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Investigates the Physiological and Biochemical Aspects of Rapeseed (Brassica napus L.) Cultivars under Drought Stress and Delayed Planting Date

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Rapeseed is a valuable oil crop due to its high nutritious value and ample oil content. Water deficit is one of the crucial limiting factors which reduce crop growth and productivity.

OBJECTIVES: The present study aimed to evaluate rapeseed cultivars in the irrigation cut-off conditions in the final stage of reproductive growth (pod formation until full maturity) and planting date delay in selecting the best cultivars for recommendation in autumn rapeseed planting.

METHODS: This experiment was implemented in two crop years of 2015-2016 and 2016-2017 as a factorial split-plot in the form of a randomized complete block design with three replications in Karaj region, Iran. In the present study, irrigation in two levels, including well-watered irrigation and irrigation cut-off from the sowing and pod formation stages in two levels, including September 27 and October 27 as the main plot and four winter rapeseed cultivars, including Tassilo, Elvise, Neptune and Okapi were placed in subplots.

RESULT: The highest content of chlorophyll in both planting dates (September 27 and October 27, respectively, with values of 1.59 and 1.88 $mg.g^{-1}$ Fw) and under normal irrigation conditions belonged to Elvise cultivar. Irrigation cut-off reduced relative water content of leaves and increased leaf proline, soluble protein content and soluble carbohydrate content. Elvise and Neptune cultivars had the highest seed yield under normal irrigation conditions with 3346 and 3220 $kg.ha^{-1}$, respectively, and under irrigation cut-off conditions, with a mean of 3211 and 3081 $kg.ha^{-1}$, respectively. According to the stress tolerance index (STI), Elvise cultivar was identified as the most tolerant cultivar under dehydration stress in pod formation stage.

CONCLUSION: Elvise cultivar can be recommended as a suitable cultivar for planting in areas similar to the experimental area where moisture stress is likely to occur in the late stages of growth due to its yield indices in drought stress and non-stress conditions highest seed yield.

KEYWORDS: *Canola, Irrigation, Seed yield, Soluble protein, Sowing date.*

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1. BACKGROUND

The rapeseed is one of the most important crop species of the genus Brassica and belongs to the Cruciferae family. Drought is one of the non living environmental stresses considered the most crucial factor is limiting the growth and production of crops in most parts of the world and Iran (Mariani and Ferrante, 2017). The results of investigating the destructive effects of abiotic stresses on rapeseed showed a decrease in the photosynthetic efficiency, stomatal conductance, carboxylation efficiency, weakened electron transfer rate in photosystems and reduced seed yield by up to 0.8% and damage to seed oil composition (Especially under heat stress) (Elferjani and Soolanayakanahally, 2018). These unfavorable conditions increase the leakage of electrons into molecular oxygen by increasing reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2), and hydroxyl (OH). However, overproduction of reactive oxygen can cause oxidative stress that damages photosynthetic pigments, membrane lipids, proteins, nucleic acids, and normal metabolism (Salehi Shanjani *et al*., 2015). Proline is involved in sweeping oxygen free radicals that damage cell membranes (Anjum *et al*., 2014). Increasing proline content under stress protects cell membranes, proteins, cytoplasmic enzymes, inhibits reactive oxygen species, and eliminates free radicals.

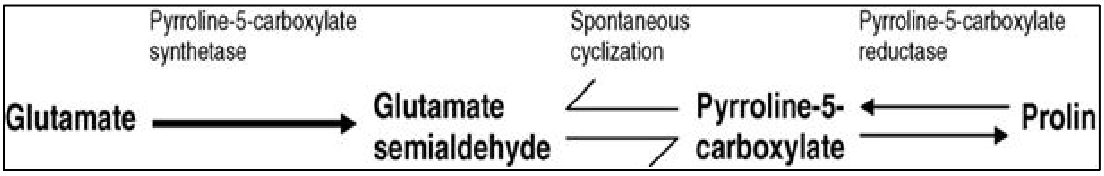

Fig. 1. Proline biosynthetic pathway in cells under drought stress (Delayoni and Verma).

Accumulation of soluble sugars under stress conditions and physiological roles such as energy supply and prevention of plant death reduces the osmotic potential and keeps the relative water level higher through osmotic regulation, and thus plays a significant role in the drought tolerance mechanism (Sperdouli and Moustakas, 2012). The rate of change in carbohydrates as osmotic regulators is one of the critical indicators in plant metabolism under water stress. The increase in protein in drought stress increases some genes related to primary metabolism, osmotic regulation, structure change, protein breakdown, and elimination of toxicity and Late Embryogenesis Abundant (LEA) protein (Jiang and Zhang, 2002). Kusvuran (2011) reports showed that drought susceptible cultivars in rapeseed had less stomatal conductance than more tolerant cultivars. Drought stress will increase the canopy temperature, which affects photosynthesis by affecting the stomatal resistance and the entry of CO_2 into the leaf (Jensen *et al*., 1996). Fischer and Maurer (1978) presented the relative susceptibility index by examining the effect of potential yield on yield under drought stress conditions in wheat. As the stress susceptibility index for a genotype under suitable moisture conditions and limited moisture conditions (water stress) decreases, that genotype will be more resistant. Behmaram *et al*. (2006) investigated the drought resistance of spring rapeseed. They found that the stress tolerance index (STI) is a more appropriate criterion for assessing drought resistance than the stress susceptibility index (SSI). The proper combination of genotype and planting date in crops is one of the most important factors to achieve high and economic yield. At the optimum sowing date, the vegetative and reproductive phases of the plant are adapted to the favorable environmental conditions and the efficiency of photosynthesis, transfer and storage of photosynthetic materials in the seeds, resulting in increased yield of the plant (Safari *et al*., 2010). In general, the effect of drought depends on factors such as genotype efficiency, intensity and duration of stress, climatic conditions, growth and developmental stage of rapeseed (Robertson and Holland, 2004). Delay in proper planting date of rapeseed caused the plant to mature in high ambient temperature, resulting in increased respiration rate of the pods, reduced photosynthetic material, and a significant weight reduction, and finally, a significant reduction in plant yield (Gan *et al*., 2004).

