Agrotechniques in Industrial Crops

Journal Homepage: https://atic.razi.ac.ir

The Physiological Response of Rapeseed (*Brassica napus* L.) Genotypes to Drought Stress

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ARTICLE INFO

Original paper

Article history:
Received: 17 Nov 2023
Revised: 12 Jan 2024
Accepted: 27 Feb 2024

Keywords:
Cell membrane stability
Canopy temperature
Relative leaf moisture content
Grain oil content

ABSTRACT

Drought stress is one of the most impactive factors of severe modification in plant physiology eventually leading to a reduction in crop productivity. A split plot-factorial experiment was conducted at the Agricultural Sciences and Natural Resources University of Khuzestan in the 2021-2022 growing season to investigate the effects of irrigation interruption and plant density on spring rapeseed genotypes. The experiment was set up in a randomized complete block design with three replications. The experimental treatments included three levels of irrigation as main plot: (I) (1. Control: no interruption of irrigation, 2. Interruption of irrigation at the beginning of flowering (phenology code 60) until the formation of 50% of the pods (phenology code 75) and 3. Interruption of irrigation at the stage of panicle formation (phenology code 99) until the stage harvest (phenology code 99)) and three levels of plant density (D) (80, 110 and 140 plants per square meter) and canola genotypes (G) (Hayola 4815 and Aram) were arranged in subplots. The study evaluated various sensitivity and tolerance indices to stress, and results showed that the triple interaction effect of IDG on cell membrane stability, photosynthetic pigments, grain protein content, grain yield, and canopy temperature was significant. Significant interaction effects were also observed between IG on grain oil content, DG, and I×D on relative water content leaf (LRWC). The genotype that demonstrated superior tolerance to drought stress had higher values for indices such as STI, YI, MP, GMP, and HM. In general, the highest grain yield was observed in control treatment and density of 110 plants M^2 and genotype of Hayola4815 (1572.6 kg. ha^{-1}) and the lowest was observed from interruption of irrigation at the stage of pods formation until harvest and plant density of 140 plants per M^2 and Aram genotype (661.87 kg. ha^{-1}).

DOI: 10.22126/ATIC.2024.9989.1127

1. Introduction

Rapeseed cultivation in Iran, particularly in tropical regions, is subject to environmental stress such as drought and high air temperature. Rapeseed is better adapted to areas with heavy rainfall and its yield significantly drops under drought stress (Majdi Nasab *et al*., 2014). In these areas, water deficit is common during critical stages of plant growth, leading to reduced photosynthetic capacity and leaf chlorophyll levels. This can disrupt plant cell growth and metabolism by reducing cell membrane stability and relative water content (Liu *et al*., 2016; Davami *et al*., 2021). The interaction between genotypes and the environment, which causes yield instability in different environments, is a complex process affected by climate, crop management, and genetic factors (Annicchiarico, 2002). Water deficit in different phenological stages of rapeseed has varying effects on grain oil and protein content, and research indicates differences in the plant's response to drought in relation to grain oil production (Seyed Ahmadi *et al*., 2015). Din et al. (2011) found that cutting off irrigation for all cultivars not only reduced grain yield but also grain oil content while significantly increasing seed protein percentage. Jabbari et al. (2020) reported that drought stress had no significant effect on oilseed oil in

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different irrigation treatments but did affect oil yield per unit area. They attributed this to genetic factors controlling grain oil content and emphasized the importance of grain yield in determining oil yield. Plant genetic breeders have employed different methods to investigate plant responses to environmental stress. To successfully modify genotypes adapted to environmental stress, they must identify effective indicators of cultivar yield stability under stress conditions and use them as a selection scale. Different indices have been introduced to evaluate genotype reaction under stress and non-stress conditions.

Rosielle and Hamblin (1981) introduced the tolerance index and average productivity index, which represent average productivity under stress and non-stress conditions and indicate the tolerance index of yield difference between the two conditions. Fischer and Maurer (1978) presented the stress sensitivity index, with low values indicating minimal variations in yield under stress and non-stress conditions, reflecting high tolerance to stress. Fernandez (1992) introduced the stress tolerance index and average harmonic mean, with the stress tolerance index comparing yield in stress and favorable conditions, and the harmonic mean evaluating the yield of all plants under optimal conditions, with high values indicating greater stability.

The objective of this study is to investigate the effect of deficit irrigation at critical stages of plant growth and suitable plant density per unit area under drought stress conditions on rapeseed yield and quality in order to provide recommendations for improving crop production and efficiency in areas affected by environmental stresses.

