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Screening of Drought Tolerant Genotypes in Rapeseed (*Brassica napus* L.) Using Agro-Physiological Traits

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ABSTRACT

This study was conducted to introduce physiological and agronomic traits and factor analysis method for screening drought tolerant rapeseed genotypes. Eighteen rapeseed genotypes were evaluated under non-stressed and water deficit stress conditions from flowering and seed filling stages to seed maturity for two years (2012-2014) at the Agricultural and Natural Resources Research and Education Center of East Azarbaijan, Iran. Occurring drought from flowering stage, significantly decreased pod length, plant height, pod numbers per plant, seeds in a pod, seed yield and stomatal conductance. Also drought stress from seed filling stage, significantly decreased 1000-seeds weight, seed oil, seed yield and increased canopy temperature. Occurring drought stress from flowering and seed filling stages, decreased the mean seed yield by 1426 and 696 Kg h^{-1}, respectively. Rapeseed genotypes were more sensitive to water deficit stress from flowering stage than seed filling stage. Thus, under water limiting conditions, irrigation at flowering stage, could be quite effective to support seed and oil yields. According to the results of factor analysis, genotypes 'HW101' and 'L183' with having higher yields, were recognized as the promising ones for non-stressed and water deficit stress conditions. Correlations among canopy temperature, stomatal conductance, pod length, pod numbers per plant, seed and oil yields, were significant. Therefore, these agronomic and physiologic traits and factor analysis method can be suggested to screen water deficit tolerant rapeseed genotypes.

Keywords: *canopy temperature, factor analysis, pod numbers per plant, stomatal conductance, water deficit.*

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INTRODUCTION

Drought among the different environmental stresses, is a major constraint in reducing crop yields (El Hafid et al., 1998). In water deficit condition, farmers are increasingly utilizing growth stage timed irrigation management as regulated deficit irrigation, where the crop is stressed at special growth stages in order to reduce crop transpiration (Dejonge et al., 2015). Grewal (2010) indicated that rapeseed may be a better option to grow for higher water use efficiency in sodic vertisols with higher subsoil NaCl salinity. Canola plants indicate the highest water use efficiency (WUE) under moderate levels of irrigation and more or less water supplying leads to decreasing WUE (Hamzei, 2011). Latifi et al. (2016) by studying Brassica oleracea L. var 'botrytis' under water deficit indicated that drought reduced leaf chlorophyll concentration and relative water content (RWC) significantly. Namvar and Khandan (2014) showed that using 150 Kg N ha-1 with 50 Kg S ha-1 in rapeseed can increase RWC and cell membrane stability. These physiological traits will increase the ability of rapeseed for drought tolerance. Occurring drought stress (depletion of 50% FC) during growth period, decreased seed yield significantly via diminishing RWC, leaf area, shoot height, root length and plant water soluble sugar in rapeseed genotypes (Heidari et al., 2015).

Infrared thermometry is a suitable method to determine drought stress in that it is non-destructive, scalable from single plants to whole fields, and is easier than many alternative methods (Dejonge et al., 2015). Takele (2001) indicated that canopy temperature is suitable trait for the selecting of the water deficit tolerant cultivars of teff. Dejonge et al. (2015) reported a linear relationship between canopy temperature and crop water stress index (CWSI) with the soil water deficit at high temperature in maize and they concluded that canopy temperature and CWSI can be used to monitor drought stress in maize crops as a nondestructive, cheap and simple method.

Water deficit stress during pod filling stage at rapeseed reduced the number of seeds per m2, oil percent and seed yield (Jensen et al., 1996). Peltonen-Sainio and Jauhiainen (2008) reported that environmental variations markedly affected seed yield, seed numbers per square meter and flowering duration in rapeseed. Generally, seed weight in relation to environmental factors is less influenced than the number of pods per unit area and number of seeds in a pod in rapeseed (Keiller and Morgan 1988; Jensen et al., 1996). Evaluation of rapeseed genotypes in Mediterranean climate, revealed that seed yield was strongly correlated with harvest index, the total number of pods per plant, 1000-seeds weight, plant height and number of primary branches. However, the number of seeds in a pod was not significantly correlated with seed yield (Gunasekera et al., 2006). Rapeseed genotypes with higher seed oil content produced higher dry matter at the end of the seed filling stage (Hua et al., 2012).

