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Short and Long Periods of Water Stress During Different Growth Stages Of Canola (*Brassica napus* L.): Effect on Yield, Yield Components, Seed Oil and Protein Contents

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Abstract: The reproductive growth of oilseed rape crops is exposed to drought stress in many areas of Iran. Our objective was to examine the effects of short and long periods of water stress during different growth stages of canola on yield, yield components, seed oil and protein contents. In a pot experiment, Hyola-308, PF-7045/91 and Heros cultivars were planted in eight treatments including short and long periods of water stress during different growth stages. These three cultivars were also exposed to different water stress intensities in a biennial field experiment. The greatest seed yield reduction was observed when water stress occurred at flowering (30.3%) and then at silique development (20.7%). Seed yield reduction by short-term water stresses during stem elongation, flowering and silique development were mostly associated with the reduction of silique number per plant, but by short-term water stress during seed development was due to the reduction of seed weight. A little compensation was observed by seed weight when water stress occurred before flowering. The number of siliques per plant was the most sensitive yield components under long-term water stress. Seed oil content decreased by water stress but protein content increased.

Key words: *Brassica napus*, water stress, growth stages, yield and yield components, seed quality

INTRODUCTION

Drought, salinity, heat and freezing are environmental conditions that cause adverse effects on the growth of plants. Water deficit more than other stresses limits the growth and the productivity of crops (Yamaguchi-Shinozaki *et al*., 2002). Without sufficient water to maintain transpiration, leaf temperature can rise above their optimum for metabolism (Mahan and Upchurch, 1988). Therefore, plants under low water availability are more prone to heat stress, too. Seed yield of *Brassica napus* (Jensen *et al*., 1996; Kumar and Singh, 1998), *B. juncea* and *B. rapa* (Sharma and Kumar, 1989; Shrama, 1992; Wright *et al*., 1995) decreased due to drought stress. The effect of drought stress is a function of genotype, intensity and duration of stress, weather conditions, growth and developmental stages of rapeseed (Robertson and Holland, 2004). The occurrence time is more important than the water stress intensity (Korte *et al*., 1983).

It is known that the most sensitive growth stage to drought stress is seed filling in bean (*Phaseolus vulgaris* L.) (Nielsen and Nelson, 1998), heading and flowering in wheat (*Triticum aestivum* L.) (Moustafa *et al*., 1996), seed filling in soybean (*Glycine max* L.) (Brevedan and Egli, 2003), flowering and seed filling in pea (*Cicer arientinum* L.) (Singh, 1991), 2-3 weeks after silking in maize (*Zea mays* L.) (Grant, *et al*., 1989), flowering and anthesis in rice (*Oryza sativa* L.) (Lilley and Fukai, 1994).

Water deficiency has adverse effect on vegetative and reproductive stages of oilseed rape crops. Mailer and Cornish (1987) demonstrated that adverse effect of water stress was more during reproductive growth of rapeseed than vegetative growth. Rao and Mendham (1991) and Richard and Turling (1978) found that flowering of rapeseed is a critical stage to water stress.

Under dryland conditions, Henry and MacDonold (1978) reported that severe drought decreased oil and increased protein content of rapeseed. Jensen *et al*.

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(1996) found that under low evaporative demands (2-4 mm day^{-1}) oil and seed yields were not influenced by soil drying. Under high evaporative demands (4-5 mm day^{-1}) oil and seed yields were significantly decreased, but protein content was not. Thompson (1978) observed little effect of water stress on seed protein content in soybean, whereas Hobbs and Muendel (1983) reported that drought stress increased protein content.

The occurrence time and intensity of drought differ annually in field. Thus, it is very important to determine critical stages of oilseed rape crops against drought stress. The growth, especially reproductive growth of rapeseed is exposed to drought stress in many areas of Iran. Hence, the objective of these pot and field experiments was to examine the effects of short and long periods of water stress during different growth stages of canola on yield, yield components, seed oil and protein contents.