2. Materials and methods

This study was carried out as a split-plot-factorial in the form of a randomized complete block design with three replications in the growing season of 2021-2022 in the research farm of Khuzestan University of Agricultural Sciences and Natural Resources. This area is located at a latitude of 31° 36′ N, longitude of 48° 53′ E, and an altitude of about 22 meters above sea level, at a distance of 35 kilometers from the north of Ahvaz, located in Khuzestan province, Iran.

The experimental treatments included three levels of irrigation as main plot: (1. Control: no interruption of irrigation, 2. Interruption of irrigation at the beginning of flowering (phenology code 60) until the formation of 50% of the pods (phenology code 75) and 3. Interruption of irrigation at the stage of panicle formation (phenology code 99) until the stage harvest (phenology code 99)) and three levels of plant density (D) (80, 110 and 140 plants per square meter) and canola genotypes (G) (Hayola 4815 and Aram) were arranged in subplots. The mentioned genotypes are of the spring type and the seeds needed for planting were obtained from the Iran Seed and Plant Breeding Research Institute. Planting was done on November 21. Each experimental plot was 2.5×2.30 meters and included six planting lines. The distance between the lines was 30 cm and the distance between the plants was 4 cm. Irrigation was done during the vegetative growth period based on plant needs and autumn rainfall, and in the final stages of growth, irrigation was stopped based on the growth stage of each cultivar.

During the growing season, weeds were controlled by hand weeding (at the stages of rosette initiation and flowering). It is noteworthy that each of the stages of irrigation interruption was done according to the growth stage of each genotype. At the time of application of the irrigation cut treatment, due to the possibility of rain, a plastic rain shield was used using a looser, and plastic was used when there was a possibility of rain (in order to prevent the effect of rain on the irrigation treatments). Climatic conditions as well as farm soil characteristics are shown in Tables 1 and 2, respectively. Based on soil test results, triple superphosphate fertilizer 100 kg/ha was added to the land before planting. Nitrogen fertilizer in the form of urea was used at the rate of 200 kg per hectare two times (Five-leaf stage and early budding stage).

Table 1. Climate data of experimental were taken from of Ahvaz metrological data

Month	Max temperature (°C)	Min temperature (°C)	Precipitation (mm)	Evaporation (mm)
November	31.3	15.2	8.7	152.7
December	23.7	10.7	44.1	87.8
January	18.2	6.8	65.1	55.3
February	20.6	6.5	13.4	93.6
March	25.1	11.7	4.3	147.4
April	32.5	14.7	0	231.0
May	36.3	20.1	9.8	302.6

Table 2. Physicochemical characteristics of soil of experimental site

Depth of Soil (cm)	EC (ds/m)	N (%)	P (mg/kg)	K (mg/kg)	pH	Soil Texture
0-30	3.7	0.02	9.38	214	7.4	Clay Silt

The content of chlorophyll a, chlorophyll b, and total chlorophyll were measured by Arnon (1949) method at the stage of pods and calculated in milligrams per gram of fresh weight. These pigments were extracted using 80% acetone and the soluble optical absorption was measured at wavelengths of 663 and 645 nm using spectrophotometer (Equation 1-3).

(1) Chla = [12.7(663nm) – 2.69 (645nm)] v / (×1000 w)

(2) Chlb = [22.9 (645nm) – 4.69 (663 nm)] v / (×1000 w)

(3) Chlt (total) = Chla + Chlb

In these relationships, "v" is the extracted sample volume and "w" is the fresh weight of the sample (Ashraf *et al*., 1994). The cell membrane stability index (CMSI) was measured through the method of Lutts et al. (1996) and at the stress stage of flowering to silique formation and silique formation to harvest (Equation 4).

(4) $CMSI = (EC_1/EC_2) \times 100$

In this equation, EC_1: primary electrical conductivity (primary leakage), EC_2: secondary electrical conductivity (secondary leakage). The measurement of the relative water content of the leaves in both phases of irrigation interruption was also calculated through the method of Ritchie et al. (1990) and using the following formula (Equation 5).