The objectives of this study were to evaluate some of the physiological and agronomic traits and the efficiency of the factor analysis method in relation to the drought tolerance of rapeseed genotypes imposed at water deficit stress.

Materials and Methods

The study was carried out at the Agricultural and Natural Resources Research and Education Center of East Azarbaijan, Iran (46□2□E, 37□58□N) during growing seasons of 2012-2014. It was conducted on loamy soil as split plot based on a randomized complete block design with three replications. Eighteen cold tolerant rapeseed genotypes were evaluated under non-stressed and water deficit conditions from flowering and seed filling stages to seed maturity. Plants were irrigated at 30-35 and 70-75 percent available soil water depletion in non-stressed and stressed plots, respectively (Table 1). The plot size was 5□1.8 meters. Rows space was 30 cm and plants were fixed at 7 cm spacing from each other in a row. Seeding time was September 14 during two experiment years.

Table 1. Characteristic of the soil water content in the experimental field

Soil depth (cm)	FC (%)		PWP (%)		AWC (%)	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
0-30	26.7	25.2	12.6	12.8	14.1	12.4
30-60	20.8	20.4	10.0	11.4	10.8	9.0
60-90	13.7	13.1	7.5	8.0	6.2	5.1

FC= Field capacity, PWP= Permanent wilting point, AWC= Available water capacity.

Stomatal conductance (Kl) was determined by an AP4 prometer (Delta-T Devices, UK). An infrared thermometer (Class 2, Testo, Germany) was used to measure canopy temperature. Measurements were made at 1200 to 1400 h (Dejonge et al., 2015 and Takele, 2001).

Plants were harvested in June 18 and 21, of the first and second years of research, respectively. To control boarder effects, plants were removed from the sides of each plot, prior to harvest. Finally, pod length, plant height, seed yield, number of pods per plant, number of seeds in pod and 1000-seeds weight traits were measured. Ten plants from each plot were used to determine plant height and components of seed yield. The seed oil content was also determined by NMR (nuclear magnetic resonance) method. Statistical evaluations of the data were performed using the SPSS service pack. 16 software package.

Results

Yield, related characteristics and Physiological indices

Significant differences were observed in all of the traits except for pod length and seed oil between the two years of the experiment (Table 2). It can be due to the freezing temperatures that occurred during the fall and winter in second year that resulted in lower plant density per plot. The effects of drought stress on all of the traits under study were significant (Table 2). Among the winter rapeseed genotypes, significant differences were observed for pod length, seeds in a pod, oil yield and stomatal conductance (Table 2). Interaction effects between drought stresses by genotypes were also significant on pod length and stomatal conductance. Also triple interaction effects of stress, genotype and year were significant on seed and oil yields (Table 2). Finally, the values of study traits on genotypes at three water deficit stress levels are shown in table 3. Drought during flowering stage significantly decreased pod length, plant height, pod numbers per plant, seeds in a pod, seed yield and stomatal conductance. Also drought during seed filling stage, significantly decreased 1000-seeds weight and seed oil and increased canopy temperature. Water deficit during flowering and seed filling stages, decreased mean seed yield 1426 and 696 Kg h-1, respectively (Table 3). Among the studied rapeseed genotypes, ‘L73’, ‘L72’, ‘L183’and ‘HW101’with having higher yield components, stomatal conductance and lower air temperature, did have higher seed and oil yields for all of the water deficit and non-stressed conditions (Table 3).

Table 2. Analysis of variances of traits measured on rapeseed genotypes

Source	df	Mean squares: Pod length	Plant height	Pods per plant	Seeds in a pod	1000-seeds weight
Year (Y)	1	0.19	40334.0**	29967.4**	937.0**	30.61**
Replication/Y	4	0.96	261.9	2296.7	19.1	00.68
Stress (S)	2	21.35**	3709.8**	20670.8**	12.1*	0.95**
S ×Y	2	0.26	096.5**	2184.8**	18.2**	1.09**
Genotype(G)	17	0.56**	116.4	316.2	6.2**	0.11
G × Y	17	0.05	131.7	350.3	2.0**	0.06
S × G	34	0.38*	129.2	244.6	3.8	0.14
S ×G × Y	34	0.03	112.4	254.4	3.4	0.09
Error	212	0.23	120.2	220.9	2.9	0.09
C.V (%)		8.96	9.87	16.99	6.02	8.51

