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The impact of drought stress at different stages of development on water relations, stomatal density and quality changes of rapeseed (*Brassica napus* L.)

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ABSTRACT- To investigate the effect of draught stress on water relations, stomatal density, chlorophyll content and yield of rapeseed, an experiment was done with four levels of drought stress including L_1 (Field Capacity, FC), L_2 (70% Available Water Content, AWC), L_3 (50% AWC), and L_4 (30% AWC), within three growth stages-including stem elongation (T_1), onset of flowering (T_2) and silique formation period (T_3) at the University of Maragheh in 2013. The results showed that the lowest relative water content and leaf water potential were obtained at 30% AWC and silique development stage. Meanwhile, the highest water use efficiency (WUE) was observed during flower bud and silique development stages and 70% AWC. Furthermore, the results demonstrated that stomatal was only influenced by the levels of applied stresses and the highest stomatal density was recorded in 30% AWC. Implementation of 30% AWC in silique development stage diminished chlorophylls *a*, *b*, and total chlorophyll content to their lowest points so that compared to field capacity (L_1), they decreased about 59, 67 and 62 percent, respectively. Likewise, the least grain yield belonged to stress application at flower bud development stage and 30% AWC stress level. Also, the grain yield loss in $L_4 \times T_3$ (30%AWC in silique formation period) treatment in comparison with the L_1 (Field Capacity, FC) was 46.2 percent. Seed protein content was adversely affected by stress level and any decrease in AWC led to a concomitant decrease in protein content. At the same time, seeds oil content was influenced by stress application times. Water deficit stress during flower bud formation had the greatest adverse effect on seeds oil content. Overall, it was concluded that severe water deficit (30% AWC) led to the decrease of chlorophylls *a*, *b*, total chlorophyll, seed protein, oil content and yield.

INTRODUCTION

Rapeseed (*Brassica napus* L.) is the most important oil seed plant source and the third plant oil in the world after soybean (*Glycine max*) and palm oil (*Elaeis guineensis* L.) (FAO, 2013). New varieties naturally contain 40-45% oil which is used as raw materials to produce industrial and hydraulic oil, cleanser, soap and biodegradable plastics (Friedt et al., 2007). After extracting the oil, the remainder which contains 38-44% high-quality proteins, is used for animal nutrition (Walker and Booth, 2007). Its oil also has potential for developing biodiesel market. The leaves and stems of oilseed rape provide high quality forage because of its low fiber and high protein content and because it can be milled into animal feeds (Ban uelos et al., 2002).

Drought is one of the most important environmental stresses which limits farm products in almost %25 of world's lands (Morison et al., 2008). Water deficit stress due to drought, salinity or extremes in temperature are the main limiting factors for plant growth and productivity resulting in large economic losses in many regions of the world (Moradshahi et al., 2004). Plants respond to water stress through a number of biochemical, physiological and developmental changes (Morison et al., 2008; ShiraniRad and Zandi, 2012). These included a decrease in photosynthetic carbon assimilation due to stomatal closure and losses in chloroplast activity (Parry et al., 2005), down-regulation of Photo system II activity (Sharkey et al., 1988), an increase in O_2 consumption by Mehler-peroxidase reaction and photorespiration (Badger, 1985; Biehler and Fock, 1996), an increase in leaf ABA concentration and induction of many stress-responsive genes by ABA (Shinozaki and Yamaguchi Shinozaki, 1996, 1997), the modification of the lipid matrix of the plasma membrane as well as the changes in the physical organization of the membrane (McKersie et al., 1996), the sum of leaf water potential, the overall osmotic potential of leaf, osmotic adjustment, relative water content (RWC), leaf water deficiency rate, elasticity coefficient and canopy temperature (Matin et al., 1989) and accumulation of osmoprotectants such as sugar alcohols, amino acids and organic acids (Holmstrom et al., 1996). The types of responses observed depend on several factors such as severity and duration of the stress and the plant genotype.

