Influence of drought stress and Chitosan on fatty acid compounds of rapeseed varieties

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Abstract

In order to investigate the changes in fatty acids composition of rapeseed lines under Chitosan application, a factorial split-plot experiment was conducted based on RCBD in 3 replications during 2 years (2014-2016) in Karaj, Iran. Irrigation and Chitosan as main plots and cultivar in sub-plots were considered. In this study, treatments included irrigation at 2 levels (normal irrigation and cut off irrigation after flowering stage), Chitosan at 2 levels (pure water and foliar application of Chitosan 250 mg/l), and rapeseed cultivars (BAL104, DIE710/08, BAL101, BAL102, QUIE03/11, and OKAPI). The result of ANOVA showed irrigation and cultivar had a significant effect on fattiest acids. Results exhibited cut off irrigation after the flowering stage reduced the oleic acid content compared to normal irrigation. Results also indicated that erucic acid and grain glucosinolate contents were different among the mentioned varieties and they were influenced by irrigation regimes. Cut off irrigation at the flowering stage increased erucic acid and grain glucosinolate in all varieties. On the other hand, the lowest erucic acid and grain glucosinolate were observed in cv. Okapi under normal irrigation, and it might be considered useful rapeseed to include in the diet as it contains low specific glucosinolate and erucic acid. Moreover, var. DIE710/08 had the lowest erucic acid and grain glucosinolate under drought stress conditions.

Keywords: erucic acid; irrigation regime; unsaturated fatty acids; rapeseed hybrids

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Introduction

Rapeseed (*Brassica napus* L.) is the most important oilseed plant source and the third plant oil in the world after soybean (Glycine max) and palm oil (*Elaeis guineensis* L.)1. New varieties naturally contain 40-45% oil which is used as raw materials to produce industrial and hydraulic oil, cleanser, soap, and biodegradable plastics2. Rapeseed (*Brassica napus* L.) production in the Mediterranean region is often limited by sub-optimal moisture conditions. This crop is the main oilseed crop in the agricultural systems of many arid and semiarid areas where its yield is often

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restricted by water deficit and high temperatures during the reproductive development. Seed yield can be mainly limited even by the relatively short period of soil moisture shortage during the reproductive growth3.

Water shortage is considered to be one of the most adverse abiotic stress factors influencing plant growth. Physiological and biochemical aspects of water deficit adversely impact the social and economic life of mankind4, and impair crop production5. In the water deficit condition, tolerant varieties show better adaptation capability. Improvement of productivity of rapeseed genotypes under drought stress has rarely been included in breeding programs6.

Chitosan is a biopolymer, a chitin derivative, a compound that is completely safe for the environment. This compound is characterized by unique properties, such as bioactivity and biocompatibility7. Studies indicate that Chitosan can increase the yield8, reduce transpiration,9 and induce a range of metabolic changes as a result of which, plants become more resistant to viral, bacterial, and fungal infections10. Chitosan can be used both in vivo and in vitro and can be sprayed on plant aerial organs to induce the accumulation of bioactive secondary metabolites11, 12. It was shown that Chitosan reduced plant transpiration in pepper plants, resulting in a 26%-43% reduction in water use without a reduction in dry matter yield13. These results suggested that Chitosan might be an effective anti-transpiratory agent for reducing the consumption of irrigation water in agriculture.

The objective of this study was to determine the changes in the fatty acid composition of oil saturated and unsaturated lipids of six new rapeseed hybrids as a response to water stress and Chitosan treatment.

Material and Methods

The study was performed during 2013-2015 at the research farm of Seed and Plant Breeding Institute, Karaj, Iran. The site of the conducted experiment was characterized with latitude 35.49°N and longitude 51.6°E with an elevation of 1321 m above the sea level. Mean precipitation per year was 243 mm and mean temperature per year was 13.5 °C. The experimental soil was silt-loam textually with a pH of 7.2-7.9 which was slightly alkaline and EC of 1.24-1.45) × 10 ds m^{-1}.

