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Study of Genotypic and Phenotypic Correlations and Stepwise Regression Analysis in Maize Under the Influence of Combinations of Nitrogen Fertilization and Humic Acid

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P A P E R   I N F O

A B S T R A C T

An experiment was commducted in the fields of Ibn Al-Bitar Preparatory Vocational School in Al-Hussainiya District-Holy Karbala governorate during the spring season 2021. Split-plot arrangement with in randomized complete blocks design (RCBD) with three replications was applied.The objective of this experiment was to identify the traits most related to the grain yield and determin its predictive equation for six maize genotypes (5018, Bahouth 106, Al-Maha, Fajr 1, Al-Furat and Sarah) under the effect of four levels of fertilization (160 N kg.ha^{-1}, 160 N kg.ha^{-1} with humic acid, 320 N kg.ha^{-1} and 320 N kg.ha^{-1} with humic acid) where the genotypes of maize represented the subplots and fertilization as the main plots.

Several traits of growth, quality, characteristics of the crop and some of its components were studied, in addition to studying some traits of the tassel and vitality of pollen grains. The genotypic and phenotypic correlations were studied, and an equation predicting the grain yield was found through a stepwise regression analysis*.*

The analysis of the phenotypic and genotypic correlations showed that the values of the genotypic correlations were higher than the values of the phenotypic correlations for most traits*.* Grain yield was connected with a highly significant positive phenotypic correlation with the characteristics of leaf area, the 500 grain's weight and yield efficiency, and a highly significant positive genetic correlation with the 500 grain's weight (0.97) and yield efficiency (0.71).

It became clear from the stepwise regression analysis that the independent traits that are closely associated with the dependent variable (yield) were, according to the predictive equation for yield, the 500 grain's weight, yield efficiency and leaf area, as these traits were responsible for 95% of the yield variance*.*

We conclude from the foregoing that it is possible to use the 500 grain's weight as a selective criterion to achieve the highest genotypic correlation with grain yield, and the stepwise regression analysis confirms this.

1. INTRODUCTION

Corn is one of the most important cereal crops in the global economy as a food source for humans and fodder for animals. It has a high yield that cannot be rivaled by any other cereal crop, and therefore it is called "the king of crops". [1,2]. Due to the exponantial increase in population, meeting food needs and other life needs through horizontal expansion by cultivating new lands is not possible, It seems that the available solution is through vertical expansion by increasing the yield of maize by increasing the yield of genotypes this is done by applying plant breeding methods and deriving new genotypes with high production capacity,

good quality, and more environmentally friendly. Also the application of integrated nutrient management is integrated with genotypes in achieving the desired goals. Grain yield is a complex trait that is controlled by different morphological and physiological traits [3]. Thus, genetic control of yield is indirectly influenced by traits that are associated with productivity, increasding it, and improved genetic characteristics.

Understanding the relationships between the yield and its components and determining the type of relationship between them can increase grain yield [4]. Yield is a complex trait determined by several variables, so it is necessary to reveal the traits that have the greatest influence on yield and their relative contributions to yield variance. This is useful in designing and evaluating special breeding programs for crops, especially maize various methods that are used to achieve this. Among these methods used by plant

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breeders is the estimation of genotypic and phenotypic correlations, and stepwise regression analysis [5].

It is important to note that the associations between traits are not sufficient to describe the importance of each trait that contributes to grain productivity [6]. As a result, it is important to conduct in-depth studies on trait relations to fully understand the contribution of each trait and then rank its importance in the targeted test.Thus, we tackled stepwise regression analysis.

Stepwise regression is a method for estimating the value of a (dependent) quantitative variable in relation to its relationship with one or more other (independent) quantitative variables. This relationship makes it possible to determine the best return prediction equation. In this method, the gradual regression analysis identifies the appropriate traits for selection in breeding programs through which the yield can be increased [7].

Based on the foregoing, this study aimed at :
1. Estimating the genotypic and phenotypic correlations, identifying the traits most closely related to grain yield, and counting them as selective evidence for improving grain yield.
2. Determining the predictive equation for predicting grain yield to know the traits that control the yield by analyzing stepwise regression.

