






Seed yield and physiological responses to deal with drought stress and late sowing date for promising lines of rapeseed (*Brassica napus* L.)

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Abstract. The introduction of new genotypes of crop plants is among the most strategic research programmes, especially in arid and semi-arid regions. To study the effect of drought stress on seed yield and some physiological traits of promising lines of rapeseed at different sowing dates, an experiment was conducted for two years (2015-2017) in a semi-arid region of Iran. In this research, two conventional sowing dates were set in October 12 and November 1 (late sowing). Irrigation was carried out at two levels: normal irrigation (control) and irrigation interruption from the silique formation stage to the next stage (late-season drought stress). The genotypes included four promising lines (L1112, L1091, L1093, L1206), and a cultivar (Okapi) as a control. Results showed that delayed sowing and drought stress increased carbohydrate content and decreased seed yield, with the highest carbohydrate content and highest yield loss in L1112 and the lowest carbohydrate and lowest yield loss in the L1206 line. Among the physiological traits measured, stomatal resistance had the highest degree of correlation and the highest direct negative effect on seed yield, which declined with increasing stomatal resistance. L1112 had the highest stomatal resistance (52.76 s cm^{-1}) in delayed sowing and drought stress conditions. Therefore, L1206 and L1112 were revealed to be resistant and sensitive lines, respectively.

Keywords: *Brassica napus* L., carbohydrate, correlation, drought stress, seed yield, proline

INTRODUCTION

Due to the rainfall deficit in various regions of the world, which has caused drought, releasing the varieties/lines of plants which are resistant to drought stress has a particular importance in the regions of the world with low levels of precipitation. The rapeseed (*Brassica napus* L.) crop contains 40-44% oil and is considered to be one of the most important edible oilseeds (Ashkiani *et al.*, 2020; Cashin *et al.*, 2014; FAO, 2018), being the third annual oil crop in the world after soybean and oil palm (Enjalbert *et al.*, 2013). One of the most critical factors that inhibits the development of the area under cultivation and prevents the successful production of rapeseed in semi-arid regions is moisture deficiency at the end of the growth period (reproductive stage) to which it is very susceptible (Nasiri *et al.*, 2017). Among the various abiotic stresses, drought stress is the most severe, destructive and significant restricting environmental factor for sustainable agricultural production (Ardakani *et al.*, 2009; Davaran Hagh *et al.*, 2016; Pace and Benincasa, 2010; Yuncai and Schmidhalter, 2005).

Hammac *et al.* (2017) assessed the effect of drought stress on seed yield, as well as the protein and fatty acids content of the rapeseed and reported that drought stress at the flowering stage had significant effects on unsaturated fatty acids, protein, and seed yield. Photosynthesis limitation through metabolic destruction is a complicated phenomenon, in which pigments have a protective task (Jaleel *et al.*, 2009; Khan *et al.* 2010). Chlorophyll loss under drought stress causes damages due to the formation of free oxygen radicals (Gaspar *et al.*, 2002; Hoseinzade *et al.*, 2016; Ilkaei *et al.*, 2011). Drought stress mediated by soil moisture deficiency in flowering and silique development stages decreased chlorophyll a and b contents in various rapeseed lines compared with the irrigation treatment (Din *et al.*, 2011; Grewal, 2010; Saranga *et al.*, 2001).

Due to the water deficiency problem in arid and semi-arid regions across the globe such as Iran, the feasibility of late rapeseed and the simultaneous cultivation of the crop with spring crops may be considered as a management strategy for the optimal use of irrigation water. Additionally, the irrigation of rapeseed at the late growth season coincides with the first irrigations of spring crops after rapeseed. It is also very important to select superior promising lines that can potentially respond well to delayed cultivation and water deficiency problems, which was the aim of this study.

MATERIALS AND METHODS

This experiment was conducted in the research farm of the Seed and Plant Improvement Institute (Karaj, Iran) during 2015-2017. The geographic coordinates of the experimental farm were 35°49' N and 51°6' E, with an altitude of 1321 m above sea level. This region has a warm and dry Mediterranean climate zone, as well as a dry moisture regime due to the cold and humid winters and warm and dry summers, there are from 150 up to 180 dry days per year based on meteorological information. The average regional yearly precipitation is 243 mm. Rainfall occurs mainly late

in the fall and early in spring. The average maximum annual temperature is 28°C in July, and an average minimum temperature of 1°C occurs in January. The average 30-year soil temperature is 14.5°C. The ambero-thermic graph for the two years of the experiment is illustrated in Fig. 1.

The soil was sampled before fertilization from a depth of 0-30 cm. The soil had the following characteristics: organic carbon (0.64%), electrical conductivity (EC) of 1.70 ds m⁻¹, soil nitrogen (0.06%), and absorbable phosphorus and potassium (10.3 and 275 mg kg⁻¹ of soil, respectively). The soil texture was clay loam (sand 28.80%, clay 41.88%, and silt 29.32%).

