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Evaluating the effect of sowing date and drought stress on morphological and functional characteristics of three genotypes of winter oilseed rape (*Brassica napus* L.)

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ABSTRACT

To assess the effects of drought stress and sowing date on phenological, morphological, and yield traits of three different cultivars of winter oilseed rape (*Brassica napus* L.), this study was conducted in research farm of Sarayan agricultural college- University of Birjand in 2016-2017 growing season. Experiment was conducted in a split-factorial based on the randomized complete block design with drought stress in the main plots and three sowing date (September 22, October 6, and October 22) along with three cultivars of canola (‘Homolious’, ‘Hayola50’, and ‘DK7070CL’) in the subplots in three replications. The results of analysis of variance and means comparison analysis showed significant and negative effect of drought stress on seed yield and biological yield traits of investigated cultivars of canola. The interaction effect of drought stress × sowing date × cultivar was only significant on leaf twisting trait at 1 % probability level. ‘Homolious’ was assigned as the most drought tolerance cultivar, based on SI, SSI, RDI, TOL, MP, STI, GMP, YI, YSI, and HARM drought tolerance indexes, whereas ‘Hayola50’ was assigned as most drought sensitive cultivar of oilseed rape**.**

Key words: canola; drought; sowing date; selection criteria; tolerance index

IZVLEČEK

OVREDNOTENJE UČINKA DATUMA SETVE IN SUŠNEGA STRESA NA MORFOLOŠKE IN FUNKCIONALNE LASTNOSTI TREH GENOTIPOV OZIMNE OLJNE OGRŠČICE (*Brassica napus* L.)

Za ovrednotenje učinka datuma setve in sušnega stresa na fenološke in morfološke lastnosti ter lastnosti pridelka treh sort ozimne oljne ogrščice (*Brassica napus* L.) je bila v rastni sezoni 2016-2017 izvedena raziskava na kmetijski šoli Sarayan, Univerze v Birjandu, Iran. Poskus je bil izveden kot popolni naključni bločni poskus z deljenkami, s sušnim stresom na glavnih ploskvah in tremi datumi setve na podploskvah (September 22, Oktober 6, and Oktober 22), s tremi sortami oljne ogrščice (‘Homolious’, ‘Hayola50’, and ‘DK7070CL’) s tremi ponovitvami. Rezultati analize variance in primerjava poprečij so pokazali značilne negativne učinke sušnega stresa na pridelek semen in parametre biološkega pridelka vseh preučevanih sort oljne ogrščice. Medsebojni učinek sušnega stres, datuma setve in sorte je bil značilen samo za lastnost zvijanja listov pri 1 % verjetnosti. Sorta ‘Homolious’ je bila prepoznana kot na sušni stres najbolj odporna na osnovi parametrov kot so SI, SSI, RDI, TOL, MP, STI, GMP, YI, YSI in HARM-ov indeks tolerance na sušo, sorta ‘Hayola50’ je bila prepoznana kot na sušni stres najbolj občutljiva sorta oljne ogrščice.

Ključne besede: oljna ogrščica; suša; datum setve; selekcijski kriteriji; indeks tolerance

1 INTRODUCTION

The growing populationof the world along withmore requests for vegetable oils leads to more cultivation of oil seed crops. Winter oilseed rape (*Brassica napus* L.) is one of the most important oil seed crops that economically compete with cereal crops (Diepenbrock, 2000). Oilseed rape is the third source of vegetable oil in the world (USDA, 2016a). In the last decades, the yield stability of oilseed rape has not improved, beside its increased seed yield (Weymann et al., 2015). One of the main barriers to plant growth and yield is abiotic stress, especially the drought stress. Prolonged water deficit is a major abiotic stresses (Farooqet al., 2009).

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Breeding for a quantitative trait, such as drought resistance, with low heritability, is complicated using certain criterions that quantify the level of the desired quantitative trait and is more suitable than a direct selection (Farshadfar and Sutka, 2002). In this situation, plant breeders prefer to use drought indices that provide a measure of drought based on yieldloss under drought condition in comparison to normal condition (Mitra, 2001). Several selection indices have been suggested by various researchers based on a mathematical relationship between favorable and stress conditions (Clarke et al., 1984; Huang, 2000). Indices such as tolerance (TOL) (McCaig and Clarke, 1982; Clarke et al., 1992), mean productivity (MP) (McCaig and Clarke, 1982), stress susceptibility index (SSI)(Fischer and Maurer, 1978), geometric meanproductivity (GMP) (Fernandez, 1992), harmonic mean (HARM) (Schneider et al., 1997), relative drought index (RDI) (Fischer and Wood, 1979) and stress tolerance index (STI) (Fernandez, 1992) have been used by researchers under different conditions.