2. MATERIALS AND METHODS

Afield experiment was conducted in Ibn Al-Bitar Agricultural Preparatory School in Al-Husseiniyah district, which is about 17 km northeast of the holy city of Karbala for the season 2021 AD during the spring season. Six genotypes of maize were planted, and the seeds of these genotypes were obtained from the Agricultural Research Department/Research Department Maize and Sorghum .

The soil was prepared for cultivation in terms of perpendicular plowing, smoothing and leveling.

The genotypes were planted in mixed clay soil on 15/3/2021 with an area of 3×3 m^2 for the experimental unit. The distance was 75 cm apart, and 25 cm between hills leaving a distance of 1.5 m between main plots. A split-plot arrangement was used with in a Randomized Complete Block Design (RCBD) and with three replications.

The fertilizer levels of nitrogen and humic acid, were assigned in main plot that have values of 160 kg N.ha^{-1}, 160 kg N.ha^{-1} with humic acid, 320 kg N.ha^{-1} and 320 kg N.ha^{-1} with humic acid. On the other hand, the genotypes which were six (5018, Bahouth 106, Al-Maha, Fajr 1, Furat, Sarah) were assigned in sub plots.

Phosphate fertilizer was adminstered at a rate of 200 kg P2O5.ha^{-1} in a single dose in the form of Dab fertilizer (18N 46P$_2$O$_5$) during the prepration process of the land for cultivation. The rest of the nitrogen fertilizer was added in two stages.The first after ten days of germination and the second at the silking in the form of urea fertilizer 46% N. Potassium fertilizer was also added at a rate of 80 kg K$_2$O.ha^{-1} in the form of potassium sulfate in two stages with urea fertilizer and according to the scientific recommendation [8].

Humic acid was added at a concentration of 99%, according to the recommendation of the manufacturer, at a rate of 4.81 kg.ha^{-1}, by spraying on the soil, by four sprays, when the land was prepared for cultivation, after 10 days of germination, after twenty-five days of germination, and at silking.

The traits studied are

Number of days from planting to 75% tasseling, Number of days from planting to 75% silking, plant higher, leaf area (cm^2), the percentage of nitrogen in grain, the percentage of protein in grain, percentage of oil, vitality of pollen grains, number of branches in tassel, total lengths of the branches of the tassel, Number of ears per plant, number of grains per ear, the 500 grain's weight (g), yield efficiency, and grain yield kg.ha^{-1}.

3. STATISTICAL ANALYSIS

The values of the phenotypic and genotypic correlation coefficients according to the equations [9, 10].

$$rPxy = \frac{cov.Pxy}{\sqrt{(\sigma^2 Px)(\sigma^2 Py)}}$$
$$rGxy = \frac{cov.Gxy}{\sqrt{(\sigma^2 Gx)(\sigma^2 Gy)}}$$

whereas:
x and y: the traits studied.
$\sigma^2 P$ and $\sigma^2 G$: phenotypic and genetic variation, respectively.
$cov.P$ and $cov.G$: phenotypic and genetic covariance, respectively.
rP and rG: phenotypic and genetic correlations, respectively.

The stepwise regression was estimated to determine a model for describing the traits. A stepwise regression analysis was performed for the obtained data to choose the most significant one of the independent variables that affect the dependent variable (grain yield) based on [11] according to the formula:

$$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n + \varepsilon$$

whereas:
$\hat{Y}$: the dependent factor.
β_0: the constant variable (regression constant).
$\beta_1, \beta_2, \dots, \beta_n$: regression coefficients.
$X_1, X_2, \dots, X_n$: the independent variables.
ε: experimental error.

4. RESULTS AND DISCUSSION

4.1 Genotypic and Phenotypic Correlates

The yield is associated with many genotypic and phenotypic traits and the components of the yield that influence it. The study of the association of these traits with the yield helps plant breeders and workers in the development of grain crops in selection for the trait of the yield or early prediction of the yield by estimating the values of the phenotypic and genetic traits associated with it.

