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OIL QUALITY OF CANOLA CULTIVARS IN RESPONSE TO WATER STRESS AND SUPER ABSORBENT POLYMER APPLICATION[1]

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RESUMO

QUALIDADE DE ÓLEO DE CULTIVARES DE CANOLA EM RESPOSTA AO ESTRESSE HÍDRICO E APLICAÇÃO DE POLÍMERO HIDROABSORVENTE

O estresse hídrico limita significativamente o crescimento de plantas e a produtividade da cultura. Por isto, o manejo eficiente da umidade no solo e o estudo de alterações metabólicas que ocorrem em decorrência do estresse hídrico são importantes para a agricultura. O presente estudo objetivou avaliar o efeito de seis genótipos de canola (Rgs003, Sarigol, Option500, Hyola401, Hyola330 e Hyola420), cultivados com e sem estresse hídrico e com e sem a presença de polímero hidroabsorvente, na qualidade e teor do óleo extraído de suas sementes. Utilizou-se delineamento em blocos casualizados, com parcelas subdivididas, em esquema fatorial 2x2x6 (estresse hídrico x polímero x genótipos), com três repetições. A pesquisa foi desenvolvida em área pertencente ao Seed and Plant Improvement Institute, em Karaj, Irã. Observou-se efeito significativo para os níveis de estresse hídrico, presença de polímero e genótipos, no teor e composição do óleo e no teor de glucosinolato. O estresse hídrico reduziu os teores de óleo e ácido linoleico, mas aumentou o teor de glucosinolato e de ácido esteárico. A aplicação do polímero hidroabsorvente aumentou o teor de ácido linoleico, mas diminuiu outros componentes. Foi possível concluir que, sob estresse hídrico, a aplicação de polímero hidroabsorvente, em razão de sua maior retenção de água, aumentou a capacidade de armazenamento desta no solo, o que contribuiu para um aumento no período vegetativo da planta, favorecendo a qualidade do óleo, por diminuir o teor de ácidos graxos saturados e aumentar o de insaturados.

PALAVRAS-CHAVE: *Brassica napus* L.; hidrogel; ácidos graxos; glucosinolato.

ABSTRACT

Water stress significantly limits plant growth and crop yield. Hence, the efficient management of soil moisture and the study of metabolic changes which occur in response to drought stress are important for agriculture. The present study was conducted to evaluate the effect of six oilseed rape (*Brassica napus* L.) genotypes (Rgs003, Sarigol, Option500, Hyola401, Hyola330, and Hyola420), with and without drought stress, and with and without the use of super absorbent polymer, on oil quality and content. A complete randomized blocks design, with a split-plot arrangement, in a 2x2x6 factorial scheme (drought stress x polymer x genotypes), with three replications, was used. The research was carried out in a farm owned by the Seed and Plant Improvement Institute, in Karaj, Iran. Results showed a significant difference for drought stress levels, presence of super absorbent and genotypes on oil content and composition, as well as on glucosinolate content in the oil. Drought stress conditions decreased the oil and linoleic acid contents, but increased the glucosinolate and stearic acid contents. The use of super absorbent polymer increased the linoleic acid content, but decreased other components. It was possible to conclude that, under drought stress conditions, the super absorbent polymer application, for reserving higher amounts of water in itself, increased the soil ability to store water, what increased the plant vegetative period and consequently the oil quality by decreasing saturate fatty acids and increasing unsaturated fatty acids.

KEY-WORDS: *Brassica napus* L.; hydrogel; fatty acids; glucosinolate.

INTRODUCTION

Canola oilseed species currently hold the third position among oilseed crops and are an important source of vegetable oil (Ashraf & McNeilly 2004), but its oil shows low quality, due to a high concentration of erucic acid and glucosinolate.