2. OBJECTIVES

The present study aimed to evaluate rapeseed cultivars in the irrigation cut-off conditions in the final stage of reproductive growth (pod formation until full maturity) and planting date delay in selecting the best cultivars for recommendation in autumn rapeseed planting.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

The present study was carried out during the two crop years of 2015-2016 and 2016-2017 in Pars Water and Soil Field, located in Karaj (Iran) with a longitude of 50˚ 57' 50.2'' E, a latitude of 30˚ 46' 53.3'' N 1821 m above sea level. It was conducted as a factorial plot split experiment in a randomized complete block design with three replications. The mean annual precipitation in the region was 243 mm and the precipitation was mainly in late autumn and early spring. The selected experiment site was under wheat cultivation in the previous year. Before land preparation, field soil was sampled at two depths of 0-30 and 30-60 cm to determine the physical and chemical properties of the soil. The results of field soil decomposition are listed in table 1 and the amber-thermal curve of precipitation and temperature at the test site during the two crop years are listed in figure 2 and 3. Treatments, including normal irrigation (I_1) and irrigation cut-off (I_2) from the pod formation stage onwards were placed in the main plots and planting date in two levels including September 27 (D_1) and October 27 (D_2) and four rapeseed cultivars with winter growth type including Tassilo with German origin and Elvise, Neptune and Okapi, with France origin, were placed in sub-plots as factorial. The tested cultivars' traits are listed in table 2.

3.2. Farm Management

The irrigation cycle was based on 80 mm of evaporation from a Class A evaporation pan. In each irrigation, 80% of evaporated water (64 mm or 640 m^{-3} ha^{-1}) was measured by a volume meter and entered the plots. Irrigation was completely cut-off to apply drought stress from the pod formation stage to physiological maturity. In other words, before this stage, irrigation for drought stress treatments was quite similar to well-watered treatment. Generally, the total irrigation number in well-watered and drought stress was eight and six stages, respectively.

Table 1. Physical and chemical properties of field soil

Sampling depth (cm)	0-30	30-60
EC ($ds.m^{-1}$)	2.2	1.7
Soil acidity	7.7	7.8
Saturation percentage	36	39.5
OC (%)	0.53	0.42
Absorbable phosphorus (ppm)	9.7	4.52
Absorbable potassium (ppm)	168	175
Percentage of total nitrogen	0.09	0.07
Percentage of clay	29	27
Percentage of silt	45	46
Percentage of sand	26	27
Soil texture	Clay loam	Clay loam

The amount of water used for control treatment was 5120 m^{-3} ha^{-1}, and for stress treatment, it was about 3840 m^{-3} ha^{-1}. The fertilizer was applied base on the results of soil analysis and fertilizer recommendation. Phosphorus and potassium fertilizers (before preparing the substrate) and urea fertilizer were added to the soil at a rate of 200 kg in three stages (100 kg as a base, 50 kg at the stem stage and 50 kilograms at the flowering stage). The spacing between the blocks was 5 m, and the spacing between the main plots was 2 m. The dimensions of each experimental plot were 1.8 × m with an area of 9 m^2, which included six five-meter planting lines with two sidelines as a margin. Seeds were screened before sowing based on two determining planting dates to be uniform in size. Seed planting operation was carried out manually on both sides of each ridge. The spacing of rows was 2 cm, and the spacing between the plants on the planting lines was 5 cm. Four seeds were planted linearly at a depth of 0.5-1.5 cm inside each hole. Butisan Star herbicide (41.6% suspension) was used after planting and before emergence at the rate of 2.5 litres per hectare to control a wide range of narrow-leaved and broad-leaved weeds, especially the rapeseed family weeds. To enhance the power of early vegetative growth, maintain uniformity in the development of rapeseed leaves and overcome weed shading and achieve the desired plant density (45 plants per square meter), the field thinning was performed in the 4 to 6-leaf stage.

Table 2. Tested cultivars' traits

Cultivars name	Source	Cultivar type	Planting regions	Growth type	Oil content (Percentage)	Oil quality
Tassilo	German	hybrid	Moderate cold and cold	winter	45	00
Elvise	France	hybrid	Moderate cold and cold	winter	44	00
Neptune	France	hybrid	Moderate cold and cold	winter	46	00
Okapi	France	op	Moderate cold and cold	winter	43	00