A factorial split-plot experiment was conducted in a completely randomized design with three replications over two years (2015-2017). The sowing dates were October 12 (conventional date) and November 1 (late sowing) with irrigation as the variable in the main plots, this took place at two levels: normal irrigation (control) and discontinuing irrigation from the silique forming stage (development of fruit stage, BBCH: 71) to the next stage (late-season drought stress). The subplots included promising new lines of open-pollinated rapeseed, that were L1112, L1091, L1093, L1206, which were originally released in Iran according to the national breeding programs for oilseeds crops and an open-pollinated cultivar, Okapi (French origin) as a control to be compared with the new lines. Each plot included six rows, with a length of 6 m, a distance of 30 cm between the lines, and a plant distance of 4 cm on the lines. The two outer rows were considered to be the border, and the four inner lines were used to evaluate various plant traits.

During both cropping years, fertilization was carried out by using nitrogen (urea 150 kg ha⁻¹, one third was applied at sowing time, one third at the stem elongation stage and one third at the beginning of the flowering stage), phosphorus (100 kg ha⁻¹ Triple-Superphosphate at sowing time) and potassium (50 kg ha⁻¹ Potassium Sulphate at sowing time).

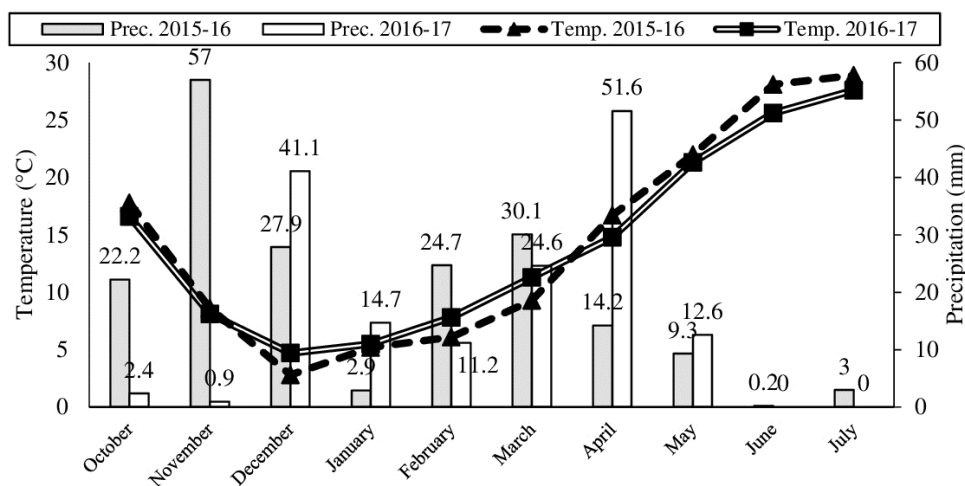


Fig. 1. Climatology curve (ambero-thermic graph) for Karaj province from October 2015 to July 2017.

Treflan herbicide was evenly sprayed at a volume of 2.5 l ha⁻¹. The fertilizers and herbicide were incorporated into the soil with a light disc. Pests, in particular the mealy aphid, were controlled through the application of Metasystox pesticide (1.5 l ha⁻¹). Irrigation was implemented using siphon pipes based on 80 mm evaporation from the surface of a Class A evaporation basin and water deficiency stress treatments consisted of eight common irrigation steps, of which only six steps were carried out and irrigation cessation took place from the silique formation stage to the next stage and the amount of water used in these treatments was 5 120 and 3 840 m³ ha⁻¹, respectively (with due consideration of precipitation during the growth period for both years). Ten plants were randomly selected from each experimental plot to determine the plant traits. The relevant physiological traits were evaluated through the random selection of the youngest developed leaves of the third internode from the top of the plant per experimental plots during the fruit development stage (BBCH: 75). In order to measure the leaf relative water content (LRWC) 10 leaf samples were randomly selected from each experimental plot and this parameter was calculated according to Ferrat and Loyal (1999) using Eq. (1):

$$LRWC(\%) = \frac{FW - DW}{TW - DW} 100, \quad (1)$$

where: *FW*, *DW*, and *TW* denote leaf fresh weight, dry weight, and the weight of inflammation, respectively.

Stomatal resistance (SR) was determined using a porometer device (Automatic Porometer AP4). Canopy temperature (CT) was measured using an infrared thermometer with an emission coefficient of 0.99. In order to measure the leaf proline content (LPC), 20 well-developed leaves were randomly selected from each experimental plot, and the amount of LPC was measured according to Bates *et al.* (1973). Carbohydrate of leaf solution (CLS) was calculated by randomly selecting 20 well-developed leaves from each experimental plot according to Dubois (Dubois *et al.*, 1956). Chlorophyll content (CC) was determined based on Arnon (1949) using Eq. (2):

$$CC: (0.020) (OD 645) - (0.008) (OD 663) V/W, \quad (2)$$

where: CC represents the total chlorophyll content (mg g⁻¹ of leaf fresh weight), OD denotes the absorbance of light at corresponding wavelengths, V shows the volume (ml) of acetone 80%, and W indicates leaf fresh weight. In order to measure seed yield, the margin and 0.5 m were removed from the top and bottom of the sowing rows, plants from the four middle rows of each plot were surface-cut in an area of 6 m².

Compound data analysis covering the two-year experiment was completed using SAS software (Ver. 9). Excel software was used to plot the graphs. The mean values were compared using Duncan's multiple range test at a 5%

probability level. Correlation and stepwise regression were analysed with SAS software (Ver. 9), and a path analysis was implemented using PATH software.