Canola has the lowest saturated fat content among vegetable oils and thus presents an increasing demand for diet-conscious consumers (Grombacher & Nelson 1992). Erucic acid and glucosinolate are considered toxic for both human and animals' health, in addition to its bitter taste (Muhammad et al. 1991). Safe limits for these compounds have been

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described as less than 2% of erucic acid in oil and less than 30 μmol g^{-1} of glucosinolate in oil free meals (Grombacher & Nelson 1992). The tender leaves of these cultivars can be consumed as vegetable and their seed as a cooking oil source. The residue left after the oil extraction is rich in proteins and can be used for livestock feeding (Khalil et al. 1995).

Zahedi & Tohidi Moghdam (2011) reported that antioxidant activities increased in many physiological cycles, such as the response to water deficit stress. The fact that water stress effects on growth and yield are genotype-dependent is well known (Bannayan et al. 2008).

In Iran, water is a scarce resource, due to the high rainfall variability. The water stress effects depend on deficit timing, duration and magnitude (Pandey et al. 2001). The identification of the critical irrigation timing and scheduling based on a timely and accurate basis to the crop is the key to conserving water and improving the irrigation performance and sustainability of irrigated agriculture (Ngouajio et al. 2007). Igbadun et al. (2006) showed that the crop yield response was very much dependent on the amount of water applied at different crop development stages than the overall seasonal water applied. This approach may save water with little or no negative impact on the final crop yield. In arid and semi-arid environments, both efficient use of available water and a higher safflower yield and quality are in demand (Lovelli et al. 2007, Dordas & Sioulas 2008, Koutroubas et al. 2008).

Drought stress significantly limits plant growth and crop yield. However, in certain tolerant/adaptable crop plants, morphological and metabolic changes occur in response to drought, which contribute towards adaptation to such unavoidable environmental constraints (Sinha et al. 1982, Blum 1996).

The efficient soil moisture management is important for agricultural production in the light of scarce water resources. Soil conditioners, both natural and synthetic, contribute significantly to provide a reservoir of soil water to plants on demand in the upper layers of the soil, where the root systems normally develop. These polymeric organic materials and hydro gels, apart from improving soil physical properties, also serve as buffers against temporary drought stress and reduce the risk of plant failure, during its establishment (De Boodt 1990, Johnson & Leah 1990). This is achieved by means of an evaporation reduction caused by a restricted water movement from the sub-surface to the surface layer (Ouchi et al. 1990).

Drought stress invariably leads to oxidative stress in plant cells, due to a higher leakage of electrons towards O_2, during the photosynthetic and respiratory processes, leading to the enhancement in the reactive oxygen species (ROS) generation (Asada 1999). ROS such as superoxide radical (O^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^*), and singlet oxygen ($1O_2$) can directly attack membrane lipids and inactive metabolic enzymes and damage nucleic acids, leading to cell death (Mittler 2002). The reaction of plants to water stress differ significantly at various organizational levels, depending upon stress intensity and duration, as well as plant species and its development stage (Chaves et al. 2003).

Environmental stresses, including drought and temperature, affect nearly every aspect of the plant physiology and biochemistry, and significantly diminish yield. Many arid and semi-arid regions in the world own soils and water resources that are too saline for most of the common economic crops, which affect plants through osmotic effects, ion specific effects, and oxidative stress (Munns 2002, Pitman & Lauchli 2002). In addition, plants are subjected to the interaction of two or more environmental stress factors under natural conditions, and many studies have been carried out to evaluate separately the effects of these stress factors on plant metabolism.

Therefore, the aim of this study was to investigate the effect of long-term drought stress on oil content and quality in canola plants and also whether a super absorbent polymer supplied to plants might be a strategy for increasing drought tolerance and unsaturated fatty acids under drought stress conditions.