(5) LRWC = (FW –DW) / (TW – DW) × 100

In this equation, LRWC: relative water content of leaves, FW: Fresh weight of the leaf samples, DW: dry weight of the leaf samples and TW: weight of the leaf samples in the turgor state. The temperature difference of plant canopy during the stages of stopping irrigation from flowering up to 50% of flowering and fruiting until harvest was measured from 11:00 to 13:00 using an infrared thermometer. When measuring the temperature of the plant canopy, the thermometer was placed at an angle of 30 degrees to the horizon at a height of one meter from the ground and about half a meter from the plant. The temperature difference of plant canopy was calculated from the following equation (Equation 6).

(6) CTD = Ta – Tc

In this equation, "Ta" represents the air temperature (daily maximum temperature) and "Tc" represents the plant canopy temperature.

In order to determine grain yield, plant harvesting from 2 m of two middle planting rows from each plot was performed. The seeds were weighed after separating from the siliques. Grain yield was calculated with 9% moisture content in kilograms per hectare. Oil content was measured using Soxhlet extractor. Grain nitrogen content was calculated by Kjeldal. Finally, Genotypes were evaluated for drought tolerance using the following indices (Table 3):

Table 3. Stress tolerance indices studied in this research

Index	Calculation formula	Reference
Stress Susceptibility Index	SSI = [1-(YS/YP)] / [1-(Y'S/Y'P)]	Fischer and Maurer (1978)
Tolerance Index	TOL =YP-YS	Rosielle and Hamblin (1981)
Mean Productivity	MP = (YP+YS)/2	Rosielle and Hamblin (1981)
Harmonic Mean	HM= (2YP.YS) / (YP+YS)	Rosielle and Hamblin (1981)
Geometric Mean Productivity	GMP = (YP.YS) 1/2	Fernandez (1992)
Stress Tolerance Index	STI = (YS.YP)/Yp'2	Fernandez (1992)
Yield Index	YI= YS/Y'S	Gavuzzi et al. (1997)
Sensitive Drought Index	SDI= [(YS.YP)/Y'P]1/2	Gavuzzi et al. (1997)
Yield Stability Index	YSI= YS/YP	Bouslama and Schapaugh (1984)
Yield Reduction Index	YRI= [(YP-YS)/YP].100	Choukan et al. (2006)

In the above relationships, Yp is the average seed and oil yield of each variety under stress-free conditions, Ys is the average seed and oil yield of each variety under stress conditions, Y'P is the average yield of all genotypes under stress-free conditions, Y'S is the average yield of all genotypes under stresst conditions.

2.1. Statistical analysis

Analysis of variance and mean data comparison were performed using SAS v.9.4 software. The mean was compared with the Least Significant Difference Test (LSD) at the probability level of 5%.

3. Results and discussion

3.1. Stability of leaf cell membranes

The stability of leaf cell membranes was significantly affected by the triple interaction of experimental factors (Table 4). In general, the highest membrane stability was observed in control treatment and density of 80 plants per M^2 and genotype of Aram (74.15%) and the lowest was observed from interruption of irrigation at the stage of pods formation until harvest and plant density of 140 plants per M^2 and Aram genotype (44.16%). The increased leakage of cell electrolytes is also proved by increasing soluble electrical conductivity in measuring this parameter. In tolerant genotypes, less ion leakage is due to higher stability of the cell membranes in them. Research conducted by Rashtbari et al. (2012) reported that drought stress in rapeseed cultivars caused a significant increase in ion leakage percentage compared to control treatment; these researchers evaluated the response of the cultivars in terms of this trait. They announced different, reports that confirm the results of the present study. These researchers reported a different response of cell membrane stability in rapeseed cultivars to drought stress; these results confirm Rashtbari et al. (2012) study.

3.2. Photosynthetic pigments

The results of the analyses of variance showed that the interaction of experimental factors on photosynthetic pigment content was significant (Table 4). In the treatment interruption of irrigation the beginning of flowering until the formation of 50% of the pods at a density of 80 plants per M^2, Aram genotype had the highest chlorophyll b and total chlorophyll (Table 5). Also, in the treatment interruption of irrigation the beginning of flowering until the formation of 50% of the pods at a density of 110 plants per M^2, Hayola 4815 genotype had the highest chlorophyll a, b and total. However, in treatment of 140 plants per M^2, Aram genotype had the highest amount of chlorophyll a, b and total (Table 5). The results of present experiment showed a relationship between grain yield and leaf chlorophyll content. Genotypes with the highest content of chlorophyll often had the highest grain yield (Table 5).