RESULTS AND DISCUSSION

The results of the ANOVA for LRWC showed the significance of the main effects of the year, sowing date, irrigation, and lines. Also, the interactions between the year, sowing date, and irrigation, as well as the interactions of the year, planting date, and lines were significant with regard to the LRWC traits ($p < 0.01$) (Table 1).

The interactions between the year, sowing date, and irrigation showed that delayed sowing and drought stress significantly reduced the LRWC for both of the experimental years. The lowest LRWC (78.21%) occurred on the sowing date of November 1 with irrigation interruption from the silique formation stage in the first year, and the highest LRWC (93.74%) was achieved on the sowing date of October 12 and with normal irrigation (Table 2). In this experiment, irrigation enhanced water absorption by the rapeseed roots due to raising the available moisture to the roots, thereby raising the LRWC. On the other hand, water elimination as a result of the silique formation stage decreased LRWC. Also, precipitation undergoes high oscillations in the Karaj meteorological data; hence, it is probable that some of the important growth stages of the rapeseed crop were affected by reduced soil water potential due to water deficiency in this experiment. Genetically, drought resistance is correlated with LRWC and osmotic regulation (Cardoso *et al.*, 2019; Reynolds *et al.*, 2016). Khan *et al.* (2010) declared that LRWC was reduced in 5 rapeseed cultivars including Con-III, Hyola, Shiralee, Rainbow and two mutants of Rainbow cultivar under drought stress at the flowering, silique formation and maturity stages. The LRWC was uppermost (84.05%) for L1206 (Table 3). It seems that L1206 was more compatible with the climatic condition of this study, but L1112 had less compatibility with the late sowing date and climatic condition of the presented research due to the lower LRWC in the same situation. On the other hand, the LRWC is a physiological trait that has frequently been proposed as a selection criterion for drought tolerance in rapeseed (Wu *et al.*, 2018).

Analysis of variances in this study showed that SR was influenced significantly by the sowing date and irrigation treatments. Furthermore, significant differences were observed between the two years and among the studied lines ($p < 0.01$) (Table 1). The interaction of the treatments was significant with regard to SR ($0.01 < p < 0.05$) (Table 1) and, thus, mean values were compared according to the interactions of year, sowing date, irrigation treatment, and lines (Fig. 2). Results showed that drought stress in general increased SR significantly (Fig. 2). Photosynthesis

Table 1. ANOVA for leaf relative water content (*LRWC*), stomatal resistance (SR), canopy temperature (CT), chlorophyll content (CC), leaf proline content (LPC), carbohydrate of leaf solution (CLS), and seed yield of the studied lines (2015-2017)

S.O.V.	df	M.S.						
		<i>LRWC</i>	SR	CT	LPC	CLS	CC	Seed yield
Year	1	256.6 **	46.99 **	29.12 **	199.1 **	395.5 **	0.138	6.68 × 10 ⁶ **
Error	4	0.18	0.97	0.43	1.006	0.05	0.027	5.12 × 10 ⁴
Sowing date (a)	1	2533 **	3852 **	1339 **	1364 **	7146 **	11.54 **	1.06 × 10 ⁷ **
Year × a	1	1.02 **	19.80 **	12.31 **	2.257 **	55.12 **	0.003	8.55 × 10 ⁴
Irrigation (b)	1	755.3 **	905.4 **	365.3 **	274.8 **	1647 **	2.594 **	4.32 × 10 ⁷ **
Year × b	1	2.84 **	0.22	3.38 **	0.077	10.28 **	0.004	9.21 × 10 ⁴
a × b	1	28.80 **	62.05 **	0.81	13.60 **	4.04 **	0.070 *	8.35 × 10 ⁵ *
Year × a × b	1	76.27 **	1.02 *	2.70 **	37.92 **	28.69 **	0.007	6.32 × 10 ⁵
Error	12	0.04	0.21	0.23	0.169	0.28	0.008	1.41 × 10 ⁵
Line (c)	4	14.94 **	16.48 **	5.60 **	6.562 **	26.81 **	0.036 **	2.01 × 10 ⁶ **
Year × c	4	10.36 **	13.80 **	5.19 **	5.22 **	28.29 **	0.041 **	2.10 × 10 ⁶ **
a × c	4	5.13 **	6.61 **	1.18 *	2.68 **	6.91 **	0.010	4.00 × 10 ⁵
Year × a × c	4	7.34 **	5.51 **	2.25 **	3.177 **	8.97 **	0.010	2.96 × 10 ⁵
b × c	4	0.21	0.16	0.07	0.063	1.04 *	0.003	5.40 × 10 ⁴
Year × b × c	4	0.04	0.28	0.14	0.160	1.65 **	0.003	2.12 × 10 ⁴
a × b × c	4	0.10	0.53	0.06	0.051	0.90	0.005	1.50 × 10 ⁴
Year × a × b × c	4	0.30	0.79 *	0.09	0.041	0.80	0.002	1.10 × 10 ⁴
Error	64	0.12	0.22	0.40	0.31	0.37	0.005	4.58 × 10 ⁵
C.V. (%)		0.41	2.37	2.31	3.47	1.55	5.42	19.1

Significant at *0.01 < p < 0.05, **p < 0.01.

reduction under drought stress has been reported by other researchers, this phenomenon is associated with non-stomatal factors (Papanatsiou *et al.*, 2019; Trueba *et al.*, 2019).