The experiment was arranged as a factorial split-plot based on RCBD with three replications for 2 years. Irrigation at 2 levels (normal irrigation and cut off irrigation after flowering stage), Chitosan at 2 levels (water as control and Chitosan foliar application) as factorial in main plots and 6 cultivars (BAL104, DIE710/08, BAL101, BAL102, QUIE03/11, and OKAPI) in subplots were considered. The foliar application of Chitosan (5 g. L^{-1}) was done at two steps (stemming and flowering stages).

Each plot consisted of 6 rows of 6 m long, spaced 30 cm apart using a seeding rate of 6 kg ha^{-1}. The experimental fields were moldboard plowed and seed-bed preparation consisted of two passes with a tandem disk. Seeds were planted 1 to 1.5 cm deep at a rate of 80 seeds m^{-2} on October 12th, 2014 and 2015. For all treatments, N: P:K fertilizers were applied at rates of 150:60:50 kg ha^{-1}, respectively. All of P, K fertilizers and one-third of N fertilizer were incorporated and added to soil pre-sowing. Another two-thirds of N fertilizer was split equally at the beginning of stem elongation and flowering stages. Weeds were controlled by application of haloxyfop- R-methyl ester (Gallant Super, 10% EC) at 0.5 L ha^{-1}. Broadleaf weeds were also hand weeded during the season. Final harvests were conducted out on June 5th, 2015 and June 3rd, 2016.

Oil extraction and fatty acid composition analysis

The studied characteristics included: Oleic acid, Linolenic acid, Linoleic acid, Palmitic acid, Erucic acid and grain glucosinolate contents which were measured as follows: The bags contain the seeds of each plot (150 g grain) transported to the laboratory. One ML soluble of H_2SO_4 (2.4%) and Methanol (98%) was added per sample (40.1 v/v) and heated under 80° C for 1 hour. After cooling, 500 µL Hexane with 1.5 ML NaCl (0.9) (w/v) were mixed and added to sample until methyl fatty acid was extracted (FAME). Then, the sample and its supernatant (including hexane-FAME) were separated by 4000 rpm centrifuge (Sigma 8k) at 10 Min, before injection into gas-liquid chromatography device to determine the fatty

Table 1
Variance analysis of fatty acids compounds of canola

S.O.V	D.f	Oleic acid	Linolenic acid	Linoleic acid	Palmitic acid	Erucic acid	Grain glucosinolate
Year	1	12.369	17.002	0.689	4.347	59.985	19.765
Error	4	8.156	0.265	0.205	1.265	2.741	3.082
Irrigation	1	588.911 **	556.803 **	260.445 **	87.610 **	13231.901 **	1747.170 **
Year × Irrigation	1	1.383	0.595	0.084	1.103	3.240	3.977
Chitosan	1	1.666	1.613	0.590	0.198	43.758	4.131
Year × Chitosan	1	0.0001	0.0001	0.005	0.0001	1.051	0.0001
Irrigation × Chitosan	1	0.388	0.492	0.108	0.051	8.123	1.394
Year × Irrigation × Chitosan	1	0.002	0.005	0.0001	0.001	0.865	0.009
Error	12	34.311	7.125	0.970	0.301	16.592	4.319
Cultivar	5	3.617	3.117 **	1.472 **	0.450 **	90.917 **	9.780 **
Year × Cultivar	5	0.005	0.010	0.007	0.018	2.006	0.063
Irrigation × Cultivar	5	4.411	4.346	1.793 **	0.568 **	108.177 **	8.799 **
Year × Irrigation× Cultivar	5	0.009	0.003	0.005	0.018	1.333	0.016
Chitosan × Cultivar	5	0.077	0.074	0.019	0.011	1.765	0.327
Year ×Chitosan × Cultivar	5	0.002	0.001	0.001	0.001	0.096	0.007
Irrigation ×Chitosan × Cultivar	5	0.077	0.084	0.029	0.009	2.257	0.266
Year × Irrigation × Chitosan × Cultivar	5	0.002	0.001	0.002	0.0001	0.045	0.003
Error	80	2.271	0.857	0.067	0.060	2.791	0.662
CV (%)	-	2.37	4.89	4.37	4.80	5.33	6.67

**and * significant at 1 and 5 %, respectively

acid composition profile 14 using an Agilent Technologies 6890N gas chromatograph.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) at $p<0.01$ and comparison among mean values of treatments was made by Duncan's multiple range test 15. SPSS software ver. 16.0 was used to calculate correlation coefficients between traits.