Chlorophyll content can be considered as a criterion to measure the effect of environmental stress such as drought stress, which is different in crop species and cultivars. Drought stress is associated with the breakdown of chloroplasts and the reduction of chlorophyll and the cessation of its production. In this study, the genotype with higher chlorophyll content showed greater resistance to drought stress.

This finding is consistent with previous research showing that lower chlorophyll content can be due to decreased chlorophyll synthesis and its degradation by increasing plant density. Drought stress reduced total chlorophyll in rapeseed cultivars (Khayat Moghadam *et al*., 2021) and Safflower (Mohammadi *et al*., 2016).

3.3. Leaf relative water content (LRWC)

Table 4 presents the leaf relative water content (LRWC) percentages in response to various factors, including irrigation interruption and planting density, as well as plant density and genotype. The mean comparison indicates that the highest LRWC was observed in the control treatment at planting densities of 80 and 110 plants per M^2, with averages of 81.59% and 78.84%, respectively. Moreover, in the two stages of irrigation interruption stress, the highest LRWC was achieved with an average of 72.07% and 75.84%, respectively, at a density of 80 plants per M^2. On the other hand, the lowest LRWC values in both control and stress treatments during the stage of pod formation until harvesting were observed at a density of 140 plants per M^2 (Fig. 1).

Regarding the interaction between plant density and genotype, the highest LRWC was obtained at a density of 80 plants per square meter and the Aram genotype, followed by the treatments of 80 plants per M^2 and Hayola 4815 genotype, and 110 plants per M^2 and Hayola 4815 genotype and Aram. Conversely, the lowest LRWC value was recorded at a density of 140 plants per M^2 and the Aram genotype (Fig. 2).

The Leaf relative water content (LRWC) serves as a reliable indicator of the equilibrium between water supply to the leaf, leaf water potential, and the overall water potential status of the plant, as it is closely associated with cell size (Martinez-Carrasco, 2005). Under drought stress, LRWC typically declines due to stomatal water loss and irreparable cell volume reduction from cell membrane damage (Blackman *et al*., 1995). The reduction in LRWC during drought stress can be attributed to insufficient soil moisture availability, resulting in a decrease in leaf water potential that is proportional to the severity of drought stress (Heidari *et al*., 2015). Numerous studies have reported decreased LRWC under stress conditions, which aligns with the findings of this research (Molnár *et al*., 2005; Liu *et al*., 2016; Davami *et al*., 2021).

Table 4. Analysis of variance of measured traits

S.O.V	df	Mean Square								
		Cell membrane stability	Chlorophyll a	Chlorophyll b	Total chlorophyll	Leaf relative water content	Canopy temperature difference	Grain yield	Grain oil	Grain protein
Block	2	13.98^{ns}	0.0022^{ns}	0.076^{ns}	0.002^{ns}	6.61^{ns}	0.065^{ns}	32224^{ns}	11.16*	34.01**
Interruption of irrigation (I)	2	899.36**	0.097*	0.27**	0.63*	258.79**	14.73**	642311**	2.16^{ns}	52.13**
Block × I (E_a)	4	15.09	0.015	0.010	0.043	5.37	0.47	22132	2.33	0.92
Planting Density (D)	2	153.84**	0.047*	0.045**	0.128**	85.98**	0.197^{ns}	443800**	19.05*	33.14*
Genotype (G)	1	3.38^{ns}	0.058*	0.091**	0.0036^{ns}	36.45*	0.010^{ns}	423234**	298.68**	6.21^{ns}
I×D	4	115.94**	0.077**	0.204**	0.35**	46.56**	0.46*	177750**	4.13^{ns}	32.23*
I×G	2	95.98**	0.044*	0.105**	0.25**	6.35^{ns}	0.260^{ns}	92089**	32.01**	20.84^{ns}
D×G	2	111.75**	0.07*	0.034**	0.016^{ns}	36.07*	1.045**	13996^{ns}	1.68^{ns}	1.85^{ns}
I×D×G	4	82.84**	0.141**	0.126**	0.49**	12.02^{ns}	0.399*	159942**	5.43^{ns}	67.61**
Block×I×D×G (E_b)	30	11.79	0.014	0.0027	0.019	7.64	0.186	13455	4.07	9.73
C.V (%)		5.84	9	9.47	7.40	3.72	17.76	11.29	5.51	14.82

ns, * and ** are non-significant and significant at the 1% and 5% levels, respectively

Table 5. Threefold interaction effect interruption of Irrigation × Density × Genotype of yield, and some traits of rapeseed