One of the wide-spread problems that seriously influence the production and quality of rapeseed is drought stress. However, the lack of effective selection criteria is hampering the development of resistant cultivars (Shiranirad and Abbasian, 2011). Sowing date is another important factor that plays a major role in determining the seed yield and the quality of rapeseed (Ozer et al., 2003). The objective of the present study was to investigate the interaction effect of drought stress and sowing date on yield and yield components traits of three cultivars of rapeseed and find the drought resistance genotype based on different drought indices.

2 MATERIALS AND METHODS

2.1 Plant materials

Three new and superior genotypes of winter oilseed rape, including ‘Homolious’, ‘Hayola50’ and ‘DK7070CL’ were cultivated in the experimental field of Sarayan agricultural college-Birjand University, in South Khorasan province-Iran in 2016-2017 growing season.

2.2 Fields Experimental conditions

The experiment was carried out in the form of split-factorial based on randomized complete block design (RCBD) with drought stress in the main plots and three sowing date (September 22, October 6 and October 22) along with three mentioned cultivars of oilseed rape in the subplots in three replications. All investigated genotypes were cultivated in their allocated subplots. Each subplot was consisted of four rows with 2 m length and with 20 cm distance between lines. In the normal experimental field, normal irrigation (one time per 10 days) of oilseed rape was applied but in drought stress environment, irrigation was interrupted in the flowering stage of genotypes.The volume of irrigated water for each subplot was calculated according to below equation (Moghadam and Fanay, 2016):

$$D = (Fc\ \theta) \times \rho_b \times D/100$$

Which d is the depth of irrigation water (mm), FC is the percentage of soil moisture content at from the field capacity, θ is the percentage of soil moisture content before irrigation, ρ_b is the bulk density of farm soil (g cm^{-3}), and D is the maximum depth of root development (m). A precise meter was used to enter the calculated amount of water in subplots (4800 m^3).

The drought stress was applied using water holding method (Bao et al., 2009). The irrigation of each flowering genotype was delayed for five days (one time irrigation per 15 days). The amount of irrigation water for each subplot was same to normal irrigation (4800 m^3).

Phenological assessments including days to flowering (DF) and days to maturity (DM) were collected during the growing season. Morphological characteristics of plant height (PH) and chlorophyll content (CC) along with number of siliques per plant (SP), number of seeds per silique (SS), 1000-seed mass (TW), biological yield (BY), seed yield (SY), harvest index (HI), plant height (PH), number of branches (NB), stem diameter (SD), silique length (SL), root length (RL), first branch height (FBH), and leaf twisting (LT) were recorded for each combination of treatments at the end of growing season, separately.Ten plants per plot were randomly selected to measure the mentioned characteristics. The chlorophyll content was measured using a SPAD 502 chlorophyll meter apparatus before the yellowing and drying of plants. The total dry matter of harvested plants (root + shoot) was considered as biological yield. To measure the seed yield, two middle rows of each plot was select and their plants were harvest. The seeds were left in fresh air to reach their moisture to 12 % and then the seed yield was measured.

2.3 Drought tolerance indices

To calculate the drought tolerance indicators, potential yield of each genotype in normal (the calculated seed yield in normal irrigated plots) (Y_p) and drought stress environment (Y_s), the average performance of all investigated genotypes in normal ($\overline{Y}_p$) and drought stress environment ($\overline{Y}_s$), were measured and then TOL, MP, GMP, SSI, HARM, RDI, and STI were calculated according to below equations, respectively:

$$TOL = Yp - Ys$$ (1) (McCaig & Clarke, 1982; Clarke et al., 1992),

$$Mp = \frac{Yp + Ys}{2}$$ (2) (McCaig & Clarke, 1982),

$$GMP = \sqrt{Yp \times Ys}$$ (3) (Fernandez, 1992),

$$SSI = \frac{1-(Ys/Yp)}{1-(\overline{Ys}/\overline{Yp})}$$ (4) (Fischer & Maurer, 1978),

$$HARM = \frac{2(Yp \times \overline{Ys})}{(Yp + Ys)}$$ (5) (Schneider et al., 1997),

$$RDI = \frac{(Ys/Yp)}{(\overline{Ys}/\overline{Yp})}$$ (6) (Fischer & Wood, 1979),

$$STI = \frac{Ys \times Yp}{\overline{Y}_p^2}$$ (7) (Fernandez, 1992),

Yield Index (YI) and Yield Stability Index (YSI) were calculated using below equations:

$$YI = \frac{Ys}{\overline{Ys}}$$ (8) (Gavuzzi et al., 1997),

$$YSI = \frac{Ys}{Yp}$$ (9) (Bouslama & Schapaugh, 1984).