Nasri et al. (2008) observed that applying drought stress caused a significant reduction in the number of siliquae per plant, the number of seeds per siliqua, 1000-seed weight, seed yield, the seed oil content, and the oil yield of five rapeseed cultivars. Water stress in particular stages of rapeseed phenology affects seed qualitative properties such as oil and protein percentage and the amount of glucosinolate (Strocher et al., 1995). Shirani Rad and Zandi (2012) reported that applying water deficit stress reduced the seed oil percentage (oil content) and the oil yield by 2.6% and 25%, respectively in comparison with the normal irrigation treatment. Tesfamariam et al. (2010) observed that the well watered control gave the highest value for leaf area index of 8, water use of 709 mm, seed yield of 3831 kg ha^{-1}, and seed oil content of 398 g kg^{-1}. Rapeseed stressed at flowering gave the lowest values for seed yield of 1361 kg ha^{-1}, seed oil content of 340 g kg^{-1}, and water use of 332 mm. Dry matter production per unit water use at seed filling was only a third of its value during the vegetative and flowering stages. Oil and protein content of rapeseed plants are main quality characteristics strongly affected by water deficit stress (Istanbulluoglu et al., 2010). Tesfamariam et al. (2010) reported that drought stress during green flower bud stage reduced the seed oil content of rapeseed plants. However, there are contradictory reports on the effects of water stress on seed protein content (Jensen et al., 1996; ShiraniRad and Zandi, 2012). Champolivier and Merrien (1996) noted that at the beginning of anthesis, the oil concentration reduced (budding stage, by 6%). The drought applied at ripening (seed enlargement in the pod and brown seeds in the pod) significantly affected oil concentration 12 and l0%, respectively. Concerning the importance of oilseed cultivation, especially rapeseed in Iran with the growing trend of its cultivated land and limited water resources, this study was undertaken to examine the effects of drought stress at different growth stages on water relations, stomatal density, chlorophyll content, oil concentration and protein content of rapeseed.

MATERIALS AND METHODS

The experiment was conducted under greenhouse conditions in Agricultural Faculty of Maragheh University, Iran, during 2012-2013. Seeds of rapeseed (cv. Cobra, an autumn cultivar) were pretreated at refrigerator for 30 days before planting. Then, they were planted in 5-liter pots filled with a homogenous silty loam soil in greenhouse with temperature, relative humidity and light intensity of 20-30°C, 40%-50% and 500 μmol $m^{-2}s^{-1}$, respectively. The experiment was arranged as factorial based on RCBD with four replications. Water deficit treatments were imposed on plants during three successive growth stages: stem elongation (T_1) onset of flowering (T_2) and silique formation period (T_3). Water deficit stress levels were also included: field capacity (L_1), 70% (L_2), 50% (L_3) and 30% (L_4) of soil available water content (AWC) and evaluated according to Topp and Ferre (2002) Eq. (1, 2, 3, 4):

$$L = (1 - PAW_t / AWC)*100 \quad (1)$$

$$AWC = FC - WP \quad (2)$$

$$PAW_t = SMC - WP \quad (3)$$

$$FC \geq SMC_t \geq WP \quad (4)$$

where PAW is available water content in defined time (t) in day, WP: wilting point, SMC: soil water content in time $_t$ (in day) during growth season and FC is field capacity. FC and WP were afforded from a pressure chamber. Pot irrigations were carried out daily based on weighing method. Measurement of the consumed water was achieved by calculating the whole water added to the pots by the end of the experiment. Water use efficiency was calculated using formula WUE= DM/ET, where DM stands for the dry matter, and ET represents the sum of evapo-transpirated water through plant and pot soil (Morantmanceau et al., 2004). First, the Fresh Weight (FW) of samples was measured. Then, the samples were put in distilled water and after 24 hours, the Turgid Wight (TW) was measured. After putting the samples in 75˚c oven, the Dry Weight (DW) was also measured. Finally, the leaf relative water content was measured in the following way:

The leaf water potential was measured in several times using pressure chamber (ELE, 3005, UK). The following formula was employed to calculate RWC (LazcanoFerrat and Lovatt, 1999);

$$RWC=100 \, (Wf-Wd)/ \, (Wt-Wd) \quad (4)$$

where Wf means fresh weight, Wd, dry weight, and Wt, turgor weight of leaf disks.