MATERIAL AND METHODS

The experiment was carried out in the Seed and Plant Improvement Institute (35°59´N, 50°75´E and altitude of 151 m above the sea level), in Karaj, Iran, in 2008/2009, in a complete randomized blocks design, with a factorial split-plot arrangement and three replications. This region has a semi-arid climate (354 mm rainfall yearly). The soil of the experimental site was a clay loam one, with a montmorillionite clay type, low in nitrogen (0.06-0.07%), low in organic matter (0.56-0.60), and alkaline in reaction with a pH of 7.2 and Ec = 0.66 dS m^{-1}. The soil texture was sandy loam, with 10% of neutralizing substances.

The irrigation strategy [80% of evaporation as control (I_1), with water deficit stress starting from the flowering stage (I_2)] and the super absorbent application [non-application of super absorbent as control (S_1) and application of super absorbent with a 7% concentration level] were allotted to main plots. The genotypes Rgs003 (V_1), Sarigol (V_2), Option500 (V_3), Hyola 401(V_4), Hyola330 (V_5), and Hyola420 (V_6) were allotted to sub-plots.

The soil amendment used in this study was a hydrophilic polymer (SUPERAB-A200) produced by the Rahab Resin Co. Ltd., under license of the Iran Polymer and Petrochemical Institute. It is a white granular powder with a 90% active ingredient, 75-1,000 μm particle size, and 0.60 g cm^{-3} bulk density, which swells to form a gel in water. A 7% super absorbent concentration was noticed for each plot. After calculation, the necessary super absorbent amounts were poured separately into each pail and sufficient water was added. Thirty minutes later, the super absorbents had already absorbed completely the water and were slowly and accurately poured on the whole plot. After settling, each plot was covered with soil.

The irrigation of the control group was carried out with seven days apart, as flooding in between plant rows. The measured parameters were oil content; glucosinolate content in oil; saturated fatty acids, such as stearic acid (C18:0) and arachidic acid (C20:0); and unsaturated fatty acids, such as linoleic acid (C18:2), α linolenic acid (C18:3), and gadoleic acid (C20:1).

Soxhlet extraction was employed to determine the total oil concentration of the canola seed. In the soxhlet extraction procedure, 10 g of milled seeds (20 mash) were packed in a paper extraction thimble and oils were extracted by using 300 ml of petroleum benzene (bp 40-60ºC, obtained from Merck Chemical Co., Germany) in a soxhlet extractor, for 4 hours, and the solvent was then evaporated. Oils were filtered and dehydrated by using Whatmann products (two filter paper, anhydrous sodium sulfate, Buchner funnel, suction flask, and vacuum pump). Based on the whole seed, the oil concentration was expressed as mg g^{-1}. Fatty acids were transformed to their methyl esters (FAME), following the Metcalf et al. (1966) method, and were determined by using a gas chromatograph (Unicam 4600, Cambridge, England) equipped with a FID detector. A fused silica capillary column BPX70 (30 m × 0.22 mmi.d.), with a 0.25 m film thickness (from SGE), was used as the stationary phase. One microliter of the FAME sample was injected into the chromatograph, by using a microliter syringe. Helium was the carrier gas with a head pressure of 18 psi. The injector, detector, and oven temperatures were 240ºC, 200ºC, and 180ºC, respectively. The FAME samples were positively identified by matching their retention time data and mass spectra with those of the standards from Aldrich or Sigma (USA). The fatty acid composition was calculated from the total identified fatty acid area and the values were averages of two injections.

Random seed samples from each plot were collected and analyzed for glucosinolate, according to the FOSS Routine Near Measurement System (35RP-3752F) TR-3657-C, Model 6500 (Maryland, USA), at the oilseed laboratory. Near infrared reflectance (NIR) spectroscopy is a rapid, non-destructive whole seed scanning technique, which does not require any sample preparation or chemicals (Daun et al. 1994).

All data were analyzed by using the SAS software (SAS Institute Inc. 1997). Each treatment was analyzed in three replications. When ANOVA showed significant treatment effects, the Duncan's multiple range test was applied to compare the means, at $p < 0.05$ (Steel & Torrie 1980).

