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Multivariate Statistical Analysis to Yield of Canola under Drought Stress and Spraying of Gibberellin and Salicylic Acid

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ABSTRACT

BACKGROUND: Drought stress is one of the most critical factors in the reduction of yield among plant growth factors. Salicylic acid is a regulator that plays a vital role in the physiological processes of the plant. Gibberellin is one of the plant growth-regulating hormones that have different effects on the growth and development of many plants during growth stages.

OBJECTIVES: The purpose of this research is to identify the traits that have a strong and productive relationship with the performance of canola under conditions of drought stress and levels of Gibberellin acid and salicylic acid application.

METHODS: An experiment was conducted as a split-split plot based on randomized complete block design (RCBD) with three replications during the cultivation season of 2017-2018 in Darreh-Shahr city, Ilam Province. The experimental factors were four levels of irrigation, including normal irrigation, cut of irrigation in stem elongation, cut of irrigation at the start of flowering. The cut of Irrigation at the beginning of the pod were allocated as main plots. Sub factors and sub-sub factors were two levels of spraying and non-spraying of Gibberellin hormone and salicylic acid, respectively.

RESULT: Based on the results of correlation coefficients between the traits, it was determined that all of the studied traits had a significant correlation with grain yield. Investigation of regression coefficients using t-test showed that only the effects of three traits such as the number of flowering branches, 1000-seed weight, and harvest index were significant, but other characteristics in the model had a feeble impact on grain yield prediction. The number of flowering branches and harvest index has a more substantial contribution than other characters in predicting grain yield because one unit of increase causes the grain yield to increase by 42% in the standard deviation scale.

CONCLUSION: According to the results, to improve the yield of rapeseed, three attributes of 1000 seed weight and number of flowering branches and harvest index have to be emphasized that directly or indirectly affect this trait.

KEYWORDS: *Correlation, Hormone, Irrigation, Growth regulator, Regression.*

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1. BACKGROUND

Canola is one of the essential oily plants in Iran, which is cultivated widely in the country (Arabi Safari *et al*., 2018; Modhej *et al*., 2013). The yield of this plant is a complex trait that is controlled by multiple mechanisms. Its performance depends on the capacity of the variety, the weather conditions, the type of the soil and agriculture management, the genetic and agronomic factors that determine the growth and development of the plant and thus the yield of the seed (Kuchtova *et al*., 1996: Koocheki and Khajehossini, 2008). Drought stress is one of the most critical factors in the reduction of yield among plant growth factors (Farooq *et al*., 2016; Jaberi *et al*., 2015). Drought stress is defined as an external factor that hurts the plant and also reduces the availability of nutrients in the soil (Modhej *et al*., 2017). Therefore, plant management in drought stress is one of the essential issues in the production of crops (Fathi *et al*., 2017). The effect of drought on any of the components of the performance can lead to a change in its efficiency (Modhej *et al*., 2017). In the absence of adequate water, plant growth is reduced not only due to the lack of water, but also due to the shortage of available nutrients (Kumar *et al*., 2015). Salicylic acid is a regulator that plays a vital role in the physiological processes of the plant. The induction of flowering, growth, development, ethylene synthesis and effect of open and closed mechanisms of stomata and respiration are essential roles of salicylic acid (Hayat *et al*., 2010; Karami Chame *et al*., 2016). The use of salicylic acid increases plant tolerance to drought stress and modifies the effect of water deficit and also increases some growth parameters (Sahraei *et al*., 2018; Yazdanpanah *et al*., 2011). Gibberellin is also necessary to break down the seeds of dormancy and start germination (Stebert *et al*., 2001). Gibberellin is one of the plant growth-regulating hormones that have different effects on the growth and development of many plants during growth stages. The use of Gibberellin at high concentrations increases the growth of some of the plants (Abbasi *et al*., 2019). Gibberellin in addition to stimulating plant growth, increases the power of photosynthesis, leaf length growth, and tolerance to drought stress (Ashraf *et al*., 2002). Measurement of the correlation coefficients between different traits with function makes it possible to decide on the relative importance of these traits and their value as selection criteria. But there are the negative correlations between characteristics related to performance (Leilah and Al-Khateeb, 2005). Due to the complex relationships of traits with each other, final judgment cannot be made solely based on simple correlation coefficients. Therefore, it is necessary to use different statistical methods to better understanding the relationships between traits and to broaden the data (Maleki *et al*., 2017). Regression analysis allows the researcher to predict the variation of performance (dependent variable) through independent variables (attributes investigated) and determine the contribution of each independent variables in explanation of performance