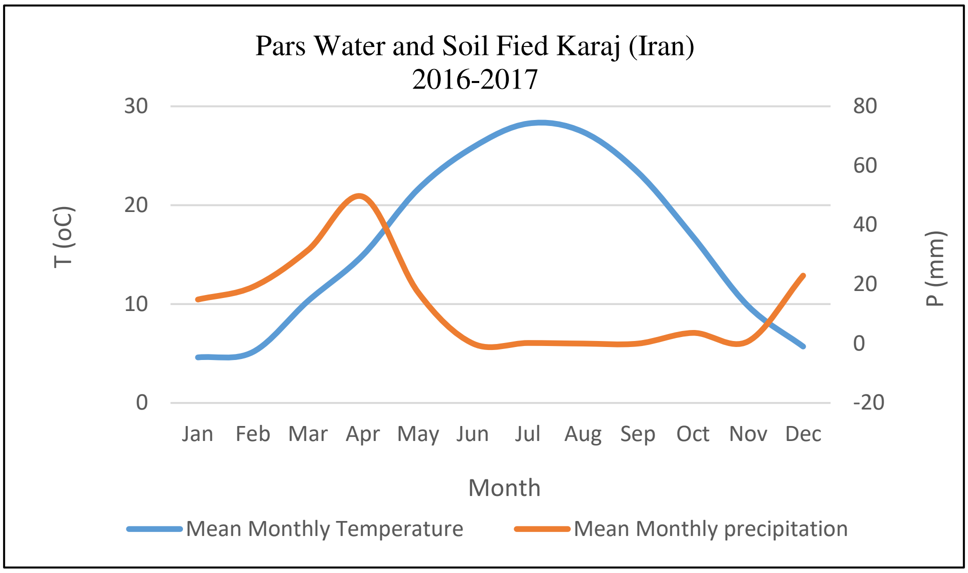

Fig. 2. Climatic profile of the test site

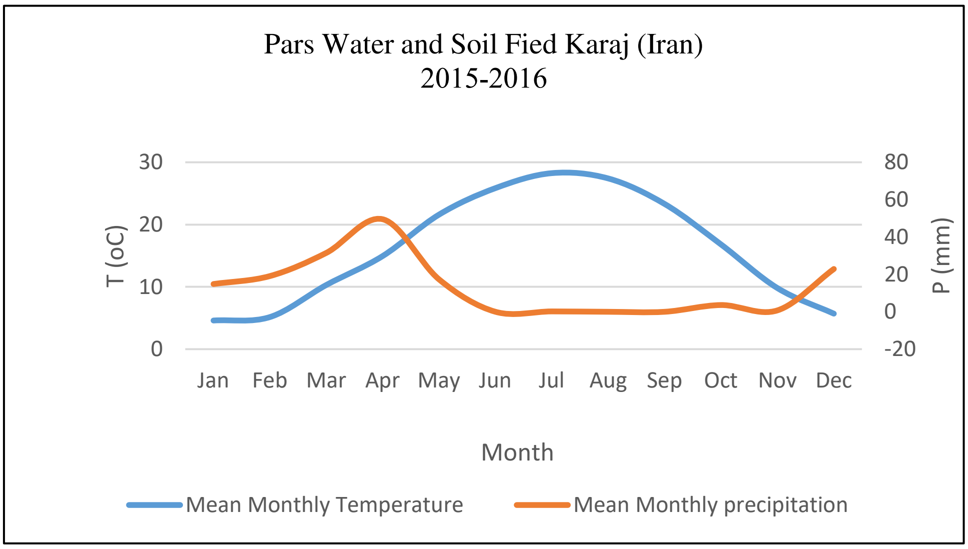

Fig. 3. Climatic profile of the test site

3.3. Measured Traits

3.3.1. *Photosynthetic pigments assay*

Measurement of chlorophyll a, chlorophyll b and total chlorophyll (a+b) was performed by the Arnon method (1967), and in the pod formation stage and fifteen days after stress, 100 mg of fresh tissue of young and developed leaves was immediately frozen by liquid nitrogen and transferred to the laboratory. Depend on the numbers obtained in the spectrophotometer; chlorophyll content was calculated in $mg.g^{-1}$.FW using equations 1 to 3. In these equations, V is the volume of the filtered solution, W is the fresh weight of the used sample in gram, and A is the light absorption at wavelengths of 661 and 60 nm.

Equ.1. Chl a ($mg.g^{-1}.FW$) = (19.3 A_{663} – 0.86 A_{645}) V/ 1000 W

Equ.2. Chl b ($mg.g^{-1}.FW$) = (19.3 A_{645} – 3.6 A_{663}) V / 1000 w

Equ.3. Chl total ($mg.g^{-1}.FW$) = Chla + Chlb

3.3.2. *Relative leaf water content assay*

The relative leaf water content was measured by Ritchie *et al.* (1999) method. For this purpose, 15 days after applying stress in the pod formation stage, three young and developed leaves were removed from the top three rows of each plot at 7.00 am and transferred to the laboratory. The fresh weight (FW), saturated weight (Sw) and dry weight (DW) were weighed immediately, and the relative content of leaf water was obtained in percentage using Equ.4.

Equ.4. RWC= (FW–DW/SW–DW) ×100

3.3.3. *Extraction of leaf proline*

The content of leaf proline was measured by Bates *et al.* (1973) method. Fifteen days after the application of stress, 3.5 grams of fresh plant material (leaf) was weighed. After performing different stages and extraction, the proline content was measured at the wavelength of 200 nm in the spectrophotometer and read in $mg.g^{-1}.FW$.

3.3.4. *Total protein content assay*

Bradford's (1976) method was used to measure protein content. For this purpose, 0.1 g of root and leaf tissue was ground in 10 ml of 50 mM phosphate buffer, and the extract was centrifuged for 10 minutes at 10 rpm. 1 ml of Bradford buffer (100% Coomassie Brilliant *Blue* G-250 reagent with 3 ml of 90% v/v ethanol) was mixed with 1.1 ml of protein extract. After 3 minutes, the sample was read by a spectrophotometer at 299 nm. Total protein content was expressed in $mg.g^{-1}$ .FW.

3.3.5. *Soluble carbohydrates content assay*

Sheligl's (1986) method was used to measure the soluble carbohydrates content. The leaf samples were dried and ground in an oven at 70 °C for 48 hours. 0.1 g of the ground sample was weighed and was added a tube containing 15 ml of 80% ethanol and was filtered by filter by Whatman paper. Forty-five minutes after preparing the solutions and stabilizing the color of solutions using spectrophotometer at 485 nm and carbohydrate content was calculated by standard glucose solutions in $mg.g^{-1}.DW$.

3.3.6. *Measurement of drought stress resistance indices*

Using the seed yield of genotypes under drought stress (Ys) and seed yield of genotypes under non-stress (Yp) conditions, quantitative indices of water stress (susceptibility) were calculated using Equations 5 and 6 provided by Fischer and Maurer (1978) and the tolerance index was calculated using Equation 7 provided by Fernandez (1992).

Equ.5. $SSI = \left(1 - \left[Ys \div Yp\right]\right) / SI)$

Equ.6. $SI = 1 - (\bar{Y}s \div \bar{Y}p)$

Equ.7. $STI = (Yp \times Ys) / (\bar{Y}p)^2$

After physiological maturity, to determine the seed yield from an area of 9 m^2 of each experimental plot was separated and kept in the open air for one week for final drying and reaching a moisture content of 12%. After separating the seeds from the pods, the seed weight was calculated with an accurate weighing scale, and seed yield was expressed in kg.ha^{-1}. To determine the 1000-Seed weight, some seeds of each experimental plot were randomly selected, and the number of 1000 seeds was counted with a seed counter and weighed in grams using an accurate laboratory scale.

3.4. Statistical Analysis

After performing the Bartlett test for homogeneity of variances, combined analysis of variance was performed in SAS and MSTAT-C software. Comparison of means was also estimated at a 5% probability level using the LSD test.

