



Early sowing date as a cultivation strategy to alleviate drought effects on yield components of different canola genotypes

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Abstract

Water shortage or drought stress is an environmental factor that strongly affects crop productivity in various regions of the world. Sowing date as a factor affecting the growth and yield of oilseed crops can alleviate drought stress occurring in reproductive stage. To assess this, agronomic and physiological responses of five canola genotypes (L1030, L1204, L1110, L1114, and Okapi) to drought stress were evaluated at two early and late sowing dates (7th and 27th October, respectively) as a two-year field experiment. Results showed that drought stress significantly reduced the seed yield and yield components of all genotypes at both sowing dates, which was a consequence of damage to photosynthetic pigments, i.e. reduction of relative water content. However, early sowing was able to alleviate the effect of drought stress as seed yield of different genotypes at early sowing were 38-47% higher than late sowing. Moreover, the oil content was not influenced by drought stress and sowing date, so the highest oil contents at both sowing dates were recorded in L1204 genotypes. Finally, results showed that sowing suitable genotype in the proper date could be an appropriate approach to canola cultivation in semi-arid areas.

Keywords: drought stress, oil content, rapeseed, seed yield, podding stage

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Introduction

Canola (*Brassica* spp.) is considered as an economic oilseed crop across the world. Its oil is of premium quality with low erusic acid and glucosinolate contents (Din et al., 2011).

In past decades, the crop has become a dominant

(Fischer et al., 2014). However, erratic rainfall and scarcity of water for irrigation during the growing season seriously reduces the yield and quality of oilseed crops in semiarid areas (HongBo et al., 2005). Canola seed yield is dependent on genotype under semiarid climates (Djaman et al., 2018). Canola yield and its components are mainly affected by water shortages, which occur during the stage from flowering to the end of the seed

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oilseed and its sowing has doubled in the world

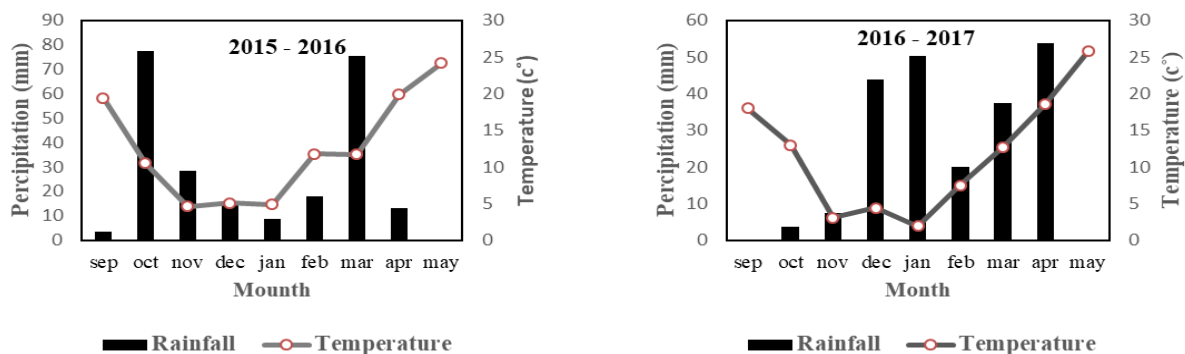


Fig. 1. Variation of temperature and rainfall in Karaj meteorology station during 2015-2017 growing seasons

Table 1

Physicochemical properties of soil collected from site study

Depth (cm)	EC (dS m ⁻¹)	pH	Organic Carbon (%)	N (%)	P (ppm)	K (ppm)	Texture
0-30	2.22	7.24	0.58	0.06	12.6	256	Clay loam

filling (Chaghakaboodi and Zebarjadi, 2012). Drought, negatively affected the photosynthetic capacity, yield, and oil quality of canola when imposed during the flowering and silique-filling stages (Elferjani and Soolanayakanahally, 2018). Previous studies also revealed that drought stress significantly decreased the seed oil content of canola (Sianaki, 2007). However, both agronomic and breeding interventions can be used to mitigate the adverse effects of drought. In this respect, sowing date has been reported as a factor affecting the growth and yield of canola (Uzun et al., 2009). Canola yield might increase with appropriate water supply and early planting (Djaman et al., 2018). Canola genotypes are slow growing especially in winter and most of them complete their life cycle in 210 to 270 days (Sharghi et al., 2011). In late sowing, the short vegetative growth period and the water deficit at reproductive phase lead to reduced canola yield. Therefore, planting at the right time provides sufficient growth to achieve optimal yield and alleviates drought stress (Uzun et al., 2009).

