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Morpho-physiological response of rapeseed (*Brassica napus* L.) genotypes to drought stress

B. Pasban Eslam[1], H. Monirifar[2] and A. R. Sadeghi Bakhtavari[3]

[1 and 2] East Azarbaijan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Tabriz, Iran.
[3]Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran.
[*]Corresponding author Email address: b_ pasbaneslam@yahoo.com

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ABSTRACT

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This study was conducted to evaluate the physiological indices in relation to screening of rapeseed genotypes for drought tolerance, and to study seed yield and seed yield components under early and late season drought stress. Six rapeseed genotypes were evaluated under non-stressed and water deficit conditions during rosette and seed filling stages. The experiments were conducted in East Azarbaijan Agricultural and Natural Resources Research and Education Center, Iran for 2 years (2010-2011 and 2012-2013). According to the results, canopy temperature (T_c) was increased, stomatal conductance (K_l) and relative water content (RWC) were decreased significantly by drought stress during the rosette and seed filling stages. Correlations among the aforementioned physiological traits at seed filling stage with each other and with seed yield were significant. It seems that these physiological traits can reflect the drought effects in rapeseed genotypes. Among the genotypes, significant differences were observed for K_l at seed filling and RWC at rosette stages. While, Okapi and Licord having the highest seed yield, presented the highest K_l during seed filling and RWC at rosette stages. Therefore, T_c, K_l and RWC can be used to screen rapeseed genotypes for tolerance to drought stress. Drought stress during the seed filling stage significantly decreased plant height, the number of siliques per plant, 1,000-seeds weight and seed yield. Results of path analysis indicated that plant height and yield components indicated positive direct effect on seed yield. Plant height had the highest direct effects on seed yield. Among the genotypes, Okapi and Licord were more suitable for cultivation in normal and drought conditions.

Keywords: Physiological index, Seed yield, Water deficit, Yield components.

INTRODUCTION

Among the different environmental stresses, drought is the major constraint reducing crop yields. Crop plants when subjected to this constraint manifest a wide range of behaviors varying from great sensitivity to high tolerance. Rapeseed is no exception to the rule (El Hafid *et al.*, 1998). Grewal (2010) indicated that rapeseed might be a better option for sustaining crop production and higher water use efficiency on sodic soils with high subsoil NaCl salinity. The most critical time for water supply in rapeseed is reported to be during flowering and seed filling stages (Richards and Thurling, 1978). Since yield and drought tolerance are controlled by separate gene loci (Morgan, 1984), it may be possible to identify and transfer the physiological traits responsible for drought resistance to high-yielding and agronomically acceptable cultivars (Kumar and Singh, 1998). Inter specific and intra specific variations were found in *Brassica napus* L. in response to drought (Rao and Mendham, 1991). As water deficit develops, stomata close progressively, transpiration decreases and canopy temperature rises. Kumar *et al.* (1984), Singh *et al.* (1990), and Kumar and Singh (1998) believe there is a close association between osmotic adjustment and both stomatal conductance and canopy temperature in oilseed Brassica species. Singh *et al.* (1990) stated that transpirational cooling (canopy temperature minus air temperature) could effectively be used as a technique to screen Brassica genotypes for drought tolerance under receding soil moisture conditions. Pasban Eslam *et al.* (2000) reported that late-season drought reduces rapeseed temperature stability (differences between daily minimum and maximum air to canopy temperatures). They also indicated positive correlations of canopy temperature stability with stomatal conductance, water potential, relative water content and seed yield. Kumar and Singh (1998) showed significant correlations of seed yield with osmotic adjustment, transpirational cooling and stomatal conductance in oilseed Brassica species. Lehman *et al.* (1993) by studying bentgrass clones suggested that relative water content would better predict growth maintenance under increasing water deficit than the simple measure of leaf water potential. Richards and Thurling (1978) found that late-season drought leads to abortion of more than 50 percent of the siliques in *B. napus* L. and *B. rapa* L., however, the remaining siliques produced more and heavier seeds. Jensen *et al.* (1996) reported that water deficit stress occurring during both vegetative growth and seed filling stages in rapeseed, decreased number of seeds per m2, oil content, harvest index and seed yield. Irrigation after anthesis in rapeseed increased seeds per silique and harvest index and thus produced a higher seed yield (Rao and Mendham, 1991). The main portion of seed yield variations in rapeseed can be related to the number of siliques per plant, the number of seeds per silique and 1,000-seeds weight (Chen, 1994). Peltonen-Sainio and Jauhiainen (2008) reported that environmental variation markedly affected seed yield, seed numbers per square meter and duration of flowering in rapeseed. Generally, seed weight in relation to environmental factors is less influenced than the number of siliques per unit area and the number of seeds in the silique in rapeseed (Keiller and Morgan, 1988; Jensen *et al.,* 1996). Pasban Eslam (2009) disclosed that fall season rapeseed cultivars (Okapi and SLM046) were more tolerant to late season water deficit stress. Evaluation of rapeseed genotypes in a Mediterranean-type environment revealed that seed yield was strongly correlated with harvest index, the total number of siliques per plant, 1,000-seeds weight, the final height of the plant, and the number of primary branches. However, the number of seeds in the Siliques was not significantly correlated with seed yield (Gunasekera *et al.*, 2006). Rapeseed genotypes with higher seed oil content produced higher dry matter in reproductive organs at end of the seed filling stage (Hua *et al.*, 2012). In spite of several reports about the effects of drought stress on *B. rapa* L. and *B. juncea* L. genotypes especially in temperate and warm areas, limited studies have been reported on cold tolerant rapeseed genotypes.