Stomata density and dimensions were calculated by microscopic observations, and plant leaves were bleached in a 30% sodium hypochlorite solution during 24 h to remove the mesophyll. The abaxial surface of bleached leaves (one leaf per plant) was observed in a microscope (Olympus CH-2, Olympus Optical Co., Ltd., Tokyo, Japan), and the total number of stomata and other epidermal cells were counted in three fields of view per leaf (0.065 mm^2 per field of view) (Ogaya et al., 2011).

Leaf chlorophyll contents (chlorophyll *a* and *b* and total chlorophyll concentrations) were assayed by Arnon modified method which was newly employed by Ando and Ouguchi (1989) according to the following Eq. (5, 6, 7):

$$chl_a(mg/ml)= (0.0127\times E_{633})-(0.00259\times E_{645}) \quad (5)$$

$$chl_b(mg/ml)= (0.0229\times E_{645})-(0.00469\times E_{663}) \quad (6)$$

$$chl_a+Chl_b=Chl_{tot}= (0.00805\times E_{663}) + (0.0203\times E_{645}) \quad (7)$$

where, Chl_a, Chl_b, and Chl_{tot} mean chlorophyll *a, b* and total chlorophyll and E_{645}, and E_{663} relate to absorption rates at 645 and 663 nm wave lengths, respectively.

Therefore, 1 gr of total leaf samples of each sub-plot was prepared and with 5 ml acetone 80% were beaten in the porcelain mortar. Obtained extract was purified by filter paper. Remained leftovers in the mortar were completely washed by 10 ml of acetone and passed through from filter paper. Obtained samples were completely homogenized liquid to 10 ml volume.

The total oil concentration was determined on dry milled seeds by hexane extraction and weighing using a Soxhlet apparatus. Seed analyses were performed by standard methods. Crude protein concentration was estimated by applying the factor N×6.25 to the total nitrogen content determined after mineralization (350$^{°C}$ during 8 h) of dry ground seeds with sulfuric acid and 5% salicylic acid added, according to the calorimetric method of Berthelot modified by Mann (1963).

Seed yield was measured at the harvest time. Data were subjected to variance analysis by MSTATC software. Mean comparisons were carried out by LSD test at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of RWC and leaf water demonstrated that in both criteria, interaction effects were significant (Table 1). The lowest amounts of RWC and leaf water potential were recorded when applying L_4 in T_3 growth stage (Table 5). T_1 and T_2 were placed afterwards respectively. The greatest amounts of these traits were obtained in L_1 water level. In an experiment by Li et al. (2002) on corn and by Ashraf and Mehmood (1990) on four *Brassica* species, it was understood that any decrease in soil water content led to further decline in leaf water potential. The leaf water potential decrease in *B. napus* was much more considerable than that of other species. According to a report by Wright et al. (1996) on *B. napus* and *B. juncea*, water potential of plants remained constant under mild stress. Meanwhile, both species demonstrated great water potential loss during severe water deficit conditions. Rahimi et al. (2010) and Jensen et al. (1996) also claimed that drought stress reduced water potential and RWC in *Brassica napus*. The same conclusion was verified by Mathur et al. (1995) on *Brassica napus* as well. Moorby et al. (1975) stated that there was a strong correlation between leaf water potential and RWC and that any decrease in these two traits corresponded with a similar decrease in photosynthesis rate. However, it seems that the data related to water content and RWC might be different considering the plant species and environmental conditions. Norouzi et al. (2008) concluded that all leaf water relation parameters decreased with imposed water stress. Leaf water potential (ψ_w) was significantly ($P \leq 0.05$) reduced under water stress in all the genotypes. Among genotypes, Elite and SLM046 had higher decreases in ψ_w and Opera showed less decrease compared with others under water deficit condition. The osmotic potential (ψ_s) of all genotypes was significantly reduced under water stress ($P \leq 0.01$) but it was more pronounced in Orient and Okapi.

