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Estimation Genetic Diversity by Using Cluster, D^2 and Principle Component Analysis of Maize Inbred Lines (*Zea mays* L.)

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ABSTRACT

Key Words :
Zea mays , genetic divergenece , Cluster analysis,D^2, Principal Components.

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A field experiment carried out by using fourteens inbred lines of Maize (local line(1) , 839(2) ,C.V890(3) , Iq10001(4) , ZM4L(5), ZM51L(6) ,ZP-301(7) ,ZM19L(8) , OH(9) , UN440(10) , IK-58(11) ,IK-8(12) , SH(13) and R-153(14)) at the agricultural field of Kirkuk Agricultural directorate during 2017 Autumn season by using Randomized Completely Block Design with three replications to estimate genetic distance using cluster , $D^2$and principal component analysis for grain yield , it's components and qualitative traits. The Cluster analysis classified lines in to two main groups depending on the morphological marker traits. So that the largest genetic distance was between local line and C.V890 line (9547033.93) which can be utilized in hybridization for exhibiting hybrid vigor. The D^2 analysis revealed that the largest distance was between the second group lines (C.V890 , Iq10001 , ZM4L , ZM51L and ZP-301) and third group lines (OH and SH).Principle components analysis explained 88% of total variances among lines , however the number of grains.ear^{-1} regarded the major trait which has largest contribution of the total variations followed by days to silking , no.rows.ear^{-1} ,grain yield and protein content.ZM19L ,OH and SH lines can be considered as a source for improving grain yield ,while local adapted line for improving ear length and other grain components also, Iq10001 line was important for improving quality traits and total digestible nutrients.

تقدير التباعد الوراثي بأستخدام التحليل العنقودي وتحليل D^2 و المكونات الأساسية لسلالات من الذرة الصفراء (*Zea mays* L.)

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الخلاصــة

الكلمات المفتاحية:
الذرة الصفراء، التباعد الوراثي، التحليل العنقودي، D^2 ، المكونات الاساسية.

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أجريت تجربة حقلية بأستخدام 14 سلالة نقية من الذرة الصفراء .*Zea mays* L و هي (سلالة محلية (1) وسـلالة 839 (2) وC.V890 (3) وIq10001 (4) وZM4L (5) و ZM51L (6) و ZP-301 (7) وZM19L (8) وOH (9) و UN440 (10) وIk-58 (11) وIk-8 (12) وSH (13) و (14) R-153) في الحقل الزراعي لمديرية زراعة محافظة كركوك خلال الموسم الخريفي 2017 و بأستخدام تصميم القطاعات العشوائية الكاملة (R.C.B.D) بثلاثة مكررات بهدف تقدير التباعد الوراثي بين السلالات باستخدام التحليل العنقودي و تحليل الـD^2 والمكونات الأساسية لصفات الحاصل الحبوب و مكوناته و لصفات النوعية . صنف التحليل العنقودي السلالات الى مجموعتين رئيستين اعتمادا على بيانات المؤشرات المظهرية و أن أكبر بعد وراثي كان ما بين السلالة المحلية و السلالة C.V890 و بلغ 9547033.93 و التي يمكن استغلالها في التهجين لابراز قوة الهجين . كما أظهر تحليل الـD^2 أن أكبر مسافة كانت بين سلالات المجموعة الثانية (C.V890 و lq10001 و ZM4L و ZM51L و ZP-301) وسلالات المجموعة الثالثة (OH وSH) و قد فسر تحليل المكونات الأساسية ما نسبته 88% من التغايرات بين السلالات و تعد صفة عدد الحبوب بالعرنوص المسؤولة عن النسبة الأكبر للتغايرات بين السلالات تلتها صفة عدد الأيام للتزهير الأنثوي وعدد الصفوف بالعرنوص و الحاصل الحبوبي و نسبة البروتين. يمكن اعتبار السلالات (ZM19L وOH و SH) كمصدر لتحسين الحاصل الحبوب و بينها السلالة المحلية لتحسين طول العرنوص ومكونات الحاصل الأخرى و سلالةIq10001 كمصدر لتحسين الصفات النوعية والعناصر الغذائية المهضومة.