The objectives of this study were to evaluate the physiological traits related to the drought tolerance of cold season rapeseed genotypes; their seed yields and seed yield components under early and late season drought stress.

MATERIALS AND METHODS

The experiment was conducted at East Azarbaijan Agricultural and Natural Resources Research and Education Center, Iran (46°2′E, 37°58′N) during the 2010-2011 and 2012-2013 growing seasons. The prevailing weather characteristics during the growing seasons are summarized in Table 1. The experiment was conducted on the loamy soil as factorial, based on randomized complete block design with three replications. Six highly yielding and cold tolerant rapeseed genotypes including Karaj 1, Karaj 3, Opera, Okapi, Licord, and Modena were evaluated under non-stressed and water deficit conditions at the rosette and seed filling stages. Plants were irrigated at 30-35 and 70-75 percent available soil water depletion in non-stressed and stressed plots, respectively (Table 2). To avoid the effect of probable precipitation on stressed plots, polyethylene rain shelters were used during periods of rain. The plot size was 5 m×1.8 m. Seeds were sown at the furrows in a 30+60 cm system (one pair of rows in each furrow with 30 cm spacing, and 60 cm spacing between two paired rows) on September 12 during two years of experiments. Plants were thinned to a spacing of 7 cm within rows four weeks after sowing. Crop management practices were performed as needed during the growing season.

The youngest fully expanded leaves were used for their various measurements and the characters were measured at the rosette and seed filling stages for stressed and non-stressed plots. Stomatal conductance (K_l) was determined with an AP_4 prometer (Delta-T Devices, UK). Relative water content (RWC) was obtained by floating the leaf discs (3 discs from each leaf with 20 mm diameter) on distilled water for 4 h at 5°C under dim light. The turgid weight (TW) was then determined after floating and the dry weight (DW) after the samples were dried for 24 h at 75°C. Fresh weight (FW), TW and DW were used to calculate RWC as RWC= FW-DW/TW-DW (Jensen *et al.*, 1996; Lazcano-Ferrat and Lovatt, 1999). A portable eld infrared thermometer (Class 2, Testo, Germany) was used to measure canopy temperature (T_c). For this characteristic, five measurements were taken on each plot and averaged for statistical analysis (Ray *et al.*, 1998). Measurements of K_l, RWC and T_c were made at 1200 to 1400 h when Brassica species may tend to show the greatest genetic variability in response to drought stress (Singh et al., 1990).

Plants were harvested on June 20 and 24, in the first and second years, respectively. To control border effects, prior to harvest, plants were removed from the sides of each plot. Finally, plant height, seed yield, number of siliques per plant, number of seeds in siliques and 1,000-seed weight were measured. Ten plants from each plot were used to determine plant height and components of seed yield. Also, seed oil content was determined by a nuclear magnetic resonance (NMR) instrument. Statistical evaluations of the data were performed using MSTAT-C and SPSS service pack.16 software packages. Path analysis was used to measure both the direct and indirect effects the yield components may have had on the seed yield of rapeseed genotypes.