(Maleki *et al*., 2017). Since multivariate regression has interactive effects among variables, it is necessary to eliminate minor variables by methods such as stepwise regression of the model (Maleki *et al*., 2017). Sometimes the syntax is more complicated than a simple relation between two variables. Therefore, in these conditions, one can judge the relationship between predictor variables by examining the quantities called the Variance inflation factor and coefficient of tolerance, which is the image of this quantity (Leilah and Al-Khateeb, 2005). Investigating the relationship between different traits with grain yield in 12 bread wheat genotypes under normal conditions and drought stress showed that in non-stress terms, harvest index and biological yield had a more active role in justifying grain yield so that these two traits account for 78% of variations. The grain yield was justified, but under stress conditions, three characters of biological yield, straw yield and 1000-grain weight justify 87% of variation of yield (Maleki *et al*., 2008). Regarding the results of various experiments, it seems that regression analysis of the effective traits on yield leads to a better understanding of the role of different factors in improving the yield of canola.

2. OBJECTIVES

The purpose of this research identify traits that have strong and productive relationship with the performance of canola under conditions of drought stress and levels of Gibberellin acid and salicylic acid application.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

An experiment was conducted as a split-split plot based on randomized complete block design (RCBD) with three replications during cultivation season of 2017-2018 in Darreh-Shahr city, Ilam Province. The experimental factors were four levels of irrigation including normal irrigation, cut of irrigation in stem elongation, cut of irrigation at the beginning of flowering. The cut of Irrigation at the beginning of the pod were allocated as main plots. Sub factors and sub-sub factors were two levels of spraying and non-spraying of Gibberellin hormone and salicylic acid, respectively.

3.2. Farm Management

Before running tests to determine soil sampling was conducted from the depth of 30 cm of soil and its characteristics were examined. Based on test results from the depth of 30 cm of soil, clay loam soil texture, organic carbon 0.91 %, acidity 3.7, EC 3.1 $dS.m^{-1}$, 0.12 % nitrogen, phosphorus 10 ppm and potassium was 242 ppm. Land preparation and seed bed preparation and planting were carried out in the first half of November. Each experimental plot consisted of six planting lines at 40 cm intervals. Each replicate consisted of 16 plots and the intervals were 1.5 m and the interval between treatments was 1 m. The area of each plot was 10 m 2 (2.5 m wide and 4 m long). To apply the treatments, a furrow was planted in middle of each line and seeds were sown in furrows and covered with soil.

During the plant growth period no disease or pest was observed in the field. To apply drought stress, a one-stage irrigation was performed at the time of treatments. Gibberellin acid at 100 ppm and salicylic acid at 150 ppm (in a single step and in the shoot step) were applied by the hand sprayer treatments. Triple super phosphate fertilizer (90 $kg.ha^{-1}$) was applied before planting and urea fertilizer (150 $kg.ha^{-1}$) was applied two times pre-planting and simultaneously with irrigation in the field. According to the results of field soil test, there was no need for potash fertilizer.

3.3. Measured Traits

To measure grain yield, all the plants in the area of 1 m 2 were removed from each plot by removing the marginal effect and placed for drying and reaching 12% moisture in the oven for 48 hours and then manually seeded. Harvested seeds of each plot were weighed separately with accurate laboratory scales and data were generalized to hectares and total yield was calculated.

3.4. Statistical Analysis

In this experiment, the regression analysis of SPSS V.19 and data analysis of variance were performed using Excel software (Kardoni and Fathi, 2016).