Table 1. ANOVA of rapeseed studied attributes in response to stages and levels of drought stress

		Mean square						
SOV	df	RWC	LWP	WUE	WC	Abaxial stomata density	Adaxial stomata density	Adaxial stomata length
R	3	0.966	0.85	0.03	49403	76.63	38.63	1.7
T	9	21**	262**	0.5**	13806958**	1042^{ns}	237.3**	14.45**
L1versus others	1	22**	603**	1.06**	42587740**	2243**	473.5**	7.99**
Stress levels	2	76**	815**	1.11**	39031786**	3538**	817.5**	57.76**
Stress stages	2	3.41*	51**	0.51**	1615244**	21.09^{ns}	10.97^{ns}	2.11^{ns}
Levels*Stages	4	1.98*	7.1**	0.05^{ns}	95290^{ns}	4.94^{ns}	1.22^{ns}	0.57^{ns}
Error	27	0.678	1.014	0.03	103151	29.81	12.37	0.97

** Significant at 0.01 probability levels; * Significant at 0.05 probability level; ns= not significant. WUE: water use efficiency, RWC: Relative water content, LWP: Leaf Water Potential, WC: Water Consumption.

Previous studies showed that by increasing drought stress, the amount of RWC reduced (Loon, 1981; Nasri et al., 2008). Loon (1981) stated that RWC values for irrigated plants were between 100% and 80% and for not-irrigated plants were between 76% and 87%. In the present experiment, the influence of water stress levels and growth stage on the amount of consumed water as well as water use efficiency (WUE) was significant (Table 1) so that the highest amount was for consumed water concerning stress time related to T_2 and T_3, and the lowest quantity belonged to T_1 (Table 2).

Diminishing the soil available water led to the decrease of consumed water amount in a way that the lowest recorded data for this trait were observed in L_4 (Table 3). Also, taking into account the water use efficiency, the highest WUE was achieved in L_2 stress severity. L_3, L_1, and L_4 were respectively ranked afterwards (Table 3). Likewise, regarding stress time, the most efficient water use was found in stage T_1. T_3 and T_2 stages were positioned at the next level.

Nielsen and Nelson's (1998) experiment on bean showed that water use efficiency of plants was greatly influenced by stress treatments and the lowest WUE was observed during reproductive growth stage. Water use efficiency of seed filling and vegetative growth stages showed a significant difference as well. This condition was changed when the plants were exposed to severe stress. In such a condition, all the treatments were faced a decrease in water use efficiency concerning any stress application time. The high WUE may be due to low leaf water loss, or low plant growth rate (Ashraf and Harris, 2013). Vafabakhsh et al. (2009) noted considerable differences between rapeseed cultivars regarding WUE and yield analyses. In addition, the ability of oil production by rapeseed cultivars under drought stress is not followed by seed yield. This ability to produce seed yield is so less than oil content.

Table 2. The effect of drought stress stages on rapeseed water relationships

Stress stages	WUE (mg/g)	Water Consumption (g)
T1	4.498	9770
T2	4.035	10270
T3	4.180	10230
LSD 5%	0.2182	217.02

WUE: water use efficiency

Table 3. The effect of drought stress levels on rapeseed stomatal characteristic and water relationships

Stress levels	WUE (mg/g)	Water Consumption (mg/g)	Abaxial stomata density (N. per μm^2)	Adaxial stomata density (N. per μm^2)	Abaxial stomata length (µ)	Adaxial stomata length (µ)
L1	4.083	12670	16.27	22.88	29.22	29.7
L2	4.562	10960	23.37	30.95	26.56	26.27
L3	4.299	9374	42.76	52.05	23.27	24.36
L4	4.006	7358	57.6	68.66	20.35	22.18
LSD5%	0.252	250.8	4.96	2.784	1.381	0.736

