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Effect of Drought Stress on Agronomic Traits of Different Rapeseed Lines

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Abstract: To study the crucial agronomic traits of rapeseed under stress conditions, 100 haploid lines derived from the cross spring line "Yudal" and winter line "Darmor" along with the parents were evaluated using a simple lattise design with two replications under drought stress conditions in University of Gonbad during 2010-2011. Measured traits were days to flowering, plant height, height of lowest primary effective branch, length of main inflorescence, seed yield, 1000-seed weight, seeds per pod, pod length, pod beak length, pod thickness and pod density. There was a large variation in the population for different traits which was due to the fact that their parents had multi forms and a different genetic background. Correlation analysis showed that days to flowering and height of lowest primary effective branch had the highest negative and positive correlation with grain yield, respectively. The regression and path analysis showed that days to flowering, height of lowest primary effective branch and plant height as important components had the highest direct effect on grain yield. The reductions in grain yield and 1000-kernel weight were affected mostly by drought stress. Nonetheless, some agronomic traits such as pod thickness, pod length and beak length were not significantly changed under drought stress. Doubled haploid lines were grouped using principal components analysis based on drought tolerance indices. The first two principal components explained 98 percent of data variation.

Key words: Rapeseed · Yield and yield components · Correlation analysis · Path analysis · Drought

INTRODUCTION

Investing on oilseeds including rapeseed has gained much attention in the government. *Brassica napus* L. is the third widely produced oilseed around the world which can assist our country in providing the required nutritive oil due to having special features. The function of the grain is the most important reformative feature which is influenced by many environmental and genetic factors. Having information about the relation between yields, its components and other variables is considered as crucial in determining the selection criteria and identifying the superior genes. Number of pods per plant and number of plants per land, number of seeds per pod and 1000-kernel weight have been reported as the criteria for the yield of rapeseed [1, 2, 3]. Environmental challenges are among the most important variables in decreasing the performance of agricultural products around the world. Drought and its challenge are among the widespread environmental challenges which have limited agricultural products and decreased the outcome of rain fed farms. The rapeseed plant which is planted in both rainfed and irrigated land, generally experience the challenge of drought in stages of its life. During the late growth season, the amount of water is highly decreased in irrigated lands and regions where surface water is used for irrigation due to a decrease in rainfalls and using water resources for beneficial spring products. Consequently, the time interval between irrigations increases and the plant faces the drought challenge in the critical period of growth in which the seeds grow. The most critical period for irrigation is the flowering stage and the early time when pods form [4].

The arboreal method can be successfully implemented for modifying the tolerance to drought in self-compatible species such as *Brassica juncea*, *Brassica carinata* and *Brassica campestris* [5] while for modifying the tolerance of non-compatible species, the periodic

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selection may be the best method [6]. According to the existing scientific sources, genetic variety in terms of performance and its elements do exist both in and among species of *Brassica* for facing the drought challenge [6-9]. The results reported by Jensen *et al.* [10] indicated that the drought challenge during seed growth period has the most negative effect on growth performance. Sing [11] believes that for modifying the genotypes tolerant of drought in *Brassica* species, features such as seed yield, 1000-kernel weight and the number of sub branches should be taken into account. Nearly two-thirds of farms are allocated to rain fed agriculture in Iran which is considered as one of the dry regions of the world. As a result, conducting researches for producing species resistant to drought is of high importance.

The present study aims to investigate the relationship between yield and its components under natural irrigation and drought stress and also the effect of drought stress on Doubled Haploid lines.

MATERIALS AND METHODS

One hundred doubled haploid lines derived from the crosses between Darmour and Youdal lines produced by Foisset *et al.* [12] in Inra, France were used in this study along with their parents. This cross is considered as a reference in Inra, France for genetic and mapping studies. Darmour is a French winter cultivar and Youdal a Korean spring cultivar which is planted with an early flowering in temperate regions such as winter types [12, 13]. The abovementioned parents show multi forms for important agronomic traits such as having short legs, early growth, quality of seed (the amount of Erucic acid and Glucosinolate) and tolerance to maladies [12]. 100 genotypes including 93 doubled haploid lines along with their parents, seven commercial cultivars (Vang, Moudna, Zarfam, Hayoula 401, Oucapi, Dante and R.G.S.003) as witnesses using a simple lattice design with two replications under normal and stress conditions in University of Gonbad, Iran during 2010-2011 seasons.