MATERIALS AND METHODS

Experiment 1: A pot experiment was conducted at Ramin Agriculture and Natural Resources University, during 2003-4. It is located in the semi-arid zone in the southwest of Iran (31°36' N latitude, 48°53' E longitude, elevation 50 m above see level). Each pot (38 cm diameter and 35 cm depth) was filled with 30 kg of clay loam field soil. A factorial experiment laid out in a Randomized Complete Block (RCB) design with three replications. Three cultivars of spring canola (Hyola-308, PF-7045/91 and Heros) were exposed to eight treatments: short-term water stress during stem elongation (S_1), flowering (S_2), silique development (S_3), seed development (S_4), long-term water stress from stem elongation to seed ripening (L_1), from flowering to seed ripening (L_2) and from silique development to seed ripening (L_3), water stress free as control (C). Watering of the control and water stress treatments occurred when 25 and 75% of the Available Water (AW) was depleted, respectively. The amount of water applied was calculated to restore the water in the pot to Field Capacity (FC) in the control and to 25% depletion of AW in water stress treatments. AW was calculated as AW = FC-PWP. FC and PWP (permanent water point) were measured by pressure plate. Phenological observations were made on a regular basis with the Sylvester-Bradley and Makepeace (1984) growth stage key. Seeds were planted 1 to 1.5 cm deep on 20 November. The pots were placed next to each other with ten plants per pot. Plants grew in natural climate. A mobile shelter excluded all rainfall. Before sowing, N:P:K fertilizers were mixed into the soil in rates of 0.5.0.6:0.5 g per pot. One gram of N was also split equally at the beginning of the stem elongation and the flowering of canola

Table 1: Monthly agro-meteorological data of the experimental site during period of the trial

Year and month	Max.. temp (°C)	Min. temp. (°C)	Rainfall (mm)	Evaporation (mm)
2003-4				
Nov.	26.9	10.8	18.4	141.7
Dec.	19.3	9.2	78.3	73.8
Jan.	19.0	9.6	163.5	79.4
Feb.	20.9	7.1	27.2	97.6
Mar.	27.8	11.1	4.6	137.4
Apr.	30.0	14.5	11.0	188.0
May	38.2	19.7	0.0	330.4
2004-5				
Nov.	26.8	13.4	17.0	126.1
Dec.	16.8	5.8	98.8	59.7
Jan.	17.2	5.8	58.9	48.8
Feb.	19.7	7.1	14.8	83.4
Mar.	24.5	10.3	41.1	122.0
Apr.	32.5	15.5	4.7	183.1
May	38.9	20.1	0.0	346.4

Experiment 2: A biennial field experiment was conducted on a clay-loam soil with pH 7.8 at Ramin Agriculture and Natural Resources University, during 2003-4 and 2004-5. A split-plot experiment laid out in a RCB design with four replications. Three water treatments: water stress free (control), moderate and high water stress during reproductive growth (from flowering to seed ripening) were as main-plot and Hyola-308, PF-7045/91 and Heros cultivars were as sub-plot. Watering of the control, moderate and high water stress treatments occurred when 25, 50 and 75% of AW were depleted, respectively. The amount of water applied was calculated to restore the water to FC, 25 and 50% depletion of AW for control, moderate and high stress treatments, respectively. FC and PWP were measured by pressure plate.

Individual plots consisted of 8 rows, 4 m long and spaced 30 cm apart. Seeds were planted 1 to 1.5 cm deep at a rate of 100 seeds m^{-2} on 20 November. For all treatments, N:P:K fertilizers applied in rates of 150:60:50 kg ha^{-1}, respectively. P, K and one-third of N were applied per plant and incorporated. Other two-third of N was split equally at the beginning of the stem elongation and the flowering. All rainfall were excluded by mobile shelter during reproductive growth. Grass weeds were controlled by application of Gallant-Super (Haloxyfop r-methyl ester) at 0.6 L ha^{-1}. Broad-leaf weeds were also hand weeded during the season.