Table 1. Weather records for two growing seasons during experimental periods.

Year	Month	Mean minimum air temperature (°C)	Mean maximum air temperature (°C)	Mean of total temperature (°C)	Rainfall (mm)
2010	September	16.3	31.1	23.7	0.0
	October	9.8	26.4	18.1	2.4
	November	3.5	17.7	10.6	7.5
	December	0.4	13.2	6.8	0.0
2011	January	-4.6	5.5	-0.6	9.4
	February	-5.1	5.5	0.18	17.4
	March	-1.1	10.2	4.6	39.3
	April	5.2	13.8	11.8	34.2
	May	16.1	21.2	18.7	129.4
	June	21.0	28.7	13.2	5.0
2012	September	16.1	30.4	23.2	3.4
	October	10.6	25.1	17.8	6.7
	November	5.6	16.6	11.1	35.9
	December	-0.1	8.6	3.8	42.9
2013	January	-4.9	3.8	-0.5	14.8
	February	-0.8	8.0	3.6	34.3
	March	1.2	11.7	6.4	21.5
	April	5.1	18.1	11.6	33.3
	May	8.4	19.9	14.1	34.7
	June	13.2	27.9	20.6	43.0

Table 2. Characteristics of soil water content in the experimental field.

Soil depth (cm)	FC (%)		WP (%)		AWC (%)	
	2010-11	2012-13	2010-11	2012-13	2010-11	2012-13
0-30	27.3	25.0	13.7	13.7	13.6	11.3
30-60	21.3	29.0	10.6	14.3	10.7	14.7
60-90	14.8	28.7	7.4	14.0	7.4	14.7

FC= Field capacity, WP= Wilting point, AWC= Available water capacity.

RESULTS

Physiological indices

Drought stress during the rosette and seed filling stages in rapeseed genotypes increased T_c and decreased K_l and RWC significantly (Tables 3, 4 and 5). Among the genotypes, significant differences were observed for K_l and RWC at seed filling stage (Table 3). Okapi and Licord had higher K_l and RWC (Tables 6 and 7) and Modena had the lowest amount of RWC at seed filling stage in both years of research (Table 7).

Yield and related characteristics

Drought stress during the seed filling stage in rapeseed genotypes significantly decreased plant height, siliques per plant, 1,000-seed weight and seed yield (Tables 3, 4, 5 and 8). Among the genotypes, significant differences were observed for plant height, siliques per plant, seeds in the silique, 1,000-seed weight and seed yield (Table 3). Licord and Karaj 3 had higher plant height and a higher number of seeds in silique belonged to Okapi, Karaj 1, Modena and Licord respectively. Okapi and Licord produced the highest seed yield (Table 6). Opera produced higher 1,000-seed weight during 2010-2011. However, all genotypes stood in the same group in 2012-2013. The results also indicated that the number of siliques per plant in all genotypes, at normal irrigation and water deficit stress at the rosette stage, were similar, whereas it decreased an average of 23.8 and 29.4 at first and second years of research respectively (Table 8).

Correlation among physiological traits and seed yield

Negative correlations were found among T_c with K_l and RWC, also K_l was correlated positively with RWC at the rosette stage (Table 9). The correlation coefficients of T_c at seed filling stage with K_l, RWC and seed yield were negative. K_l and RWC at seed filling stage were positively correlated with each other and seed yield.

Path analysis

The direct and indirect effects of four traits on seed yield estimated by path coefficients are shown in Table10. All traits in the model indicated positive direct effect on seed yield. Plant height and 1,000-seed weight had the highest and lowest direct effects on seed yield, respectively. Although the plant

Table 3. Analysis of variance for measured traits on rapeseed genotypes.