2.4 Statistical analysis

Statistical analyses including the analysis of variance (ANOVA) and means comparison analysis were carried out using the SAS software (Ver. 9.2). Means comparison analysis was conducted using Duncan's multiple range tests at 5 % probability level. All drought tolerance indices were calculated using Excel 2010 software. Simple Pearson correlation analysis was carried out to calculate the correlation of investigated plant characteristics and estimated drought tolerance indexes using SAS software.

3 RESULTS AND DISCUSSION

3.1 Analysis of variance and means comparison

The results of analysis of variance of split-factorial design (Table 1) showed the significant effect of drought stress on seed yield and leaf twisting traits at 5 % and on biological yield trait at 1 % probability level, respectively. The main effect of drought stress was not significant on other investigated traits. The genetic analysis of grain yield of twenty one F_2 progenies of oilseed rape using diallel cross analysis revealed that the additive gene effects are more effective than non-additive effects (Amiri-Oghanet al., 2009) and a S_1 recurrent selection program can help improve the seed yield of investigated cultivars of oilseed rape under drought stress.

The main effect of sowing date was only significant for the day to flowering and day to maturity traits at 1 % probability level. It is obvious that these two important phenological traits can be affected by sowing date. Amiri-Oghan et al (2009) reported 81.99 % heritability for days to maturity in 21 F_2 progenies of oilseed rape. Therefore, it is a trait with high heritability and direct or recurrent selection can be helpful to select the desired genotypes for this trait.

The interaction effect of drought stress × sowing date had significant effect on1000-seed mass, stem diameter, first branch height, chlorophyll content, and days to maturity at 5 % probability level. The results of analysis of variance revealed significant differences among three investigated cultivars of oilseed rape for 1000-seed mass, biological yield, seed yield, plant height, stem diameter, silique length, first branch height, chlorophyll content, day to flowering, and day to maturity at 1 % probability level and for root length at 5 % probability level. The number of seeds and their average mass are the parameters that affect the seed yield, therefore the improvement of1000-seed mass can improve the seed yield of investigated genotypes of oilseed rape (Labra et al., 2017).There was no significant difference between investigated cultivars of oilseed rape for harvest index, anyway it is obvious that a greater harvest index can leads to higher seed yield in canola (Diepenbrock, 2000). Shiranirad and Zandi (2012) also reported non-significant effect of drought stress on harvest index of twenty cultivars of spring rapeseed.

Based on the results of analysis of variance, the interaction effect of drought stress × cultivar was only significant for number of branches at 1 % probability level (Table 1).

The interaction effect of sowing date × cultivar was significant for leaf twisting at 1 % probability level and fornumber of branches, first branch height, and seed yield at 5 % probability level (Table 1). Azizi (2015) also reported the different response of two investigated cultivars of oilseed rape (‘Hyola401’ and ‘RGS003’) in different sowing date for number of branches per plant and number of siliqua per plant. Based on the results of analysis of variance, three ways interaction effect of drought stress × sowing date × cultivar was only significant for leaf twisting trait at 1 % probability level (Table 1).

The results of means comparison analysis using Duncan’s multiple range tests at 5 % probability level showed the adverse effect of drought stress on seed yield and biological yield of three investigated cultivars of oilseed rape under three different sowing date (Table 2).

Table 1: Analysis of variance of investigated traits of oilseed rape under drought stress and different sowing dates

		Means of squares					
S. O. V	df	Number of siliques per plant	Number of seeds per silique	1000-seed weight	Seed yield	Biological yield	Harvest Index
Block	2	92166.50^{ns}	47453985.7^{ns}	0.19^{ns}	8284.04^{ns}	224788.85*	10.68^{ns}
Drought Stress	1	33908.18^{ns}	9170609.7^{ns}	1.80^{ns}	92865.43*	1457781.33**	70.99^{ns}
Error a	2	46033.68	42062174.3	0.60	3583.32	6281.41	12.98
Sowing date	2	51151.31^{ns}	11216812.6^{ns}	0.10^{ns}	3635.54^{ns}	111737.25^{ns}	3.05^{ns}
Drought Stress × Sowing date	2	61056.82^{ns}	12628299.4^{ns}	0.54*	5486.26^{ns}	481462.13^{ns}	2.25^{ns}
Error b	8	38015.90	31571885.3	0.06^{s}	1575.62	151174.58	5.00
Cultivar	2	13219.55^{ns}	37133130.6^{ns}	1.68**	14493.77**	1352266.10**	9.44^{ns}
Drought Stress × Cultivar	2	10305.26^{ns}	6420690.9^{ns}	0.22^{ns}	599.38^{ns}	50258.78^{ns}	8.37^{ns}
Sowing date × Cultivar	4	32689.63^{ns}	11086189.0^{ns}	0.03^{ns}	6288.07*	181704.66^{ns}	10.55^{ns}
Drought Stress × Sowing date × Cultivar	4	8478.08^{ns}	4357525.4^{ns}	0.09^{ns}	1840.96^{ns}	107095.99^{ns}	8.34^{ns}
Error c	24	31652.40	24389046.4	0.10	2138.55	95242.40	6.75
Total	53	34155.28	23069582.1	0.22	4933.52	199279.01	8.34
C.V (%)		18.3	27.45	10.07	18.23	17.21	18.25

