

<https://doi.org/10.17221/519/2019-PSE>

Change in oil fatty acids composition of winter oilseed rape genotypes under drought stress and different temperature regimes

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Citation: Gharechaei N., Paknejad F., Shirani Rad A.H., Tohidloo G., Jabbari H. (2019): Change in oil fatty acids composition of winter oilseed rape genotypes under drought stress and different temperature regimes. *Plant, Soil Environ.*, 65: 503–507.

Abstract: To assess the response of winter oilseed rape promising line genotypes to late-season drought stress in delayed cultivation conditions and select the superior genotypes, an experiment was conducted for two years (2015–16 and 2016–17) in Iran (Karaj). In this experiment, the sowing date was specified in two levels including timely cultivation (October 7) and delayed cultivation (October 27) and irrigation factor including normal irrigation and irrigation interruption from podding stage as factorial in main plots and four winter oilseed rape genotypes (L1030, L1204, L1110, and L1114) and a commercial cultivar (Okapi) were categorized in subplots. Applying drought stress after the podding stage declined the seed yield and seed oil yield, and the highest and lowest mean of these traits were observed in the L1204 and L1114 genotypes, respectively. The interaction effect of the sowing date × genotype on all the studied traits was significant. With the standard erucic acid, the genotype L1204 in both normal and delayed sowing dates had the highest seed yield of 5118 and 3015 kg/ha. Besides, with high oleic acid with a mean of 63.65% and the minimum amount of glucosinolate of seed with a mean of 21.55 μmol/g, this genotype is recommended in delayed cultivation conditions.

Keywords: *Brassica napus* L.; growing season; oil quality; semi-arid environment; water stress

Among non-living stresses, drought is the most important restricting factor of plant growth and agricultural production all over the world, in particular in arid and semi-arid areas (Sun et al. 2013).

Winter oilseed rape (*Brassica napus* L.) has the third rank in the world's vegetable oil supply, and it is the fifth rank in terms of protein (Jaberi et al. 2015). The fall, winter, and early spring rainfalls meet the winter oilseed rape's water need during the growing season; however, in most of the areas, the most critical growth stages, namely, flowering and podding stages, may have no celestial precipitation. Hence, the damage caused by drought stress in different growth stages will be different. Genetic differences also exist in tolerance to drought stress in a wide range of plants, such as winter oilseed rape (Kausar

et al. 2006). Hence, the selection of cultivars with more tolerance to drought stress at the reproductive stage may provide a bed for the development of the cultivation of this plant in Iran and other parts of the world with similar climatic conditions.

Sowing date is also a significant factor affecting seed yield and seed oil content (Shirani Rad et al. 2017). By providing the necessary growth rates of winter oilseed rape plants and reducing their vulnerability to frost, the appropriate sowing time significantly enhances the seed yield (Moradi Aghdam et al. 2019). The results of different researches 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 is obtained from the appropriate sowing date (Bashir et al. 2010).

The quality of seed oil is one of the most important traits for *Brassica* species breeding purposes in semi-arid environments, and the qualitative properties of any oil depend on the composition of its fatty acids (Safavi Fard et al. 2018). Shahsavari (2019) also reported that seed yield was influenced by water shortages from flowering until the end of seed filling, and in drought stress conditions, winter oilseed rape cultivars that we were able to maintain more water content had more seed and oil yields.

Given that in some cold and temperate cold areas, farmers do not succeed in timely culture for different reasons, investigation of the reaction and selection of winter oilseed rape genotypes with a higher yield stability in slow-growing conditions have a significant impact on the development of winter oilseed rape cultivation, in particular in areas where spring crops harvesting is taken place late.

By summing up what has been mentioned, the goal of this project is to assess the effect of treatments of irrigation interruption from the podding stage (reducing the two plant irrigation cycles and saving water consumption by 1600 m³ per hectare), the effect of delayed cultivation on the composition of oil fatty acids, and the reaction of five fall-winter oilseed rape genotypes in terms of oil quality.

MATERIAL AND METHODS

Experimental design. In order to assess the response of winter oilseed rape promising line genotypes to late-season drought stress in delayed cultivation conditions and select the superior genotypes with higher seed yield stability in these conditions, a factorial split-plot experiment was conducted in a randomized complete block design with three iterations for two cultivation years (2015–16 and 2016–17) in Iran (Karaj). In this experiment, the sowing date factor was specified in two levels including timely cultivation (October 7) and delayed cultivation (October 27) and irrigation factor including normal irrigation and irrigation interruption from podding stage (this time in normal sowing date was the early May, and in delayed sowing date it was in mid May) as factorial in main plots and four winter oilseed rape genotypes (L1030, L1204, L1110, and L1114) and a commercial cultivar (Okapi) were categorized in subplots.