Results

Results of ANOVA showed that the irrigation regime had a significant effect on oleic acid, linolenic acid, linoleic acid, palmitic acid, erucic acid, and grain glucosinolate contents. The cultivars also had significant differences regarding linolenic acid, linoleic acid, palmitic acid, erucic acid, and grain glucosinolate contents. The effect of irrigation × cultivar interaction was significant on linoleic acid, palmitic acid, erucic acid, and grain glucosinolate contents (Table 1).

The mean comparison of irrigation effect on oleic acid revealed that cut off irrigation after the flowering stage reduced the oleic acid content compared to normal irrigation (Table 2). Water stress causes a significant reduction of about 15% in the concentration of oleic acid in sunflower standard hybrid 16. This supported the previous findings showing that drought-induced decrease in oleic acid of rapeseed was associated with an increase in erucic acid content which was ameliorated by applied growth regulators17. The correlation confident analysis of data also demonstrated a significant negative relationship (-0.716**) between oleic acid and erucic acid. Besides, oleic acid had a significant negative correlation with linolenic acid and glucosinolate content. Furthermore, there was a positive relationship (0.704**) between oleic acid and grain yield (data not shown) (Table 3).

Table 2
Mean comparison of fatty acid compounds of canola

Irrigation	Cultivar	Oleic acid (%)		Linolenic acid (%)		Linoleic acid (%)		Palmitic acid (%)		Erucic acid (%)		Grain glucosinolate (mg/g.fw)	
I_1		65.5	a	20.90	a	4.584	b	5.896	a	0.21	b	8.706	b
I_2		61.4	b	16.97	b	7.274	a	4.336	b	0.40	a	15.67	a
	BAL104	63.2	a	18.68	b	6.09	a	5.015	b	0.32	a	12.65	ab
	DIE710/08	64.1	a	19.55	a	5.48	c	5.363	a	0.28	d	11.06	d
	BAL101	63.4	a	18.92	b	5.91	b	5.125	b	0.31	b	12.27	bc
	BAL102	63.1	a	18.63	b	6.10	a	5.014	b	0.33	a	12.81	a
	QIE03/11	63.1	a	18.67	b	6.13	a	5.017	b	0.32	a	12.46	ab
	Okapi	63.6	a	19.15	ab	5.84	b	5.162	b	0.30	c	11.89	c

Means within the same column and rows and factors, followed by the same letter are not significantly difference ($P < 0.05$). I_1: Normal irrigation, I_2: Irrigation cut off after flowering stage

Table 3
Pearson correlation coefficient between characteristics

Characteristics	Grain yield	Palmitic acid	Linolenic acid	Linoleic acid	Oleic acid	Erucic acid	Glucosinolate
Grain yield	1						
Palmitic acid	0.902**	1					
Linolenic acid	-0.949**	-0.886**	1				
Linoleic acid	0.864**	0.851**	-0.859**	1			
Oleic acid	0.704**	0.660**	-0.664**	0.707**	1		
Erucic acid	-0.972**	-0.907**	0.938**	-0.852**	-0.716**	1	
Glucosinolate	-0.951**	-0.902**	0.965**	-0.873**	-0.662**	0.941**	1

** significant at 1%

Results demonstrated that linolenic acid content decreased by about 25% by cutting off irrigation after flowering compared to the control (Table 2). Results also revealed that there was a significant difference among the studied cultivars regarding linolenic acid content so that the highest linolenic acid content was observed in var. DIE710/08 with a mean of 19.55% that was in the same statistical group as cv. Okapi (Table 2). Connor and Sadras (1992) reported that fatty acid composition differs between cultivars due to changes in environmental conditions18. The result of the correlation analysis revealed that linolenic acid had a vigorous negative correlation with grain yield (-0.949**). Moreover, it had a significant negative correlation with palmitic acid, linoleic acid, and oleic acid while showing a positive correlation with erucic acid and glucosinolate (Table 3).