** ,*: significant at 1% and 5% probability level, respectively.

Table1: continued

S. O. V	df	Mean of Squares									
		Plant height	No. of branches	Stem Diameter	Silique length	Root length	First branch height	Chlorophyll content	Days to flowering	Days to maturity	Leaf twisting
Block	2	21.35^{ns}	189.0^{ns}	0.01^{ns}	0.10^{ns}	2.48^{ns}	8.1^{ns}	9.33^{ns}	6.80^{ns}	0.80^{ns}	0.07^{ns}
Drought Stress	1	90.74^{ns}	9.0^{ns}	0.02^{ns}	3.65^{ns}	0.67^{ns}	41.6^{ns}	35.85^{ns}	1.50^{ns}	6.00^{ns}	3.13*
Error a	2	208.13	17.3	0.06	0.35	12.77	220.3	2.08	5.06	3.17	0.07
Sowing Date	2	316.69^{ns}	510.8^{ns}	0.16^{ns}	0.16^{ns}	5.59^{ns}	61.5^{ns}	15.67^{ns}	401.46**	550.13**	0.02^{ns}
Drought Stress × Sowing Date	2	384.57^{ns}	316.7^{ns}	0.23*	0.12^{ns}	3.13^{ns}	69.1*	36.38*	3.72^{ns}	3.39*	0.02^{ns}
Error b	8	306.91	219.2	0.05	0.27	5.85	14.7	8.13	2.29	0.45	0.13
Cultivar	2	6355.0**	150.7^{ns}	0.60**	10.19**	24.55*	572.2**	144.04**	244.13**	80.57**	0.02^{ns}
Drought Stress × Cultivar	2	21.35^{ns}	655.5**	0.08^{ns}	0.06^{ns}	0.80^{ns}	22.1^{ns}	4.71^{ns}	2.17^{ns}	0.72^{ns}	0.02^{ns}
Sowing Date × Cultivar	4	182.19^{ns}	315.6*	0.08^{ns}	0.44^{ns}	7.55^{ns}	56.5*	22.42^{ns}	2.13^{ns}	0.30^{ns}	0.57**
Drought Stress × Sowing Date × Cultivar	4	30.19^{ns}	190.6^{ns}	0.04^{ns}	0.13^{ns}	4.08^{ns}	27.1^{ns}	28.32^{ns}	0.56^{ns}	3.11^{ns}	1.24**
Error c	24	85.44	100.9	0.03	0.28	6.52	14.3	22.19	2.19	2.66	0.06
Total	53	378.49	186.6	0.08	0.70	6.59	51.8	23.79	26.60	25.75	0.25
CV		9.6	13.31	16.45	12.18	14.63	22.01	11.69	1.02	0.79	14.97

** ,*: significant at 1 % and 5 % probability level, respectively.

Table 2: Means comparison analysis of investigated characteristics of oilseed rape cultivars under drought stress condition.

Drought Stress	Seed Yield (g m^{-2})	Biological Yield (g m^{-2})	Leaf twisting
Normal irrigation	295.08 a	1957.39 a	1.815 a
Drought stress	212.15 b	1628.78 b	1.333 b

The means with the same letter(s) at each column had no significant difference at 5 % level

The adverse effect of drought stress in seed yield is obvious, in addition, it is reported that late plantings can lead to reduced seed yield in oilseed rape because of shortening the vegetative stage (Azizi, 2015).