Interruption of irrigation	Density (plant per m^2)	Genotype	Cell membrane stability	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Canopy temperature difference (˚c)	Grain yield (kg.ha^{-1})	Grain protein (%)
Control	80	Hayola 4815	63.62^{cde}	1.452^{bcd}	1.234^{a}	2.686^{a}	3.33^{ab}	1251.21^{b}	17.31^{cdefg}
	80	Aram	74.15^{a}	1.508^{abc}	0.721^{bc}	2.229^{b}	3.50^{a}	1482.69^{a}	23.75^{abc}
	110	Hayola 4815	63.65^{cd}	1.075^{h}	0.641^{cd}	1.716^{cde}	3^{abcd}	1572.67^{a}	20.52^{cdef}
	110	Aram	57.83^{ef}	1.361^{bcde}	0.569^{ed}	1.930^{c}	3.66^{a}	1460.63^{a}	16.33^{fg}
	140	Hayola 4815	64.44^{bc}	1.653^{a}	0.590^{ed}	2.243^{b}	3.16^{abc}	934.82^{cde}	14.89^{g}
	140	Aram	58.92^{cdef}	1.285^{defg}	0.401^{f}	1.687^{cdef}	2.66^{bcde}	770.38^{ef}	22.08^{bcd}
Interruption of irrigation at the beginning of flowering stage	80	Hayola 4815	61.47^{cdef}	1.075^{h}	0.381^{f}	1.642^{def}	2.33^{def}	1112.59^{bc}	25.45^{ab}
	80	Aram	61.07^{cdef}	1.361^{bcde}	0.353^{f}	1.857^{cd}	2.25^{defg}	877.03^{de}	23.77^{abc}
	110	Hayola 4815	63.33^{cde}	1.284^{defg}	0.602^{ed}	1.886^{cd}	2.50^{cde}	1216.79^{b}	16.58^{fg}
	110	Aram	69.71^{ab}	1.178^{efgh}	0.324^{f}	1.502^{ef}	3^{abcd}	633.17^{f}	22.77^{abc}
	140	Hayola 4815	57.12^{f}	1.147^{fgh}	0.367^{f}	1.836^{cd}	3.50^{a}	759.42^{ef}	23.81^{abc}
	140	Aram	58.03^{def}	1.546^{ab}	0.750^{b}	2.296^{b}	2.16^{efg}	887.33^{de}	16.87^{efg}
Interruption of irrigation in the stage of formation of pods until harvest	80	Hayola 4815	47.29^{gh}	1.097^{gh}	0.382^{f}	1.479^{ef}	1.16^{h}	1208.21^{b}	22^{bcd}
	80	Aram	49.55^{gh}	1.326^{cdef}	0.554^{de}	1.880^{cd}	1.33^{h}	779.99^{ef}	20.50^{cdef}
	110	Hayola 4815	64.35^{bc}	1.281^{defg}	0.596^{ed}	1.877^{cd}	1.50^{gh}	924.72^{cde}	21.56^{bcde}
	110	Aram	48.25^{gh}	1.374^{bcde}	0.520^{e}	1.895^{c}	1.50^{gh}	896.01^{de}	19.37^{cdef}
	140	Hayola 4815	50.33^{g}	1.305^{def}	0.531^{e}	1.836^{cd}	1.50^{gh}	1062.24^{bcd}	24.20^{abc}
	140	Aram	44.16^{h}	1.066^{h}	0.388^{f}	1.455^{f}	1.66^{fgh}	661.87^{f}	27.00^{a}

Mean in each column followed by similar latter (s) are not significantly different at 5% probability level, using LSD test

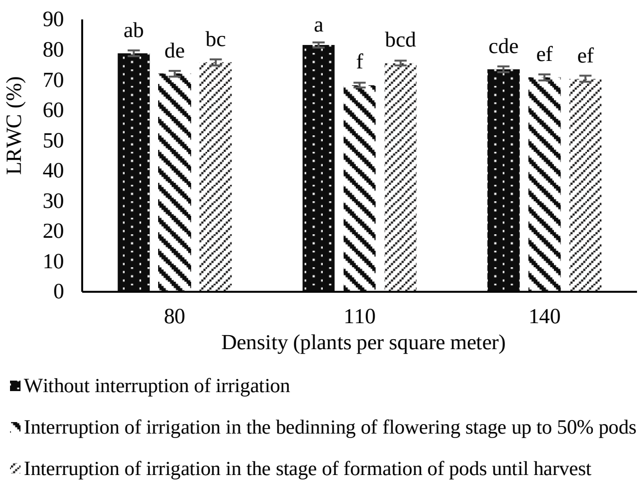

Figure 1. Effect of planting density and irrigation treatments on leaf relative water content of rapeseed