Data recording: Meteorological data were collected 0.5 km from the experiment site. Maximum and minimum temperatures, rainfall and class A pan evaporation data for the experimental period are presented in Table 1.

Final harvests were carried out at the end of April. 10 plants per pot in pot experiment and 1.8 m^2 area of the two inner rows of each plot in field experiment harvested. Dry matter was determined after drying at 72°C for at least 48 h. The following measurements were carried out: biological (above-ground), straw and seed yields, harvest of siliques per plant (with at least one seed), the number index (seed yield divided by biological yield), the number of seeds per silique and 1000-seed weight. Seed oil and protein contents were determined by the Nuclear Magnetic Resonance (NMR) and the Kjeldahl (protein = 6.25×N) methods, respectively.

RESULTS

Experiment 1

Biological, straw and seed yields: Biological, straw and seed yields reduced significantly under all short and long-term water stress treatments (Table 2). Among short-term water stress treatments, the lowest biological yield obtained at S_2 for Hyola-308 and PF-7045/91 and at S_1 for Heros. In terms of seed yield, flowering was the most sensitive stage to drought. Water stress during flowering reduced 23.6, 29.2 and 38.7% seed yield in Hyola-308, PF-7045/91 and Heros, respectively (Table 2). The second sensitive stage was silique development, as 15.1, 20.7 and 27.4% of seed yield decreased due to water stress during this stage in Hyola-308, PF-7045/91 and Heros, respectively. Biological, straw and seed yields and harvest index more decreased when water stress lengthened (Table 2).

Yield components: The effect of long-term water stress on all yield components was significant (p = 0.01). The effect of short-term water stress on the silique number per plant and seed weight was significant, too, but on the seed number per silique was not (Table 2). Among short-term water stress treatments, the least siliques per plant and seeds per silique obtained at S_2 and then at S_3 treatments (Table 2). S_4 treatment did not have effect on the silique number per plant and the seed number per silique. The lowest seed weight was observed at S_4. The earlier treatments had heavier individual seed weight. The number of siliques per plant and seeds per silique more decreased when water stress lengthened, as the number of siliques per plant and seeds per silique were at L_3>L_2>L_1. 1000-seed weight at L_3 was less than L_1 and L_2 (Table 2).

Seed quality: Cultivars were different (p = 0.01) in terms of oil content and oil yield, but were not in protein content (Table 2). Short and long-term water stresses decreased the seed oil content and oil yield. Only long-term stress treatments affect on the protein concentration. Among short-term water stress treatments, the lowest oil content observed at S_3, but the lowest oil yield obtained at S_2. In the same experiment, the lowest oil and the highest protein contents obtained by water stress during flowering and pod development, respectively (Champolivier and Merrien, 1996). The oil content decreased when water stress lengthened. The seed protein content was more at all water stress treatments than the control. The most protein content obtained at L_3.

Experiment 2

Biological, straw and seed yields: Biological yield decreased 20.7 and 31.2% under moderate and high water stresses compared to the control, respectively. Straw dry matter production also reduced 21.2 and 30.6%, respectively (Table 3). Heros was the most sensitive cultivar to water stress in terms of biological, straw and seed yields. Hyola-308 produced the most seed yield. Seed yield decreased 19.4 and 32.8% at the moderate and high stresses compared to the control, respectively. In a field experiment on *B. napus* and *B. juncea* in the west Australia, Gunasekara *et al.* (2003) observed that the mean biological yield was decreased 17.9 and 32.1% and the mean seed yield was decreased 18.5 and 38.7% by moderate and high water stresses during reproductive growth compared to the control, respectively.