Furthermore, var. QIE03/11 had the highest level of linoleic acid compared to other varieties (Fig. I.a). The result of correlation analysis demonstrated that linoleic acid had a positive

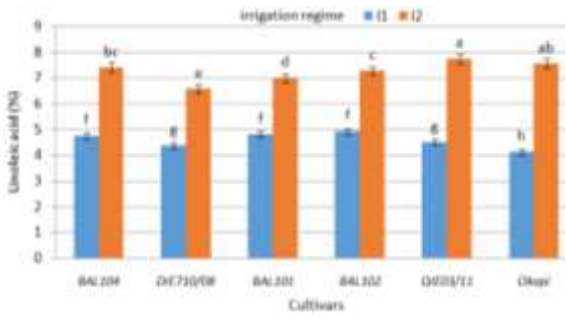

Fig. I.a. Interaction of irrigation and cultivar on linoleic acid content; I1: Normal irrigation; I2: Cut off irrigation after flowering stage; error bars represent the standard deviations of the means.

correlation with grain yield, palmitic acid, and oleic acid while it showed a negative correlation with linolenic acid, erucic acid, and glucosinolate content (Table 3).

In this study, irrigation cut off at the flowering stage provided a decrease in Palmitic acid about 26.5% (Table 2, Fig. I.b). The result showed cv. Okapi had the highest palmitic acid content under normal irrigation, but var.

DIE710/08 had the highest level under water stress conditions (Fig. I.b).

Results of this experiment indicated that erucic acid and grain glucosinolate contents were the meaningfully different among tested varieties and they were influenced by irrigation regimes. Irrigation cut off at the flowering stage increased erucic acid and grain glucosinolate in all varieties. On the other hand, the lowest erucic acid and grain glucosinolate were observed in cv. Okapi under normal irrigation, that it might be considered useful rapeseed to include in the diet as it contains low specific glucosinolate and erucic acid. Moreover, var. DIE710/08 had the lowest erucic acid and grain glucosinolate under irrigation cut off after flowering treatment (Fig. I. c and d). The result of correlation analysis suggested that both erucic acid and grain glucosinolate had a positive correlation as well as with linolenic acid. They had also an inverse correlation with grain yield, linoleic, palmitic acid, and oleic acid (Table 3).

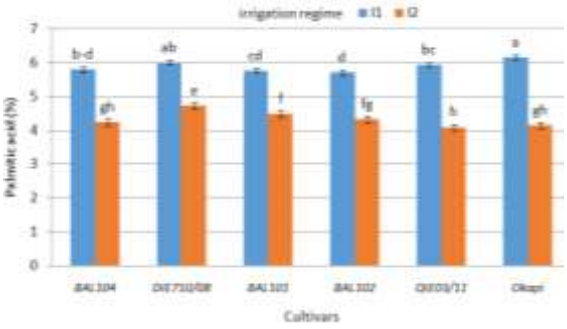

Fig. I.b. Interaction of irrigation and cultivar on palmitic acid content; I1: Normal irrigation; I2: Cut off irrigation after flowering stage; error bars represent the standard deviations of the means.

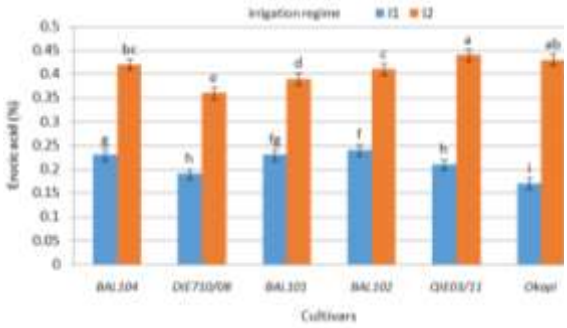

Fig. I.c. Interaction of irrigation and cultivar on erucic acid content; I1: Normal irrigation; I2: Cut off irrigation after the flowering stage; error bars represent the standard deviations of the means.

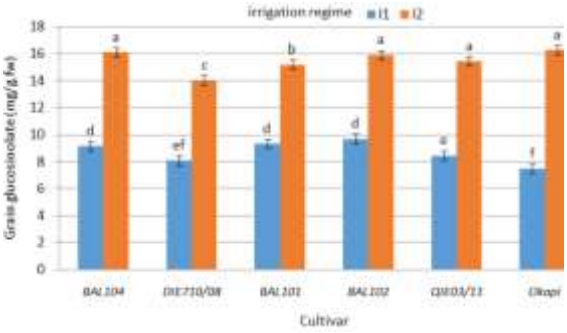

Fig. I.d. Interaction of irrigation and cultivar on grain glucosinolate content; I1: Normal irrigation; I2: Cut off irrigation after the flowering stage; error bars represent the standard deviations of the means.