Source	df	Mean squares					
		T_c (rosette)	T_c (seed filling)	K_l (rosette)	K_l (seed filling)	RWC (rosette)	RWC (seed filling)
Year (Y)	1	13.021**	939.28	1.025**	0.001	0.018**	0.002
Replication(R)/Y	4	1.653	3.78	0.001	0.001	0.001	0.001
Stress (S)	2	182.480**	184.94**	0.415**	1.066**	0.157**	0.185**
Y ×S	2	10.577**	0.78	0.120**	0.001	0.001	0.006**
Genotype(G)	5	0.626	1.01	0.001	0.017**	0.002	0.011**
Y × G	5	1.586	1.40	0.001	0.001	0.002	0.003*
S × G	10	1.005	1.20	0.001	0.001	0.001	0.001
Y ×S × G	10	0.962	1.13	0.001	0.001	0.001	0.001
Error	68	0.844	0.62	0.001	0.001	0.001	0.001
C.V (%)		5.67	3.19	4.23	3.07	3.96	2.96

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

Table 3 continued.

Source	df	Mean squares					
		Plant height	Siliques per plant	Seeds in silique	1000-seeds weight	Seed yield	Oil content
Year (Y)	1	3852.08**	2955.78**	23.14*	0.025	19845.33	610.13**
Replication(R)/Y	4	421.296	624.56	1.71	0.003	3685470.07	3.57
Stress (S)	2	1404.39**	7876.25**	7.44	1.547**	7218306.73**	2.65
Y ×S	2	63.19	371.59*	0.25	0.170*	22259.19	6.16
Genotype (G)	5	165.23**	443.32*	10.34*	0.433**	2948919.90**	5.16
Y × G	5	83.75	199.18	2.32	0.454**	34475.22	2.26
S × G	10	26.62	104.54	5.43	0.009	132986.04	2.31
Y ×S × G	10	59.86	256.7**	1.27	0.013	51485.88	3.39
Error	68	47.52	98.80	3.49	0.036	182306.64	2.17
C.V (%)		6.01	8.58	6.36	5.76	10.53	3.59

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

Table 4. Means of some measured traits on rapeseed genotypes at different stress levels.

Stress levels	T_c (seed filling) (°C)	K_l (seed filling) (cm s^{-1})	RWC (rosette)	Plant height (cm)	Seed yield (kg ha^{-1})
Non-stressed	23.6 b	0.571 a	0.77 a	118.9 a	4348 a
Stress during rosette stage	23.5 b	0.584 a	0.66 b	117.6 a	4274 a
Stress during seed filling stage	27.5 a	0.280 b	0.78 a	107.5 b	3538 b
LSD (P=0.01)	0.611	0.019	0.019	4.306	266.7

Means for each variable followed by the same letter are not significantly different.

Table 5. Means of some measured traits on rapeseed genotypes at different stress levels over two years.

Year	Stress levels	T_c (rosette) (°C)	K_l (rosette) (cm s^{-1})	RWC (seed filling)	1,000-seed weight (g)
2010-11	Non-stressed	14.8 c	0.566 c	0.77 a	3.32 b
	Stress during rosette stage	19.7 a	0.501 d	0.77 a	3.42 ab
	Stress during seed filling stage	15.0 c	0.562 c	0.67 b	3.13 c
2012-13	Non-stressed	14.8 c	0.687 a	0.78 a	3.43 a
	Stress during rosette stage	17.8 b	0.606 b	0.77 a	3.48 a
	Stress during seed filling stage	14.9 c	0.697 a	0.63 c	3.01 d
LSD (P=0.01)		0.611	0.027	0.027	0.089

Means for each variable followed by the same letter are not significantly different.

Table 6. Means of some measured traits on rapeseed genotypes.

Genotypes	K_l (seed filling) (cm s^{-1})	Plant height (cm)	Seeds per silique	Seed yield (kg ha^{-1})
Karaj 1	0.477 bc	112.5 ab	29.9 ab	3854 b
Karaj 3	0.485 bc	117.5 a	28.9 bc	3989 b
Opera	0.457 cd	110.8 b	28.2 c	3769 b
Okapi	0.517 a	113.9 ab	30.4 a	4651 a
Licord	0.500 ab	118.9 a	29.4 a-c	4439 a
Modena	0.431 d	114.4 ab	29.5 a-c	3620 b
LSD	0.027	6.09	1.244	377.2

Means for each variable followed by the same letter are not significantly different.
LSD at 5% level for RWC and seeds in pod; at 1% for other traits.