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

Table 2 Continue

Source	df	Mean squares: Seed yield	Seed oil	Oil yield	Canopy temperature	Stomatal conductance
Year (Y)	1	188522053**	1.81ns	29094748**	693.4**	0.008**
Replication/Y	4	2162751	122.37	676034	36.3	0.001
Stress (S)	2	54937849**	35.85**	9396529**	784.4**	4.404**
S ×Y	2	6063339**	15.04**	1088666**	834.8**	0.003*
Genotype(G)	17	716338	1.35	128941*	2.0	0.006**
G × Y	17	393124	1.50	70542	1.5	0.000
S × G	34	601083	1.68	99399	2.2	0.005**
S ×G × Y	34	733151*	2.11	119171*	2.9	0.000
Error	212	438069	3.13	73690	2.9	0.001
C.V (%)		22.32	4.46	22.87	7.29	5.26

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

Table 3. Means of traits measured on rapeseed genotypes at different stress levels

Stress levels	Genotype	Pod length (cm)	Plant height (cm)	Pods per plant	Seeds in a pod	1000-seeds weight (g)	Seed yield (Kg h-1)	Seed oil (%)	Oil yield (Kg h-1)	Canopy temperature (°C)	Stomatal conductance (cm s-1)
Non-stressed	HW113	5.61	115.83	107.83	27.83	3.65	3382.0	41.56	1403.4	20.66	.6900
	RS12	5.55	112.50	90.50	28.83	3.53	3343.2	41.51	1379.7	20.83	.6750
	Karaj1	5.58	115.00	101.83	28.00	3.68	3554.8	39.60	1410.5	21.58	.6483
	KR18	5.88	113.33	110.17	27.00	3.60	3960.5	40.28	1583.0	22.08	.7117
	L73	5.81	116.66	101.50	29.66	3.65	4101.8	40.73	1672.4	19.33	.7100
	L72	5.93	118.33	107.00	28.33	3.35	3881.3	41.08	1598.2	19.66	.6783
	HW101	5.91	118.33	109.17	28.16	3.26	4004.2	40.91	1628.0	21.00	.7167
	L146	4.95	121.66	92.83	28.83	3.88	2805.2	40.00	1121.5	21.33	.6700
	L210	5.40	115.00	89.66	29.00	3.68	3551.5	40.30	1435.6	21.50	.5983
	L183	5.28	120.00	102.50	28.83	3.66	4243.8	40.45	1712.3	20.83	.7133
	SW101	5.70	119.16	102.17	30.16	3.60	3714.0	40.31	1489.5	19.66	.6767
	L5	5.78	119.16	102.83	27.83	3.58	3964.7	40.86	1612.3	21.08	.6800
	L201	5.48	115.00	103.83	29.50	3.48	3279.2	40.73	1330.4	21.33	.6650
	HW118	5.05	118.33	90.66	28.83	3.93	3604.3	40.53	1471.5	20.16	.6600
	KR4	5.33	120.83	96.66	28.83	3.46	3866.7	40.95	1584.0	20.75	.6950
	Karaj2	5.36	115.00	112.00	29.16	3.71	4028.8	40.33	1619.6	20.83	.6767
	Karaj3	5.45	113.33	100.17	27.16	3.91	3394.0	38.88	1338.7	21.33	.6683
	KS7	5.23	115.83	97.00	29.50	4.08	3421.0	40.93	1395.1	21.50	.6717
LSD 5%		0.4453	10.1815	13.8025	1.5814	0.2786	614.64	1.6429	252.09	0.7907	0.0293

Table 3 Continue

Stress levels	Genotype	Pod length (cm)	Plant height (cm)	Pods per plant	Seeds in a pod	1000-seeds weight (g)	Seed yield (Kg h-1)	Seed oil (%)	Oil yield (Kg h-1)	Canopy temperature (°C)	Stomatal conductance (cm s-1)
Stress from flowering	HW113	4.70	101.66	67.50	28.00	3.66	2396.7	39.73	958.88	23.66	.2933
	RS12	5.00	103.33	79.50	27.16	3.68	2194.5	39.41	870.40	24.00	.2850
	Karaj1	4.55	100.00	64.16	29.16	3.56	2129.3	39.20	837.50	24.16	.2833
	KR18	4.83	98.33	73.33	28.50	3.53	2079.7	39.78	830.32	24.50	.2633
	L73	5.06	110.00	65.00	28.33	3.58	2333.5	40.70	953.17	24.00	.2783