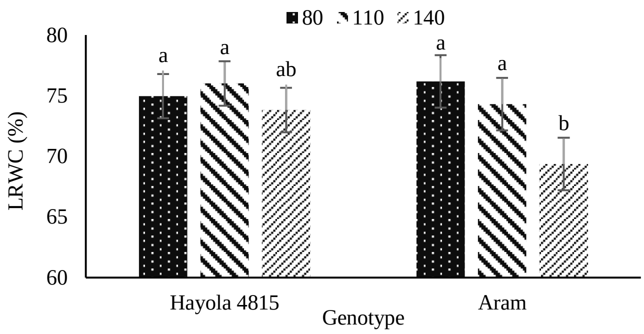

Figure 2. Effect of planting density (plant per m^2) and genotypes on leaf relative water content of rapeseed

3.4. Canopy temperature difference (CPD)

The three-way interaction effect of the experimental factors on canopy temperature difference (CPD) was found to be significant (Table 4). The mean comparison revealed that the highest CPD was associated with the irrigation interruption treatment at a density of 80 and 110 plants per M^2 meter and the Aram genotype (3.66). In both levels of irrigation interruption, the highest CPD was observed at a density of 80 plants per square meter and the Hayola 4815 genotype, while the lowest CPD was recorded under both levels of irrigation treatment and a plant density of 140 plants per square meter and the Aram genotype (Table 5).

Irrigation interruption after flowering was found to elevate canopy temperature, leading to a reduction in the difference between canopy temperature and air temperature. Towards the final growth stages, a decline in the relative content of leaf water (LRWC) and adverse weather conditions contributed to the reduction in CPD. The researchers reported that under stress conditions, cultivars exhibiting lower canopy temperatures tend to have higher photosynthesis rates, which can be attributed to improved water relationships in plants, such as transpiration (Gardner *et al*., 1986). The research findings of Pinter et al. (1990) also demonstrated that soil water condition has a direct impact on wheat canopy temperature, and the difference between crop cultivars increases with drought stress.

3.5. Grain yield (GY)

The interaction between irrigation interruption, cultivar and plant density on grain yield was significant (Table 4). In the control treatment with a density of 80 plants per M^2, Aram genotype had an average of 1482.62 kg ha^{-1} and Hayola4815 genotype had an average of 1572.67 and 934.82 kg ha^{-1} respectively. By interruption of irrigation at the beginning of flowering until the formation of 50% of the pods at stage 80 and 110 plants per M^2, Hyola4815 genotype had more grain yield than other treatments: 1112.59 and 1216.79 kg/ha^{-1} respectively (Table 4). By interruption of irrigation at the stage of panicle formation until harvest and density of 80, 110 and 140 plants per M^2, Hayola4815 genotype had more yield than other treatments: 1208.21; 924.73; 1062 kg/ha respectively (Table 4). In general, the highest grain yield was observed in control treatment and density of 110 plants per m^2 and genotype of Hayola4815 and the lowest was observed from interruption of irrigation at the stage of pods formation until harvest and plant density of 140 plants per m^2 and Aram genotype.

The Hayola 4815 genotype showed higher yields under both the interruption of irrigation levels than the Aram genotype, which indicates that the Hayola genotype had earlier flowering and maturity, lower canopy temperatures, and retained LRWCs compared to the Aram genotype under water limitation stress by reducing turgor pressure and photosynthesis. This study concluded that water limitation stress by reducing the turgor pressure and photosynthesis mainly caused stomata to close, reducing chlorophyll content and oxidative damage to cell membranes and organelles, which resulted in lower grain yield (Raza *et al*., 2017). In this regard, Joozi et al. (2017) reported that increasing density up to 100 plants per M^2 increased grain yield in rapeseed.

3.6. Grain oil content

The highest percentage of grain oil in the control treatment was from Hayola 4815 genotype (39.55 percent), which was at the same statistical level by interruption of irrigation at the flowering stage up to 50 percent of silique formation and Hayola 4815 genotype (40.22 percent), and the lowest grain oil percentage in the stage of interruption of irrigation at the stage of pods formation until harvest and Aram genotype (32.22%) (Fig. 3). Reducing the amount of carbohydrates for oil synthesis due to drought stress causes a decrease in oil percentage (Ashrafi and Razmju, 2014). Also, studies have shown that in addition to genetic factors, environmental factors also affect the percentage of grain oil (Koocheki *et al*., 2020).