Another surprising result of means comparison analysis was that leaf twisting in normal irrigation was higher than in drought stress condition (Table 2). These results indicate that leaf twisting is not a drought tolerance strategy in investigated cultivars of oilseed rape. The results of means comparison analysis for the main effect of sowing date using Duncan's multiple range test at 5 % probability level revealed that the highest means of days to flowering was achieved in September 22 cultivation date, and later sowing dates lead to significant decrease in day to flowering of investigated cultivars of oilseed rape (Table 3). It is reported that both additive and non-additive genetic effects involved in controlling flowering and maturity time of oilseed rape, however additive effects are more important than non-additive genetic effects (Amiri-Oghanet al., 2009). Therefore, the direct selection for shorter flowering period under drought stress condition can be effective.Though our results are obtained in winter oilseed rape, however, in spring rapeseed it is reported that early sowing dates gave higher yields than late sowings and yield differences in this situation can related to the changes in branch numbers, silique numbers per plant, and 1000 seed mass (Ozer, 2003). The results of means comparison analysis for the main effect of cultivar using Duncan's multiple range test at 5 % probability level revealed that the highest means of number of seed per silique, 1000-seed mass, biological yield, plant height, stem diameter, root length, chlorophyll content, days to flowering, and days to maturity were related to 'Homolious' of oilseed rape, whereas the highest means of silique length was related to 'Hayola50' cultivar (Table 4).The growth rate and duration of the growing period are the two main factors that affect the biological yield of oilseed rape (Diepenbrock, 2000). These results were also evident in the present study, as the 'Homolious' with the highest days to maturity showed the highest mean of biological yield.

The means comparison analysis using Duncan's multiple range test at 5 % probability level showed that the highest mean of 1000-seed mass was obtained in normal irrigation condition and in September 22 sowing date, anyway there was no significant difference between this interaction effect and interaction effect of normal irrigation × October 6 sowing date for 1000-seed mass trait at 5 % probability level (Table 5).These results indicate to it that normal irrigation in early sowing date can improve 1000-seed mass of oilseed rape but in delayed sowing date providing sufficient soil moisture is not very helpful to improve this trait. Andersen et al (1996) reported that drought stress during flowering did not affect seed mass of oilseed rape, whereas the earlier drought stress adversely affects the seed mass of oilseed rape.

The lowest mean of 1000-seed mass was achieved from drought stress condition and latest sowing date (October 22) (Table 5). These results indicate that drought stress and delayed sowing date can significantly reduce 1000-seed mass of investigated cultivars of oilseed rape in South-Khorasan province of Iran. These results are completely agreed with the findings of Andersen et al (1996). The growth rate and duration of the growing period are the main factors that involved in biological yield, both of which indicate the potential for improvement in yield (Diepenbrock, 2000).

The highest and lowest means of first branch height were achieved from normal irrigation × Septamber 22 and drought stress × October 22 treatments, respectively (Table 5).

Table 3: Means comparison analysis of phonological characteristics of three investigated cultivars of oilseed rape under different sowing data

Sowing Date	Days to flowering
September 22	150.44a
October 6	145.83b
October 22	141.00c

The means with the same letter(s) at each column had no significant difference at 5 % level

Table 4: Means comparison analysis of phonological, morphological, and yield traits in three investigated cultivars of oilseed rape.

Cultivar	1000-seed mass	Biological Yield (g m^{-2})	Plant Height (cm)	Stem Diameter (cm)	Silique length (cm)	Root length (cm)	Chlorophyll content	Days to flowering	Days to maturity
Homolious	3.40 a	2109.54 a	117.28 a	1.32 a	3.86 b	18.78 a	42.88 a	149.83 a	207.06 a
Hayola50	3.15 a	1638.58 b	82.33 b	1.00 b	5.22 a	17.02 ab	37.27 b	144.78 b	203.17 b
DK7170CL	2.79 b	1631.12 c	87.83 b	1.01 b	3.99 b	16.57 b	40.74 b	142.67 b	203.67 b

The means with the same letter(s) at each column had no significant difference at 5 % level

Table 5: Means comparison analysis of interaction effect of drought stress and sowing date on investigated traits of three oilseed rape cultivars

Drought stress × Sowing Date	1000-seed mass(g)	First branch height(cm)	Chlorophyll content	Days to maturity
Normal irrigation ×September22	3.52 a	23.20 a	43.41 a	207.44 a
Normal irrigation × October6	3.46 a	13.80 b	37.78ab	203.67 b
Normal irrigation ×October22	2.91bc	16.89ab	42.14ab	203.78 b
Drought stress ×September22	3.28ab	23.76 a	42.34ab	206.67 a
Drought stress × October6	2.84bc	11.84 b	36.77 b	202.67 b
Drought stress ×October22	2.68 c	13.02 b	39.33ab	203.56 b

The means with the same letter(s) at each column had no significant difference at 5 % level

The identification of the primary and secondary yield components can help to analyze seed yield and improve it under different condition (Diepenbrock, 2000).

The highest and lowest means of chlorophyll content were achieved from normal irrigation × September 22, and drought stress × October 6, respectively (Table 5). The small photo synthetically active area can limit the source and affects source and sink relation and therefore affects the seed yield of oilseed rape (Diepenbrock, 2000).