	L72	5.60	108.33	91.66	28.83	3.70	2937.5	39.68	1187.20	23.83	.3517
	HW101	4.61	112.50	86.50	28.16	3.88	2902.7	40.34	1177.30	24.83	.3333
	L146	4.96	106.66	67.00	27.33	3.76	1754.3	40.08	705.12	24.00	.2567
	L210	4.83	102.50	85.16	27.83	3.75	2465.2	40.13	981.82	24.50	.3100
	L183	5.33	106.66	81.00	27.33	3.68	2630.7	40.10	1061.30	23.66	.2983
	SW101	4.80	114.16	74.83	28.66	3.58	1953.7	39.61	778.27	24.66	.2717
	L5	4.61	102.50	67.00	27.33	3.60	2060.8	39.28	818.28	24.16	.2767
	L201	4.76	104.16	61.16	28.16	3.68	2121.5	39.10	838.48	25.50	.2717
	HW118	4.36	103.33	67.00	27.16	3.71	1945.0	39.10	767.50	24.00	.2650
	KR4	4.51	98.33	72.00	28.33	3.63	2063.2	39.83	823.98	25.00	.2617
	Karaj2	4.88	110.83	71.33	29.00	3.68	1863.5	39.63	735.12	23.50	.2700
	Karaj3	4.88	105.00	77.33	27.00	3.48	2525.7	40.36	1020.6	24.16	.2833
	KS7	4.95	104.16	69.16	27.33	3.71	2070.2	39.76	829.05	23.50	.2767
LSD 5%		0.4453	10.1815	13.8025	1.5814	0.2786	614.64	1.6429	252.09	0.7907	0.0293

Table 3 Continue

Stress levels	Genotype	Pod length (cm)	Plant height (cm)	Pods per plant	Seeds in a pod	1000-seeds weight (g)	Seed yield (Kg h^{-1})	Seed oil (%)	Oil yield (Kg h^{-1})	Canopy temperature (°C)	Stomatal conductance (cm s^{-1})
Stress from seed filling	HW113	5.90	107.50	89.50	29.00	3.30	2729.0	39.13	1074.2	26.16	.5400
	RS12	5.58	115.00	87.50	29.33	3.46	3069.3	39.61	1213.6	25.66	.6300
	Karaj1	5.73	109.16	88.16	27.50	3.46	2766.0	39.13	1084.0	26.00	.5400
	KR18	5.58	114.16	87.00	28.50	3.63	2635.5	39.85	1044.4	26.00	.5450
	L73	5.81	111.66	87.33	29.00	3.38	3266.7	39.60	1298.5	26.83	.6050
	L72	6.06	97.50	90.83	30.00	3.65	3015.3	38.50	1170.4	26.33	.5233
	HW101	5.68	112.50	85.50	28.16	3.48	3179.8	39.93	1278.4	26.50	.5917
	L146	5.93	117.50	87.16	26.33	3.63	3588.8	39.91	1427.2	26.16	.6067
	L210	5.10	104.16	93.00	28.50	3.51	2782.0	39.33	1094.8	26.66	.5183
	L183	5.81	113.33	89.16	28.50	3.51	3157.0	39.35	1245.3	26.33	.5733
	SW101	5.46	102.50	82.00	29.33	3.41	3150.0	39.06	1228.6	27.00	.5650
	L5	5.91	115.83	81.83	26.66	3.68	3023.7	39.91	1202.5	26.00	.5533
	L201	5.55	109.16	83.00	28.33	3.30	3075.7	39.58	1216.8	26.33	.5717
	HW118	5.83	120.00	88.66	28.16	3.43	2973.8	39.11	1165.7	25.66	.5333
	KR4	5.81	108.33	88.83	30.00	3.48	2611.2	39.35	1022.2	25.66	.5483
	Karaj2	5.55	115.83	94.83	27.83	3.71	2958.7	38.98	1150.5	26.66	.5683
	Karaj3	5.58	117.50	93.33	28.16	3.38	2493.0	39.96	9946.2	26.33	.5017
	KS7	5.50	113.33	88.66	28.33	3.43	3095.7	39.43	1223.3	25.16	.6000
LSD 5%		0.4453	10.1815	13.8025	1.5814	0.2786	614.64	1.6429	252.09	0.7907	0.0293

Correlation among traits

Simple correlation coefficients among measured traits are shown in table 4. Positive correlations were found among seed and oil yields with each other and with pod length, pod numbers per plant, seeds in a pod and 1000-seeds weight. The seed and oil yields indicated negative correlations with plant height. The correlation among seed oil with pod numbers per plant, 1000-seeds weight and seed yield were positive. Pod length and numbers per plant indicated significant correlations with seed and oil yields, its components, canopy temperature and stomatal conductance. The correlation among seed and oil yields with canopy temperature and stomatal conductance were negative and positive, respectively.