Moreover, there are wide variations among canola genotypes with respect to seed and oil yields at different planting dates as well as irrigation regimes (Sharghi et al., 2011). Genetic variations among genotypes with various drought tolerance have been reported in different crops (Kauser et al., 2006). Ul Haq et al. (2014) reported

that three genotypes of *Brassica napus* showed different growths and yields in response to irrigation treatments. Various morphological and metabolic activities in tolerant canola genotypes resulted in higher seed yield under drought stress conditions (Jabbari et al., 2016). Thus, reduction of adverse effects of drought stress and improvement of canola yield in semi-arid area can be achieved by identification of drought tolerant genotypes. In addition, various genotypes show different efficiency depending on sowing date. Little attention has been paid to assessing the drought tolerance of various canola genotypes at different sowing date as well as irrigation regimes. Hence, the aim of this study was to evaluate the drought tolerance of various canola genotypes at different sowing dates as well as under different irrigation regimes.

Materials and Methods

Site description and experimental design

The experiment was carried out at experimental field of Seed and Plant Improvement Institute (SPII) in Alborz province, Iran (35° 59' 12" N, 50° 75' 33" E and 1313 m). Local climatic and soil characteristics are summarized in Fig. (1) and Table 1, respectively. To evaluate the effect of drought stress on canola, a two-year field experiment was conducted during growing seasons of 2015-2016. The experiments were set out in a randomized

complete block split plot design, including two irrigation regime (normal and interrupting at silique stage), two early and late sowing date (7th and 27th October), and five canola genotypes (L1030, L1204, L1110, L1114, and Okapi). Seeds were sown in six rows at 4 cm intervals (row length: 5 m, row distance: 30 cm) during October. At physiological maturity stage samples were collected from middle lines and two sidelines of each plot were removed to control for marginal effects in order to evaluate agronomic and physiological traits.

Agronomic traits

At maturity stage, seed yield and its components including silique number, seed per silique, and 1000 seeds weight were measured. Harvest index was calculated as the ratio of seed yield to the total plant biomass.

Physiological traits

Young and developed leaves were sampled at noon and the relative water content (RWC) was calculated using the equation:

$$\text{RWC}\% = ((\text{FW}-\text{DW})/(\text{TW}-\text{DW})) * 100$$

where FW represented the fresh weight of the sample leaf, TW their overnight turgid weight, and DW their weight after oven drying (Cornic, 1994). Stomatal resistance of leaves was determined using a portable automatic diffusion porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK). Free proline content was extracted from 0.5 g leaf samples in 3 % (w/v) aqueous sulphosalicylic acid and estimated using ninhydrin reagent according to the method described by Bates et al. (1973). Ground fresh sample (0.5 g) was extracted with 80% (v/v) aqueous acetone to measure total chlorophyll (Chl). The absorbance of the resulting supernatant was recorded at 663 and 646 nm using an UV-visible spectrophotometer (Cary300, Varian, Inc.). Total Chlorophyll was calculated according to the following equation (Ianculov et al., 2005):

$$\text{Chl. Total} = 7.32. A_{646} + 7.18. A_{663}$$

Oil content

Oil content was determined by an NMR spectrometer at 25 °C, fitted with a permanent magnet of 0.23 T (9 MHz for ¹H) and a 13 mm × 30 mm catheter of useful area, using the Condor IDE software with CPMG pulse sequence with Qdamper (Colnago et al., 2011), expressed on a dry basis (DB%).

Statistical Analysis

Routines implemented in the SAS statistical analysis software v9.2 package (SAS Institute, Cary, NC, USA) were used to derive analyses of variance. Before performing combined analysis, Bartlett's test was applied to confirm variance homogeneity. The least significant difference (LSD) test was used to compare treatment means, applying a p threshold of 0.05 to declare significance.

Results

Yield and yield components

The ANOVA analysis showed that seed yield and its components were significantly different in different genotypes, irrigation regimes, and sowing dates. Among the genotypes, L1204 showed highest seed yield (5118 kg ha⁻¹) at both irrigation regimes and sowing dates. In addition, silique number, seed per silique, and one thousand seed weight of L1204 were significantly higher than those of the other genotypes (Table 2). Drought stress significantly reduced seed yield at all genotypes and sowing dates. The highest reduction rate occurred at L1114 genotype (31.7%). The reduction for silique number, seed per silique, and thousand seed weight were 30%,

Table 2
Interaction effects of sowing dates and genotypes on canola agronomic and physiological traits