4. RESULT AND DISCUSSION

4.1. *Chlorophyll a, b and a+b*

The results of analysis of variance showed that the main effect of irrigation, planting date, cultivar and interaction the effect between planting date and cultivar on chlorophyll a was significant at the probability level of 1% (Table 3). Comparison of the mean interaction effects of planting date × cultivar showed no significant difference between the studied cultivars in any of the determined dates (September 27 and October 27) so that the highest chlorophyll a content was obtained from Elvise cultivar (1.45 mg.g^{-1} FW) on planting date of September 27. The main effect of irrigation, cultivar, and interaction effect of planting date × cultivar and irrigation × planting date × cultivar on chlorophyll b level was significant at the probability level of 1% (Table 3). The results obtained from the interaction of irrigation × planting date × cultivar on chlorophyll b showed that Neptune cultivar had the highest level of chlorophyll b in both planting dates (September 27 and October 27) with values of 0.34 and 0.31 mg.g^{-1}.FW, respectively, and under normal irrigation and irrigation cut-off conditions (Table 4). One of the causes of chlorophyll content reduction under drought stress conditions is the inhibitory effect of chlorophyll biosynthesis (Chegeni *et al*., 2016). Our experimental findings were consistent with the results reports of Din *et al*. (2011), who reported a significant difference between rapeseed cultivars in terms of chlorophyll a and b content. The analysis of variance demonstrated that the total chlorophyll content was affected by the effect of irrigation, planting date, cultivar, and interaction of irrigation× cultivar, planting date× cultivar and irrigation× planting date× cultivar at a probability level of 1% and the effect of the year became significant only at the level of 5% (Table 3). By examining the results of the combined interaction of irrigation× planting date× cultivar on the total chlorophyll content, Elvise cultivar showed the highest total chlorophyll in both planting dates (September 27 and October 27) with values of 1.59 and 1.88 mg.g^{-1}.FW, respectively, and under normal irrigation conditions and Tassilo cultivar in the irrigation cut-off conditions and earlier planting date provided the lowest total chlorophyll content (0.77 mg.g^{-1}.FW) (Table 4). Chlorophyll reduction in plants has a compatibility aspect. With the decrease in electrons excited during photosynthesis processes, the damage caused by the formation of oxygen-free radicals decreases (Kranner *et al*., 2002). Continuity of the photosynthesis process and maintenance of leaf chlorophyll capacity under drought stress is one of the physiological indicators of stress resistance and is proposed as a criterion for selecting resistant genotypes (Pessarkli, 1999). So investigating photosynthesis and fluorescence of chlorophyll furthermore water status and cell membrane stability under drought stress conditions can provide appropriate physiological perspectives for researchers to justify the behaviour of drought stress in this plant (Su *et al*., 2015).

4.2. *Relative leaf water content (RWC)*

The results of analysis of variance showed that the main effect of irrigation, cultivar and planting and interaction effect of cultivar× irrigation, planting date× cultivar and third interaction between irrigation, planting date and cultivar on relative leaf water content was significant at the probability level of 1% (Table 3). Comparison of the means of irrigation interaction× planting date× cultivar showed that the highest and lowest relative leaf water content (RWC) was 90.99% and 66.06%, respectively, under normal irrigation and irrigation cut-off conditions in Tassilo cultivar on the planting date of October 27. However, under normal irrigation conditions, no significant difference was observed among cultivars at different planting dates in terms of this trait (Table 4). The high relative leaf water content will maintain the inflammation of plant cells under drought stress conditions, and plant growth will continue in these conditions (Shirani Rad *et al*., 2010). Differences in cultivars in RWC are due to physiological and morphological mechanisms such as the closure of pores, increasing cell sap concentration, more water absorption through root development or leaf size and angle (Abdoli *et al*., 2013). Reduction in the relative leaf water content under water stress can be due to the plant's lack of access to soil moisture content, which leads to a decrease in leaf water potential due to the intensity of water stress (Heidari *et al*., 2015).

4.3. *Proline accumulation rate*

The results obtained from the analysis of variance showed that the proline accumulation rate was significantly affected by interactions irrigation× cultivar, planting date× cultivar and irrigation× cultivar and irrigation× planting date× cultivar at a probability level of 1%. The main effect of planting date and the interaction effect between irrigation and planting date was significant only at the 5% level (Table 3). The results of comparing the means of interaction of irrigation× planting date × cultivar on proline accumulation showed that under irrigation cut-off conditions, the highest proline accumulation with 18.88 $mg.g^{-1}.FW$ belonged to Elvise cultivar at an earlier planting date. In contrast, Tassilo cultivar showed a lower proline accumulation after applying the irrigation cut-off conditions on both planting dates (Table 4). Accumulation of compatible solutions under drought and salinity stress is an important mechanism to maintain the turgor pressure in the cell and reduce the water potential in the plant (Ashraf and Foolad, 2007). All plants store proline in their tissues under stress and vary between 2 and 100 times depending on the plant type and stress intensity (Mortezaie Nejad and Jazi Zadeh, 2017). Lotfi *et al*. (2015) reported that the content of proline measured in rapeseed cultivars increased under drought stress conditions compared to normal irrigation conditions. Still, the rate of increase or decrease in proline could not be considered as a measure for resistance and superiority among cultivars.

4.4. *The content of soluble proteins*

The results of analysis of variance showed that the content of soluble proteins in rapeseed was significantly affected by irrigation, planting date, cultivar, and irrigation interactions × cultivar and irrigation × planting date × cultivar at the probability level of 1%. Also, the interaction of planting date × cultivar on the content of soluble proteins was significant only at the probability level of 5% (Table 3). Comparison of the means of interaction of irrigation × cultivar showed that Elvise cultivar with a significant difference of 8.59 mg.g^{-1}.FW had more soluble protein than other cultivars under irrigation cut-off conditions. This cultivar was not significantly different from other cultivars under normal irrigation conditions except Tassilo cultivar, which had the lowest protein content (Fig. 3). Regarding seed protein content, which is one of the important components of seed quality, there are contradictory results. Sinaki *et al*. (2007) also reported that they received high nitrogen for protein production after irrigation cut-off of growing pods. The soluble protein content was affected by the third interaction effect between irrigation, planting date, and cultivar.