During normal condition, the lines under study did not experience drought stress during the irrigations but under stress condition, irrigation was cut after flowering. Each replication consisted of 12 blocks each of which having four 1.5 meter lines with a 25 centimeter distance and 80-100 plants per square meter. Fertilization and spraying were implemented as usual. Ten plants were selected from among each block and the following traits were recorded: the beginning of flowering (5% of plants have open flowers), full flowering (90% of plants have open flowers), the end of flowering (only 10% of the plants have open flowers and 90% of inflorescences have become pods), the height of the plant (the height from ground up to the main inflorescence), height of lowest primary effective branches (height from ground up to the lowest primary effective branches), the length of main inflorescence (the height from the highest primary branches up to the end point of the plant), the density of the pod in the main inflorescence (dividing the number of pods in the main inflorescence by its length) and the length of the pod (the length of 10 pods in the mid part of the main inflorescence).

The general heritability of the traits was estimated using relation No. 1:

$$h^2 = \sigma^2 g / \left[\sigma^2 g + \sigma^2 e / r \right) \Big] \tag{1}$$

In the above equation, $\sigma^2 g|$ which is the genetic variance, was obtained out of relation No. 2. In this relation σ^2e| is the environmental variance (σ^2e=MSe)| and r stands for the number of experiments.

$$\left[\sigma^2 g = 1/r \left(MSg - MSe \right) \right] \tag{2}$$

In the population of a doubled haploid, a dominant variance does not exist and the genetic variance includes only the increasing variance. In such populations, the genetic (increasing) variance is twice the size of the genetic variance of a base population (11). Consequently, relation No. 3 was used for estimating the heritability:

$$h^2 = 1/2\sigma^2 g / \left[1/2\sigma^2 g + (\sigma^2 e / r) \right] \Big] \tag{3}$$

The experiment performed based on simple lattice with four replications. Factors included as genotypes (100) and drought in two levels (Normal irrigation and drought after flowering). Analysis variance done by SAS software.

RESULTS AND DISCUSSION

Variance Analysis, Simple Correlation, Partial Correlation and Path Analysis: The results of variance analysis for the traits under study using a simple lattice design indicated that the blocks had no significant difference for any of the traits analyzed with two replications. As a result, the average of squares used in random sampling design was used for variance analysis of the data which is hidden in simple lattice design. The results showed that the difference between the genotypes was significant for all traits under normal and stress conditions (Table 1). A great variety was found for all the measured traits under normal and stress conditions (Table 2). Taking into account the morphologic and genetic multi shapes of their parents, the existence of variety was expected in the population.

The range of seed's yield was 1093-8527 kilograms per hectare under normal conditions and 1447-8167 kilograms under stress conditions. The yield of parental lines i.e. Darmour and Youdal was 3520 and 7233 kilograms per hectare under normal conditions, respectively which was 2400 and 6480 kilograms under stress conditions. The results clearly show that the yield of spring parental line (Youdal) is remarkably more than winter parental line (Darmour) under both normal and stress conditions. The results of phenotypic correlation analysis (Tables 2 and 3) indicated that days to flowering and growth had the most significant and negative correlation with seed yield under both normal and stress conditions. In other words, those lines which had characteristics similar to that of winter parents and were hibernal had a lower yield. Whereas, winter cultivars has a higher potential for yield and the cross between spring and winter lines is sometimes used for transferring parts of winter lines' genomes to spring lines cause a better yield [14, 15].

A lower yield for winter lines in comparison with spring lines is due to the genetic background of evaluated materials. In other words, since the winter line (Darmour) was incompatible on the one hand and the spring line (Youdal) had positive features on the other hand, the yield of Youdal which is a spring line was remarkably more than Darmour line. The thickness of the pod, the height of lowest primary effective branch and the height of the plant had the most positive correlation with the seed yield respectively under normal irrigation conditions. Kis *et al.* [16] also reported similar results for the correlation between the height of the plant and the seed yield and also the correlation between the height of the plant and the height of lowest primary effective branch. Nonetheless, a few studies reported a negative correlation between the height of the plant and seed yield [17, 18].

Under drought stress conditions, 1000-seed weight, pod length and pod thickness had the highest positive correlation with seed yield (Table 4). Other studies have also mentioned the importance of 1000-seed as one of the main elements of seed yield [1, 2, 3]. The correlation between number of pods per main branches and days to flowering and growth was positive and significant. In fact, the lines with a winter type had possessed a higher number of pods in the main branch. In spite of the fact that many studies have pointed to the significance of the number of pods in a plant, no phenotypic correlation was found between the number of pods in the

Table 1: The square means and heritability of traits under study in normal and drought stress for rapeseed.