A comparison of the rapeseed crop responses in the two years of the study revealed that the SR level was lower in the first year while it increased significantly in the second year (Fig. 2). Considering the results, it seems that inappropriate environmental conditions, such as an undesirable temperature and unequal distribution of precipitation in the second year, have brought about increased SR due to the negative effect on rapeseed internal metabolism. On the other hand, the lower SR in the first year are a result in part of the optimal climate conditions and precipitation in the first year (Fig. 1).

In almost all of the test plots, late sowing significantly amplified the SR (Fig. 2). With the November 1 sowing date, rapeseed could desirably stabilize the status of the stomatal cells since it had a shorter growth period and

encountered fewer unsuitable environmental conditions than on October 12 (Henry *et al.*, 2019). The mechanisms (such as stomatal closure and leaf area decrease) that bring about resistance to drought through diminishing water losses usually induce the reduction of carbon dioxide absorption. Osmotic pressure increases resistance to drought through the maintenance of crop turgor, but the enhancement of soluble substance concentrations, besides energy consumption, might have an undesirable effect on osmotic regulation. The highest SR value (52.76 s cm⁻¹) was recorded in the second year and on the November 1 sowing date in L1112 under drought stress (irrigation interruption from the silique formation stage to the next stage). L1206 had the lowest SR (49.43 s cm⁻¹) among the other examined lines in the unfavourable second year experiment with delayed cultivation (November 1) and drought stress (irrigation interruption from the silique formation stage). With open stomata under adverse conditions and

Table 2. Interactions of year, sowing date, and irrigation on leaf relative water content (*LRWC*), canopy temperature (CT), leaf proline content (LPC), and carbohydrate of leaf solution (CLS) of the studied lines (2015-2017)

Year	Treatments		<i>LRWC</i> (%)	CT (°C)	LPC ($\mu\text{mol g}^{-1}$ fresh weight)	CLS (mg g^{-1} fresh weight)
	Sowing date	Irrigation				
2015- 2016	October 12	Control	92.91 ^b	24.82 ^g	20.68 ^b	25.85 ^h
		Drought stress	85.01 ^c	27.84 ^f	15.90 ^d	32.07 ^f
	November 1	Control	80.96 ^f	30.73 ^d	11.86 ^f	42.04 ^d
		Drought stress	78.21 ^h	34.01 ^b	10.68 ^g	49.47 ^b
2016- 2017	October 12	Control	93.74 ^a	24.53 ^g	21.90 ^a	29.28 ^g
		Drought stress	89.65 ^c	28.82 ^e	19.28 ^c	38.62 ^e
	November 1	Control	85.35 ^d	32.32 ^c	15.89 ^d	44.71 ^c
		Drought stress	80.03 ^g	35.68 ^a	12.36 ^e	51.35 ^a

Means in a column and a treatment followed by the same letter are not significantly different at 5% level.

Table 3. Interactions of year, sowing date, and line on leaf relative water content (*LRWC*), canopy temperature (CT), leaf proline content (LPC), and carbohydrate of leaf solution (CLS) of the studied lines (2015-2017)

Year	Treatments		<i>LRWC</i> (%)	CT (°C)	LPC ($\mu\text{mol g}^{-1}$ fresh weight)	CLS (mg g^{-1} fresh weight)
	Sowing date	Line				
2015-2016	October 12	L1112	87.60 ^g	26.85 ⁱ	17.72 ^f	29.96 ^l
		L1091	91.33 ^c	25.25 ^k	19.73 ^{de}	26.89 ⁿ
		L1093	86.91 ^h	27.32 ^{hi}	16.62 ^g	31.34 ^k
		L1206	90.72 ^{de}	25.70 ^k	19.44 ^e	27.51 ⁿ
		Okapi	88.23 ^f	26.53 ^{ij}	17.93 ^f	29.12 ^m
	November 1	L1112	79.59 ^o	32.48 ^{ef}	11.23 ^{kl}	45.83 ^e
		L1091	80.26 ⁿ	31.68 ^g	11.69 ^k	43.72 ^g
		L1093	78.96 ^p	33.07 ^{de}	10.93 ^l	47.65 ^{cd}
		L1206	79.17 ^p	32.80 ^{de}	11.00 ^{kl}	47.11 ^d
		Okapi	79.95 ^{no}	31.82 ^{fg}	11.52 ^{kl}	44.46 ^f
October 12	L1112	90.56 ^e	27.72 ^h	19.66 ^{de}	36.50 ^h	
	L1091	91.03 ^{cd}	27.13 ^{hi}	20.25 ^{cd}	34.76 ⁱ	
	L1093	91.43 ^c	26.89 ⁱ	20.52 ^{bc}	34.38 ⁱ	
	L1206	92.99 ^a	25.69 ^k	21.38 ^a	31.78 ^{jk}	
	Okapi	92.47 ^b	25.94 ^{jk}	21.15 ^{ab}	32.33 ^j	
2016-2017	November 1	L1112	81.45 ^m	34.97 ^a	13.13 ^j	49.74 ^a
		L1091	82.59 ^k	33.97 ^{bc}	14.20 ⁱ	48.21 ^c
		L1093	83.48 ^j	33.39 ^{cd}	14.83 ^{hi}	46.99 ^d
		L1206	84.05 ⁱ	33.14 ^{de}	15.01 ^h	46.20 ^e
		Okapi	81.89 ^l	34.51 ^{ab}	13.44 ^j	49.01 ^b

Means in a column and a treatment followed by the same letter are not significantly different at 5% level.

