowest glucosinolate contents by 16.6 and 14.0

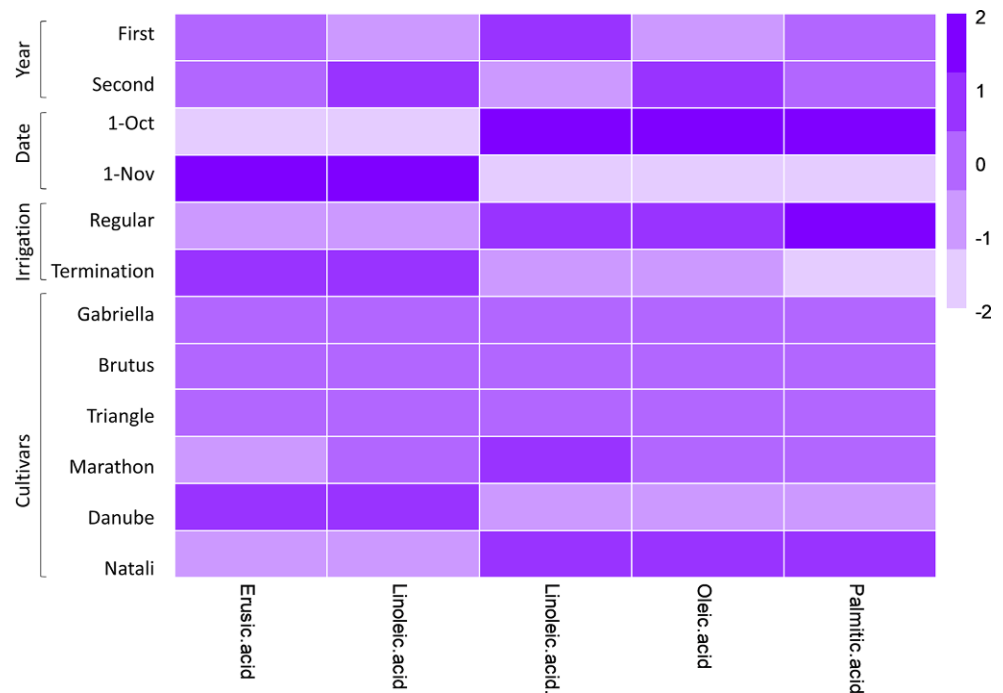
on Oct. 1 and 24.7 and 22.2 mg g⁻¹ on Nov. 1, respectively (Table 6).

Results of analysis of variance showed that the main effects of cropping year, planting date, irrigation and canola cultivars on unsaturated fatty acids of canola seeds were significant at 1% probability level. The interaction effect of irrigation regimes and planting date was significant on erucic acid and linoleic acid and the interaction of planting date and cultivars significantly affected oleic acid content (Table 3). Mean comparison results showed that the content of unsaturated fatty acid in canola seed at the second cropping year significantly increased compared to the first year, except for linoleic acid which the increased amount for erucic, linolenic, oleic acids at the second year compared to the first year was 6, 6.3, and 1%, respectively. In comparison, the

Table 6 Mean comparison results of the interaction effects of planting date and cultivars on seed oil and composition and leaf proline content of canola. Means with different letter in a row are statistically different (Tukey, $p \leq 0.05$)

Planting date	Cultivar	Proline in the leaf ($\mu\text{mol/g}$)	Palmitic acid (%)	Oleic acid (%)	Seed glucosinolate content (mg/g)
October 1	Gabriella	21.34 ab	5.25 b	64.2 ab	14.6 f
	Brutus	20.22 bc	5.15 c	46.04 ab	15.7 f
	Triangle	19.96 c	5.12 c	63.9cd	16.1 d
	Marathon	21.62 a	5.28 b	64.25 a	14.3 f
	Danube	19.08 c	5.06 d	63.8 b	16.6 d
	Natali	21.97 a	5.34 a	64.3 a	14.0 f
November 1	Gabriella	12.85 e	4.44 e	62.6 c	24.4 a
	Brutus	13.96 d	4.54 e	62.9 c	22.9 bc
	Triangle	13.37 e	4.48 e	62.8 c	23.5 b
	Marathon	14.04 d	4.56 e	63.0 c	22.4 c
	Danube	12.65 f	4.42 ef	62.5 c	24.7 a
	Natali	14.33 d	4.59 e	63.1 c	22.2 c

Means having similar letters have no significant difference at 5% probability level