	Square mean			Square mean		
Trials [BE2]	Treatment	Error	h^2	Treatment	Error	h^2
	80088**	26770	0.5	60564**	29451	0.35
Grain yield (kg/ha)	0.5**	0.11	0.64	0.32**	0.098	0.53
1000-grain weight (g)	0.46**	0.12	0.59	0.44**	0.12	0.57
Siliqua thickness (mm)	0.58**	0.05	0.84	0.64**	0.065	0.81
Siliqua length (mm)	11.9**	4.7	0.43	18.3**	11.5	0.23
Seed number per siliqua	7.4**	0.6	0.85	7.2**	1	0.76
Length of siliqua top	23.2**	1.9	0.85	18.7**	3.3	0.7
Day to maturity (day)	41.1**	4.95	0.78			
Days to flowering (day)	588.9**	28.7	0.91			
Plant height (cm)	443.8**	48.6	0.8			
Height of the first branch (cm)	53.6**	30.1	0.28			
Length of main florescence(cm)	146.7**	86.2	0.26			
Number of siliqua per main florescence	0.099**	0.034	0.49			
Density of siliqua in main florescence						

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Table 2. Phenotypic correlation coefficients of traits under normal irrigation conditions for rapeseed.

	Seed yield (kg/ha) (1)	Siliqua thickness (mm) (2)	Seed number per siliqua (3)	Length of siliqu a top (4)	Siliqua length (mm) (5)	1000 -seed weight (gr) (6)	Height of the first branch (cm) (7)	Length of main florescence (cm) (8)	Number of siliqua per main florescence (9)	Plant height (cm) (10)	Density of silik in main florescence (11)	Days to flowering (day) (12)	Day to maturity (day) (13)
1	1												
2	0.41**	1											
3	0.02	0.14	1										
4	0.27**	0.4**	0.29**	1									
5	0.21*	0.15	0.32**	0.18*	1								
6	0.33**	0.49**	-0.13	0.12	0.45**	1							
7	0.44**	0.29**	0.075	0.1	0.23**	0.35**	1						
8	-0.11	-0.18*	-0.07	-0.14	-0.16*	-0.05	-0.1	1					
9	-0.08	-0.21*	0.013	-0.14	-0.32**	-0.24**	0.19*	0.5**	1				
10	0.35**	0.22**	0.06	0.04	0.22**	0.35**	0.89**	0.13	0.28**	1			
11	0.011	-0.007	0.04	-0.03	-0.22**	-0.23**	0.32**	-0.26**	0.69**	0.23**	1		
12	-0.57**	-0.56**	-0.06	-0.37**	-0.4**	-0.47**	0.012	0.17	0.53**	0.08	0.46**	1	
13	-0.38**	-0.52**	-0.19	-0.39**	-0.36**	-0.29**	0.07	0.26**	0.52**	0.15	0.37**	0.85**	1

Table 3: Phenotypic correlation coefficients of traits under drought stress conditions for rapeseed.

	Grain yield (kg/ha) (1)	Siliqua thickness (mm) (2)	Seed number per siliqua (3)	Length of siliqu a top (4)	Siliqua length (mm) (5)	1000 -seed weight (gr) (6)	Height of the first branch (cm) (7)	Length of main florescence (cm) (8)	Number of siliqua per main florescence (9)	Plant height (cm) (10)	Density of silik in main florescence (11)	Days to flowering (day) (12)	Day to maturity (day) (13)
1	1												
2	0.45**	1											
3	0.13	15	1										
4	0.25**	0.3**	0.18*	1									
5	0.35**	0.3**	0.36**	0.16	1								
6	0.46**	0.48**	-0.08	0.19*	0.51**	1							
7	0.32**	0.38**	0.21	0.09	0.2*	0.23**	1						
8	-0.03	-0.18*	0.1	-0.036	-0.05	-0.06	-0.1	1					
9	-0.09	-0.33*	0.11	-0.9	-0.3	-0.27**	0.19*	0.5**	1				
10	0.32**	0.24*	0.22**	0.04	0.21*	0.25**	0.89**	0.13	0.28**	1			
11	-0.1	-0.19*	0.045	-0.063	-0.31**	-0.24**	0.32**	0.26**	0.69**	0.23**	1		
12	-0.58**	-0.65**	-0.054	-0.32**	-0.45**	-0.47**	0.012	0.17*	0.53**	0.08	0.46**	1	
13	-0.26**	-0.51**	0.09	-0.25**	-0.33**	-0.22**	0.1	0.24**	0.52**	0.19*	0.4**	0.8**	1

* and ** significantly in 0.05 and 0.01 probability level., respectively.