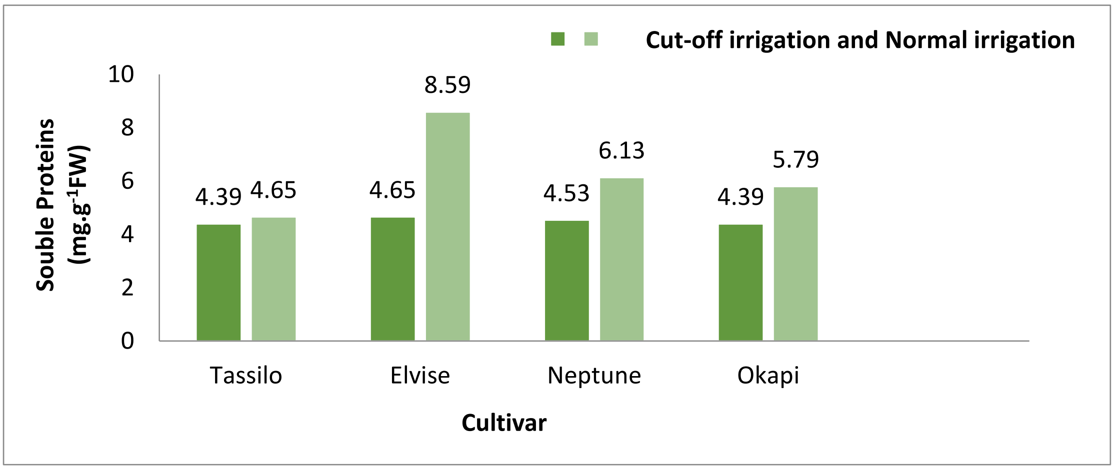

Fig. 3. Interaction effect between irrigation and cultivar on the soluble proteins content in rapeseed leaves. The means that their difference is larger than LSD are significantly different at the 5% level. (LSD5%=0.24)

So, under the irrigation cut-off conditions, the highest soluble protein content with 9.7 mg.g^{-1}.FW belonged to Elvise cultivar on the earlier planting date. While under the normal irrigation conditions, Tassilo cultivar showed a lower accumulation of soluble proteins at 0.80 mg.g^{-1}.FW on the late irrigation date (Table 4).

4.5. *Soluble carbohydrates content*

Analyzing the results obtained from the analysis of variance of the data revealed that the effect of irrigation and the interaction of year and irrigation on soluble carbohydrates were significant at the level of 1% (Table 3). Si *et al.* (2003) stated that the increase in soluble carbohydrates is directly related to resistance to stress conditions. The interaction between year and irrigation on the soluble carbohydrates content showed that under the drought stress conditions, the highest soluble carbohydrates content with 1.78 mg.g^{-1}.DW was obtained in the first experiment year. The Lowest soluble carbohydrates content was obtained in the second experiment year and under normal irrigation conditions (Fig. 4).

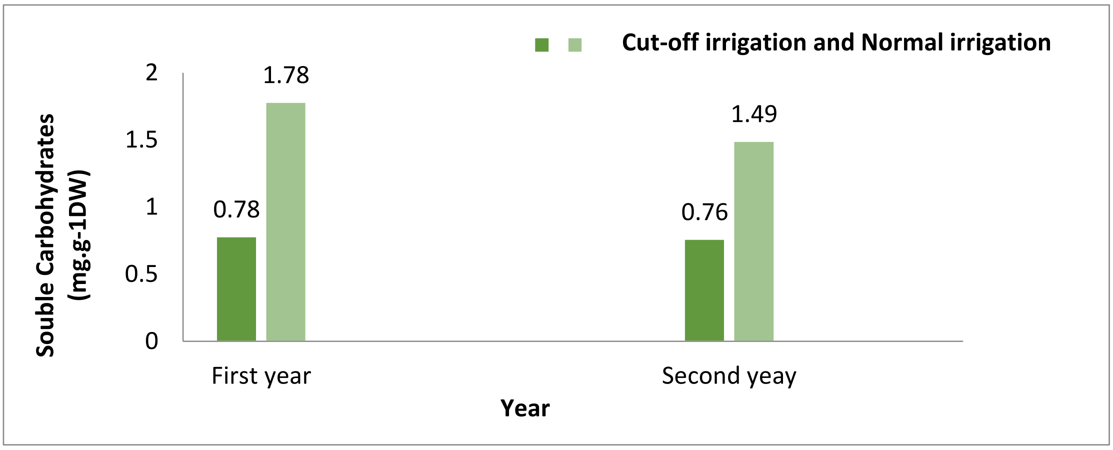

Fig. 4. Interaction effect between year and irrigation on the soluble carbohydrates content in rapeseed leaves. The means that their difference is larger than LSD are significantly different at the 5% level. (LSD5%=0.03)

Farooq *et al.* (2009) reported soluble carbohydrates decreased due to drought stress, which is inconsistent with results of this study. This inconsistency may be considered a defense mechanism in rapeseed under drought conditions. Reducing soluble carbohydrates due to environmental stresses can be attributed to an increase in anaerobic respiration due to increased stress intensity. Plants with anaerobic respiration prefer to maintain their survival by converting sugars (Hosseini and Hassibi, 2011). Increase in soluble sugars under drought stress may be due to the increased activity of alpha-amylase enzyme and hydrolyzing starch to simpler sugars, and reducing transfer of sugars from leaf to other parts of plant (Zhang *et al.*, 2012).

4.6. *Drought susceptibility*

The results of analysis of variance of year data showed that the drought susceptibility index in rapeseed was significantly affected by irrigation, cultivar and irrigation × cultivar interaction at the probability level of 1% (Table 3). Comparing the means of irrigation × cultivar showed that the lowest drought susceptibility in normal irrigation conditions belonged to Elvise cultivar and Okapi cultivar showed the highest susceptibility to drought under irrigation cut-off conditions (Fig. 5).