WUE: water use efficiency

Drought stress influenced the stomata density in every level as well as in both abaxial and adaxial surface of the leaves (Table 1). The highest stomata density was observed in the final levels of stress and the lowest density was observed in L_1 (Table 3). Regarding the stomata dimensions of abaxial surface of the leaf, the lengths of stomata were affected by the levels of draught stress, but the impacts of levels and the times of draught were not significant on stomata width (Table 1). Likewise, stomata length of abaxial surface of the leaves decreased by intensifying the stress. The stomata maximum and minimum length were observed in L_1 and L_4, respectively (Table 3). Dimensions of adaxial stomata were the same as abaxial ones (Tables 1, 4). In an experiment conducted by Xia (1994) and Xu et al. (2008) on broad bean, it was clarified that drought stress in different stages of growth increased the number of stomata. Likewise, the present study also showed that the stomata width decreased in different periods of draught stress. Naik et al. (1993) and Shaneka et al. (2014) in their study on sugar-cane and Arabidopsis reported that mild and severe stress led to increased stomata numbers. Also, Jagtap et al. (1992) and Drak et al. (2012) in a work on sugarcane represented that drought tolerance was positively correlated with stomata length and density but negatively corresponded with stomata width. It seems that there is a positive correlation between stomata density and drought severity.

Considering the leaf chlorophyll content, the interactive effects of stress time and levels were significant on chlorophyll '*a*' and '*b*' content, the Chl_a/Chl_b, and total chlorophyll content (Table 4). In our study, L_4 stress level in T_3 highly decreased chlorophyll content of plants (Table 5). The lowest Chl_b and Chl_a concentration was observed in T_3 and L_4 interactions. The lowest amount of chl_{tot} was extracted in L_4 at T_3 growth stage. Interactive effects of different stress levels in their application times were significant on Chl_a/Chl_b ratio.

The highest amount for this ratio was observed when applying L_4 level at T_3 stage. In a study on different *Brassica* species, Ashraf and Mahmood (1990) noted that chlorophyll content of plants decreased after draught stress, despite the fact that the decrease of total chlorophyll content and 'Chl_a and Chl_b in *B. napus* was less than that of other *Brassica* species. It seems that those differences were related to the variation in the activities of some enzymes responsible for chlorophyll biosynthesis. Drought not only causes dramatic loss of pigments but also leads to disorganization of thylakoid membranes; therefore, a reduction in chlorophyll contents is expected (Ashraf and Harris, 2013).

Table 4. ANOVA of rapeseed studied attributes in response to stages and levels of drought stress

SOV	df	Mean Square						
		Abaxial stomata width	Adaxial stomata width	Chl t	Chl *a*	Chl *b*	Chl *a*/Chl *b*	Yield
R	3	0.24	0.23	0.045	0.012	0.014	0.019	94.96
T	9	1.32^{ns}	1.51^{ns}	0.157**	0.422**	0.327**	1.485**	1099**
L1 versus others	1	1.04^{ns}	1.12^{ns}	0.194**	0.544**	0.600**	2.111**	453.8**
Stress levels	2	1.34^{ns}	1.71^{ns}	0.227**	1.479**	0.936**	4.903**	992.5**
Stress stages	2	1.88^{ns}	1.12^{ns}	0.147**	0.094**	0.116**	0.451**	2504**
Levels*Stages	4	1.1^{ns}	1.51^{ns}	0.117**	0.028**	0.059**	0.135**	612.1**
Error	27	0.95	1	0.110	0.004	0.002	0.005	42.86