Sowing date	Genotype	Silique Number	Seed Number Silique ⁻¹	1000 seed weight (g)	Seed yield (kg ha ⁻¹)	Oil content (%)	Stomatal Resistance (S cm ⁻¹)	Total chlorophyll (mg g ⁻¹ FW)
7th October	L1030	168.7b	22.1b	4.56b	4809b	44.1a	12.99h	1.68b
	L1110	147.0c	19.1c	3.99c	4165c	43.8b	14.75g	1.63c
	L1114	135.3e	17.5e	3.71e	3854d	43.6b	15.35f	1.59d
	L1204	179.6a	23.6a	4.84a	5118a	44.2a	12.42i	1.72a
	Okapi	141.5d	18.0d	3.88d	4013cd	43.5c	16.43e	1.55e
27th October	L1030	83.1g	8.4h	2.48h	2531fg	41.5fg	26.67b	0.99h
	L1110	84.0g	9.2g	2.55g	2560f	41.6e	25.17d	1.07f
	L1114	75.6h	8.0i	2.26i	2297h	41.6ef	25.93c	1.02g
	L1204	97.9f	10.9f	2.97f	3015e	41.8d	24.88d	1.09f
	Okapi	76.2h	7.56j	2.23i	2322gh	41.4g	27.10a	0.96i

Mean values of the same category followed by different letters are significant at p<0.05 level.

34% and 25%, respectively (Fig. II). Results showed that late sowing (27th Oct) drastically decreased seed yield from 38% to 59% in different genotypes. Moreover, under drought stress seed yield components were significantly higher at early sowing (7th Oct) compared with late sowing date (Table2).

Harvest index (HI), as the ratio of seed yield to the total plant biomass, was significantly affected by the interaction of genotypes, irrigation regimes, and sowing dates. HI of different genotypes reduced under drought stress at both sowing dates. However, the HI of some genotypes under drought stress were significantly higher at early sowing date. The highest HI under drought stress was observed in L1110 genotypes (26.84%) at

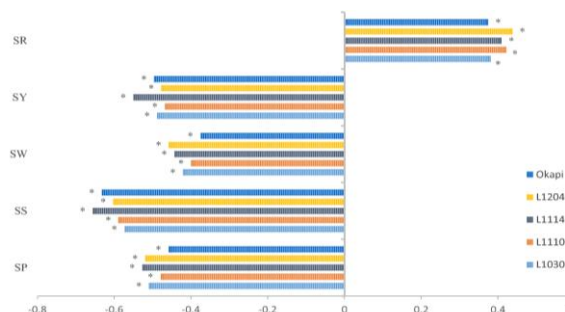


Fig. II. Log 2-fold change of yield and yield components of canola genotypes in drought stress condition compared to control; asterisk indicates significantly change (P< 0.05) in comparison with the control.

early sowing. In contrast, the lowest level of HI was recorded in L1114 genotype (25.59%) at late sowing date (Fig. III).

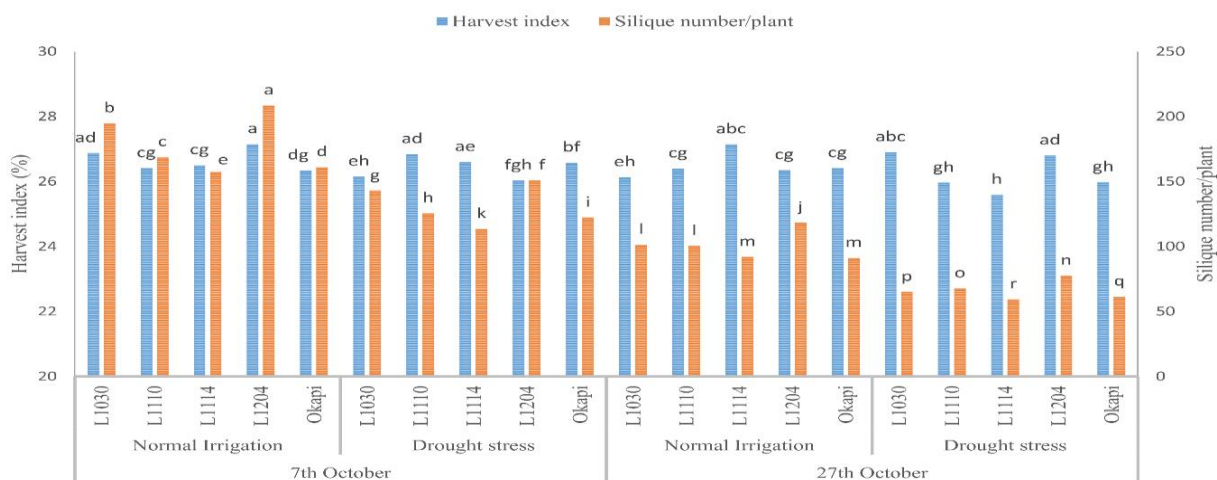


Fig. III. The effect of sowing date, irrigation regime and genotype interaction on some agronomic traits of canola. Bars sharing the similar lower case letters indicate that data are not significantly different from each other according to least significant different test (P = 0.05).