Yield components: The number of siliques per plant was the most sensitive yield components to drought stress during reproductive growth. The silique number per plant and seed weight decreased significantly when water stress intensified (Table 3). A little compensation was observed at the moderate water stress by seeds per silique, but there was not under high stress. The number of siliques per plant in Heros was more at the control, but fewer at the moderate and high stresses compared to other cultivars. The number of seeds per silique and 1000-seed weight in Hyola-308 were significantly higher than other cultivars. This superiority compensated the deficiency of siliques per plant, as Hyola-308 finally produced higher seed yield. The number of siliques per plant had no significant correlation with seeds per silique (r = -0.03) and with 1000-seed weight (r = -0.01), but a significant correlation was observed between the number of seeds per silique and 1000-seed weight (r = 0.52).

Seed quality: The effect of water stress intensity was significant (p = 0.01) on the seed oil and protein contents (Table 3). The effect of water stress was more important on the oil and protein yield than their concentrations. For example, the oil concentration decreased only 0.39 and

Table 2: Biological (BY), straw (StY) and seed (SY) yields per plant , harvest index (HI), the number of siliques per plant (Si/Pl), the number of seeds per silique (S/Si), seed weight (SW), oil content (OC), protein content (PC) and oil yield (OY) in short and long periods of water stress during different growth stages of canola (for the pot experiment)

Treatment	BY (g/plant)	StY (g/plant)	SY	HI (%)	Si/Pl	S/Si	SW (mg)	OC (%)	PC (%)	OY (g/plant)
C	20.51^{a}	13.99^{a}	6.52^{a}	32.3^{b}	109.5^{a}	20.7^{a}	3.01^{a}	45.1^{a}	23.0^{c}	2.94^{a}
S_1	15.27^{cd}	9.23^{c}	6.03^{a}	39.4^{a}	100.2^{b}	20.1^{ab}	3.02^{a}	44.6^{ab}	23.8^{abc}	2.69^{a}
S_2	14.50^{d}	9.95^{bc}	4.54^{b}	31.7^{b}	80.5^{d}	18.7b^{cd}	2.93^{ab}	43.2^{bc}	23.9^{cab}	1.96^{b}
S_3	16.15^{c}	10.99^{b}	5.16^{b}	32.3^{b}	92.3^{c}	19.6^{abc}	2.83^{bc}	42.2^{cd}	24.3^{abc}	2.17^{b}
S_4	19.45^{b}	13.20^{a}	6.24^{a}	32.4^{b}	107.7^{ab}	20.1^{ab}	2.69^{c}	42.6^{c}	26.7^{abc}	2.65^{a}
L_1	8.40^{f}	6.37^{e}	2.02^{d}	23.3^{c}	53.7^{e}	17.7^{d}	2.75^{bc}	39.9^{e}	23.9^{abc}	0.81^{c}
L_2	10.40^{e}	7.71^{d}	2.69^{c}	26.5^{c}	60.5^{e}	18.2^{cd}	2.81^{bc}	40.7^{de}	24.6^{ab}	1.08^{c}
L_3	14.72^{d}	9.73^{c}	4.98^{b}	34.4^{b}	89.4^{c}	19.3^{abc}	2.71^{c}	42.2^{cd}	25.0	2.09^{b}
LSD	1.05	1.13	0.59	4.33	7.85	1.49	0.1	61.47	1.14	0.27
Hyola-308	14.29^{b}	8.59^{c}	5.69^{a}	39.6^{a}	82.9^{b}	23.0^{a}	2.94^{a}	41.6^{b}	23.6^{a}	2.39^{a}
PF-7045/91	14.34^{b}	10.00^{b}	4.34^{b}	29.6^{b}	86.1^{b}	17.3^{b}	2.81^{b}	42.3^{b}	24.0^{a}	1.85^{b}
Heros	16.14^{a}	11.85^{a}	4.29^{b}	25.4^{c}	91.1^{a}	17.5^{b}	2.79^{b}	43.8^{a}	24.4^{a}	1.90^{b}
LSD	0.64	0.69	0.36	2.65	4.81	0.91	0.1	0.9	0.9	0.17