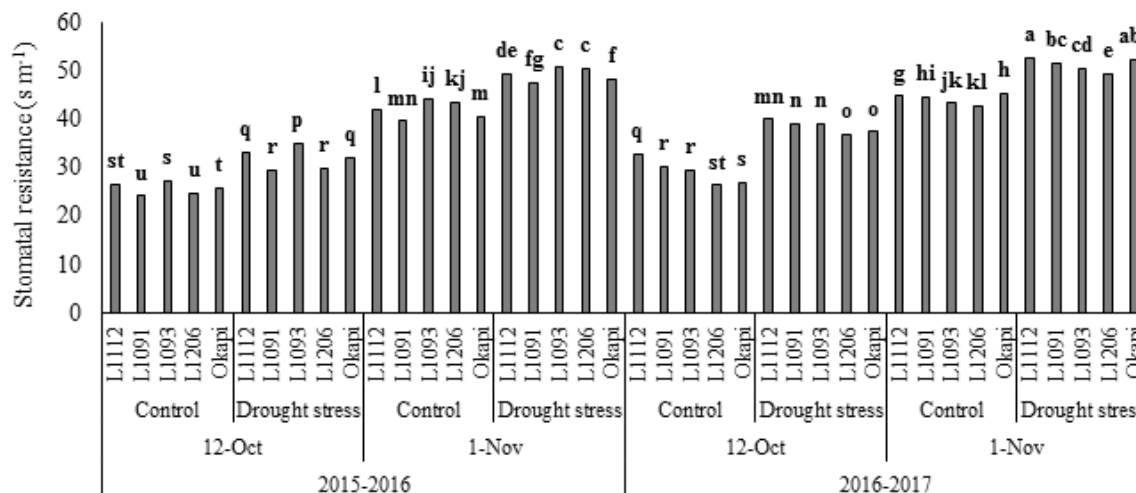


Fig. 2. Interactions of year, sowing date, irrigation, and line on stomatal resistance (SR) of the studied lines (2015-2017). Means with the same letter are not significantly different.

drought stress, the line received further CO₂ and continued photosynthetic activity, therefore, L1206 had a superior performance in such conditions. However, L1112 had the highest SR with faster closure of stomata and reduced photosynthesis under adverse conditions (delayed planting date and drought stress). Research on three pea genotypes suggested that water deficiency caused a significant increase in SR (Krouma, 2010). Hence, drought stress and delayed sowing resulted in a rise in SR, and more resistant lines presented lower SR values.

The main effects of the year, sowing date, and irrigation were significant with regard to CT and also there were significant differences between the lines. The interaction of the effect of year, sowing date and irrigation was significant on CT ($p < 0.01$) (Table 1). The results of mean comparisons in the interaction effect of the year, sowing date and irrigation showed that the second year had less favourable weather conditions (Fig. 1) and resulted in an increase in CT (35.68°C) with delayed sowing (November 1) and drought stress (irrigation interruption from the silique formation stage). CT was lowermost with the October 12 sowing date and normal irrigation in both experimental years (Table 2). Delayed sowing with drought stress probably resulted in more rapid stomatal closure and lower levels of gas exchange, eventually increasing CT in the plant.

The results showed that the interaction of the sowing date for the two years and lines had a significant effect on CT ($p < 0.01$) (Table 1). A comparison of mean values revealed that the CT of the studied lines increased significantly through late sowing in both years of the experiment and that the highest CT value was observed in the second year for late sowing in L1112 (34.97°C), but L1206 (33.14°C) had the lowest CT in the same situation (Table 3). For the first sowing date, the crop was able to regulate its internal reactions in a way that CT did not increase to any significant extent. Increasing CT for the delayed sowing date is indicative of inappropriate environmental

conditions for optimal crop growth. L1112 revealed a lower compatibility with the conditions of the experiment due to a relatively higher CT and, in comparison with the other lines, was not able to desirably overcome the environmental conditions. Araus *et al.* (2008) and Balota *et al.* (2007) believe that lines with lower CT are more appropriate for planting in arid regions and would have increased resistance in these conditions.

The results of ANOVA showed that the main effects of year, sowing date, and irrigation treatments were significant for LPC and that there were notable differences between lines. LPC was also significantly influenced by the interactions of year, sowing date, and irrigation, as well as by interactions of year, sowing date, and lines ($p < 0.01$) (Table 1).