The earliest sowing date (September 22) lead to the highest number of days to maturity in both normal and drought stress condition (Table 5), whereas there were no significant differences among other interaction effects of drought stress and sowing date for this trait (Table 5). In short-season areas and also in stressful conditions determination of optimum sowing date is likely to be of critical importance because delayed sowing limits the size to which the crop grows before the change from vegetative to reproductive development which in turn controls yield potential (Gross, 1963; Ozer, 2003). Our results indicate that earlier sowing date can lead to longer vegetative period in both normal and drought environments; therefore the earlier sowing date is not a perfect strategy to escape from terminal drought stress.

The results of means comparison analysis for interaction effect of drought stress × cultivar showed that the highest and the lowest means for number of secondary branches were achieved from drought stress × 'Hayola50' and drought stress × 'Homolius', respectively (Table 6). There was no significant difference between number of branches in 'Homolius' under normal condition and in 'Hayola50' cultivar under drought stress condition at 5 % probability level (Table 6).

The results of means comparison analysis for interaction effect of sowing date × genotype using Duncan's multiple range tests at 5 % probability level revealed that the highest and the lowest means of seed yield were achieved from September 22 × 'Homolious' and September 22 × 'Hayola50' (Table 7). Except for September 22 × 'Hayola50', there were no significant differences among cultivars for this trait in different sowing dates at 5 % probability level (Table 7).

The results of means comparison on analysis showed that the highest and the lowest means of number of branches were achieved from October 22 × 'DK7170CL' and September 22 × 'Hayola50', respectively (Table 7). Although it is reported that delayed sowing led to the lowest effective branching in winter oilseed rape (Momoh and Zhou, 2001), however in the present study the number of branches in delayed sowing date can be related to the effect of drought stress in field capacity condition, whereas in transplanting method the plantlets are not faced with drought stress.

For first branch height trait, the highest and the lowest means were corresponded to October 6 × 'Homolious' and September 22 × 'Hayola50', respectively (Table 7).

For leaf twisting trait, the highest and the lowest means were achieved from October 6 × 'Hayola50' and September 22 × 'Hayola50', respectively (Table 7). These results indicated that the sowing date can significantly affect leaf twisting of Hayola50 cultivar of oilseed rape and delayed cultivation can significantly increase its leaf twisting properties.

As it shown in Table 1, the interaction effect of drought stress × sowing date × cultivar was only significant for leaf twisting trait, therefore in this step means comparison analysis was conducted for this trait.

Table 6: Means comparison analysis of interaction effect of drought stress and cultivar on number of secondary branch trait of oilseed rape

Drought stress × Cultivar	No. of branches
Normal irrigation × Hayola50	71.33 b
Normal irrigation × DK7170CL	72.81 b
Normal irrigation × Homolius	80.92 a
Drought stress × Hayola50	84.96 a
Drought stress × DK7170CL	71.96 b
Drought stress × Homolius	70.59 b

The means with the same letter(s) at each column had no significant difference at 5 % level

Table 7: Means comparison analysis of interaction effect of sowing date and genotype on investigated traits of oilseed rape cultivars under drought stress condition

Sowing Date × Cultivar	Seed Yield ($g\ m^{-2}$)	No. of branches	First branch height (cm)	Leaf twisting
Sept. 22 × Homolious	299.23 a	76.88 ab	24.00 ab	1.83 ab
Sept. 22 × Hayola50	181.95 b	65.67 b	11.03 d	1.33 c
Sept. 22 × DK7170CL	233.33 ab	69.55 ab	11.43 d	1.50 bc
October 6 × Homolious	279.68 a	74.22 ab	27.20 a	1.33 c
October 6 × Hayola50	264.88 ab	79.61 ab	14.37 cd	2.00 a
October 6 × DK7170CL	225.03 ab	69.33 ab	15.77 cd	1.50 bc
October 22 × Homolious	277.99 a	83.33 ab	19.23 bc	1.50 bc
October 22 × Hayola50	247.93 ab	71.89 ab	13.07 cd	1.50 bc
October 22 × DK7170CL	272.50 a	88.39 a	17.67 bcd	1.67 abc

The means with the same letter(s) at each column had no significant difference at 5 % level

Ozer (2003) also reported not significant effect of sowing date × cultivar × nitrogen on yield and yield component traits of two cultivars of spring rapeseed. The results of means comparison analysis using Duncan's multiple range test revealed that there were no significant differences among most of the investigated three ways interaction effects at 5 % probability level for leaf twisting, anyway the lowest means of this trait were achieved from drought stress × October 6 × 'DK7170CL', drought stress × October 22 × 'Homolious', and drought stress × October 22× 'Hayola50' (Table 8). It is obvious that delayed sowing date can decrease this trait in three investigated cultivars of oilseed rape under drought stress condition.