Table 7. Means of RWC and 1,000-seed weight in rapeseed genotypes over two years.

Years	Genotypes	RWC (seed filling)	1000-seeds weight (g)
2010-11	Karaj 1	0.75 a-c	3.13 bc
	Karaj 3	0.74 a-c	3.35 b
	Opera	0.71 c-e	3.90 a
	Okapi	0.77 a	3.14 bc
	Licord	0.76 ab	3.13 bc
	Modena	0.68 e	3.07 c
2012-13	Karaj 1	0.76 ab	3.32 bc
	Karaj 3	0.74 a-d	3.29 bc
	Opera	0.73 a-d	3.32 bc
	Okapi	0.73 a-d	3.33 bc
	Licord	0.73 a-d	3.32 bc
	Modena	0.69 e	3.33 bc
LSD		0.039	0.237

Means for each variable followed by the same letter are not significantly different.
LSD at 5% level for RWC; at 1% for 1,000-seed weight.

height had a positive effect on seed yield, its indirect effect through seeds in the pod was negative. Seeds in the pod had a negative indirect effect on seed yield through plant height, too.

DISCUSSION

Since Tc was increased and Kl and RWC were decreased significantly with water deficit stress during the rosette and seed filling stages in both research years (Tables 3, 4 and 5), it seems that these physiological traits can reflect the effects of drought in rapeseed genotypes. Significant correlations were observed among the above-mentioned physiological traits measured at seed filing stage with each other and seed yield (Table 9). Significant differences among the genotypes were observed for K_l and RWC at the seed filling stage (Table 3). Okapi and Licord, with the highest seed yield, presented higher K_l and RWC at seed filling stage (Tables 4, 6 and 7). Therefore, T_c (at the seed filling stage), K_l, and RWC can be used to screen rapeseed genotypes for tolerance against water deficit stress, especially during seed filling stage. It is reported that the most critical time to supply water to rapeseed is during the flowering and seed filling stages (Richards and Thurling, 1978). Kumar and

Table 8. Means of traits measured on rapeseed genotypes at different levels of water deficit stress over two years.

Year	Water deficit stress	Genotype	Siliques per plant
2010-11	Non-stressed	Karaj 1	136.3 a
		Karaj 3	104.0 d-i
		Opera	122.3 a-e
		Okapi	127.3 a-d
		Licord	119.3 a-g
		Modena	120.0 a-f
	Stress at rosette stage	Karaj 1	112.0 a-h
		Karaj 3	113.0 a-h
		Opera	113.0 a-h
		Okapi	115.7 a-h
		Licord	128.3 a-d
		Modena	129.0 a-d
	Stress at seed filling stage	Karaj 1	90.7 hi
		Karaj 3	94.0 g-i
		Opera	99.7 e-i
		Okapi	107.7 b-i
		Licord	86.0 i
		Modena	99.3 e-i
2012-13	Non-stressed	Karaj 1	127.7 a-d
		Karaj 3	127.3 a-d
		Opera	131.3 ab
		Okapi	130.7 a-c
		Licord	130.7 a-c
		Modena	133.3 ab
	Stress at rosette stage	Karaj 1	137.3 a
		Karaj 3	128.7 a-d
		Opera	130.3 a-c
		Okapi	134.7 a
		Licord	130.0 a-c
		Modena	128.0 a-d
	Stress at seed filling stage	Karaj 1	105.0 d-i
		Karaj 3	96.3 f-i
		Opera	101.7 e-i
		Okapi	98.0 e-i
		Licord	103.7 d-i
		Modena	103.7 d-i
LSD (P=0.05)			21.51

Means for each variable followed by the same letter are not significantly different.

Table 9. Simple correlation coefficients among traits measured on rapeseed genotypes.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Trait	T_c (rosette)	T_c (seed filling)	K_l (rosette)	K_l (seed filling)	RWC (rosette)	RWC (seed filling)	Seed yield
(2)	-0.47						
(3)	-0.97**	0.25					
(4)	0.47	-0.96**	-0.35				
(5)	-0.97**	0.25	0.95**	-0.35			
(6)	0.40	-0.91**	-0.44	0.96**	-0.42		
(7)	0.29	-0.75**	-0.29	0.79**	-0.24	0.79**	

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

Table 10. Path analysis showing direct and indirect effects on seed yield in rapeseed genotypes.