Fig. 5 A heat map illustrating the variation in fatty acids composition of canola in different cropping years, planting dates, irrigation regimes, and cultivars

linoleic acid content decreased by 3.7%. While the erusic and linoleic acid content at the Nov. 1 planting date increased compared to the Oct. 1, the linolenic and oleic acid contents showed a decrease, so that the highest oleic acid and linoleic acid were obtained from the Oct. 1 planting date which showed an increase of 2% and 15%, respectively, compared to Nov. 1. Also, the highest amounts of linolenic acid and erusic acid were obtained from the planting date of Nov. 1 (6.9 and 0.42%, respectively), which showed a 15 and 48% increase compared to October 1 (Fig. 5). The highest amount of oleic acid and linoleic acid was obtained from fully irrigated treatments, which showed a 2 and 8% increase in these fatty acids compared to when

the irrigation was cut at the flowering stage, respectively (Fig. 5). In the studied cultivars, the highest oleic acid and linolenic acid were found to be 63.7% and 16.3% in Natali, and the lowest amount of these fatty acids was in Danube cultivar (63.2% and 15.5%, respectively). Also, the highest linolenic and erusic acids obtained in the Danube cultivar by 6.38% and 0.36%, respectively; the lowest related values were observed in the Natali cultivar by 5.91% and 0.29%, respectively. Results of mean comparison of the interaction effect of irrigation and planting dates on linolenic acid showed that irrigation termination and delayed planting date on Nov. 1 had the highest linolenic and erusic acid by 7.5 and 0.48% (Table 5). Also, the interaction effect of plant-

ing date and canola cultivar on the oleic acid indicated that Natali cultivar and planting date on Oct. 1 had the highest percentage of oleic acid (64.3%), which increased by 2% compared to the same cultivar at the planting date Nov. 1 (Table 6).

ANOVA analysis showed that palmitic acid content was significantly affected by all studied treatments and their interaction (Table 3). Mean comparison results showed that planting the canola plants at the earlier date (Oct. 1) resulted in the highest palmitic acid content in the seeds at 5.2% which was 14% higher than that of Nov. 1 (4.5%). The fully-irrigated treatment, caused a 10% increase in the saturated fatty acid content in the seeds compared to the irrigation termination treatment (Table 4). Among the studied cultivars, the highest palmitic acid content was found in Natali (4.96%) cultivar followed by Gabriella and Brutus with 4.84% and the Danube cultivar had the lowest palmitic acid content (4.73%; Table 4). Mean comparison results of the interaction of irrigation regimes and planting dates showed that the fully irrigated and planting date of Oct. 1 had the highest palmitic acid content (5.5%) which had a significant difference with other treatment and the lowest content was recorded for delayed planting and irrigation cut at the flowering stage (4.3%; Table 5). Also, according to the results of mean comparison of planting date and canola cultivar, Natali cultivar had the highest amount of palmitic acid at the planting date of Oct. 1 (5.34%), followed by Marathon and Gabriella cultivars (5.28 and 5.25%, respectively) under the same irrigation treatments (Table 6).

Discussion

Water deficit at the end of the growing season and the water deficit in canola, in accordance with the flowering time and seed filling, leads to a shortened flowering period and consequently a decrease in seed yield (Gan et al. 2007). Reduction of seed yield in response to water deficit stress may be due to the supply of the required materials for seed filling, reduction of reservoir capacity for adsorption of photosynthetic materials, and also the reduction of the seed growth period, and the early events associated with the growth of seeds, including cell division and formation of smaller reservoir size which are affected by water deficit stress (Karami Chame et al. 2016; Safavi Fard et al. 2018). Foadvand et al. (2017) reported the significant impact of planting date on canola plant grain yield. Alteration in planting date could change the plant growth and development schedule and might alleviate negative impacts of water and temperature stresses (Chen et al. 2003). Also, the results illustrated the genetic variation between the cultivars in their efficiency for the conversion of dry matter to economic performance in this result is involved. Temperature

drop, cold and freezing occurring at the beginning of the growing season caused more challenging conditions for the cultivars, and the Natali cultivar could perform better than other varieties. Similar findings have been reported by other authors (Foadvand et al. 2017; Safavi Fard et al. 2018). The difference between cultivars might be due to the difference in cultivars resistance to environmental stresses and growth habits (ShiraniRad and Zandi 2012). Tesfamariam et al. (2010) reported that water deficit stress at the flowering stage reduced the oil content of canola. Also, Mumpton (1999) stated that the reduction in seed oil percentage and oil yield of canola cultivars under water deficit stress conditions at flowering stage might be due to the oxidation of some unsaturated fatty acids and the reduction of the ability to convert carbohydrates into oil in stress conditions. Results are consistent with the findings reported by Ahmadi and Bahrani (2009).