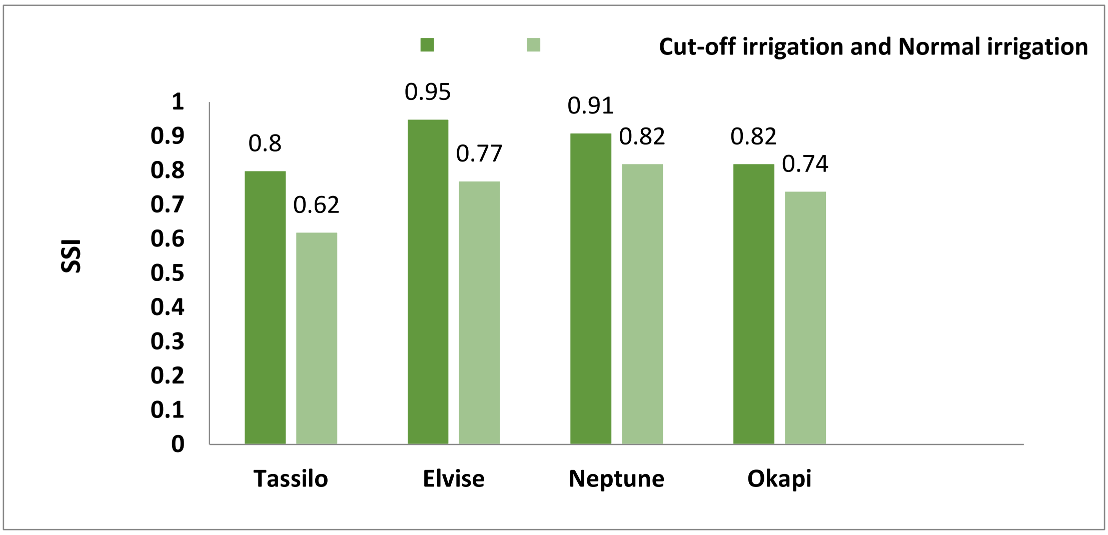

Fig. 5. Interaction effect between irrigation and cultivar on drought susceptibility index in rapeseed. The means that their difference is larger than LSD are significantly different at 5% level. (LSD5%=0.02)

4.7. *Drought tolerance*

The drought tolerance index in rapeseed cultivars was significantly affected by irrigation, cultivar and interaction effect between irrigation and cultivar at the probability level of 1%, and the effect of planting date on drought stress resistance at 5% level was significant (Table 3). Comparing the means of interaction effect between irrigation and cultivar showed that the highest drought resistance in well-watered treatment belonged to Elvise cultivar and Okapi cultivar showed the lowest one (Fig. 6). Elvise cultivar can be suitable for drought stress conditions because of the optimal yield under well-watered, and drought stress the lowest drought stress susceptibility. According to Fernandez's (1992) theory, the most suitable index can have a relatively high and significant correlation with yield in optimal irrigation and drought stress conditions. Rashidi *et al.* (2017) reported that drought tolerance and drought susceptibility criteria to identify drought-resistant lines are not appropriate criteria, confirming the present study results.

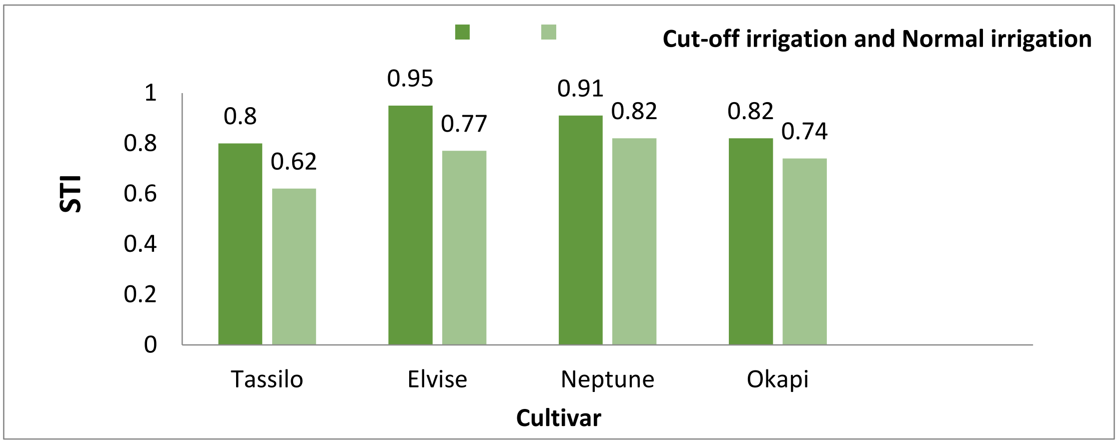

Fig. 6. Interaction effect between irrigation and cultivar on drought tolerance index in rapeseed. The means that their difference are larger than LSD are significantly different at the 5% level. (LSD5%=0.03)

Table 3. Combined analysis of variance of studied traits

S.O.V	df	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Relative water content (RWC)
Year	1	0.01 ns	0.001 ns	0.02*	3.84 ns
Replication (year)	4	0.03	0.00003	0.0003	0.07
Irrigation	1	0.76**	0.03 **	1.06**	9448.6**
Year × irrigation	2	0.001 ns	0.000009	0.001 ns	0.001 ns
Error I	4	0.001	0.0007	0.001	2.31
Planting data	1	0.74 **	0.00008	0.76**	3.48 ns
Year× planting date	1	0.002 ns	0.000001 ns	0.0002 ns	0.05 ns
Irrigation × planting date	1	0.01 ns	0.00005 ns	0.01 **	1.26 ns
Year × irrigation × planting date	1	0.0003 ns	0.0000015	0.0003 ns	0.0004 ns
Cultivar	3	1.73 **	0.08 **	1.99 **	11.71**
Year × cultivar	3	0.0004 ns	0.00008 ns	0.0006 ns	0.05 ns
Irrigation × cultivar	3	0.007 ns	0.00002 ns	0.07 **	23.15 **
Planting date × cultivar	3	0.04 **	0.008 **	0.02 **	22.68 **
Year × Irrigation × cultivar	3	0.0008 ns	0.00005 ns	0.001 ns	0.04 ns
Year × Planting date × cultivar	3	0.0005 ns	0.000005	0.0005 ns	0.05 ns
Irrigation × Planting date × cultivar	3	0.007 ns	0.0004 **	0.008 **	23.73 **
Year × irrigation × planting Date × cultivar	3	0.0005 ns	0.00004 ns	0.0006 ns	0.04 ns
Error II	56	0.0009	0.00004	0.0008	0.95
CV (%)	-	7.62	9.72	7.2	1.23

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 3.