** Significant at 0.01 probability levels; ns= not significant.

The decrease in chlorophyll content under water stress is a commonly observed phenomenon (Chaves et al., 2003; Reynolds et al., 2005). Therefore, our results are in agreement with those of Kauser et al. (2006). The decrease in chlorophyll under drought stress might be due to reduced synthesis of the main chlorophyll pigment complexes encoded by the *cab* gene family (Allakhverdiev et al., 2000) or destruction of chiral macro-aggregates of light harvesting chlorophyll 'a' or 'b' pigment protein complexes (CHCIIs) which protect the photosynthetic apparatus (Shirani Rad and Zandi, 2012) or due to oxidative damage of chloroplast lipids, pigments and proteins (Tambussi et al., 2000). Din et al. (2011) concluded that chlorophyll *a* and *b* content of all the Napus genotypes declined due to drought stress at both growth stages. Genotypes Rainbow showed the least reduction (12%) in chlorophyll content during the flower initiation and silique filling stage. Taize and Zeiger (1991) expressed that water losses cause the increase of contraction cells which, as a result, cause the increase of cell concentration solution. Mild stress may increase the concentration of chlorophyll per unit leaf area, but severe stress will stop making chlorophyll.

The results of the present study revealed that the highest yield loss was observed in stage T_3 at L_4 (61 %) compared to control). Stage T_2 in L_4, and T_3 in L_3 level showed less yield reduction (Table 5). Mingeau (1974) noted that the most critical growth stage of rapeseed plant to drought stress was from anthesis to two weeks later. Ahmadi and Bahrani (2009) and Mailer and Wratten (1987) stated that the highest decrease in *Brassica napus* yield occurred while drought stress was imposed on plant from flowering to seed ripening stage. Also, Champolivier and Merrin (1996) declared that flowering till the end of seed set were the most influential growth stages of plants regarding negative effects of drought stress on yield and yield components reduction. Ahmadi and Bahrani (2009) reported that flowering was the most sensitive stage for water stress damage resulting in a drastic reduction in seed and oil yields by 29.5% and 31.7%, respectively.

Table 5. The interaction between levels (L) and stages (T) of drought stress

		RWC (%)	LWP (bar)	Chl t (mg/fw)	Chl *a* (mg/fw)	Chl *b* (mg/fw)	Chl *a*/Chl *b*	Yield (kg/m^2)
L1		96.75	-3.500	3.111	1.825	1.286	1.420	12.36
L2	T1	96.33	-7.575	2.898	1.760	1.138	1.549	12.24
	T2	93.70	-7.375	2.798	1.745	1.053	1.660	11.07
	T3	90.85	-8.675	2.725	1.737	0.987	1.762	11.21
L3	T1	87.68	-15.60	2.432	1.465	0.967	1.515	11.00
	T2	86.18	-16.08	2.295	1.385	0.910	1.522	6.93
	T3	87.07	-19.80	2.128	1.278	0.85	1.504	8.63
L4	T1	87.35	-21.60	2.092	1.235	0.857	1.440	8.64
	T2	82.93	-23.42	1.398	0.855	0.542	1.576	4.73
	T3	80.95	-27.92	1.173	0.732	0.440	1.655	6.64
LSD 5%		1.171	1.435	0.0788	0.0643	0.0321	0.0643	0.7609

RWC: Relative water content, LWP: Leaf Water Potential.

Tesfamariam et al. (2010) concluded that rapeseed and oil yield were most sensitive to water stress at flowering and less sensitive during the vegetative and seed-filling stages. Rashidi et al. (2012) attributed the reason why grain yield reduces in different genotypes to the level of used stress and its effect on some yield components such as silique per plant, seeds per silique, and the weight of thousand seeds. Rapeseed and oil yield are more sensitive to water stress at flowering and less sensitive during the vegetative and seed-filling stages (Reynolds et al., 2005). Quality attributes (protein and oil content) of *Brassica napus* plants were affected by treatments (Table 6).