Since oil yield is positively correlated with seed yield, its increase and decrease is a function of seed yield. Jensen et al. (1996) investigated the effect of water deficit stress on the seed oil content of canola oil and reported that water stress reduced oil yield by 17%. Oil yield was significantly different between the studied cultivars and the Natali cultivar was the superior cultivar. Bellaloui et al. (2013) stated that water deficit stress significantly decreased soybean oil due to the high sensitivity of lipid accumulation during water deficit stress and reduction of current photosynthesis and photosynthetic material supplied for seed filling and also the decrease in seed filling period. Tohidi-Moghadam et al. (2011) showed a decrease in seed oil content under water deficit stress which might be attributed to the oxidation of some unsaturated fatty acids and reduced functionality conversion of carbohydrates to oil under water deficit stress conditions. Ahmadi and Bahrani (2009) reported differences between canola cultivars in oil yield. Plants can protect themselves from mild stresses with osmolyte accumulation (Lotfi et al. 2015). During water shortages, a plant needs to maintain its water potential for continuing growth and development, through the osmotic regulation mechanisms due to the association of compounds such as proline and carbohydrates in the cytoplasm (Ajithkumar and Panneerselvam 2013). Similarly, decreasing proline content with the aging of plants has been reported by Sharma and Kuhad (2006). Tohidi-Moghadam et al. (2011) stated that under water deficit stress conditions, the mean glucosinolate increased by 15%. The amount of glucosinolate content in canola seeds is one of the critical qualitative components of canola and is a common factor in assessing the quality of canola meals. So increasing glucosinolates in the seeds will decrease the beneficial effect of canola oil on the feeding of livestock because they create harmful toxic compounds for livestock. Good commercial canola seeds for the European

Union should have less than $20\mu\text{mol g}^{-1}$ of glucosinolate (Jensen et al. 1996).

Composition of fatty acids is one of the essential characteristics of oilseed crops, and fatty acids content determines the oil nutritional value (Dogan and Akgul 2005). The environment can significantly affect the quality of canola oil (Enjalbert et al. 2013). Bellaloui et al. (2013) also stated that although oil production and fatty acid composition are under genetic control, changes in the composition of fatty acids between genotypes and various environmental conditions such as water deficit stress and temperature are observed. In canola, lipid biosynthesis is highly susceptible to water shortage during flowering, and increases the production of oleic acid against linolenic acid and erusic acid, but varies in different cultivars (Bouchereau et al. 1996). Enjalbert et al. (2013) stated that the decrease in linoleic acid content in drought stress is due to the reduction of grain filling time. Laribi et al. (2009) showed a significant decrease in the total content of fatty acids and their composition under water shortage conditions which might be due to a decrease in oleic and linoleic fatty acids and the increase in palmitic and stearic acid contents. It seems that a water shortage caused a decrease in plant growth which is effective on transferring the materials into seeds and consequently decreases the fatty acids content (Safavi Fard et al. 2018). Canvin (1965) reported the significant impact of plating date on the fatty acid composition of oilseed seeds. Stefanoudaki et al. (2001) reported that moisture stress increases saturated fatty acids, especially palmitic acid. High oleic, linoleic, and low levels of stearic and palmitic acid under water deficit stress, reflect the response of plants to persistent dryness and water stress because persistent stresses allow the plant to adapt to the dryness and may produce with high quality. The results of Ashrafi and Razmjoo (2010) showed that stearic acid and palmitic acid in safflower oil seeds significantly decreased under water stress. They also stated that under water deficit stress, the plant might allocate saturated fatty acids (palmitic acid and stearic acid) to aerial parts instead of seeds and increase water deficit tolerance. In water shortage, decreasing the growth period reduces the amount of saturated fatty acids (Shekari et al. 2016).

Conclusion

The results of the present study showed that oleic acid content of canola oil varied from 63–64% and linoleic acid varied from 14–16%. Irrigation termination from the flowering stage, because of imposing severe water deficit stress, prevented the supply of required material for filling the seeds and the metabolism of the seed compounds and reduced the growth period of the seed. Therefore, a reduction in oleic

and linoleic acid and proline was observed. Also, water deficit stress caused an increase in glucosinolate content of the seed. The reaction of canola cultivars was different in terms of fatty acids so that the Natali cultivar had higher palmitic acid, oleic acid, and proline in both planting dates compared to the other cultivars. Fully irrigated and early planting yielded the highest amount of seed and oil yield, palmitic acid, proline content and the lowest glucosinolate content. Generally, the Natali cultivar and planting date of Oct. 1 and fully irrigated treatment are highly recommended in the studied area regarding qualitative traits and yield of canola.

Conflict of interest A. Khalatbari, A. Shirani Rad, S.A. Valadabady, S. Sayfzadeh and H. Zakerin declare that they have no competing interests.

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