Mean followed by the same letter(s) in each column (between two horizontal lines) are not significantly different (Dancan 5%)

Table 3: Biennial mean comparison of biological (BY), straw (StY) and seed (SY) yields, harvest index (HI), the number of siliques per plant (Si/Pl), the number of seeds per silique (S/Si), seed weight (SW), oil content (OC), protein content (PC) and oil yield (OY) in different water stress intensities, cultivars and their interaction (for the field experiment)

Treatment	BY (g m^{-2})	StY (g m^{-2})	SY (g m^{-2})	HI (%)	Si/Pl	S/Si	SW (mg)	OC (%)	PC (%)	OY (g m^{-2})
Courol (W_1)	1128.6^{a}	808.9^{a}	319.6^{a}	28.4^{a}	103.6^{a}	20.7^{a}	3.37^{a}	48.4^{a}	20.3^{b}	155.0^{a}
Mild stress (W_2)	894.9^{b}	637.3^{b}	257.5^{b}	28.8^{a}	78.8^{b}	20.9^{a}	3.17^{b}	48.0^{a}	20.7^{b}	124.0^{b}
High stress (W_3)	775.7^{c}	561.1^{c}	214.6^{c}	27.6^{b}	63.6^{c}	19.8^{a}	3.16^{b}	46.3^{b}	22.9^{a}	99.7^{c}
LSD	77.02	44.36	18.0	1.46	6.06	1.59	0.15	0.71	1.28	6.53
Hyola-308 (V_1)	935.9^{a}	614.2^{b}	321.6^{a}	34.5^{a}	77.0^{b}	23.2^{a}	3.73^{a}	48.3^{a}	19.6^{b}	155.6^{a}
PF-7045/91 (V_2)	933.7^{a}	694.1^{a}	239.6^{b}	25.6^{b}	84.7^{a}	18.9^{b}	2.99^{b}	46.3^{a}	22.2^{a}	11.3^{b}
Heros (V_3)	929.7^{b}	699.0^{a}	230.6^{b}	24.7^{b}	84.1^{a}	19.3^{b}	2.98^{b}	48.2	22.2^{a}	11.7^{b}
LSD	70.64	41.64	14.69	1.14	6.94	1.17	0.17	0.55	0.7	5.32
V_1 . W_1	1106.5^{a}	730.4^{b}	376.1^{a}	34.1^{a}	92.5^{c}	23.5^{a}	3.88^{a}	48.7^{a}	19.1^{c}	183.4^{a}
V_1 . W_2	905.6^{b}	590.3^{de}	315.2^{b}	34.8^{a}	77.1^{de}	23.2^{a}	3.59^{b}	48.7^{a}	18.8^{c}	153.6^{b}
V_1 . W_3	795.5^{cd}	521.9^{e}	273.6^{d}	34.5^{a}	61.6^{fg}	22.8^{a}	3.71^{ab}	47.5^{b}	21.0^{b}	130.1^{c}
V_2 . W_1	1100.2^{a}	831.7^{a}	286.4^{cd}	26.0^{b}	104.0^{b}	19.5^{bc}	3.08^{cd}	47.2^{b}	20.9^{b}	135.3^{c}
V_2 . W_2	912.0^{b}	676.2^{bc}	235.7^{e}	25.9^{b}	81.1^{d}	19.3^{bc}	2.99^{cd}	46.7^{b}	21.9^{b}	110.2^{d}
V_2 . W_3	789.0^{cd}	592.3^{de}	196.7^{f}	25.0^{b}	69.1^{ef}	17.9^{c}	2.91^{cd}	44.8^{c}	23.6^{a}	88.4^{e}
V_3 . W_1	1179.0^{a}	882.6^{a}	296.4^{c}	25.1^{b}	114.2^{a}	19.0^{bc}	3.14^{c}	49.3^{a}	21.0^{b}	146.3^{b}
V_3 . W_2	867.3^{bc}	645.4^{cd}	221.8^{c}	25.6^{b}	78.2^{d}	20.2^{b}	2.93^{cd}	48.7^{a}	21.4^{b}	108.1^{d}
V_3 . W_3	742.7^{d}	569.0^{de}	173.6^{g}	23.4^{c}	60.1^{g}	18.7^{bc}	2.85^{cd}	46.5^{b}	24.2^{a}	80.8^{c}
LSD	86.51	72.13	17.99	1.36	8.5	1.44	0.21	0.95	1.21	9.21