The mean comparison revealed that LPC was affected by the interaction of the sowing date and irrigation treatments in both years of the study (Table 2). As shown in Table 2, delayed sowing and drought stress decreased the LPC. Rapeseed lines may have used a pathway other than the LPC elevation pathway to cope with the stress they were subjected to because LPC production is, in turn, an energy-intensive pathway for the plant and this would reduce optimal plant yield. In stressful situations, the plant first tries to use less energy-intensive methods to cope. The highest LPC (21.90 $\mu\text{mol g}^{-1}$ leaf fresh weight) was obtained in the second year, October 12 sowing date, and normal irrigation, and the lowest level (10.68 $\mu\text{mol g}^{-1}$ leaf fresh weight) was observed in the first year, November 1 sowing date, and drought stress. Drought stress leads to stomatal closure and the accumulation of LPC (Drake *et al.*, 2013). Sanchez-Rodriguez (2010), however, reported a decreased amount of LPC in a stress-resistant cultivar of tomatoes. The interaction of the year and sowing date on different lines showed that the amount of LPC was significantly higher in the second year for most of the lines planted when the two years are compared, and late sowing

caused a significant decrease in LPC (Table 3). As mentioned above, it seems that the studied lines did not use an energy-intensive proline pathway to cope with stress conditions. However, L1206 had a higher LPC ($15.01 \mu\text{mol g}^{-1}$ leaf fresh weight) than the other lines for the delayed sowing date of November 1 and in the second year. LPC acts as an osmotolerant factor and as a nutritional source (Chun *et al.*, 2018).

The ANOVA for CLS in the silique development stage showed that this parameter was significantly influenced by the sowing date and irrigation treatments. There were significant differences between the two years of the experiment and also between the lines. Interactions of the year, sowing date and irrigation; interactions of the year, sowing date and lines; interactions of the year, irrigation, and lines also had a significant influence on CLS content ($p < 0.01$) (Table 1). Concerning the climate condition in the first year of this research, precipitation dispersion was desirable early in the growth season, and the elevated vegetative growth led to diminish CLS.

The interactions of the year, sowing date and irrigation treatments showed that the highest amount of CLS (51.35 mg g^{-1} leaf fresh weight) were observed in the second year, late sowing (November 1), and drought stress treatments (Table 2). By creating unfavourable conditions for the normal activities of the plant, drought stress and delayed sowing enable the plants to cope with these adverse conditions, hence, increasing CLS is one of the coping strategies (Yan *et al.*, 2016).

The interactions between the year, planting date, and lines showed that the highest amount of CLS (49.74 mg g^{-1} leaf fresh weight) obtained in L1112 on the November 1 sowing date in the second year, indicated the higher susceptibility of this line to delayed sowing. The lowest level of CLS (46.20 mg g^{-1} leaf fresh weight) was observed in L1206 (Table 3). A comparison of the mean interactions between year, irrigation and lines also showed that L1112 contained the highest amount of CLS (46.44 mg g^{-1} leaf fresh weight) with delayed sowing and irrigation interruption from the silique formation stage in the second year. In the same conditions (delayed sowing and drought stress in the second year), the lowest level of CLS (43.26 mg g^{-1} leaf fresh weight) was observed in L1206 (Table 4).

As mentioned in the LPC section, the production of LPC is very energy intensive for the plant to cope with adverse conditions. Therefore, the presented lines used less energy-intensive osmoregulation and increased CLS strategies to cope with the unfavourable conditions of delayed sowing and drought stress. Among the investigated lines, L1112 was able to cope with these conditions through a further synthesis of CLS due to its higher sensitivity to experimental situations.

Table 4. Interactions of year, irrigation, and line on carbohydrate of leaf solution (CLS) of the studied lines (2015-2017)

Year	Irrigation	Line	CLS (mg g^{-1} fresh weight)
2015-2016	Control	L1112	34.34 ⁱ
		L1091	32.02 ^k
		L1093	35.90 ^h
		L1206	34.24 ⁱ
		Okapi	33.23 ^j
		L1112	41.45 ^d
	Drought stress	L1091	38.60 ^f
		L1093	43.08 ^c
		L1206	40.38 ^e
		Okapi	40.35 ^e
		L1112	39.81 ^e
		L1091	37.58 ^g
2016-2017	Control	L1093	36.53 ^h
		L1206	34.72 ⁱ
		Okapi	36.33 ^h
		L1112	46.44 ^a
	Drought stress	L1091	45.39 ^b
		L1093	44.84 ^b
		L1206	43.26 ^c
		Okapi	45.01 ^b

Means in a column and a treatment followed by the same letter are not significantly different at 5% level.

The main effects of the sowing date and irrigation treatments were significant for CC and there was also a significant difference between the lines ($p < 0.01$). The CC was also affected by the interaction of the sowing date and irrigation ($0.01 < p < 0.05$) (Table 1).

A comparison of the mean interactions between the sowing date and irrigation indicated that the amount of CC decreased for the delayed sowing date of November 1 (0.87 mg g^{-1} leaf fresh weight) as the plant hibernates with a weaker rosette, this disrupts the activity of the chloroplasts and CC and finally reduced photosynthetic activity. Also, drought stress causes chloroplast degradation and CC reduction by irrigation interruption from the silique formation stage to the next stage (Fig. 3). A study concerning rapeseed suggested that drought stress could influence chlorophylls a and b, as well as the total CC (Din *et al.*, 2011).