From Table (1) the genotypic and phenotypic correlations, the following can be seen:

Number of days up to 75% tasseling was connected phenotypically, positively, and highly significant with the number of ears per plant 0.74 and genetically with each of the height of the plant and the number of grains per ear (0.74, 0.77), respectively.The trait was also positively, significantly and genetically linked with the number of hairs per plant 0.4., and it was significantly negatively associated with the 500 grain's weight -0.48. In [12] indicated that the number of days up to 50% of tasseling were significantly positively correlated, both genotypic and phenotypically, with plant height 0.44 for each and with the number of earwigs (0.46, 0.47), separately. The characteristic of the number of days from planting to 75% silking was highly significant negatively associated phenotypically and genetically with pollen viability (-0.67, -0.83), respectively.It was also highly significant negatively genetically associated with the yield efficiency characteristic -0.75.

The characteristic of plant height was positively and highly noteworthy correlated with the characteristics of the tasseling (number of branches and total lengths of branches) and the number of hairs (0.70, 0.67, 0.65), individually.The trait was highly significant and positively associated genetically with the characteristics of the tasseling (number of branches and total lengths of branches) (0.99, 0.99), respectively.This agrees with [13], who found that there is a strong positive genetic correlation between tassel branches and plant height of 0.74. Plant height was negatively highly significant, phenotypically, with leaf area, weight of 500 seeds, and yield efficiency (-0.83, -0.94, -0.61), respectively, and genetically highly significant negatively with each of weight of 500 grains and yield efficiency (-0.99) for each.

The leaf area trait was phenotypically highly significant with a weight of 500 grains (0.96) and genotypically positively highly significant with a weight of 500 grains 0.71.On the other hand, the leaf area trait was associated with a highly significant negatively significant phenotypic correlation with plant height and number of grains per ear (-0.83, -0.84), respectively, phenotypically and genetically with the number of earlobes (-0.93, -0.99), correspondingly, and the trait had an important negative correlation with the characteristics of the tasseling (the number of branches of the tasseling and the total lengths of branches in the tasseling (-0.54, -0.55), respectively.

The characteristic of the percentage of nitrogen in grains was associated with a highly significant positive phenotypic and genetic correlation with the weight of 500 grains (0.65), for both, and a highly significant positive genetic correlation with the percentage of protein, the percentage of oil, and the 500 grain's weight amounted to (0.65) for each. The trait was also associated with Highly significant negative correlation phenotypically with the number of ears and the number of grains per ear (-0.83, -0.99), respectively.

The characteristic of the percentage of protein in grains showed a high positive phenotypic and genetic correlation with the weight of 500 grains 0.65 each.This is not consistent with what [14] found, as the results of the genotypic and phenotypic correlation of his experiment showed a non-significant correlation between protein concentration and the weight of 500 grains. The trait was also highly significant negatively, phenotypically and genetically, with the number of grains per ear reaching (-0.83, -0.99), respectively. The characteristic of the percentage of oil in the grains was associated with a highly significant negative correlation, phenotypically and genetically, with the characteristic of the number of grains per ear reaching (-0.76, -0.99), respectively.

The characteristic of the number of branches of the tasseling was associated with each of the weight of 500 seeds and the yield efficiency was negatively highly significant (-0.68, -0.75), respectively.In addition,it was genetically highly significant negatively associated with the weight of 500 grains and the yield efficiency (-0.74, -0.99), respectively.

The sum of the lengths of the tasseling branches showed a highly significant positive correlation, both visually and genetically, with each of the plant height (0.67, 0.99), respectively, and the number of tasseling branches (0.90, 0.99), individually. The characteristic of the total lengths of the tasseling branches was associated with a highly significant negative phenotypic and genetic correlation with the weight of 500 grains (-0.66, -0.74), separately, and the yield efficiency (-0.92, -0.99), correspondingly. Pollen vitality was highly significant, phenotypically and genetically negatively associated with number of days from sowing to 75% silking (-0.67, -0.83), respectively.