Table 3
The effects of sowing date, irrigation regime, and genotype on some physiological traits of canola

	Treatments	Proline content ($\mu\text{M g}^{-1}$ FW)	Relative water content (%)	Total chlorophyll (mg g^{-1} FW)
Sowing date	7th October	19.83a	89.43a	1.63a
	27th October	12.95b	80.33b	1.03b
Irrigation	Normal Irrigation	17.94a	87.36a	1.47a
	Drought stress	14.85b	82.39b	1.18b
Genotypes	L1030	16.58b	85.20b	1.33c
	L1110	16.53b	84.92b	1.35b
	L1114	16.17b	84.35bc	1.31d
	L1204	17.29a	86.29a	1.40a
	Okapi	15.39c	83.63c	1.25e

Mean values of the same category followed by different letters are significant at $p \leq 0.05$ level.

Table 4
Mean comparison of the interaction effects of genotypes and irrigation regimes on stomatal resistance in plant

Irrigation	Genotype	Stomatal Resistance (SR)
Normal Irrigation	L1030	17.23h
	L1110	17.03h
	L1114	17.76g
	L1204	15.82i
	Okapi	18.99f
Drought stress	L1030	22.42d
	L1110	22.89c
	L1114	23.52b
	L1204	21.46e
	Okapi	24.54a

Mean values of the same category followed by different letters are significant at $p \leq 0.05$ level.

Oil content

The measured oil content of all genotypes at both sowing dates are summarized in Table 5. In both early and late sowing dates, the highest and lowest oil contents were recorded in L1204 (44.2 and 41.8%) and Okapi (43.5 and 41.4%) genotypes, respectively.

Relative water content (RWC)

The results of physiological assessment of different canola genotypes at both irrigation regimes and sowing dates have been shown in table 3. Relative water content (RWC) of different genotypes had significant difference in both irrigation regimes and sowing dates. Among the genotypes, the L1204 and Okapi genotypes have showed highest and lowest RWC, respectively. RWC significantly affected by drought stress, when occurred at reproductive stage: the reduction was 5.6% in drought condition. Early sowing was able

to mitigate the drought adverse effect on RWC, which was 20% higher in early sowing compared with late sowing date (Table 5).

Stomatal Resistance (SR)

Measured stomatal resistance (SR) in all genotypes was significantly different in both irrigation regimes (Table 4) and sowing dates (Table 2). In both irrigation regimes as well as both sowing dates, the highest and lowest SR value was recorded in Okapi and L1204 genotypes, respectively. Under drought condition, stomatal resistance significantly increased by 32% compared with normal irrigation (Fig. II). In contrast, the effect of drought stress on stomatal resistance decreased at early sowing, as recorded SR values in all genotypes were significantly lower than those in late sowing date (Table 5).

Proline content

Proline content in different genotypes was significantly different in both irrigation regimes as well as at two sowing dates. Among the genotypes, the highest proline contents were recorded in L1204 genotype (17.29 $\mu\text{M g}^{-1}\text{FW}$). In all studied genotypes, proline content markedly reduced by 17% in drought condition. Moreover, proline content was significantly affected by sowing date; therefore, late sowing led to 34% reduction in proline content in comparison with early sowing (Table 3).

Total chlorophyll (CHL)

Total chlorophyll content was significantly different in different genotypes, irrigation regimes as well as sowing dates. Total chlorophyll content significantly reduced by 20% under drought condition when occurred at seed filling stage (Table 4). In addition, late sowing led to a markedly reduced chlorophyll content so that it was 37% lower than early sowing. However, the L1204 genotype showed the highest chlorophyll content at both sowing dates. In contrast, the lowest chlorophyll content was obtained in Okapi genotype at late sowing date (Table 2).

Discussion

In plants, the damage caused by drought stress depends on the species and genotype, length and severity of water loss, as well as on the developmental stage (Silvente et al., 2012). Results of current study showed that canola genotypes exhibited significant difference in yield components and physiological traits under drought condition. There are many studies that suggest the effectiveness of agronomic adaptation and mitigation strategies including adjustments of sowing dates in relation to climate-induced yield losses in different regions (McLean and Tsyban, 2001; Wassmann et al., 2009). According to the obtained results shifting planting date to an earlier time is considered as an effective approach to mitigate the harmful effects of drought on canola yield.