Table 4. Simple correlation coefficients among traits measured on rapeseed genotypes

Trait		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1)	Pod length	0.19**	0.30**	0.04	-0.22**	0.28**	-0.04	0.27**	-0.24**	0.54**
(2)	Plant height		-0.17**	-0.41**	-0.43**	-0.25**	0.08	-0.23**	0.19**	0.31**
(3)	Pods per plant			0.32**	0.24**	0.63**	0.18**	0.64**	-0.35**	0.48**
(4)	Seeds in a pod				0.39**	0.48**	-0.02	0.47**	-0.37**	0.07
(5)	1000-seeds weight					0.32**	0.12*	0.32**	-0.36**	-0.07
(6)	Seed yield						0.13*	0.99**	-0.48**	0.47**
(7)	Seed oil							0.25**	-0.24**	0.10
(8)	Oil yield								-0.51**	0.46**
(9)	Canopy temperature									-0.21**
(10)	Stomatal conductance									

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

Factor analysis

The factor analysis divided the ten traits into four groups or factors (Table 5) and the varimax orthogonal rotation was subjected to the matrix of factor loadings after the first extraction of factor loadings. This rotation accentuated the larger loadings in the extracted factors and suppressed the minor loading which improves the opportunity of achieving meaningful interpretation of factors. Factor analysis indicated that four main factors (groups) were accounted for 82.11% of the total variation in the dependent structure (Table 5). The first factor represents 29.24% of the total variation of the data and this factor includes pod length, pod numbers per plant, seed and oil yields. The suggested name for this factor is yield components. Increasing pod length, pod numbers per plant would be the most effective way of increasing oil yield. The second factor included seed oil and stomatal conductance which accounted for 21.32% of the total variation. The third factor represents 18.87% of total variation of the data and this factor also includes plant height and 1000-seeds weight. It can be called effective factor to seed weight. The fourth factor which accounted for 13.17% of total variation of the data included only canopy temperature. This factor is a physiological index and the suggested name for this factor is canopy temperature. Figure 1 shows scattering cultivars based on first and second factors. In this plot, the factor-1 axis mainly distinguish genotypes based on pod length, pod numbers per plant, seed and oil yields traits. Factor-2 axis define cultivars mainly based on seed oil and stomatal conductance. Genotype 'HW101' had high scores of factor 1 and 2. Genotype 'L72' had the highest score of factor 1, but its score for factor 2 was low and this order was the opposite for genotype 'L73'. By selecting genotypes based on factor 1 and 2 scores, 'HW101' and 'L183' were recognized as the promising genotypes in this experiment.

Table 5. Factor analysis of all traits measured on rapeseed genotypes

Traits	Factor Loadings			
	1	2	3	4
Pod length	0.728	0.232	-0.251	0.228
Plant height	0.001	0.400	0.735	0.219
Pods per plant	0.890	-0.143	-0.060	-0.124
Seeds in a pod	0.131	0.028	-0.0739	0.418
1000-seeds weight	-0.157	-0.260	0.818	0.254
Seed yield	0.839	0.418	-0.034	0.134
Seed oil	0.101	0.882	0.003	-0.087
Oil yield	0.817	0.473	-0.042	0.139
Canopy temperature	-0.109	-0.045	-0.108	-0.932
Stomatal conductance	0.410	0.807	-0.008	0.221
Eigenvalue	4.05	1.93	1.18	1.05
Portion	29.24	21.32	18.87	13.17
Cumulative	29.24	50.56	68.94	82.11