RESULTS AND DISCUSSION

The main effects of all experimental factors were significant for traits, and also three-way interactions among them were significant for all, except for oil content (Table 1). Results of the three-way interaction among irrigation × super absorbent × genotype showed that, under water deficit stress and absence of super absorbent, the highest oil content belonged to Hyola401 and the highest glucosinolate content to the Hyola330 genotype (Table 2).

Under water deficit stress, the percentage of saturated fatty acids decreased, what could be explained by a shorter growing season, and plant oil yield also decreased. Researchers have mentioned reduced availability of carbohydrates for oil synthesis under drought stress. Bouchereau et al. (1996) showed that water stress, along the flowering stage, affected the oil concentration and fatty acid composition of canola seeds. Oil concentrations of 25-37%, in the whole safflower seeds, were also reported (Gecgel et

Table 1. Variance analysis for the experimental traits (Karaj, Iran, 2008/2009).

Treatment	D. F.	Oil content	Glucosinolate content	Stearic acid	Arachidic acid	Linoleic acid	α Linolenic acid	Gadoleic acid
		Mean square						
Replication	2	8.047^{ns}	2.047**	0.209**	0.038**	0.823**	0.248**	0.044**
Irrigation	1	401.814**	133.933**	1.840**	5.093**	1.891**	18.458**	0.008*
Super absorbent	1	19.792*	19.908**	0.203**	0.082**	10.793**	1.704**	0.229**
Irrigation*Super absorbent	1	39.768*	2.340**	0.034**	0.055**	1.683**	6.275**	0.072**
Error	6	3.170	0.004	0.0009	0.0008	0.028	0.0007	0.0009
Genotype	5	68.977**	2.532**	0.622**	2.485**	6.356**	2.883**	0.715**
Irrigation*Genotype	5	31.151**	0.027**	1.534**	2.920**	3.950**	0.740**	0.210**
Super absorbent*Genotype	5	14.032^{ns}	0.016**	0.793**	3.770**	2.510**	4.836**	0.266**
Irrigation*Super Absorbent*Genotype	5	3.268^{ns}	0.008**	1.463**	2.248**	1.598**	4.329**	0.112**
Error	40	6.529	0.001	0.003	0.002	0.025	0.003	0.002
Total	71							
C.V.		6.66	0.22	2.39	7.04	0.825	1.16	9.85

^{ns}, *, and ** : Non-significant, and significant at 5% and 1%, respectively.

al. 2007, Sabzalian et al. 2008). Zaman & Das (1991) observed that oil concentration in safflower seeds increased from 28.5% (with no irrigation) to 29.3% (with three irrigation procedures). Singh & Sinha (2005) reported that the decrease in oil concentration may be due to the oxidation of some polyunsaturated fatty acids. In the present study, this report was supported by the positive and significant relationship observed between oil concentration and linoleic concentration in seeds.

Under drought stress conditions, the average glucosinolate content increased to 15%, for all varieties. Glucosinolate contents increased with the water stress increasing. It is widely accepted that the glucosinolate content is affected by environmental factors, including climatic conditions, nutritional availability, and agronomic practices, in addition to genetic characteristics (Fenwick et al. 1989). The results are also in agreement with the findings of Fenwick et al. (1983).

With regard to oil fatty acid composition (Table 2), the highest stearic acid level belongs to the Sarigol, while the highest arachidic acid content belongs to the Hyola420 genotype. Gecgel et al. (2007) revealed that the effect of genotype on fatty acids was greater than that provided by the environment. However, under water deficit stress and absence of super absorbent, the arachidic acid (C20:0) content decreased to 18%, for all genotypes. Under these conditions, the percentage of unsaturated fatty acids (Table 3), such as linoleic acid, α linolenic acid, and gadoleic acid, decreased 16%, 7%, and 4%, respectively, in all genotypes seeds.