Experimental procedure. Each experimental plot included six six-meter lines with 30 cm spacing and plant spacing on the line was 4 cm, with two lateral lines as margins. To determine the seed yield, the

plants in the 4.8 m² area of each plot were separately submerged and precisely weighed and calculated. To determine the seed oil percentage, a sample of 5 g of seed was selected from each plot, and its percentage was specified by nuclear magnetic resonance (NMR) device (ISO 5511, 1992). After determining the seed oil percentage, by multiplying it by seed yield, seed oil yield was calculated in kg/ha. The gas chromatography method was used to measure and determine the fatty acids in seed oil (Azadmard-Damirchi et al. 2005). The amount of seed glucosinolate was measured by a spectrophotometer (Cary, USA) (Makkar et al. 2007).

Statistical analysis. By performing the Bartlett test and after determining the insignificant result of the test, combined ANOVA was performed using SAS v.9 software. Duncan's multiple range test was used at a 5% level to compare the means. Excel software (Redmond, USA) was used to draw charts.

RESULTS AND DISCUSSION

Seed yield. The effect of genotype at 1% level and the effect of sowing date and irrigation at 5% level and interaction effect of sowing date × genotype and irrigation × genotype were respectively significant at 5% and 1% on seed yield. Delayed sowing significantly reduced seed yield in all genotypes, and the L1204 genotype with the 41% reduction in yield compared to the timely sowing date had the highest mean yield. The L1114 genotype had the lowest mean seed yield in delayed cultivation conditions (Table 1). Delay in winter oilseed rape cultivation has encountered the plant ripeness period with high temperatures of the environment, increasing the rate of respiration of the pods, resulting in reduced photosynthetic and seeds weight and, finally, reducing yield (Rafiei et al. 2011). Also, by applying the late-season drought stress, seed yield decreased, and L1204 and L1114 genotypes showed the highest and lowest values of this trait with 28% and 32% decrease, respectively (Figure 1). It seems that the reduction of photosynthetic materials under stress conditions and failure to sufficiently supply them for the pods and consequently, their loss will ultimately result in reduced yields. These results have also been reported by some other researchers (Shahsavari et al. 2014a, Moradi Aghdam et al. 2019).

Seed oil yield. The profitability of winter oilseed rape depends on the amount of oil produced per area unit (Robertson et al. 2004). The simple effects of genotype, sowing date and irrigation at 1% and 5% probability levels, and interaction effects of sowing

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Table 1. Mean comparison of simple effect of irrigation on winter oilseed rape characteristics

Irrigation	Erucic acid	Linoleic acid	Linolenic acid	Glucosinolate ($\mu\text{mol/g}$)
		(%)		
Normal irrigation (control)	0.27 ^b	16.16 ^a	5.86 ^b	16.17 ^b
Irrigation interruption from podding stage	0.35 ^a	14.93 ^b	6.70 ^a	20.77 ^a

Any two means sharing a common letter do not differ significantly from each other at 5% probability

date \times genotype and irrigation \times genotype at 1% level were also significant. Delayed sowing date reduced the seed oil yield. The highest seed oil yield related to the L1204 genotype at 5118 kg/ha on the sowing date of October 7. The highest and lowest seed oil yield on October 27 was observed in L1204 and L1114 genotypes, respectively (Table 1). Temperature is one of the environmental factors affecting the seed oil yield, whose increase leads to a significant drop in oil yield. This temperature reducing oil yields are more pronounced in delayed sowing dates (Darby et al. 2013). For each degree of temperature during the flowering and seed filling period, seed oil content decreases by 1.7% (Robertson et al. 2004). Irrigation interruption from the podding stage reduced the seed oil yield. The amount of this reduction in the studied genotypes was 30% to 35%, respectively, compared to the optimal irrigation, and with the highest oil yield, L1204 genotype was the best genotype in these conditions (Figure 2). In this study, the oil yield was more affected by the shortage of soil moisture content compared to the seed oil percentage. It seems that the reason for this is the increased control of seed oil content by genetic factors and high seed

yield changes effects on oil yield compared to the oil percentage (Zarei et al. 2010).