4. RESULT AND DISCUSSION

The results of the variance analysis of studied traits showed that the irrigation effect on all traits was significant (Table 1). The results also showed that both salicylic acid and Gibberellin acid have a considerable impact on the all studied features. Interaction between irrigation and Gibberellin acid was substantial only on harvest index, while the interaction of irrigation and salicylic acid was significant on the number of flowering branches (Table 1). Based on the results of correlation coefficients between the traits (Table 2), it was determined that all characteristics had a significant correlation with grain yield. Therefore, the importance of all characteristics in explaining the grain yield was revealed. On the other hand, the correlation coefficients between two traits showed a significant linear relationship between the number of flowering branches and yield. So it is possible to predict the coexistence in the regression equation. In this experiment, the correlation coefficient of 1000 seed weight with grain yield showed that this trait had the highest correlation coefficient with yield (r =86%**). So it's the high correlation caused the effect of other characteristics and the essential traits to determine the performance can't be entered into the regression model, and the value of the Watson Camera statistic was D= 2.675 (Table 3), which was in an acceptable range, indicating the independence of the errors and the suitability of the estimated model for Functional explanation. The coefficient of the model explanation was estimated as R^2 = 0.933 (Table 3). The value of this coefficient indicates that 93 percent of grain yield changes are attributed to the traits introduced into the model, and the remaining 7 % depends on other factors and features.

Table 1. Result analysis of variance of measured traits

S.O.V	df	Plant height	Pod length	No. pods per plant	No. branches of flowering	No. seeds per pod
Replication	2	3119.47**	2.32^{ns}	958.41*	0.93	48.48*
Irrigation (I)	3	651.08*	6.10*	2305.61**	8.69**	97.74**
Error I	6	127.40	0.96	155.68	0.19	6.61
Gibberellin acid (GA)	1	2181.60**	7.98**	2692.06*	14.37**	273.89
I×GA	3	2.44^{ns}	1.92*	8.52^{ns}	0.33^{ns}	4.07^{ns}
E (GA)	8	82.00	0.38	279.19	0.24	9.84
salicylic acid (SA)	1	2030.60*	5.71**	3365.91**	14.87**	216.41^{ns}
I×SA	3	28.34^{ns}	1.59*	3.90^{ns}	0.34^{ns}	2.19^{ns}
GA×SA	1	485.78^{ns}	0.09^{ns}	527.81^{ns}	1.65*	34.92^{ns}
I×GA×SA	3	36.13^{ns}	3.00**	40.13^{ns}	0.30^{ns}	1.86^{ns}
Residual	16	273.10	0.36	178.26	0.25	7.24
C.V (%)	-	12.8	14.4	7.1	7.8	9.9

$^{ns, *}$ and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue Table 1.

S.O.V	df	Seed weight	Seed yield	Biological yield	Harvest index
Replication	2	0.78**	325051.66*	8352547.1	11.62
Irrigation (I)	3	3.54**	1961348.67**	19809911.9*	35.98*
Error I	6	0.03	47165.51	2960051.7	5.38
Gibberellin acid (GA)	1	2.87**	5103354.18**	28883468.8**	166.92**
I×GA	3	0.04*	53090.05^{ns}	1487728.6^{ns}	10.15*
E (GA)	8	0.01	37820.17	1247836.5	1.61
salicylic acid (SA)	1	1.98**	3271379.41**	9700030.3*	53.45**
I×SA	3	0.01^{ns}	17980.42^{ns}	639616.4^{ns}	10.88**
GA×SA	1	0.02^{ns}	833210.72**	8403313.0^{ns}	46.16**
I×GA×SA	3	0.04^{ns}	160108.20^{ns}	1006965.9^{ns}	20.22**
Residual	16	0.03	65867.80	1967558.9	1.41
C.V (%)	-	5.1	9.9	15.9	4.02

$^{ns, *}$ and **: no significant, significant at 5% and 1% of probability level, respectively.