Table 6. ANOVA of rapeseed quality properties in rapeseeding response to time and drought stages

SOV	df	Mean square: Protein content (%)	Mean square: Oil content (%)
R	3	1.17	136.1**
T	9	4.98**	10.183^{ns}
L1 versus others	1	16.27**	4.877^{ns}
Stress levels	2	13.78**	3.86^{ns}
Stress stages	2	0.164	33.16**
Levels*Stages	4	0.163	3.179
Error	27	0.743	6.169

** Significant at 0.01 probability levels; ns= not significant.

The results disclosed that seed protein content of plants was significantly affected by water deficit levels. Rapeseed plants with full available water had the highest protein content. Any decrease in available water content resulted in an intensified decline in protein content (Fig. 1). At the same time, stress encountered in different plant growth stages had no impact on protein content. The inferior amount for oil content was monitored during T_2 growth stage (Fig. 2).

Water deficit levels had no significant effects on this trait. In an investigation on soybean, Rose (1988) noted that depending on the stress type, three situations were predictable. 1: Under dry locations, seasons with high precipitation levels do not influence protein and oil content of plants. 2:

Under severe water deficit conditions, oil showed a decreasing pattern but protein levels of plants showed an increasing trend. 3: During initial stages of silique filling, strict drought conditions led to a large reduction in protein biosynthesis and accumulation. Shirani rad (2012) concluded that water stress reduces seed oil content and yield at a rate of 2.6 and 25%, respectively. Results also showed seed oil yield was more affected by water stress which could be due to genetic control of seed oil content trait or high influence of seed yield on seed oil yield. Champolivier and Merrien (1996) noted that oil biosynthesis was more affected by water stress than protein synthesis.

The combined reduction of yield and oil concentration decreased the oil yield by 44%, when water stress occurred during the flowering period. In this case, the oil concentration minimum threshold for marketing (40%) was not achieved, reducing crop market value.

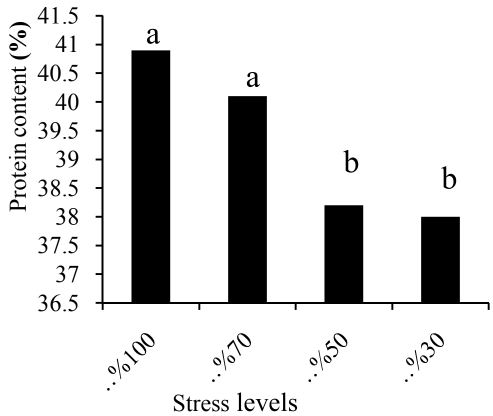

Fig. 1. Effect of drought stress levels on rapeseed grain protein content.

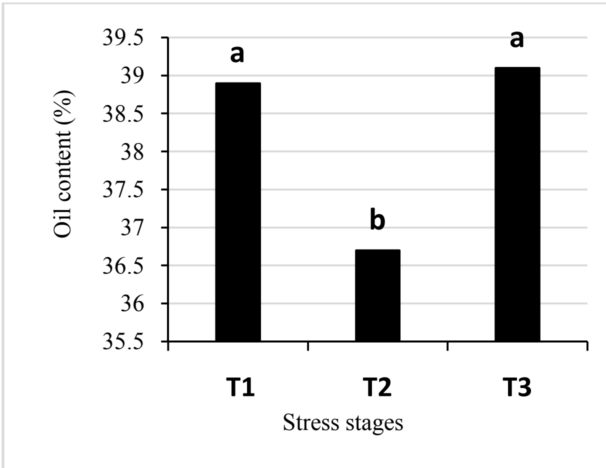

Fig. 2. Effect of drought stress stages on rapeseed grain oil content.

Under water deficit stress, the percentage of saturated fatty acids decreased, which 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 (Taize and Zeiger, 1991). Istanbulluoglu et al. (2010) concluded that increasing the number of irrigations decreased the oil content although minimally. Other results on rapeseed exhibited that initial drought which occurred during green flower bud stage reduced the seeds oil content. This may be due to the extended mega-sporangia stage (Strocher et al., 1995). Champolivier and Merrien (1996) showed that drought stress during flowering stage until ten days from seed oil content of *Brassica napus* plants. Those scientists also reported that there was a negative relationship between seed protein and oil accumulation. Moreover, Mingeau (1974) wrote that adverse effects of water deficit stress on oil biosynthesis were more strengthened than on protein biosynthesis. Jensen et al. (1996) noted that during vegetative phase of growth, drought stress had opposing effects on oil and protein content of *Brassica napus* seeds so that water stress during this growth stage led to reduced oil content and *vice versa*. Nasri et al. (2008) declared that simple correlation coefficient between RWC and drought resistance was %99. Kaiser's (1987) findings also showed sever reduction in RWC (less than 35%) that occurred under extreme stresses can cause cell death.