The characteristic of grain yield was positively and highly significant with the characteristics of leaf area, weight of 500 grains, and yield efficiency (0.81, 0.90, 0.81), respectively. It was associated with a highly significant positive genetic correlation with the weight of 500 grains and the yield efficiency (0.97, 0.71),

respectively. In [15] indicated that there is a positive and significant genotypic and phenotypic correlation between the yield and its efficiency. The yield trait was also positively and significantly genetically associated with the percentage of nitrogen in grains and the percentage of protein in grains (0.48, 0.48), respectively.This is consistent with [16]. The results of [17] found that yield was negatively associated with protein content. While the trait was negatively significant, phenotypically and genetically, with plant height (-0.92, -0.99), respectively. Likewise, the same trait was associated with a highly significant, negative, phenotypic and genetic relationship with the number of

tassel branches (-0.80, -0.96), respectivelyand the total lengths of tassel (-0.88, -0.99), respectively. With the number of grains per ear -0.66, it was also negatively associated genetically with the number of days from planting to 75% tasseling and the number of ears per plant (-0.52, -0.46), respectively.This is consistent with [12] where he reported a strong negative association at the genotypic and phenotypic level between the yield trait and tasseling.

Accordingly, it is concluded that the weight of 500 grains can be adopted as a selective criterion to improve the grain yield of yellow corn to achieve the highest genetic correlation with the characteristic of grain yield.

TABLE 1. Shows the values of genotypic (below diagonal) and phenotypic (above diagonal) correlations between the studied traits under the influence of nitrogen and humic fertilization.

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15
X1	—	0.71**	0.29	-0.50*	-0.51*	-0.51*	0.31	-0.29	0.01	-0.30	0.74**	0.35	-0.37	-0.08	-0.31
X2	0.81**	—	-0.17	0.07	0.22	0.22	0.81**	-0.39	0.03	-0.67**	0.19	-0.28	0.16	-0.24	-0.02
X3	0.74**	0.29	—	-0.83**	-0.54*	-0.54*	-0.25	0.7**	0.67**	0.29	0.65**	0.47*	-0.94**	-0.61**	-0.92**
X4	-0.01	0.50*	0.07	—	0.74**	0.74**	0.46*	-0.54*	-0.55*	0.02	-0.93**	-0.84**	0.96**	0.35	0.81**
X5	-0.78**	-0.25	-0.96**	-0.01	—	1**	0.56*	0.01	0.11	-0.41	-0.77**	-0.83**	0.65**	-0.27	0.31
X6	-0.78**	-0.25	-0.96**	-0.01	0.99**	—	0.56*	0.01	0.11	-0.41	-0.77**	-0.83**	0.65**	-0.27	0.31
X7	-0.15	0.37	-0.76**	-0.02	0.81**	0.81**	—	-0.35	-0.01	-0.35	-0.31	-0.76**	0.42	-0.32	0.11
X8	0.03	-0.07	0.99**	-0.11	-0.33	-0.33	-0.61**	—	0.90**	0.01	0.24	0.24	-0.68**	-0.75**	-0.88**
X9	0.30	0.32	0.99**	-0.11	-0.26	-0.26	-0.29	0.99**	—	-0.32	0.36	0.14	-0.66**	-0.92**	-0.88**
X10	-0.44	-0.83**	-0.01	-0.04	-0.17	-0.17	-0.23	-0.08	-0.42	—	-0.17	0.03	-0.1	0.27	0.07
X11	0.49*	-0.01	0.25	-0.99**	-0.28	-0.28	-0.01	0.02	0.14	-0.10	—	0.83**	-0.82**	-0.19	-0.65**
X12	0.77**	0.30	0.99**	0.02	-0.99**	-0.99**	-0.99**	0.60**	0.52*	-0.10	0.26	—	-0.71**	0.16	-0.39
X13	-0.48*	0.05	-0.99**	0.71**	0.65**	0.65**	0.50*	-0.74**	-0.74**	-0.09	-0.67**	-0.73**	—	0.50*	0.90**
X14	-0.67**	-0.75**	-0.99**	-0.19	0.49*	0.49*	0.21	-0.99**	-0.99**	0.35	0.30	-0.87**	0.54*	—	0.81**
X15	-0.52*	-0.18	-0.99**	0.51*	0.48*	0.48*	0.27	-0.96**	-0.99**	0.19	-0.46*	-0.66**	0.97**	0.71**	—

* Significant at the 5% level   ** Significant at the 1% level.