The interaction of the sowing date and irrigation showed that delayed sowing caused a significant decrease in the CC in both irrigation treatments, whereas drought stresses decreased CC significantly (Fig. 4). Photosynthetic pigments have a vital task in light absorbance and in the

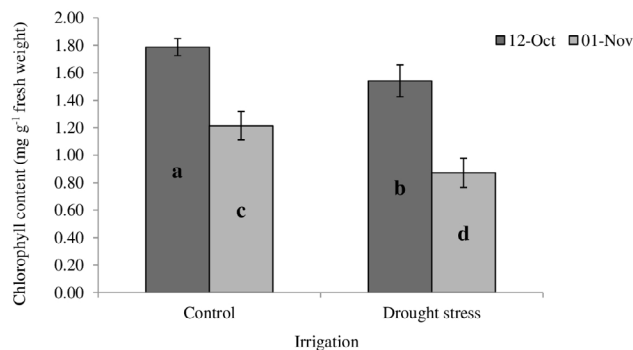


Fig. 3. Interaction of sowing date and irrigation on chlorophyll content (CC) of the studied lines (2015-2017). Means with the same letter are not significantly different.

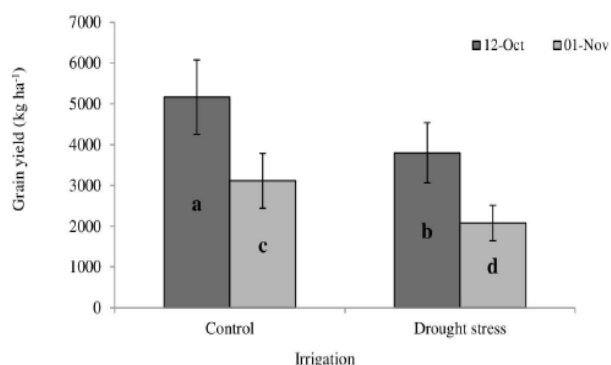


Fig. 4. Interaction of sowing date and irrigation on grain yield of the studied lines (2015-2017). Means with the same letter are not significantly different.

production of reduction power (energy) in plants, they comprise a vast range of different pigments, which directly or indirectly play a role in photosynthesis (Jaleel *et al.*, 2009). The total chlorophyll index in leaves is an effective index used to quantify photosynthetic potential and overall plant power (Hara *et al.*, 2013). A decrease in CC was reported to be an indicator of plant reaction to drought stress (Bayat *et al.*, 2009). Akram *et al.* (2018) reported that drought stress caused a significant reduction in growth, chlorophyll pigments, *LRWC*, and soluble proteins, while it also resulted in significant increases in relative membrane permeability, proline, glycine, betaine, malondialdehyde, phenolic compounds, and in the activities of catalase and peroxidase enzymes. In this research, the first sowing date raised the total CC due to the increased vegetative growth period. On the other hand, the plants cultivated on October 12 had an improved initial growth period because of the simultaneous primary growth of rapeseed seedlings with an optimal growth temperature occurring in mid-October and early November.

Drought stress harmed the total CC in rapeseed lines and reduced the total CC with an impact on the related metabolism in chlorophyll synthesis. The assessment of the

physiological properties of rapeseed in response to drought stress demonstrated that soil moisture insufficiency for vegetative growth, flowering, and the silique development stages gave rise to a noteworthy decline in CC in different rapeseed lines relative to the irrigation treatments (Din *et al.*, 2011).

The main effects of year, sowing date, irrigation, and line on seed yield were significant ($p < 0.01$). There was also a significant interaction between the sowing date and irrigation ($0.01 < p < 0.05$) (Table 1). A comparison of the mean interactions between the sowing date and irrigation showed a decreasing seed yield as a result of delayed sowing and drought stress, with the lowest seed yield (2080 kg ha^{-1}) occurring on the November 1 sowing date and irrigation interruption from the silique formation stage to the next stage. The highest seed yield (5164 kg ha^{-1}) was obtained on the October 12 sowing date and normal irrigation (Fig. 4). It is noteworthy that delayed sowing had a more pronounced effect on seed yield than drought stress because seed yield was higher under normal irrigation and sowing date (October 12) than normal irrigation and a delayed sowing date (November 1), as shown in Fig. 4. Hammac *et al.* (2017) declared that seed yield is profoundly affected by irrigation interruption at the flowering stage of rapeseed genotypes. Drought stress causing a decline in photosynthesis likely led to the inappropriate accumulation of photosynthates for seed filling and eventually reduced seed yield.

Seed yield had significant positive correlations with *LRWC*, CC, and LPC, while it presented significant negative correlations with SR, CT, and CLS ($p < 0.01$) (Table 5). The closest correlation was observed between SR and seed yield, suggesting an elevated seed yield with decreasing SR, which led to increased stomatal opening, a higher CO_2 exchange, increased photosynthetic activity, and finally, to an elevated seed yield. Pasban Eslam *et al.* (2017) studied the correlation between seed yield and physiological traits and reported that rapeseed genotypes with a higher *LRWC* and lower stomatal resistance had relatively higher seed yields under drought stress at the seed filling stage.