CONCLUSIONS

According to the results, the lowest amount of RWC and LWP were recorded in L_4 (30% AWC), applying at T_3 (silique formation period) so that the highest amount for consumed water concerning stress time was related to T_2 and T_3 and the lowest quantity belonged to T_1. The highest stomatal density was observed in the final levels of stress and the lowest density was observed in L_1. The lowest Chl_a and Chl_b concentration were observed for L_4 in T_3. Stage T_2 in L_4, and T_3 in L_3 level showed less yield reduction. Also, the results disclosed that seed protein content of plants was significantly affected by water deficit levels. Rapeseed plants with full available water had the highest protein content. Any decrease in available water content resulted in an intensified decline in protein content. The inferior amount for oil content was monitored during T_2 growth stage. But, water deficit levels had no significant effects on this trait.

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تاثیر تنش خشکی در مراحل مختلف نموی بر روابط آبی، تراکم روزنه و تغییرات کیفی کلزا (*Brassica napus* L.)

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محتوای نسبی آب

چکیده- به منظور بررسی اثر تنش خشکی بر روابط آبی، تراکم روزنه، محتوای کلروفیل و عملکرد کلزا، آزمایشی در چهار سطح تنش خشکی: L_1 (آبیاری کامل در حد گنجایش زراعی)، L_2 (۷۰ درصد میزان آب در دسترس)، L_3 (۵۰ درصد میزان آب در دسترس)، L_4 (۳۰ درصد میزان آب در دسترس) و در سه مرحله رشدی: ساقه‌روی (T_1)، گلدهی (T_2) و خورجین‌بندی (T_3) به اجرا درآمد. نتایج نشان داد که کمترین مقدار محتوای آب و پتانسیل آب برگ در تیمار ۳۰ درصد میزان آب در دسترس و دوره خورجین‌بندی بدست آمد. بیشترین کارآیی مصرف آب در زمان گلدهی و خورجین‌بندی با ۷۰ درصد میزان آب در دسترس مشاهده شد. علاوه بر این، نتایج به دست آمده نشان داد که روزنه‌ها فقط تحت تاثیر تنش خشکی قرار گرفت و بالاترین تراکم روزنه در تیمار ۳۰ درصد میزان آب قابل دسترس مشاهده شد. کمترین مقادیر کلروفیل a، b و کلروفیل کل در ترکیب تیماری تنش خشکی شدید (۳۰ درصد آب قابل دسترس) و مرحله خورجین‌بندی مشاهده گردید که نسبت به تیمار آبیاری کامل کاهشی در حدود ۵۹، ۶۷ و ۶۲ درصد را نشان داد. به همین ترتیب، پایین‌ترین مقدار عملکرد در همین تیمار مشاهده شد. به طوری‌که، کاهش عملکرد دانه در تیمار ۳۰ درصد آب در دسترس در مرحله خورجین بندی ۴۶/۲ درصد بود. محتوای پروتئین دانه به واسطه تنش خشکی تحت تاثیر قرار گرفت به گونه‌ای که کاهش مقدار آب در دسترس به کاهش مقدار پروتئین منجر شد. این درحالی بود که، درصد روغن دانه‌ها نیز تحت تاثیر تنش خشکی قرار گرفت به طوری که بیشترین تأثیر تنش خشکی شدید بر درصد روغن در مرحله گلدهی بود. در نتیجه می‌توان بیان کرد که تنش خشکی شدید باعث کاهش مقادیر کلروفیل های a، b، کلروفیل کل، پروتئین، درصد روغن و عملکرد گردید.