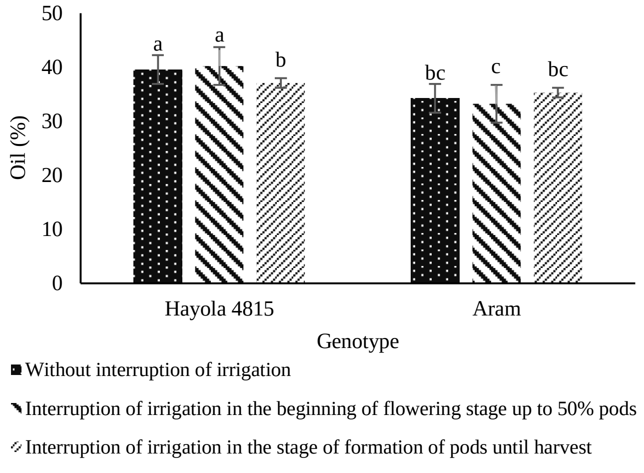

Figure 3. Interaction between interruption of irrigation and genotype on oil content in rapeseed.

3.7. Grain protein content

The three-way interaction of the experimental factors on the amount of grain protein was significant at the 1% probability level (Table 4). Results showed that Aram genotype produced the highest amount of grain protein in the treatment of interruption of irrigation at the stage pods formation until harvest and a density of 140 plants per M^2, while Hayola4815 produced the lowest (Table 5). In fact, drought stress reduces the percentage of grain oil, especially during ripening, while the percentage of protein increases, which is due to the acceleration of plant ripening. This issue does not give enough opportunity to synthesize oil from the carbohydrates stored in the grain, and therefore the percentage of oil decreases. In the research of Nawabpour et al. (2017), the relationship between oil percentage and grain protein was negative, and cultivars with high oil percentage had low protein percentage.

3.8. Indices of tolerance to drought stress

The comparison of stress tolerance index averages showed that in both stresses, the Hayola 4815 genotype had the highest stress tolerance (lowest sensitivity) in both stresses with an average of 0.86 and 0.89 and the Aram genotype had the least tolerance to stress (the most aggressiveness) towards interruption of irrigation with an average of 0.6 and 0.58, respectively. This shows that as performance increases, so does tolerance and sensitivity decrease. Considering that Hayola 4815 genotype had a higher tolerance (less sensitivity) in the condition of irrigation interruption at the end of growing season with lower shading temperature and higher moisture retention in leaves plus higher grain yield. The geometric mean production (GMP) and the mean production (MP) indices are two of the most common drought tolerance indices, both of which indicate average grain yield under optimal and drought conditions. The range of GMP was between 1165.55 (interruption of irrigation at the beginning of flowering stage up to 50% pods on Hayola 4815) and 1185.32 (interruption of irrigation in pod development stage until harvest time). A similar result was obtained for MP index with the highest value of MP for Hayola 4815 but the lowest value belonged to Aram culture. The high values of the mentioned indices, GMP, HM and MP, and their use in the selection of cultivars tolerant to drought stress indicate an increase in grain yield under stress conditions and without applying stress. Therefore, they can be suggested to identify suitable numbers for any situation. The results of this research were consistent with other studies by Salamati and Danaie (2020), Aboodeh et al. (2019) and Yousefi (2017). The TOL and SSI indices had opposite rankings compared to GMP, MP, STI and YI indices. In treatments, interruption of irrigation in the stage of pods until harvest resulted in the Aram genotype having the highest TOL index indicating its sensitivity to water deficiency. Other drought tolerance indicators such as GMP, MP, STI and YI have previously revealed the lowest values for these interruptions. Hayola 4815 in interruption of irrigation at the beginning of flowering stage up to 50% pods had a high amount of these values indicating that it is one of the most tolerant genotypes in this study while other interruptions such as Hayola 4815 had a low amount indicating that TOL and SSI indices have inverse rankings when compared with GMP and MP indices. The TOL index measures the absolute difference in yield between favorable and drought circumstances for a genotype. Similarly, the lower value of the SSI index indicates small changes in the yield of a genotype under water-stressed conditions compared to normal conditions, and therefore the stability of the genotype yield under normal conditions is higher. In the research of Sangi et al. (2021), TOL and SSI indices are not suitable indices for evaluating drought-tolerant genotypes.