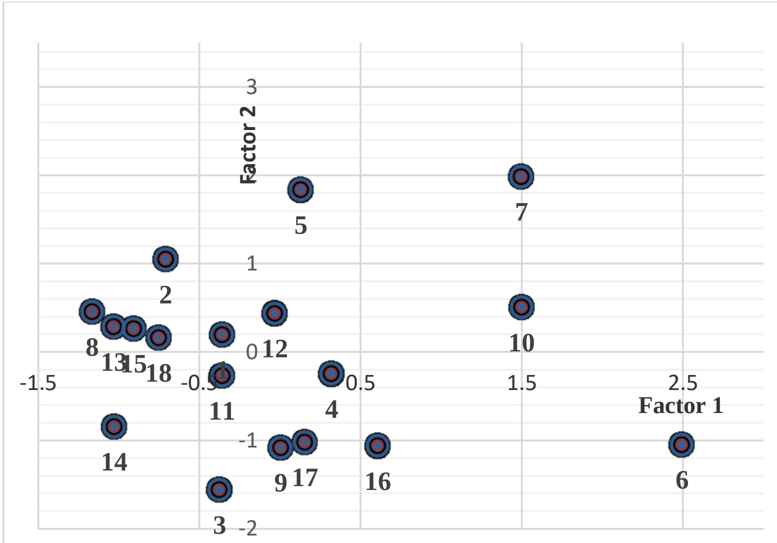

Figure 1. Scattering of the rapeseed genotypes based on their scores for factor 1 and 2 (The numbers 1 to 18 are related to HW113, RS12, Karaj1, KR18, L73, L72, HW101, L146, L210, L183, SW101, L5, L201, HW118, KR4, Karaj2, Karaj3 and KS7 genotypes, respectively)

Discusion

Water deficit during flowering and seed filling stages, decreased mean seed yields by 1426 and 696 Kg h-1, respectively. The results of the experiment indicated that cold tolerant rapeseed genotypes are more sensitive to water deficit during flowering stage than to seed filling stage. Thus, under water limiting conditions, irrigation at flowering, would be more effective to seed and oil yields stabilities. The results of studying rapeseed genotypes under drought stress indicated that, the chlorophyll a and b content of all genotypes declined due to drought stress at flowering and seed filling stages, but greater reduction in seed yield was observed when stress was imposed at flowering stage (Din et al., 2011). Darjani et al. (2013) reported that interruption of irrigation from pod development stage to seed maturity in rapeseed, significantly decreased pod numbers per plant, seeds in a pod, 1000-seeds weight and seed yield. Positive correlations were found among seed and oil yields with each other and with pod length, pod numbers per plant, seeds in a pod and 1000-seeds weight. The seed and oil yields indicated negative correlations with plant height. The correlation among seed oil with pods per plant, 1000-seeds weight and seed yield were positive. It seems that pod length, pod numbers per plant, seeds in a pod and 1000-seeds weight, have a main role to support seed yield. A significant and positive correlation between seed yield and relative growth rate in spring genotypes of B. napus L. has also been previously reported (Arvin et al., 2010). Among agronomic traits, pod length and numbers per plant indicated higher correlations with seed and oil yields, seed yield components, canopy temperature and stomatal conductance. The incidence of water deficit stress from the pod formation stage in rapeseed genotypes, negatively affected pod length, the number of pods per plant, number of seeds in a pod, 1000-seeds weight, seed oil content and seed yield (Shirani-Rad et al., 2014). Positive and significant correlations were reported among seed yield with pod numbers per plant and seeds in a pod of canola winter genotypes, under normal and drought stress conditions (Darjani et al., 2013). The correlation between seed and oil yields with canopy temperature and stomatal conductance were negative and positive, respectively. Strong negative relationship between seed yield and canopy temperature during reproductive stage of Brassica napus L. genotypes have also been reported (Faraji et al., 2009).

According to the results of factor analysis and scattering cultivars based on first and second factors, genotypes 'HW101' and 'L183' were recognized as the promising ones in this experiment. They indicated higher seed and oil yields during all of the water deficit stress and non-stressed conditions. In earlier studies (Naderi and Emam, 2010) factor analyses were used to determine structural factors related to growth traits and yield components and also for detecting factors related to environmental stresses including drought tolerance in B. napus L.

Conclusion

It can be concluded that rapeseed is more sensitive to water deficit at flowering stage than seed filling stage. Thus, water supply at flowering stage could be effective in rapeseed yield production. Pod length and numbers per plant as agronomic traits, canopy temperature and stomatal conductance as physiological traits and factor analysis method can be used to screen high yielding rapeseed genotypes for water deficit condition.

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