Discussion

Results revealed that water stress at flowering stage caused an increase in linoleic acid in all varieties compared to normal irrigation. In this regard, the effect of water stress on the content of oleic and linoleic acid may be attributed to the activity of the enzyme Δ12 desaturase16. Some researchers found that the increase in oleic acid was due to the activity of Δ12 desaturase, responsible for the conversion of oleic to linoleic acid, which was affected due to water stress16. The results obtained in the present study are confirmed by Sobrino et al. (2003) who found a strong inverse relationship between oleic and linoleic acid contents and reported that Δ12 desaturase enzyme is responsible for these responses19.

The results of our study indicated that water stress at flowering stage caused an increase in erucic acid and grain glucosinolate content in all varieties. The induction of glucosinolates accumulation by drought conditions has been reported as part of the plant response to stress through the process of osmotic adjustment20. However, contradictory results have been discovered in the literature when high drought had no effect on the concentration of total glucosinolates in *Brassica oleracea* L.21 or in Brassica napus L. under mild drought stress22. Water deprivation produced significant glucosinolate reductions in Brassica oleracea23 and in the rosette leaves of Arabidopsis thaliana L.24. Therefore, the intensity and duration of drought appear to be an important factor in the accumulation of each specific glucosinolate, as

well as the developmental stage of the plant when the stress is applied25.

Conclusions

Results suggested that the unsaturated fatty acids, i.e. oleic acid and linolenic acid were declined under drought stress at the flowering stage and also grain glucosinolate and erucic acid contents as anti-quality factors were increased under these conditions. Results also indicated that DIE710/08 variety had the best quality among studied varieties regarding fatty acid composition by having less glucosinolate and erucic acid contents and higher unsaturated fatty acids. No effect of Chitosan application was found on these attributes. Therefore, higher doses of Chitosan are recommended to determine its effects on physiological and biochemical attributes of rapeseeds under water deficit conditions.

References

Al-Hester M.Y., M.A. Zainal Abidin, M. Sariah, and **M.Y. Wong**. 2011. 'Antifungal activity of Chitosan against *Fusarium oxysporumf*. sp. cubense'. *J. Appl. Polym. Sci.,* 120: 2434-2439.

Anjum, S. A., M. F. Saleem, M. A. Cheema, M. F. Bilal, and **T. Khaliq**. 2012. 'An assessment of vulnerability, extent, characteristics, and severity of drought hazard in Pakistan'. *Pakistan J. Science*. 64 (2): 138-143.

Baldini, M., R. Givanardi and **G.P. Vanozzi**. 2000. 'Effect of different water availability on the fatty acid composition of the oil in standard and high oleic sunflower hybrids'. Proceedings of 15th International Sunflower Conference, A.79-A.84.Toulouse, France, 12-15 June.

Bitelli M., M. Flury, G.S. Campbell, and **E.J. Nichols.** 2001. 'Reduction of transpiration through foliar application of chitosan', *Agric. Forest Meteorol*. 107: 167–175.

Bouchereau, A., N. Clossais-Besnard, A. Bensaoud, L. Leport, and **M. Renard**. 1996. 'Water stress effects on rapeseed quality'. *Europ. J. Agron*., 5: 19-30.

Byczynska B and **J. Krzymanski**. 1969. 'A fast method for obtaining fatty acids methyl esters to be analyzed by the gas chromatography method (in Polish)'. *TluszczeJadalne XIII*: 108–114.

Chaghakaboodi, Z., A. Zebarjadi, and **D. Kahrizi.** 2012a. 'Evaluation of Rapeseed Genotypes Response to Drought Stress via Callus Culture'. *Biotechnology in Agriculture* 10(2): 49-58.