Table 4: Partial correlation between seed yield and other traits under normal and drought stress conditions for rapeseed.

Trials	Siliqua thickness (mm)	Seed number per siliqua	Length of siliqu a top	Siliqua length (mm)	1000 -seed weight (gr)	Height of the first branch (cm)	Length of main florescence (cm)	Number of siliqua per main florescence	Plant height (cm)	Density of silik in main florescence	Days to flowering (day)	Day to maturity (day)
Yield (stress)	0.05	0.09	0.03	0.06	0.12	0.006	-0.1	0.12	0.15	-0.09	-0.56**	0.3**
Yield (normal)	-0.04	-0.05	0.06	-0.08	-0.09	0.22*	-0.09	0.11	0.04	-0.07	-0.46**	0.1
Mean	-0.12	0.03	0.08	-0.08	0.002	0.14	-0.1	0.12	0.13	-0.08	-0.57**	0.2*

* and ** significantly in 0.05 and 0.01 probability level., respectively.

main branch and seed yield in this study. The lack of correlation between number of pods in the main branches and seed yield, may relates to the genetic background of the materials which will be discussed in the path analysis of the results.

Other studies have also pointed to the low heritability of number of pods in a plant since this trait is seriously influenced by environmental factors [3, 7, 8]. No relationship was found in this study between seed yield and number of seeds in a pod as one of the yield components under normal and drought stress conditions. In addition, only an average correlation was found between length of pod and seed yield under normal and stress conditions. The correlation between number of seeds in a pod and length of pod was positive and significant. The relationship between number of seeds per pod and pod length is crucial for modification purposes since pod length is easily measured and can be used as a criterion for selecting the seed yield. According to Chay and Thrling [19], the effect of pod length on seed yield is highly dependent on the genetic background of the evaluated materials. Having analyzed an F_2 population, Samizade Lahiji [20] also pointed to the fundamental role of pod's length in seed yield. There exists a hypothesis regarding the correlation between pod's length and number of seeds in a pod claiming that pod's length is genetically controlled and does not rely on the number of seeds and a longer pod only leads to a more distance between the seeds [30].

The role of growth type or days to flowering in seed yield is highly significant in the above correlations which can affect other correlations of traits with seed yield. Partial correlation is used for analyzing the relation between a trait and seed yield so that other variables cannot influence it [21]. The results for partial correlation between seed yield and other measured traits are shown in Table 1. It shows that partial correlation between yield and other traits is remarkably different from that of simple correlations. For example, thickness of pod and weight of 1000-seed were traits which had the most significant and positive correlations with seed yield while the results of partial correlation indicated that these traits have little effect on seed yield. Nonetheless, the common point between simple and partial correlations was a high and significant correlation between seed yield and days to flowering under both normal and stress conditions. These results prove the effect of days to flowering on seed yield. The lack of significance for partial correlations between seed yield and other traits indicated that except for days to flowering, other traits alone cannot have a remarkable effect on seed yield. The partial correlation between days to growth and seed yield was found to be significant under stress condition. These results show those days to growth has more effect on seed yield under stress conditions and this is due to the plant's attempt to escape stress conditions and reach early growth.

Since simple correlations cannot state the real nature of relations between traits, using methods such as path analysis can be used in explaining such relations [22, 23]. To do this, stepwise regression analysis was used to determine the effective variables and use them in path analysis. The results of regression analysis showed that under normal irrigation conditions, traits including days to flowering, height of lowest primary effective branch and number of pods in the main inflorescence were entered in the model and accounted for 48% of changes in seed yield. Under stress conditions however, traits including days to flowering, the height of plant and number of pods per main inflorescence were entered in regression model and accounted for 49% of changes in seed yield (Table 5). The low amount of explanation for seed yield under both normal and stress conditions shows that seed yield is a highly complex trait. The results of path analysis (Table 6) indicated that days to flowering has the most negative effect on seed yield under both normal and drought stress conditions. Under normal irrigation condition, height of lowest primary effective branch and number of pods in the main inflorescence had

Table 5: Stepwise regression for seed yield under normal irrigation and stress conditions for rapeseed.