3.2 Estimation of drought tolerance indices

The comparison of estimated seed yield under drought stress conditions (Y_s), and yield under normal conditions (Y_p), for all investigated cultivars revealed that the highest yield was achieved from 'Homolious' under drought stress condition, whereas the lowest seed yield was related to 'Hayola50' under drought stress condition (Table 9). At all, Y_s of all investigated cultivars of oilseed rape was less than Y_p, except for 'Homolious' that its Y_s were higher than Y_p (Table 9). These results indicate to it that the severity of applied drought stress was not enough; therefore, in addition to the longer period of irrigation, the amounts of entered water to each plot should also be reduced. Stress intensity [$SI = 1 - (Y_s/ Y_p)$] that shows the ratio of yield under drought stress conditions to yield under normal condition was also calculated. The highest SI was related to 'Hayola50' but this index was negative in 'Homolious' (-0.090) (Table 9), which indicate to higher Y_s than Y_p in this cultivar. The stress susceptibility index (SSI) was also negative estimated for 'Homolious' of oilseed rape, whereas the highest level of this index was corresponded to 'Hayola50' (Table 9). Genotypes that have SSI less than a unit are drought resistant, because their yield reduction under drought condition is smaller than the mean yield reduction of all genotypes (Fischer and Maurer, 1978). SSI is a suitable selection index to identify resistant cultivars against susceptible genotypes (Kutlu and Kinaci, 2010).

Based on the calculated drought tolerance indices, the highest values of RDI, MP, STI, GMP, YI, YSI, and HARM were related to 'Homolious', whereas the lowest values of these indices were achieved from 'Hayola50' of oilseed rape (Table 9). The highest value of TOL index was related to Hayola50 cultivar, whereas this index was negative in Homolious cultivar of oilseed rape (-241.733) (Table 9). Shiranirad and Abbasian (2011) used different drought tolerance indices including SSI, TOL, MP, GMP, and STI to find drought tolerance genotypes among six winter rapeseed cultivars and reported GMP, STI, and MP as the most suitable recognizing indexes.

Table 8: Means comparison analysis of interaction effect of drought stress, sowing date, and cultivar on leaf twisting trait of oilseed rape cultivars

Drought Stress × Planting Date × Genotype	Leaf twisting
Normal irrigation × September 22 × Homolious	1.67 ab
Normal irrigation × September 22 × Hayola50	1.67 ab
Normal irrigation × September 22 × DK7170CL	2.00 a
Normal irrigation × October 6 × Homolious	1.67 ab
Normal irrigation × October 6 × Hayola50	2.00 a
Normal irrigation × October 6 × DK7170CL	2.00 a
Normal irrigation × October 22 × Homolious	2.00 a
Normal irrigation × October 22× Hayola50	2.00 a
Normal irrigation × October22 × DK7170CL	1.33 bc
Drought stress × September 22 ×Homolious	2.00 a
Drought stress × September 22 × Hayola50	1.00 c
Drought stress × September 22 × DK7170CL	1.00 c
Drought stress × October 6 × Homolious	1.00 c
Drought stress × October 6 × Hayola50	2.00 a
Drought stress × October 6 × DK7170CL	1.00 c
Drought stress × October 22 × Homolious	1.00 c
Drought stress × October 22 × Hayola50	1.00 c
Drought stress × October 22 × DK7170CL	2.00 a

The means with the same letter(s) at each column had no significant difference at 5 % level

According to RDI, genotypes that show the highest value of this index can be select as drought resistant genotypes (Fernandez, 1992). Higher values of TOL indicate more sensitivity to stress. MP is the mean production under both stress and non-stress conditions (Rosielle and Hamblin,1981), so this index is based on arithmetic means and therefore it has an upward bias due to a relatively larger difference between Y_s and Y_p, but GMP is less sensitive to large extreme values (Fernandez, 1992). Anyway, based on MP and GMP, 'Homolious' of oilseed rape had more uniform performance in both stress and non-stress conditions than other investigated genotypes in the present study. STI is able to identify cultivars producing high yield under both stress and non-stress conditions (Kutlu and Kinaci, 2010), therefore this index can help to selection of drought resistance genotypes with acceptable level of seed yield in both irrigated and non-irrigated environments. YI index refers to the rate of seed yield in stress and mean stress, therefore this index ranks investigated genotypes only based on their yield under stress, but YSI is the rate of stress and non-stress a genotype, therefore genotypes that show higher YSI are expected to have high yield under both irrigated and drought stress conditions.