Stepwise regression was used to eliminate the effects of ineffective or less effective traits on seed yield in the regression model. Among the studied physiological traits, stomatal resistance, canopy temperature, and leaf proline were entered into Eq. (3), respectively (Table 6).

$$Y = 11041 - 136.7 X_2 - 129.9 X_3 - 57.53 X_5 \quad (3)$$

Y is seed yield, and X_2 , X_3 , and X_5 denote stomatal resistance, canopy temperature, and proline content, respectively. The model had an R^2 of 0.810, meaning that these traits account for more than 81% of seed yield variations. Stomatal resistance alone accounts for 79% of seed yield variations (Table 6). Stomatal resistance was introduced earlier in the model than the other traits and had the closest correlation (-0.89) with seed yield (Table 5). The addition

Table 5. Pearson correlation coefficients for seed yield, leaf relative water content (*LRWC*), stomatal resistance (*SR*), canopy temperature (*CT*), chlorophyll content (*CC*), leaf proline content (*LPC*), and carbohydrate of leaf solution (*CLS*) of the studied lines

	SY	<i>LRWC</i>	<i>SR</i>	<i>CT</i>	<i>CC</i>	<i>LPC</i>	<i>CLS</i>
SY	1						
<i>LRWC</i>	0.783 *	1					
<i>SR</i>	-0.892 *	-0.915 *	1				
<i>CT</i>	-0.889 *	-0.887 *	0.972 *	1			
<i>CC</i>	0.854 *	0.885 *	-0.957 *	-0.958 *	1		
<i>LPC</i>	0.754 *	0.984 *	-0.890 *	-0.858 *	0.859 *	1	
<i>CLS</i>	-0.885 *	-0.859 *	0.984 *	0.974 *	-0.967 *	-0.829 *	1

Significant at * $p < 0.01$.

Table 6. Stepwise regression for seed yield (dependent variable) and the other traits (independent variable)

Added trait to model	Stepwise regression stages		
	1	2	3
Intercept	7109	9635	11041
<i>SR</i>	-180.7	-99.83	-136.7
<i>CT</i>		-138.2	-129.9
<i>LPC</i>			-57.53
R ²	0.795	0.804	0.811

Table 7. Path analysis showing direct and indirect effects on the seed yield in rapeseed promising lines

	<i>SR</i>	<i>CT</i>	<i>LPC</i>
<i>SR</i>	<u>-0.675</u>	-0.376	0.157
<i>CT</i>	-0.656	<u>-0.386</u>	0.152
<i>LPC</i>	0.600	0.330	<u>-0.178</u>
Residual effect = 0.435			

Underlined values have direct effects.

of other variables to the model did not significantly influence any further increase in R². *CT* accounts for the highest grain yield variations in 30 genotypes of bread wheat under mild and intense drought stress conditions among such traits as 1000-grain weight, plant height, number of grains per panicle, and *CC* (Naroui Rad *et al.*, 2010).

SR had the greatest direct and negative effect (-0.675) on seed yield (Table 7). This trait had the most pronounced indirect effect on seed yield through *CT* (-0.376) and *LPC* (-0.157), respectively, and *LPC* exerted the least direct effect (-0.178) and the greatest indirect effect (0.930) on seed yield. It seems that high *SR* in the studied lines decreased CO₂ uptake and consequently decreased the photosynthetic rate, which ultimately lowered the seed yield. Therefore, the lines with a lower *SR* had a higher CO₂ uptake, photosynthetic rate, and yield (Taiz *et al.*, 2018). Since there was a significant positive correlation between

SR and *CT*, it appears that the transpiration rate decreased with increasing *SR*, followed by an increased *CT* in the studied lines.

CONCLUSIONS

1. Delayed sowing and late-season drought stress led to significant increases in stomatal resistance, canopy temperature, and carbohydrate of leaf solution, but significant decreases in leaf relative water content, chlorophyll content, and seed yield in the studied lines. By evaluating traits such as stomatal resistance, canopy temperature, leaf relative water content, and chlorophyll content, it is likely that stress, increased stomatal resistance, and stomatal closure led to reduced leaf relative water content, chlorophyll content, CO₂ entrance and thus the nutritional processes diminished notably in the plant, which finally reduced the seed yield. Among the studied lines, the highest leaf relative water content, chlorophyll content, and seed yield belonged to L1206 whereas L1112 contained the highest carbohydrate of leaf solution level. Thus, L1112 was able to increase carbohydrate of leaf solution in the face of adverse conditions, resulting in the loss of crop yield and greater seed yield loss in the plant, which indicates the greater susceptibility of the line to the experimental conditions.

2. The amount of leaf proline content did not increase significantly due to delayed sowing and late-season drought stress, thus indicating the following points: (1) Under stress conditions, rapeseed plants increased their resistance by increasing the amount of carbohydrate of leaf solution. (2) Since the crop needs to use amino acids and energy (glucose) to produce proline, and the final goal of rapeseed is to produce oil (seed and oil yield), the crop achieved resistance with the accumulation of carbohydrate of leaf solution instead of increasing its resistance to stress through the production of proline.

3. The results of the correlation and stepwise regression showed that among the physiological traits in this study, stomatal resistance justified most of seed yield variations, with the greatest direct and negative effect on seed yield according to the path analysis results. Therefore, the

measurement of stomatal resistance can be used as a parameter for the selection of resistant genotypes under stress conditions.

4. L1206 achieved the highest leaf relative water content, chlorophyll content and seed yield and the lowest stomatal resistance under delayed sowing and late-season drought stress. L1206 may be introduced as a superior line in order to select a line that can be cultivated in rotation with autumn crops and present favourable resistance to drought stress with the lowest yield loss at the end of the growing season in arid and semi-arid regions.

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