X1: the number of days up to 75% of tassel
X2: the number of days up to 75% of silk
X3: plant higher
X4: leaf area

X5: the percentage of nitrogen in the grain
X6: the percentage of protein in the grain
X7: the percentage of oil in the grain
X8: the number of branches of the tassel
X9: Total lengths of the tassel branches

X10: Pollen vitality
X11: the number of ears in a plant
X12: The number of grains per ear
X13: The 500 grain's weight
X14: yield efficiency
X15: grain yield

4.2 Stepwise Regression Analysis

Stepwise regression process was adapted with a semi-automated protocol that included the addition and removal of indepandent variables based on the t and f statistics of its estimated coefficients. Gradual regression was used to remove the effect of ineffective characteristics in the regression model on grain yield.

Grain yield was used as the dependent variable, and the other studied traits were used as predictive variables. A stepwise regression analysis was performed for the obtained data to test the importance of the independent

variables and their impact on the grain yield (dependent).

It is clear from Table (2) that the analysis of variance was carried out in five stages, all of which were highly significant at the 1% level.

TABLE 2. Analysis of variance for regression analysis of sequential steps for the studied traits

ANOVAª

Model	Sum of Squares	Df	Mean Square	F
1 Regression	47820809.884	1	47820809.884	94.378[b]**

	Residual	35468548.336	70	506693.548	
	Total	83289358.220	71		
	Regression	59005946.100	2	29502973.050	83.831[c]**
2	Residual	24283412.121	69	351933.509	
	Total	83289358.220	71		
	Regression	68705065.360	3	22901688.453	106.780[d]**
3	Residual	14584292.860	68	214474.895	
	Total	83289358.220	71		
	Regression	79415929.254	4	19853982.313	343.421[e]**
4	Residual	3873428.967	67	57812.373	
	Total	83289358.220	71		
	Regression	79413050.961	3	26471016.987	464.367[f]**
5	Residual	3876307.259	68	57004.519	
	Total	83289358.220	71		

** Significant at the 1% level.

a. Dependent Variable: VAR00015

b. Predictors: (Constant), VAR00013

c. Predictors: (Constant), VAR00013, VAR00012

d. Predictors: (Constant), VAR00013, VAR00012, VAR00014

e. Predictors: (Constant), VAR00013, VAR00012, VAR00014, VAR00004

f. Predictors: (Constant), VAR00013, VAR00014, VAR00004

Table (3) shows the results of the stepwise regression. whereas:

The first stage comprised the introduction of the trait the 500 grain's weight (X13) into the form, and the coefficient of determination for this stage was 0.57.The coefficient of determination increased in the second stage to 0.70 after adding the trait the number of grains per ear (X12) so that the model involved the traits weight 500 grain (X13) and the number of grains per ear (X12) together. In the third stage, the trait yield efficiency (X14) was added This led to an increase in the coefficient of determination of 0.82.Thus, the model contained the 500 grain's weight (X13), the number of grains per ear (X12), and yield efficiency (X14).

Then the trait leaf area (X4) was introduced in the fourth stage in addition to the previous traits, which increased the determination coefficient to become 0.95.

Concerning the fifth and final step, the trait the number of grains per ear (X12) was excluded and the traits weight 500 grain (X13), yield efficiency (X14) and leaf area (X4) were retained in the model because it did not affect the determination coefficient, as it remained at the same value of 0.95 with the reduction of the experimental error of estimation from 240.44 to 238.76.

The features that were entered into the prediction equation are the features that had the highest coefficient of determination and that were associated with the yiald. The prediction equation was formulated as follows:

$$\hat{Y} = -7048.23 + 7.82(X13) + 26.98(X14) + 1.20(X4); \; R^2 = 0.95$$

According to this equation, 95% of the total variance in grain yield can be linearly related to the variables accepted in the regression analysis.

Acceptable variables are the 500 grain's weight (X13), yield efficiency (X14) and leaf area (X4) as these traits were responsible for yield variance. In [18] agreed with this, who stated that serial regression analysis proved that leaf area and weight of 500 grains had the greatest effect on the total variance of maize yield in intercropping with cowpeas.

The results of [5] for stepwise regression analysis of maize revealed that the acceptable variables are the weight of 1000 grains, the number of grains per row, the number of rows per ear, and the harvest index were responsible for (82.2%, 2.9%, 2.3%, and 1.5%), respectively, of the total variance of yield.