S.O.V	df	Proline content	Soluble protein	Soluble carbohydrate	SSI
Year	1	0.02 ns	0.16 ns	0.58**	0.00002 ns
Replication (year)	4	0.3	0.29	0.0006	0.000001
Irrigation	1	1076.69**	33.25**	17.97 **	0.29**
Year × irrigation	2	0.02 ns	0.002 ns	0.44**	0.000004
Error I	4	1.42	0.41	0.006	0.002
Planting data	1	2.21*	19.17**	0.01 ns	0.0001 ns
Year× planting date	1	0.09 ns	0.008 ns	0.008 ns	0.00004 ns
Irrigation × planting date	1	2.26 *	0.1 ns	0.006 ns	0.0001 ns
Year × irrigation × planting date	1	0.07 ns	0.15 ns	0.006 ns	0.00002 ns
Cultivar	3	19.789 **	52.99 **	0.001 ns	0.09 **
Year × cultivar	3	0.07 ns	0.23 ns	0.006 ns	0.00001 ns
Irrigation × cultivar	3	15.49 **	2.29**	0.004 ns	0.009 **
Planting date × cultivar	3	2.58 **	1.4 *	0.007 ns	0.000007 ns
Year × Irrigation × cultivar	3	0.08 ns	0.26 ns	0.008 ns	0.00007 ns
Year × Planting date × cultivar	3	0.02 ns	0.19 ns	0.004 ns	0.000001
Irrigation × Planting date × cultivar	3	2.12**	1.76 **	0.008 ns	0.00002 ns
Year × irrigation × planting Date × cultivar	3	0.05ns	0.16 ns	0.005 ns	0.000006 ns
Error II	56	0.31	0.33	0.004	0.001
CV (%)	-	4.77	10.25	6.25	6.39

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

4.8. *1000-Seed weight*

The experimental results showed that the simple and interaction effects between experimental factors on 1000-Seed weight were significant. Interaction effects between irrigation and planting date showed that the planting date of October 27 in normal irrigation conditions with a mean of 4.57 g and then the planting date of September 27 under normal irrigation conditions with 4.46 g had the highest 1000-Seed weight (Table 4). Interaction effect between irrigation and cultivar indicated that the highest 1000-Seed weight belonged to Elvise cultivar with a mean of 4.25 g in normal irrigation conditions. The lowest belonged to Tassilo cultivar with 3.95 g in irrigation cut-off conditions. The interaction of planting date × cultivar indicated that the highest 1000-seed weight with a mean weight of 4.77 g belonged to an earlier planting date (September 27), and Elvise cultivar and also the lowest 1000-seed weight with a mean of 3.96 g belonged to the late planting date (October 27) and Tassilo cultivar.

Continue table 3.

S.O.V	df	STI	1000-seed weight	Seed yield
Year	1	0.00008 ns	0.003 ns	446901.04ns
Replication (year)	4	0.00006	0.005	729.17
Irrigation	1	0.42**	2.59**	3561251.04**
Year × irrigation	2	0.000001	0.0003	84.37 ns
Error I	4	0.02	0.02	4315.23
Planting data	1	0.001 ns	1.25**	2978626.04**
Year× planting date	1	0.00005 ns	0.00003 ns	759.37ns
Irrigation × planting date	1	0.0001 ns	0.03**	3384.37ns
Year × irrigation× planting date	1	0.000001	0.00007 ns	551.04 ns
Cultivar	3	0.13 **	1.37 **	489789.93**
Year × cultivar	3	0.000001 ns	0.0001 ns	201.04 ns
Irrigation × cultivar	3	0.02 **	0.08 **	5465.49 ns
Planting date × cultivar	3	0.003 ns	0.019 *	2453.82 ns
Year × Irrigation × cultivar	3	0.00002 ns	0.0013 ns	87.15 ns
Year × Planting date × cultivar	3	0.00002	0.0007 ns	89.93 ns
Irrigation × Planting date × cultivar	3	0.0009 ns	0.014 *	18039.93 *
Year × irrigation × planting date× cultivar	3	0.00001 ns	0.001 ns	14641.15 ns
Error II	56	0.002	0.003 ns	17.16.66
CV (%)	-	6.37	0.005	2.28

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Since Elvise cultivar has a high initial growth rate, does not coincide with the high temperature at the end of the season and more proper environmental factors during their seed-filling period, this cultivar caused an increase in 1000-Seed weight. One of the crucial factors in increasing seed yield is the coinciding of seed filling stages with a cooler climate, which increases seed yield by ameliorating the 1000-Seed weight (Shabani *et al.*, 2010). Results of an interaction effect between irrigation, planting date and cultivar also demonstrated that with a delay in sowing date and withholding irrigation, the rate of reduction in weight in the studied cultivars was significantly different. So that the maximum 1000-Seed weight with a mean of 4.98 g belonged to Elvies cultivar under normal irrigation conditions and earlier planting date (September 27), and the lowest one belonged to Tassilo cultivar under-withholding irrigation conditions and delay in planting date (October 27) (Table 4). Increase in 1000-seed weight can be attributed to rise in seed filling period in which tank strength plays a key role. Robertson and Holland (2004) stated the reasons for reducing 1000-Seed weight due to planting delay was increase in temperature during seed-filling period.