Mean followed by the same letter(s) in each column (between two horizontal lines) are not significantly different (Dancan 5%)

2.16% in the moderate and high water stresses compared to the control, but the oil yield decreased 20.0 and 35.6%, respectively. The protein yield also decreased despite increased protein concentration.

DISCUSSION

First, flowering, then silique development were the critical stages of canola to water stress. The seed yield reduction due to water stress during flowering (S_2) was associated with the reduction of the silique number per plant (26.5%) and the seed number per silique (9.9%). The seed yield reduction at S_1 and S_3 treatments were also associated mostly with the reduction of the siliques per plant. Water stress during seed development (S_4) reduced seed yield via reduction of seed weight. Since, water stress during seed development did not affect on the sink size (seeds per plant), decreased source capacity led to reduction of seed weight. Richard and Thurling (1978) observed that some cultivars were more sensitive at flowering and others were at silique development. Mingeau (1974) demonstrated a critical period from anthesis to anthesis +2 weeks, that seed yield was reduced (20%) due to water stress during this period. Champolivier and Merrien (1996) reported that the most sensitive period of *B. napus* to water stress was between flowering and silique development. Water stress during vegetative or early reproductive stages of soybean usually reduces yield by reduction of seed number per unit area (Korte *et al.*, 1983), while stress during seed filling reduces seed size (Mechel *et al.*, 1984; De Souza *et al.*, 1997; Brevedan and Egli, 2003).

The number of siliques per plant was the most sensitive yield components to water stress during reproductive growth in both pot and field experiments. In canola, Mendham *et al.* (1984) have argued that canola breeders should be aiming to produce plants with fewer pods but with a higher potential number of seeds per pod as this maximizes seed survival and hence increases seed number per unit area. A similar ideotype has been suggested for both canola and mustard (Bhargava and Tomar, 1990, Wright *et al*., 1995). There was not the compensation effect between yield components under long-term water stress in the pot and the field experiments. Clarke and Simpson (1978) did not observe any compensation between the number of siliques and seeds per silique, too. Kumar *et al.* (1994) demonstrated increased 1000-seed weight (a compensation effect) following water stress and reduced the seeds per plant.

Straw and seed yields were similarly influenced at S_2, S_3 and S_4 treatments, consequently harvest indices did not differ compared to the control. Harvest index was higher at S_1 treatment, because water stress during stem elongation more affected straw dry matter production than seed yield. The reduced vegetative dry matter is due to reduction of leaf area and photosynthesis rate (Wright *et al*., 1988). Long-term water stress during reproductive growth decreased harvest index in the pot experiment, but did not affect in the field experiment. Ali *et al.* (1988) observed a increased harvest index following drought stress, but Wright *et al.* (1995) obtained reduction of harvest index.

There was a significant correlation between oil and protein concentrations in the pot (r = -0.34) and the field (r = -0.58) experiments. Short and long periods of water stress decreased the oil but increased the protein concentrations of seed.

CONCLUSIONS

It is concluded from the present studies that water stress during reproductive growth of canola mainly decreases seed yield by reduction of the silique number per plant. The number of seeds per silique less change than the siliques per plant. Therefore, selection or breeding of genotypes with high seeds per silique seems better under water deficit conditions. This characteristic leads to higher seed yield with higher seed yield stability under drought stress. Even though seed weight is usually depend on genotype, heavier individual seed weight is also a good characteristic.

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