These outcomes reinforce the results of genetic associations, since the trait weight of 500 grains is the most determinant of grain yield.

As for the rest of the variables, they were removed because they had no effect on the yield.

In the regression of the stepwise, the variable added at each step was the one that achieves the largest decrease in the experimental error of estimation. It is the variable that has the highest relative contribution to the coefficient of determination with the variable dependent on the fixed values of those previously added variables.

TABLE 3. Sequential step regression analysis equations

Step	Equations	R^2	Coff. Std. Error
1	$\hat{Y} = 2715.16 + 40.67(X13)$	0.57	711.82
2	$\hat{Y} = 115.57 + 37.82(X13) + 5.13(X12)$	0.70	593.24
3	$\hat{Y} = -2660.28 + 32.31(X13) + 5.57(X12) + 12.89(X14)$	0.82	463.11
4	$\hat{Y} = -7071.94 + 7.56(X13) - 0.12(X12) + 27.15(X14) + 1.21(X4)$	0.95	240.44
5	$\hat{Y} = -7048.32 + 7.82(X13) + 26.98(X14) + 1.20(X4)$	0.95	238.76

5. CONCLUSION

The ruslts have shown that the closest related treats to the yield were the 500 grain's weight, the yield efficiency and the leaf area. This was confirmed by the stipwise regression analysis. When the highest genetic correlation was achieved between the 500 grain's weight and the grain yield, this enables us to consider this trait as a selective criterion for improving grain yield.

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Arabic Abstract

نفذت التجربة الحقلية في حقل التجارب التابع الى اعدادية ابن البيطار المهنية في قضاء الحسينية – محافظة كربلاء المقدسة خلال موسم الربيعي 2021، بهدف تحديد الصفات الأكثر ارتباطاً بمحصول الحبوب وتحديد المعادلة التنبؤية لستة تراكيب وراثية من الذرة الصفراء (5018، بحوث 106، المها، فجر1، الفرات وساره) وتقدير بعض المعالم الوراثية بتأثير اربعة مستويات من التسميد (160 N كغم.هـ-1، 160 N كغم.هـ-1 مع حامض الهيومك، 320 N كغم .هـ-1، 320 N كغم.هـ-1 مع حامض الهيومك) باستخدام ترتيب الالواح المنشقة وفق تصميم القطاعات الكاملة المعشاة RCBD بثلاثة مكررات، حيث تمثل التراكيب الوراثية للذرة الصفراء الألواح الثانوية والتسميد يمثل الالواح الرئيسية.

تم دراسة عدة صفات نمو ونوعية وصفات الحاصل وبعض مكوناته بالإضافة الى دراسة بعض صفات النورة الذكرية وحيوية حبوب اللقاح. تم دراسة الارتباطات الوراثية والمظهرية و تم ايجاد معادلة تنبؤ بالحاصل من خلال اجراء تحليل الانحدار المتسلسل (التدريجي).

تبين من تحليل الارتباطات المظهرية والوراثية ان قيم الارتباطات الوراثية اعلى من قيم الارتباطات المظهرية لأغلب الصفات. ارتبط حاصل الحبوب ارتباطا مظهرياً موجبا عالي المعنوية مع صفات المساحة الورقية ووزن 500 حبة وكفاءة الحاصل، وارتبطت ارتباطا وراثيا موجبا عالي المعنوية مع وزن 500 حبة (0.97) وكفاءة الحاصل (0.71). اتضح من تحليل الانحدار المتسلسل ان الصفات المستقلة التي ترتبط ارتباطاً وثيقاً مع المتغير التابع (الحاصل) كانت وحسب المعادلة التنبؤية للعائد هي وزن 500 حبة وكفاءة الحاصل والمساحة الورقية حيث كانت هذه الصفات مسؤولة عن 95% تباين الغلة.

نستنتج مما سبق امكانية استخدام 500 حبة معيارا انتخابيا لتحقيقه اعلى ارتباط وراثي مع حاصل الحبوب وقد اكد تحليل انحدار الخطوات المتسلسلة ذلك.