	MS					
Trials	Regression	Error	df	R^2	R2- adjective	Slope
		Normal irrigation				
Day to flowering	96738966**	1806196	1	0.27	0.27	-204
Height of the first branch)	82959219**	1328371	2	0.47	0.46	42.2
Number of Siliqua per main florescence	57989664**	1280356	3	0.49	0.48	37
		Drought stress				
Day to flowering	122644651**	1742600	1	0.33	0.33	-234
Plant height	86975837**	13910780	2	0.47	0.46	33.6
Number of siliqua per main florescence	60784603**	1341001	3	0.49	0.48	35.5

* and ** significantly in 0.05 and 0.01 probability level., respectively.

Table 6: Direct and indirect effects of some important traits on seed yield under normal irrigation and stress conditions for rapeseed.

		Indirect effect			
Trials	Direct effect	-1	-2	-3	Correlation with seed yield
		Normal irrigation			
Day to flowering (1)	-0.68	-	0.004	0.11	-0.57
Height of the first branch(2)	0.41	-0.01	-	0.038	0.44
Number of siliqua per main florescence (3)	0.2	-0.36	0.08	-	-0.08
		Drought stress			
Day to flowering (1)	-0.71	-	0.025	0.103	-0.58
Plant height (2)	0.32	-0.057	-	0.054	0.32
Number of siliqua per main florescence (3)	0.2	-0.38	0.09	-	-0.09

the most direct effect on seed yield after days to flowering. Under stress condition however, the height of plant and number of pods in the main inflorescence had the most direct effect on seed yield after days to flowering.

Since no simple correlation was found between number of pods in the main inflorescence and seed yield under normal and stress conditions, a noticeable point in regression and path analysis is the entrance of this trait into the model and its direct effect on seed yield. It seems that a higher number of pods in the main inflorescence itself increases seed yield but due to the high and fundamental effect of days to flowering on seed yield and the fact that the number of pods in the main inflorescence has a positive correlation with days to flowering on the one hand and a negative correlation between days to flowering and seed yield on the other hand, the effect of number of pods on seed yield stays unnoticed.

In fact, the results of correlation, regression and path analysis indicated that the most significant factors for seed yield were days to flowering or growth type of rapeseed, height of plant and height of lowest primary effective branch. It's worth mentioning that the abovementioned results are mostly due to the features of the population used for this study and thus may not be generalized to other populations. For example, Ali *et al.* [24] mentioned indices including harvest, 1000-kernel weight and number of pods in a plant and Murat *et al.* [25] introduced 1000-kernel weight, number of pods per plant and number of seeds per pod as the main determining factors in seed yield. This is due the fact that the parents of the abovementioned population had multi forms which is much higher for traits such as days to flowering or growth type and height of the plant. Since one of the parents had *Bzh* gene on the one hand which causes the plants to have short legs and the growth type of parents is totally different on the other hand, the relations between yield and yield components have been seriously influenced by the abovementioned factors.

Yield and Yield Components: Seed yield had the most decrease (15%) among the evaluated traits under stress conditions (Table 4). The last irrigation under stress condition was conducted when 50% of lines had been blossomed and taking into account those days to flowering was different for double Haploid lines, the effect of stress drought was different for lines with early and late flowering. As a result, the lines with early flowering were less influenced by drought stress such that their yield decreased for only 10%, while lines with late flowering were more influenced by drought stress and their yield decreased for 20% (Table 5). Considering the abovementioned results, it seems that the decrease in seed yield is less at the end of agronomic season since the physiologic growth of rapeseed is less than other fall products for about a month. 1000-kernel weight had the most reduction under stress condition with 9% decrease after seed yield. We can use 1000-kernel weight as one of the selection indices for yield since it has a crucial role in determining the seed yield both under normal and stress conditions. The length and thickness of pods and pod beak length were not significantly influenced under drought stress conditions. Number of seeds per pod was decreased a little (1.5 %) under stress condition and the average of days to growth was reduced to three to four days under stress condition.

CONCLUSION

It can be concluded that there was a high variety in evaluated doubled haploids of this study. Taking into account that rapeseed is not an indigenous product and most of the existing modifying materials have been imported from other well-known countries in planting rapeseed and there is thus a little genetic variety in the aforementioned materials, the current available materials can be used in modification programs of our country for rapeseed. According to the results of this study, it seems that spring lines had a high yield and due to their early growth and stress escape, they can be used in modification programs for introducing the species tolerant of drought.

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