The stress treatments provided a decrease in the number of days required for canola plants to reach 50% of flowering or maturity, with an average of 4-7 days, if compared with the unstressed control. Similar findings were reported for faba bean (*Vicia faba* L.), by Mwanamwenge et al. (1999). The acceleration of the flowering and/or maturity processes probably contributed to reduce the impact of drought stress in canola genotypes. The decrease in oil content and yield components, in different safflower genotypes, due to water deficiency, has also been reported by other researchers (Zaman & Das 1991, Kar et al. 2007, Lovelli et al. 2007). Under the same conditions, the higher percentage of saturated fatty acids over unsaturated fatty acids would increase, because unsaturated fatty acids are obtained from saturated fatty acids.

Bouchereau et al. (1996) reported that water stress, during the flowering stage, affected the oil concentration and fatty acid composition of canola seeds. The water stress, in the vegetative stage, was also effective on seed quality. Dwivedi et al. (1996) pointed out that the peanuts total oil and linoleic acid contents decreased under water stress, during the grain filling stage, but that total protein and stearic acid contents increased under this condition.

Results for the three-way interaction among irrigation × super absorbent × genotype showed that, under water deficit stress and super absorbent

Table 2. Canola oil percentage, glucosinolate content, and some saturated fatty acids, according to irrigation regimes (I), super absorbent concentrations (S), and genotypes (G) (Karaj, Iran, 2008/2009).

I	S	G	Glucosinolate content	Oil content	Stearic acid	Arachidic acid
			μmol g^{-1}	%		
I1			17.107b	40.69a*	2.180b	0.932a
I2			19.835a	35.97b	2.500a	0.401b
	S1		18.997a	38.85a	2.393a	0.700a
	S2		17.945b	37.80b	2.287b	0.633b
		RGS003	18.149e	35.42b	2.215d	0.302d
		Sarigol	17.994f	35.18b	2.622a	1.237a
		Option500	18.313d	38.97a	2.461c	0.217e
		Hyola401	18.608b	40.32a	2.534b	1.229a
		Hyol330	19.295a	40.32a	2.091e	0.570b
		Hyola420	18.469c	39.75a	2.116e	0.444c
I1	S1	RGS003	17.100d	34.44b	2.058d	0.030d
		Sarigol	16.873e	35.25b	1.458e	0.639b
		Option500	17.396c	39.50a	3.588a	0.417c
		Hyola401	17.626b	41.57a	2.351c	0.877a
		Hyol330	18.290a	43.14a	1.188f	0.584b
		Hyola420	17.433c	42.65a	2.625b	0.418c
	S2	RGS003	16.420e	39.30b	2.220b	0.578b
		Sarigol	16.230f	37.66b	2.517a	0.408a
		Option500	16.620d	40.92ab	1.885e	0.040c
		Hyola401	16.906b	41.95ab	2.134c	0.553b
		Hyol330	17.573a	47.40a	2.136c	0.619b
		Hyola420	16.823c	44.52ab	2.002d	0.028c
I2	S1	RGS003	20.233e	33.08c	2.191d	0.557b
		Sarigol	20.153f	33.08c	3.649a	0.178d
		Option500	20.313d	38.35ab	1.842e	0.061e
		Hyola401	20.636b	41. 87a	2.923b	0.452c
		Hyol330	21.470a	35.28bc	2.405c	0.481c
		Hyola420	20.443c	35.46bc	2.440c	0.712a
	S2	RGS003	18.843e	34. 87b	2.392d	0.043c
		Sarigol	18.720f	34.74b	2.866a	0.722a
		Option500	18.923d	37.11a	2.529cd	0.350b
		Hyola401	19.263b	35.90ab	2.726ab	0.035c
		Hyol330	19.850a	35.47ab	2.636bc	0.598a
		Hyola420	19.176c	36.39ab	1.399e	0.620a

* Means within each column of each section followed by the same letter are not significantly different ($p < 0.05$). I1: irrigation (control); I2: water deficit stress; S1: non-application of super absorbent; S2: application of super absorbent.

application, the highest oil content belonged to Option500 (Table 2).