Table 4. Mean comparison interaction effect between irrigation, planting date and cultivar on studied traits of rapeseed cultivars

Irrigation	Planting date	Cultivar	Chlorophyll b ($mg.g^{-1}$.FW)	Total chlorophyll ($mg.g^{-1}$.FW)	Relative leaf water content (%)
Normal irrigation	September 27	Tassilo	0.24	0.99	90.99
		Elvise	0.28	1.59	90.28
		Neptune	0.34	1.41	90.04
		Okapi (control)	0.28	1.21	90.34
	October 27	Tassilo	0.24	1.08	90.85
		Elvise	0.28	1.88	90.30
		Neptune	0.30	1.66	89.86
		Okapi (control)	0.32	1.38	90.06
Irrigation cut-off	September 27	Tassilo	0.20	0.77	66.06
		Elvise	0.24	1.38	74.55
		Neptune	0.31	1.23	72.26
		Okapi (control)	0.25	0.92	70.36
	October 27	Tassilo	0.21	0.92	70.84
		Elvise	0.25	1.42	70.30
		Neptune	0.25	1.42	69.64
		Okapi (control)	0.29	1.15	70.01
LSD (5%)			0.007	0.033	1.12

The means that their difference is larger than LSD are significantly different at the 5% level.

4.9. *Seed yield*

The data analysis showed that the main effect of irrigation, planting date and cultivar on seed yield was highly significant, and the interaction effect between irrigation, planting date and cultivar on seed yield was significant (Table 3). Therefore, Elvise cultivar by 3346.67 $kg.ha^{-1}$ had the highest seed yield on September 27 under normal irrigation conditions. The lowest seed yield (2540 $kg.ha^{-1}$) is related to Tassilo cultivar combined with withholding irrigation and later planting date (October 27) treatments. In general, Elvise cultivar had a higher seed yield on both planting dates. Seed yield in Elvise and Neptune cultivars was 3211.67 and 3081.61 $kg.ha^{-1}$, respectively, under irrigation cut-off conditions and on September 27 planting date (Table 4). The seed yield of rapeseed was decreased with stopping irrigation at the flowering and pods growth stages because water stress in these periods reduced photosynthesis assimilate and nutrient transfer to the seeds and, as a result, reduced pod formation and seed weight (Ghasemian-Ardestani., 2019).

Continue table 4.

Irrigation	Planting date	Cultivar	Proline ($mg.g^{-1}.FW$)	Soluble proteins ($mg.g^{-1}.FW$)	1000-seed weight (g)	Seed yield ($kg.ha^{-1}$)
Normal irrigation	September 27	Tassilo	8.68	4.95	4.31	3036.66
		Elvise	8.81	7.51	4.98	3346.67
		Neptune	8.07	4.96	4.63	3220.00
		Okapi (control)	8.68	4.70	4.37	3103.33
	October 27	Tassilo	8.82	3.83	4.18	2683.33
		Elvise	8.74	6.79	4.77	3030.00
		Neptune	8.09	4.11	4.60	2870.00
		Okapi (control)	8.59	4.07	4.27	2736.67
Irrigation cut-off	September 27	Tassilo	13.80	4.78	4.18	2913.33
		Elvise	18.88	9.74	4.56	3211.67
		Neptune	15.08	6.71	4.39	3081.61
		Okapi (control)	14.48	5.85	4.29	2976.67
	October 27	Tassilo	14.40	4.51	3.73	2540.00
		Elvise	16.62	7.44	4.26	2880.00
		Neptune	14.28	5.56	4.11	2728.33
		Okapi (control)	14.50	5.74	3.97	2603.33
LSD (5%)			0.64	0.34	0.04	38.65

The means that their difference is larger than LSD are significantly different at the 5% level.

Our results showed that with a delay of one month in rapeseed planting date, seed yield decreased by 10 to 50% depending on the cultivar. It reported that differences in the rapeseed cultivars in terms of seed yield might be due to differences in growth traits such as the number of branches that reflect the number of pods per plant and 1000-seed weight (Sharghi *et al.*, 2011). Although the delay in sowing influence on yield losses cannot be ignored, the effect of cultivar on seed yield or crop production is also crucial (Moradi Aghdam *et al.*, 2018), which further supports the present study results.

4.10. *Correlation between traits*

Correlation between different traits was examined using Pearson correlation coefficient, and the results are listed in Table 5. Seed yield in rapeseed depends on the number of pods, seed number per pod and the 1000-Seed weight (Nasri *et al.*, 2008). The seed yield correlation with physiological traits such as drought resistance index (r= 0.62**), content of carbohydrates (r= 0.59**) and proteins (r= 0.21**) was positively and significantly. Sinaki *et al.* (2007) reported an increase proteins under drought conditions.

A positive and significant correlation was observed between drought resistance index and proline. Rezayian *et al*. (2018) reported an increase in the rapeseed pyrroline content as a criterion for ameliorating drought resistance and confirming the present study results on the existence of a positive correlation between water stress resistance and increased pyrroline content. Based on different reports, cultivars with high yield have high pyrroline content and have been introduced as resistant cultivars. The experimental results showed a significant negative correlation between drought resistance index and accumulation of soluble carbohydrates in leaves (r=-0.62**). Based on these results, with increasing drought resistance in rapeseed, seeds will have a higher capacity to absorb carbohydrates. Therefore, the carbohydrate content accumulated in the leaves of more resistant cultivars will be further reduced.

Table 5. Pearson correlation coefficients between some important physiological traits and seed yield of rapeseed genotypes at different planting dates under irrigation regimes

Variables	Seed yield	Drought resistence	Proline	Soluble carbohydrates	Soluble protein
Seed yield	1	0.62**	-0.49**	0.59**	0.21**
Drought resistance		1	0.56**	-0.62**	0.11 ns
Proline			1	0.89**	0.56**
Soluble carbohydrates				1	0.32**

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

5. CONCLUSION

The effect of irrigation cut-off on physiological traits such as drought susceptibility index, drought resistance index, the relative water leaf content (RWC), proline, and carbohydrate was more significant than effect of planting date. The results of trait evaluation during two years of the experiment showed that the Elvise cultivar had the highest seed yield and good adaptability to drought stress conditions. So, this cultivar can be recommended for planting in areas similar to the experimental area where moisture stress is likely to occur in the late stages of growth due to its yield indices in drought stress and non-stress conditions highest seed yield.

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FOOTNOTES

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