Cheema K.L and **H.A. Sadaqat**. 2004. 'The potential and genetic basis of drought tolerance in canola (*Brassica napus*) I. Heterosis manifestation in some morphophysiological traits in canola. *International Journal of Agriculture and Biology*, 6: 82–85.

Connor D.J and **V.O. Sadras**. 1992.'Physiology of yield expression in sunflower'. *Field Crops Res*. 30, 333- 389.

Del Carmen Martínez-Ballesta, M., D. A. Moreno, and **M. Carvajal**. 2013. 'The Physiological Importance of Glucosinolates on Plant Response to Abiotic Stress in Brassica'. *International Journal of Molecular Sciences*, 14(6), 11607–11625.

Dias A.M.A., A.R. Cortez, M.M. Barsan, J.B. Santos, C.M.A. Brett, and **H.C. De Sousa**. 2013. 'Development of greener multi-responsive Chitosan biomaterials doped with biocompatible ammonium ionic liquids.' *ACS Sustainable Chem. Eng.*, 1(11): 1480-1492.

Duncan, D.B. 1955. 'Multiple range 'F' tests. Biometrics', 11: 1-42.

Dzung N.A., V.T.P. Khanh, and **T.T. Dzung**. 2011. 'Research on the impact of Chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee'. Carbohydr.Polym., 84: 751-755.

FAO, 2013. FAOSTAT. Food and Agriculture Organization of the United Nations.

Friedt, W., R. Snowdon, F. Ordon, and **J. Ahlemeyer**. 2007. 'Plant Breeding: Assessment of Genetic Diversity in Crop Plants and its Exploitation in Breeding'. *Progress in Botany*, 168, 152-177.

Gutbrodt B., S. Dorn, S.B. Unsicker and **K. Mody**. 2012. 'Species-specific responses of herbivores to within-plant and environmentally mediated between-plant variability in plant chemistry' .*Chemoecology*. 22:101–111.

Hamrouni I., H.B. Salah and **B. Marzouk**. 2001. 'Effects of water-deficit on lipids of sunflower aerial parts'. *Phytochemistry*. 58: 227-280.

Jensen C.R., V. Mogensen, G. Mortensen, J. Fieldsen, G. Milford, M. Andersen and **J. Thage** 1996. 'Seed glucosinolate, oil and protein contents of field-grown rape (*Brasica napus* L.) affected by soil drying and evaporative demand'. *Field Crops Res*.47:93–105.

Khan M.A.M., C.h. Ulrichs and **I. Mewis**. 2011. 'Drought stress—Impact on glucosinolate profile and performance of phloem-feeding cruciferous insects'. *Acta Hort*.917:111–117.

Lei C.Y., D.M. Ma, G.B. Pu, X.F. Qiu, Z.G. Du, H. Wang, G.F. Li, H.C. Ye, and **B.Y. Liu.** 2011. 'Foliar application of chitosan activates artemisinin biosynthesis in (*Artemisia annua* L.'), *Ind. Crop. Prod*. 33: 176–182.

Mondal M.M.A., M.A. Malek, A.B. Puteh, M.R. Ismail, M. Ashrafuzzaman and **L. Naher**. 2012. 'Effect of foliar application of Chitosan on growth and yield in okra'. *Aust. J. Crop*, 6: 918-921.

Ren H., H.X. Zhong, S.J. Dai, S.X. Chen and **X.F. Yan.** 2009. 'Water stress on glucosinolate contents in Arabidopsis rosette leaves'. *Acta Ecol. Sin*.8:4372–4379.

Schreiner M., B. Beyene, A. Krumbein, and **H. Stutzel**. 2009. 'Ontogenetic changes of 2-propenyl and 3-indolylmethyl glucosinolates in *Brassica carinata* leaves as affected by water supply'. *J. Agric. Food Chem*.57:7259–7263.

Sobrino E, A.M. Tarquis and **M.C Diaz**. 2003. 'Modeling the oleic acid content in sunflower oil'. *Agron. J.* 95, 329- 334.

Yin H., X.C. Fretté, L.P. Chrestensen, and **K. Grevsen**. 2011. 'Chitosan oligosaccharides promote the content of polyphenols in Greek oregano (*Origanum vulgare* ssp. hirtum)', *J. Agric. Food Chem.* 60: 136–143.