The ranking of investigated cultivars of oilseed rape based on their calculated drought tolerance indices is presented in Table 10. The highest values of RDI, MP, STI, GMP, YI, YSI, and HARM indexes were related to Homolious cultivar, whereas the highest values of SI, SSI, and TOL indexes were related to Hayola50 cultivar of oilseed rape. Based on all calculated drought tolerance indices, 'DK7170CL' had interstitial situation of Homolious and Hayola50 cultivars of oilseed rape (Table 10). SSI and SI can help to select drought tolerance genotypes in severe drought stress environments, whereas MP, GMP, and STI can help to distinguish drought tolerance genotypes in less severe drought stress environments. Using of MP, GMP, HARM, YI, and YSI can help to select genotypes with uniform performance in both stress and non-stress environments. Khaliliet al (2012) used eleven different drought tolerance indices and finally reported 'Hyola 308', 'Heros' and 'SW5001' as the most droughts tolerant cultivars of rapeseed by ranking the drought tolerance indices.

4 CONCLUSIONS

Significant differences were observed among investigated cultivars of oilseed rape for studied phenological, morphological, and yield properties. The main effect of drought stress was only significant on seed yield, biological yield, and leaf twisting traits. These results indicate that the applied drought stress was not severe enough. Sowing date had significant effect on days to flowering and days to maturity. There were significant differences among investigated cultivars of oilseed rape for most of the studied traits. The interaction effect of drought stress and sowing date had significant effect for stem diameter, first branch height, chlorophyll content and days to maturity. The interaction effect of drought stress and cultivar was only significant for number of branches. Number of branches, first branch height, and leaf twisting were significantly affected by the interaction effect of sowing date × cultivar. The three ways interaction effect of drought stress, sowing data, and cultivar was only significant for leaf twisting. Drought stress led to the significant decrease of seed yield and biological yield in investigated cultivars of oilseed rape. Later sowing dates led to significant reduction in days to maturity and days to flowering of three investigated cultivars of oilseed rape. The highest 1000-seed mass was achieved in normal irrigation and earlier sowing data. However drought stress and later sowing date led to significant reduction in 1000-seed mass of oilseed rape cultivars. The highest seed yield was related to earlier cultivation of ‘Homolious’ of oilseed rape. The seed yield of genotype in earlier sowing date was lower than in later sowing dates. Based on the drought stress indices, Homolious cultivar of oilseed rape was recognized as the most drought tolerance genotype that can keep its performance in severe drought stress environments, whereas ‘Hayola50’ was found as the most drought susceptible cultivar. However, the bigger Ys than Yp for ‘Homolious’ r can be related to mild applied drought stress, so that more severe drought stress can lead to rational results for this cultivar.Based on calculated MP, GMP, HARM, YI, and YSI indices, ‘Homolious’ lead to uniform performance in both stress and non-stress environments. The results of the present study can be used in future studies for seed yield improvement of oilseed rape in drought stress condition. Regarding to significant effect of sowing data × cultivar for seed yield trait, the obtained results can help to find the best sowing date for different cultivars of winter oilseed rape.

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Table 9: Drought tolerance indices and seed yield under normal and drought stress conditions measured in the three oilseed rape cultivars

Genotype	Ys	Yp	SI	SSI	RDI	TOL	MP	STI	GMP	YI	YSI	HARM
Homolious	292.404	268.231	-0.090	-0.486	1.338	-241.733	2803.178	1.090	2800.571	1.396	1.137	2797.966
Hayola50	106.850	254.844	0.581	3.134	0.515	1479.944	1808.472	0.419	1650.155	0.510	0.416	1505.698
DK7170CL	229.137	248.253	0.077	0.416	1.133	191.156	2386.956	0.923	2385.041	1.094	0.891	2383.128
Average	2094.641	2571.096	0.189	1.021	0.995	476.456	2332.869	0.811	2278.589	1.000	0.815	2228.931

Ys: Yield under drought stress conditions (g m^{-2}); Yp: Yield under normal conditions (g m^{-2}); SI: Stress Intensity; SSI: Stress Susceptibility Index; RDI: Relative drought index; TOL: Tolerance; MP: Mean Productivity; STI: Stress Tolerance Index; GMP: Geometric Mean Productivity; YI: Yield Index; YSI: Yield Stability Index; HARM: Harmonic Mean Productivity.

Table 10: Ranking of three oilseed rape genotypes in respect to different drought tolerance indices

Genotype	SI	SSI	RDI	TOL	MP	STI	GMP	YI	YSI	HARM	Mean
Homolious	3	3	1	3	1	1	1	1	1	1	1.6
Hayola50	1	1	3	1	3	3	3	3	3	3	2.4
DK7170CL	2	2	2	2	2	2	2	2	2	2	2.0

SI: Stress Intensity; SSI: Stress Susceptibility Index; RDI: Relative drought index; TOL: Tolerance; MP: Mean Productivity; STI: Stress Tolerance Index; GMP: Geometric Mean Productivity; YI: Yield Index; YSI: Yield Stability Index; HARM: Harmonic Mean Productivity.

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