Oleic acid. The studied genotypes had a significant difference in terms of fatty acid content at a 1% level. The comparison of the average interaction effect of sowing date \times genotype revealed that the highest amount of this unsaturated fatty acid was allocated to the L1204 genotype in the timely sowing date (October 7) with the mean of 64.90%. Besides, also in delayed sowing date (October 27), L1204 genotype with the mean of 63.65% and cv. Okapi, with a mean of 63.21%, had the highest and lowest amount of oleic acid ($\omega 9$) (Table 1). The oil quality is mainly determined by the amount of oleic and linoleic fatty acids (Ul-Hassan et al. 2005).

Oleic acid has the highest amount of winter oilseed rapeseed oil and plays a significant role in increasing the winter oilseed rapeseed oil quality.

Linoleic acid. The type and amount of fatty acids in the oil of the studied genotypes indicate oil quality. The simple effect of irrigation at a 5% level and interaction effect of sowing date \times genotype at a 1% level on linoleic acid was significant. Comparing the average effect of irrigation on the amount of linoleic

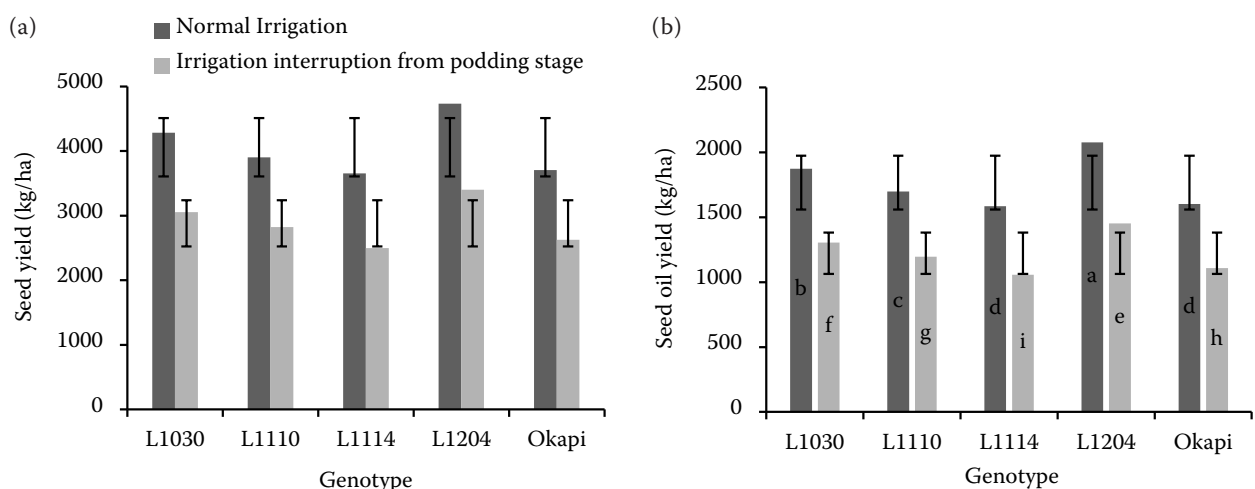


Figure 1. Mean comparisons of the interaction effect of irrigation \times genotype on (a) seed yield and (b) seed oil yield (the vertical bars indicated *LSD* (least significant difference)). The mean values with different letters across treatments are significantly different at $P < 0.05$

acid revealed that late-season drought stress caused a decrease of 7.6% of this unsaturated fatty acid compared to control treatment so that the mean linoleic acid in normal irrigation and irrigation interruptions were 16.6% and 14.93%, respectively (Table 1). Shahsavari et al. (2014b) also reported that the winter oilseed rape oil and linoleic acid content decreased under drought stress conditions. In the investigation of winter oilseed rape genotypes, the delayed sowing date decreased the linoleic acid percentage, and the L1204 genotype was the superior genotype with a 15% reduction compared to the timely cultivation, and the L1110 genotype was ranked second (Table 1). Differences have also been observed in the response of genotypes to the latency of linoleic acid in other studies (Safavi Fard et al. 2018, Moradi Aghdam et al. 2019).

Since linoleic acid is an important factor in the photosynthetic activity of the plant (Hugly et al. 1989) and the winter oilseed rape pollen development (McConn and Browse 1996), it seems that increasing its percentage in terms of delayed sowing is for achieving maximum seed yield.

Linolenic acid. Significant differences in linolenic acid genotypes in both timely sowing dates (October 7) and delayed sowing date (October 27) showed that delayed cultivation increased linolenic acid compared to the timely sowing dates (Table 2). With the value of 30.9%, cv. Okapi showed the highest amount, and the L1204 genotype with the value of 29.81% showed the lowest amount. The average linolenic acid percentage (ω 3) significantly varied at different irrigation levels, and drought stress increased linolenic acid by 14.3% compared to the normal irrigation conditions (Table 2). An increase of 1.7 to 2 percent of linolenic unsaturated

fatty acid in winter oilseed rape oil was reported due to drought stress in Mediterranean climate conditions (Aslam et al. 2009). Furthermore, Shahsavari et al. (2014b) reported that drought stress in the winter oilseed rape podding stage caused an increase of 35% in linolenic acid compared to the control treatment (normal irrigation).