The application of the super absorbent polymer under drought stress conditions resulted in a better and more effective use of water and nutrition, increasing the available water for plants and the transmission of photosynthetic substances to canola seeds, increasing its oil content and decreasing the glucosinolate content (about 7%), for all genotypes. Also under drought stress conditions, the application of the super absorbent polymer and saturated fatty acids, such as stearic acid (C18:0) and arachidic acid (C20:0), decreased 6% and 3%, respectively, for all genotypes.

Mekki et al. (1999) also showed that drought stress increased the percentage of palmitic acid and reduced unsaturated fatty acids. The fatty acid profile of all canola genotypes reveals that lipids are a good source of the nutritionally essential linoleic and oleic acids. Linoleic acid was the predominant fatty acid, which, along with the oleic acid, comprised about 90% of the fatty acid composition. Similar findings were reported for safflower (Pascual-Villalobos & Albuquerque 1996, Carvalho et al. 2006, Sabzalian et al. 2008).

Table 3. Linolenic acid, α linoleic acid, and Gadoleic acid, according to irrigation regimes (I), super absorbent concentrations (S), and genotypes (G) (Karaj, Iran, 2008/2009).

I	S	G	Linoleic acid	α Linolenic acid	Gadoleic acid
			%		
I1			19.465a*	4.471b	0.534b
I2			19.141b	5.484a	0.556a
	S1		18.916b	5.131a	0.601a
	S2		19.690a	4.823b	0.488b
		RGS003	20.041a	4.241d	0.292e
		Sarigol	19.401c	5.456a	0.976a
		Option500	17.907d	5.071b	0.358d
		Hyola401	19.637b	4.574c	0.507c
		Hyol330	19.352c	5.482a	0.642b
		Hyola420	19.480c	5.039b	0.494c
I1	S1	RGS003	20.286a	4.745d	0.041d
		Sarigol	19.368d	6.689a	1.496a
		Option500	17.472f	5.357b	0.461c
		Hyola401	19.539b	2.195e	0.611b
		Hyol330	19.216e	5.319bc	0.673b
		Hyola420	19.504c	5.216c	0.452c
	S2	RGS003	21.033b	2.217f	0.453c
		Sarigol	19.933c	3.025e	0.613b
		Option500	17.316f	4.389c	0.226d
		Hyola401	18.963e	5.478a	0.484c
		Hyol330	19.552d	5.221b	0.862a
		Hyola420	21.400a	3.803d	0.037e
I2	S1	RGS003	18.383b	5.359b	0.416c
		Sarigol	19.366a	5.499a	1.106a
		Option500	17.001c	5.101c	0.334d
		Hyola401	19.900a	5.001d	0.472c
		Hyol330	19.488a	5.560a	0.426c
		Hyola420	17.466c	5.534a	0.730b
	S2	RGS003	20.463a	4.642e	0.259d
		Sarigol	18.938f	6.613a	0.689a
		Option500	19.840c	5.437d	0.413cd
		Hyola401	20.148b	5.624c	0.462bc
		Hyol330	19.150e	5.829b	0.606ab
		Hyola420	19.548d	5.606c	0.758a

*Means within each column of each section followed by the same letter are not significantly different ($p < 0.05$). I1: irrigation (control); I2: water deficit stress; S1: non-application of super absorbent; S2: application of super absorbent.

CONCLUSION

Water deficit stress conditions decreased canola oil and linoleic and arachidic acids contents, but increased glucosinolate, stearic, α linolenic, and gadoleic acids contents. So, the application of super absorbent increased the linoleic acid content, but decreased other traits. Consequently, the super absorbent polymer used under water deficit stress conditions, with improved moisture conditions, increased sink capacity, and a longer growing period provided enough time to prepare unsaturated fatty acids from saturated fatty acids.

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