Investigating causality relationships to interpret the structure of factors affecting grain yield can be useful, although determining the relationship between the essential characteristics and grain yield is crucial. However, the correlation coefficient does not specify the nature of relationship between characteristics so that by the causality analysis, it is possible to identify their direct and indirect effects on the performance (Rahmani *et al*., 2004). In this table, the regression multiplicity correlation coefficient (R) shows the linear relationship between grain yield and traits entered into the model by 96% (Table 3). The significance of the regression model and the linearity of the relationship between characters (F calculated) are shown (Table 4). Therefore, the estimated model has enough credit to analyze the data. As can be seen in table 5, various traits are introduced as independent variables and grain yield as a dependent variable. Based on the regression coefficients (Table 5), the following equation was used to predict grain yield using the traits:

Equ. 1. $Y= -1951.755+-2.688\ (X_1) + 19.754\ (X_2) +0.907\ (X_3) + 122.091\ (X_4) + 18.881\ (X_5) + 346.820\ (X_6) + -0.011\ (X_7) + 74.440\ (X_8)$

Table 2. Correlation between studied traits of canola

Traits	Plant height	Pod length	No. pods per plant	No. branches of flowering	No. seeds per pod	1000 Seed weight	Seed yield	Biological yield	Harvest index
Plant height	1								
Pod length	0.21642	1							
No. pods per plant	0.24733	0.24293	1						
No. branches of flowering	0.56114**	0.38989**	0.63412**	1					
No. seeds per pod	0.60801**	0.39573**	0.45665**	0.84998**	1				
Seed weight	0.30101*	0.49651**	0.64894**	0.77463**	0.60174**	1			
Seed yield	0.41436**	0.37066**	0.72336**	0.86240**	0.76658**	0.8268**	1		
Biological yield	0.37360**	0.43671**	0.51054**	0.68424**	0.61123**	0.6742**	0.6149**	1	
Harvest index	0.39583**	0.07892	0.65212**	0.62352**	0.58541**	0.505**	0.813**	0.3798**	1

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively.

The weighted standard coefficients (beta coefficients) in this table indicate the importance of the role of these traits in predicting the regression model. The high beta coefficient indicates the importance and relative role of a trait in predicting grain yield (Table 5). Therefore, it can be judged here that the number of flowering branches and harvest index has a greater contribution than other traits in predicting grain yield because one unit of increase causes the grain yield to increase by 42 percent in the standard deviation scale. There was a positive and significant correlation between plant height and yield (r= 41 %**). Therefore, increasing plant height would increase the photosynthetic potential and vegetative growth of the plant and, consequently, increase grain yield. The positive and high correlation between 1000 seed weight and grain yield (r= 82 %**), which is consistent with regression coefficients, indicates that any factor that increases 1000-seed weight can be useful in increasing grain yield.

Table 3. Components and regression coefficients of the fitted model for the traits studied under experimental treatments

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.966	0.933	0.919	176.121	2.675

Table 4. Analysis of variance of the studied traits affected by yield by multivariable regression

ANOVA					
Model	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Regression	16865339.336	8	2108167.417	67.964	.000
Residual	1209729.554	39	31018.707		
Total	18075068.890	47			

Regression coefficients using t (Table 6) showed that only the effects of three traits such as the number of flowering branches, 1000 seed weight, and harvest index were significant and other characteristics in the model had a feeble effect on grain yield prediction. To find the best model and to eliminate the impact of ineffective or low impact traits on grain yield and also to determine the cumulative share of characters in determining the yield through stepwise regression, the traits significantly correlated with grain yield were selected. Grain yield as a dependent variable and traits entered in the model using the regression coefficients in table (7) are as follows:

Equ. 2. Y= -1994.708+ 154.725 + 76.516 + 371.543

The results in table (7) show that step-by-step regression analysis has only tripled progress. In the first step, the number of branches of flowering entered the equation. At this stage, the coefficient of explanation showed that at this stage, the number of flowering branches alone justified 86% of the variation of yield. Based on the fact that the number of flowering branches of the first traits entered the model, it can be said that it is the most critical factor affecting grain yield changes in response to the treatments. In this study, the studied factors were considered so that the number of branches of flowering branches in comparison with other components could be changed and would have a more severe effect on grain yield. In the second step, with the index of harvesting, the coefficient of determination increased to 93%. After the first step, the number of flowering branches was an essential trait; the second trait is of particular importance. In the third step, with the introduction of the 1000-grain weight trait into the model, the correlation coefficient of yield increased with the linear combination of characters of flowering branch number, harvest index to the explanatory factor to 96%. In other words, 96% of the variation of performance is explained by these three attributes. Leilah and Al-Khateeb (2005) reported using stepwise regression and determining the most useful traits for wheat grain yield under drought stress conditions, which were 1000 seed weight, harvest index, biological yield, number of spikes per square meter and length Spike entered the step ways model and overall justified 98.1% of the variation in performance.

Table 5. Components and regression coefficients of traits affecting yield by multivariate analysis

Coefficients							
Model	**R**	**Std. Error**	**Beta**	**t**	**VIF**	**Tolerance**	**VIF**
Constant	-1951.755	285.901		-6.827	.000		
Plant height (X1)	-2.688	1.677	-.087	-1.604	.117	.584	1.711
Pod length (X2)	19.754	26.014	.039	.759	.452	.648	1.544
No. pods per plant (X3)	.907	1.903	.031	.477	.636	.397	2.522
No. branches of flowering (X4)	122.091	56.280	.238	2.169	.036	.142	7.028
No. seeds per pod (X5)	18.881	11.049	.152	1.709	.095	.216	4.620
1000 Seed weight (X6)	346.820	78.994	.347	4.390	.000	.274	3.643
Biological yield (X7)	-.011	.019	-.037	-.602	.551	.448	2.230
Harvest index (X8)	74.440	11.249	.425	6.618	.000	.416	2.403

In the stepwise regression method, indirect relationships between attributes are also considered in determining the relations between two traits. Therefore, it should be said that other characteristics affect the performance indirectly by affecting these three performance components. The results of stepwise regression analysis were significant for confirmation of the multiple effects of linear regression analysis for different traits. But these traits differed in order of importance because, based on the beta produced in step ways analysis, the number of flowering branches was in second-degree essence which was ranked as third-degree in terms of the linear regression beta. The reason for this change is that in the previous model, the other traits that participated in the model indirectly affected the precedence of these three traits, but only with the removal of them in the stepwise regression model, these three traits were remained, thus the Beta coefficients were calculated and compared with themselves.

Table 6. Components and regression coefficients of the studied traits affecting yield in stepwise regression

Model	(R)	R Square	Adjusted R Square (R^2Ad)	Std. Error of the Estimate	Durbin-Watson
1	0.862[a]	0.744	0.738	317.32457	
2	0.932[b]	0.868	0.862	230.29116	
3	0.961[c]	0.923	0.918	177.66398	2.522

A= Number of branches of flowering, B=hi, C=1000 Seed weight

5. CONCLUSION

In this experiment, the results of regression analysis of different traits showed that the significant increase in yield under drought stress and consumption of both Gibberellin acid and salicylic acid among different morphological characteristics and vegetative components depend on the improvement of characters such as the number of flowering branches, 1000 seed weight, and index. The rest of the traits are less important, so further studies and primarily corrective actions to increase the amount of yield should be done to promote these traits.

Table 7. Regression model of traits affecting yield by stepwise regression

Model	Un standard-ized Coefficients	Std. Error	B	t	Sig.	VIF	Tolerance
Constant	-216.492	246.288		-0.879	.384		
No. branches of flowering	441.948	38.249	0.862	11.55	.000	1.000	1.000
Constant	-1634.56	281.855		-5.799	.000		
No. branches of flowering	297.895	35.506	0.581	8.390	.000	1.636	0.611
Harvest index	78.963	12.135	0.451	6.507	.000	1.636	0.611
Constant	-1994.708	226.684		-8.800	.000		
No. branches of flowering	154.725	37.401	0.302	4.137	.000	3.050	0.328
Harvest index	76.516	9.372	0.437	8.164	.000	1.640	0.610
1000 Seed weight	371.543	66.086	0.372	5.622	.000	2.506	0.399

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

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