The mean silique number per plant was negatively affected by water deficiency and late in sowing date. Reduction in silique number per plant under

drought stress is mainly associated with an increase in silique abortion and shattering which is due to reduction in photosynthetic supply (Faraji et al., 2009). Results of current study also revealed that silique per plant was less affected by drought in early sowing date. The reducing effect of drought stress on silique per plant is more remarkable when occurring in flowering stage which mainly is due to the shorter flowering stage and increase in infertile flowers per plant (Nasri et al., 2008). The most significant reduction in seeds per silique as an important factor in grain yield of canola was observed in late-cultivated plants which were affected by drought in all studied genotypes. This trait is mostly affected by genotype too (Ray et al., 2006). It has been reported that the number of seeds per plant in Brassicaceae family is remarkably impressed by the number of pod per plant and the number of seed per pod and for the reason drought influence negatively this trait (Wright et al., 1995). Results obtained by Shahsavari et al. (2014) also showed water deficit specially during the flowering stage adversely affected the number of pods in each plant and in the case of late-season drought significantly decreased the number of seeds per pod.

Drought also caused a highly significant reduction in seed weight and 1000 seed weight in all studied canola genotypes especially when the sowing date delayed. Thousand seed weight of L1204 genotype was less affected by drought in late season in comparison to other genotypes. Seed maturity period is longer in early sowing date and leads to more nutrient transport from source to seeds as sources. Moreover, late-season drought stress is a consequence of delay in sowing date which occurring leaf senescence, reduction of leaf area, and disruption in assimilating transferring to seeds will be more restrictive for seed yield (Dong et al., 2006). Our results are in agreement with Bitarafan and Shirani Rad (2012) who reported reduction in seed weight in canola in response to water deficiency.

Harvest index implies the relative distribution of photosynthesis products between economical sinks and other existing sinks in the plant (Hütsch and Schubert, 2018). It has been reported that genotypes with higher HI in stress conditions

allocate a greater part of the photosynthetic products to seeds and produce higher seed yield (Kobata et al., 2018).

Oil content in canola is a determining factor in the selection of high yield lines for breeding programs. The higher negative effect of drought on oil content in late sowing dates might be mainly because of lower photosynthetic activity due to changes in temperature, which occurs mostly in the seed filling stage. Consequently, there is less conversion of carbohydrates into lipids. The other reason might be that in the early sowing date, there is a long time between the flowering and ripening stage and as a result, lipid formation will increase (Gecgel et al., 2007).

Water deficiency was accompanied by reduction in relative water content in canola plants, which differed among genotypes. Differential changes in relative water content of canola genotypes under drought condition have been attributed to variation in their root systems (Kage et al., 2004).

Decreased levels of proline content were observed in all genotypes in response to drought stress and when sowing was delayed. Proline normally accumulates in large quantities in response to environmental stresses (Filek et al., 2015; Godarzi et al., 2017). An increase in proline content seems like a stress adaptation mechanism in the plant, mostly as an osmotic adjustment mechanism (Hare and Cress, 1997). Despite a strong correlation between stress tolerance and accumulation of proline in higher plants, this relationship may not be universal. Results of the current study also showed a negative correlation between proline accumulation and drought resistance in canola genotypes.

Growth of canola genotypes was limited under drought stress and by delay in sowing date. A

major features included decreased chlorophyll content, which indicates the destruction of chloroplasts and reduction in pigment production. Results are in agreement with Ashraf and Mehmood (1990), who also noted that the chlorophyll content of plants decreased after drought stress.

Results revealed that L1204 by using escape mechanisms was more resistant to drought stress and showed the highest seed yield by producing more silique number, seed number /silique, and 1000 seed weight in both water deficiency and delayed sowing condition (27th October). Late sowing of canola genotypes led to meeting of vegetative stage with high temperature and increased respiration of pods. This phenomenon by restriction in photosynthesis led to a decrease in grain weight (Rafiei et al., 2011). In terms of nutrient allocation competition between vegetative and reproductive organs in canola, it is necessary to consider appropriate sowing date to minimize the probability of occurrence of drought stress in the flowering stage (Sianaki, 2007).

Conclusions

Current canola genotypes showed reduced yield under drought condition. Results also revealed reduction in yield of canola genotypes by delaying in sowing date in two years' experiments, which represents the importance of early sowing as an agronomical technique to obtain optimum yield in both normal and drought condition. L1204 genotype by 5118 kg ha⁻¹ grain yield production in early sowing date was the genotype with high oil and grain yield under both sowing dates and irrigation regimes in comparison with the other genotypes, demonstrating the importance of sowing of this genotype in semi-arid areas.

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