Erucic acid. The studied genotypes were significantly different in terms of erucic acid content in different sowing dates, so that the L1204 genotype, with an average of 0.19% and 0.38%, had the lowest amount of erucic acid in both normal and delayed sowing dates (Table 2). Comparing the mean irrigation effect (Table 2) revealed that the late-season drought stress (irrigation interruption from the podding stage) caused an increase of 30% in the erucic acid amount compared to the normal irrigation. Accordingly, in all irrigation conditions, the erucic acid amount that is an important indicator for winter oilseed rape and its edible consumption was standard (less than 2%). Ullah et al. (2012) and Shahsavari et al. (2014b) reported that drought stress enhanced the percentage of winter oilseed rape erucic acid and reduced the percentage of winter oilseed rape oleic and linoleic acid.

Seed glucosinolate. The mean seed glucosinolate content at various irrigation levels significantly varied, so that the mean of this trait in normal irrigation and irrigation interruption from podding stage treatments were 16.71 and 20.77 μ mol/g of seed meal, respectively (Table 2). Comparing the mean of cultivars at different levels of the sowing date suggested that in the timely sowing date (October 7), the L1204 genotype with the mean of 13.73 μ mol/g had the lowest amount of glucosinolate. Also, in delayed sowing date (October 27),

Table 2. Mean comparisons of the interaction effect of sowing date and cultivar on winter oilseed rape characteristics

Sowing date	Genotype	Seed yield (kg/ha)	Oil yield	Oleic acid	Linoleic acid	Linolenic acid	Erucic acid	Glucosinolate (μ mol/g)
					(%)			
Oct. 7	L1030	4809 ^b	2125 ^b	4809 ^b	2125 ^b	44.07 ^a	5.14 ^b	13.98 ⁱ
	L1010	4165 ^c	1824 ^c	4165 ^c	1824 ^c	43.75 ^b	5.05 ^c	15.11 ^h
	L1114	3854 ^d	1684 ^d	3854 ^d	1684 ^d	43.63 ^b	5.02 ^d	15.43 ^g
	L1204	5118 ^a	2267 ^a	5118 ^a	2267 ^a	44.18 ^a	5.17 ^a	13.73 ⁱ
	Okapi	4013 ^{cd}	1746 ^{cd}	4013 ^{cd}	1746 ^{cd}	43.45 ^c	4.95 ^e	15.98 ^f
Oct. 27	L1030	2531 ^{fg}	1051 ^{fg}	2531 ^{fg}	1051 ^{fg}	41.45 ^{fg}	4.34 ⁱ	23.39 ^b
	L1010	2560 ^f	1068 ^f	2560 ^f	1068 ^f	41.63 ^e	4.44 ^g	21.97 ^d
	L1114	2297 ^h	958 ^{fg}	2297 ^h	958 ^{fg}	41.58 ^{ef}	4.39 ^h	22.53 ^c
	L1204	3015 ^e	1262 ^e	3015 ^e	1262 ^e	41.83 ^d	4.46 ^f	21.55 ^e
	Okapi	2322 ^{gh}	964 ^g	2322 ^{gh}	964 ^g	41.39 ^g	4.33 ^j	23.75 ^a

Any two means sharing a common letter do not differ significantly from each other at 5% probability

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L1204 and L1110 genotypes had less glucosinolate than other genotypes (Table 2). Therefore, in both timely and delayed sowing dates and all irrigation conditions, glucosinolate amount of L1204 genotype was standard and less than 30 $\mu\text{mol/g}$ of seed meal. Increasing glucosinolate decreases the quality and nutritional value of winter oilseed rape meal (Salisbury et al. 1987).

Given the results, with the superior fatty acid composition, the highest seed yield and seed oil yield, besides low and standard erucic acid and glucosinolate for reducing damage caused by delayed cultivation and drought stress at the late stages of winter oilseed rape growth, the L1204 genotype is recommended in the regions similar to the test site. Hence, by the selection of genotypes that produce reasonable economic yield and standard qualitative traits, it is possible to increase the under cultivation areas of this plant in the cold and temperate-cold regions with dry and semi-dry climates.

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Received on September 24, 2019

Accepted on October 21, 2019

Published online on October 29, 2019