In both stress conditions applied in this research, Hayola 4815 and Aram genotypes had the highest and lowest Sensitive Drought Index (SDI) values at the end of the season (Table 6). Yield Stability Index (YSI) in both stresses of interruption of irrigation in the beginning of flowering stage up to 50% pods and Interruption of irrigation in the stage of pods until harvest was most tolerant to stress for both genotypes with average values of 0.80 and 0.78, respectively. The lowest average value belonged to the most sensitive genotype with an average of 0.65 and 0.67. In case of Yield Reduction Index (YRI), results were opposite. The highest values belonged to Hayola 4815 genotype while Aram had the lowest values under both stresses (Table 6).

Based on the Yield Stability Index (YSI), genotypes with higher index values show high yield in both stressed and non-stressed conditions. The Sensitivity

Drought Index (SDI), genotypes with lower values of this index are suitable for both stress and non-stress conditions. Therefore, The SDI index is the opposite of the (YSI) Yield Stability Index. According to this index, Aram genotype has the lowest values of the drought sensitivity index. Yield Stability Index (YSI) is aimed at identifying genotypes that have the same performance despite different environmental conditions. Hayola 4815 genotype is more stable in drought stress due to its high Yield Stability Index (YSI) and minimum Yield Reduction Index (YRI).

Table 6. Comparison of the mean of grain oil yield of rapeseed genotypes with tolerance and susceptibility indices to stage stress interruption of irrigation at the beginning of flowering stage and interruption of irrigation in the stage of formation of pods until harvest

Interruption of irrigation at the beginning of flowering stage up to 50 % pods										
Genotype	STI	SSI	SDI	YI	TOL	YSI	YRI	GMP	MP	HM
Hayola4815	0.86	0.81	32.94	1.12	289.96	0.78	21.96	1165.55	1174.58	1156.59
Aram	0.6	1.2	27.48	0.87	384.28	0.67	32.47	972.4	991.32	953.85
$LSD_{0.05}$	0.15	0.35	3.23	0.13	130.64	0.09	9.66	114.51	114.81	115.27
Interruption of irrigation in the stage of formation of pods until harvest										
Genotype	STI	SSI	SDI	YI	TOL	YSI	YRI	GMP	MP	HM
Hayola4815	0.89	0.73	33.5	1.15	262.23	0.8	19.23	1185.32	1192.31	1178.37
Aram	0.58	1.29	27.14	0.84	402.11	0.65	34.2	960.15	981.38	939.4
$LSD_{0.05}$	0.23	0.52	5	0.23	150.7	0.13	13.71	177	165.9	188

4. Conclusion

The study evaluated various physiological parameters and some indices of sensitivity and tolerance to stress. The results showed that the interaction effect of irrigation, genotype and plant density on cell membrane stability, photosynthetic pigments, grain protein content, grain yield, and canopy temperature was significant. In addition, significant interaction effects were observed between irrigation and genotypes in terms of grain oil content. Interaction effect of density×genotype and irrigation×density on relative water content leaf (LRWC) was significant. Compared to other genotypes, genotype that had superior tolerance to drought stress, had higher values for indices such as STI, YI, MP, GMP, and HM. In general, the highest grain yield was observed in control treatment and density of 110 plants M^2 and genotype of Hayola4815 (1572.6 kg. ha^{-1}) and the lowest was observed from interruption of irrigation at the stage of pods formation until harvest and plant density of 140 plants per m^2 and Aram genotype (661.87 kg. ha^{-1}).

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

Funding/Support

This study was supported by Agricultural Sciences and Natural Resources University of Khuzestan, Khuzestan, Iran.

Acknowledgement

The authors are grateful to the Vice-Chancellor of Education and Postgraduate Education as well as the Vice-Chancellor of Research and Technology of Agricultural Sciences and Natural Resources University of Khuzestan, for supporting this research.

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HOW TO CITE THIS ARTICLE

Aboodeh H., Bakhshandeh A.M., Moradi Telavat M.R., Siadat S.A., Moosavi S.A., Alami Saeid Kh. 2024. The Physiological Response of Rapeseed (*Brassica napus* L.) Genotypes to Drought Stress. *Agrotechniques in Industrial Crops* 4(1): 38-47. 10.22126/ATIC.2024.9989.1127