	Indirect effects			
Traits	Plant Height	Seeds in pod	Seeds in pod	1,000- seed weight
Plant Height	(0.473)	0.0694	-0.0340	0.0013
Pod in plant	0.0983	(0.3340)	0.1243	0.0035
Seeds in pod	-0.0624	0.1609	(0.2580)	0.0011
1000 seeds weight	0.1097	0.1963	0.0503	(0.0060)

Values in parenthesis are direct effects.

Singh (1998) showed significant correlations among seed yield with osmotic adjustment, transpirational cooling and stomatal conductance in oilseed Brassica species. The Strong negative relationship between seed yield and air temperature during the reproductive stage of *Brassica napus* L. genotypes has also been reported (Faraji *et al.*, 2009). Golestani Araghi and Assad (1998) reported that leaf temperature and stomatal resistance are the proper drought resistance indicators in wheat.

The onset of water deficit stress during the seed filling stage in rapeseed genotypes decreased plant height, siliques per plant, 1,000-seed weight and seed yield significantly (Tables 3, 4, 5 and 8). It seems that drought occurring at this stage decrease seed yield, mainly by thoroughly reducing the plant height and seed yield components. A limited supply of carbohydrates to the siliques leads to silique abortion and a reduction in 1,000-seed weight. Also, plant height in rapeseed as the indeterminate crop can be affected by limited assimilates production during the drought period. A shortage supply of carbohydrates to siliques may also lead to silique abortion in rapeseed during late flowering and silique filling stages (Habekotte, 1993). The results of a study on rapeseed genotypes indicated that the time of occurring drought stress and its duration have a significant effect on seed yield and its components (Sheikh *et al.*, 2006). Drought occurring at the flowering stage significantly increases the rate of silique abortion, thus decreasing the final seed yield in soybean (Liu *et al.*, 2003; Liu *et al.*, 2004). Among seed yield with siliques per plant and 1,000-seed weight, positive correlations were seen. These two components of rapeseed yield showed the highest contribution to seed yield formation than other components. The main portion of seed yield variations in rapeseed can be attributed to the number of siliques per plant, the number of seeds in silique and 1000-seeds weight (Chen, 1994). Faraji (2013) reported the positive and significant effect of siliques per plant and number of seeds in the silique on seed yield in rapeseed genotypes. The result of path analysis indicated that plant height and yield components indicated positive direct effect on seed yield. Plant height and 1000-seeds weight had the highest and lowest direct effects on seed yield, respectively (Table 10). It seems that taller rapeseed plants with more pods per plant manifest more seed yield. A significant and positive correlation between seed yield and relative growth rate in spring genotypes of *B. napus* L. has been previously reported (Arvin *et al.*, 2010).

Licord and Karaj 3 had higher plant height, and the higher number of seeds per siliques were found in Okapi, Karaj 1, Modena and Licord. Okapi and Licord had the highest seed yield (Table 6). Karaj 3 and Opera produced a higher 1,000-seed weight than others during 2010 and 2011, than all of the genotypes located in the same group in 2012-2013.

It may be concluded that all of the genotypes under study presented acceptable performance in seed yield, but Okapi and Licord were more promising than others to grow under both non-drought and drought conditions. Canopy temperature (T_c) at seed filling stage, stomatal conductance (K_l) and relative water content (RWC) can reflect the drought stress effects in rapeseed genotypes and they may be used to screen rapeseed genotypes for tolerance against water deficit stress especially during the seed filling stage.

CONCLUSIONS

The results indicated that canopy temperature was increased while, stomatal conductance and relative water content (RWC) were decreased by drought stress during the rosette and seed filling stages. Also correlation among these physiological traits at seed filling stage and with seed yield was significant. The genotypes with having higher amounts of stomatal conductance and RWC and lower canopy temperature in case of occuring stress at seed filling stage indicated higher seed yield. It is concluded that canopy temperature at seed filling stage, stomatal conductance, and RWC can be used to selecting rapeseed drought tolerant genotypes.

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