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[1] The research selected from the theses for the first author

Introduction:

Maize Croup is one of the most important cereal crops which considered third cereal groups followed wheat and rice respecting of cultivated area, productivity and consumption(White and Johanson ,2003).Maize genotypes differ significantly in their agronomic and quality traits that regard as the raw material of plant breeder of breeding and improving programs. Preseeding studies revealed significant differences among genotypes in maize traits especially in days to silking and plant height (Al-Fahdawi et al ,2006 and Al-Kaisy , 2013) and grain yield and it's components (Al-Assafiy ,2002 ; Hamadan and Baktash ,2011 ; Al-Zuhairy and Al-Zubaidy, 2012 and Al-Talibany ,2016) besides grain quality traits (Martin et al ,2012 and Rodrigus et al ,2014).Production super hybrids and synthetic varieties of Maize are very important in increasing productivity and improving quality that depends on select different parents for utilizing hybrid vigor (Ashish et al ,2007).Selected parents should be differ from each other in what about genetic, morphological and agronomical traits.It not related with the previous statement many biometrical methods can be used by plant breeders to assesment similarity and dissimilarity among genotypes such as cluster analysis (Hamadallah ,2011) which classify genotypes according to genetic distance (Stuckburger ,2011) in respect of different traits such as grain yield and it's components and quality traits(Baktash and Abdelhameed ,2015 ; Al-Talabany ,2016 and Kumar et al ,2016).Wheanever large genetic distance among lines mean large differences from each other(Hamadalluh ,2011 ;Al-Dawoody ,2016 and Al-Joboory ,2016). D^2 analysis is the another important technique which used for evaluating genetic diversity within and between formed groups to understand the nature of similarity and dissimilarity among lines (Alam et al ,2013 and Seshu et al ,2014).Principle component analysis technique widely used for determining contribution of each trait in the total divergence among specific genotypes (Afuape et al ,2011 ; Ajmal et al ,2013 and Malik et al ,2014).The estimate genetic divergence by using cluster ,D^2 and Principle component analysis are important in screening different lines and diminished effort, time and costs and directed breeding program towards right path (Al-Zuhairy and Al-Zubaidy,2012 and Jackson ,1991).Therefore, the study aim to evaluate maize lines and estimating genetic diversity in grain yield and it's components and quality by using cluster,D^2 and principle component analysis.

Materials and Methods:

A field experiment was carried out in the field farm of the agricultural Kirkuk directory \Kirkuk Province at the 2016 Autumn season by using 14 maize lines as shown in the table (1) (obtained from College of Agriculture \ University of Baghdad) for evaluating and estimating genetic diversity among studied lines.

Randomized Completely Block Design with three replications was used after soil plowing , harrowing and leveling. Seeds were sown at 20 × 75 cm within and between .Other crop management applicated as demand. The studied traits were: days to silking ,plant height(cm), No.ear.plant^{-1},No.grains.ear^{-1},ear length(cm) ,1000grain weight(gm),specific weight(hectoliter) ,and percentage of each protein, oil, ash, fiber, nitrogen free extract and total digestable nutrients. The significant differences trait means was estimated according to Dawood and Abdulyas (1990). Then cluster analysis used for grouping lines according to pattern responses which starts from formation approximates matrix followed by dendogram depending on UPGMA method (Sneath and Sokai ,1973 and Williams ,1976).Euclidean distances calculated in this method represent the degree among average groups means of approximates matrix. It was calculated from the following formula: $\text{Distance}(x,y)=[\sum(xi-yi)^{\wedge 2}]^{\wedge 0.5}$ (Punitha et al,2010).Cluster analysis performed by using SPSS program. The genetic distances and diversity between and within formation groups of D^2 statistical analysis calculated by using Microsoft excel program. Canonical method was used when the significant dispersion of D^2 was exist. After means transformation the distances calculated through matrices and plotting diagram of Z1 against Z2: Z1=[y][V1] , [y]:transformed mean , [V1]:first vector ,[V2]:second vector (Singh and Chaudhary ,2007).Principle component analysis achieved by using Minitab statistical program depending on the correlation relationships among traits for determining the trait that caused of the large portion of divergence from the total variation at each score.Also plotting C1: Coordinate 1 against C2: Coordinate 2 for checking if the analysis in the right way (Jackson ,1991).

Table (1) Names and cods of studied lines

Cod	Lines name
G1	local line
G2	Line No.839
G3	C.V.890
G4	Iq10001
G5	Zm4L
G6	Zm51L
G7	ZP-301
G8	Zm19L
G9	0H
G10	Un440
G11	IK-58
G12	IK-8
G13	SH
G14	R-153

Results and Discussion:

Significant differences among most studied traits except specific weight were appeared in the analysis of variance(table 2).G9 line has shortest duration: days to 50%silking and lasted 56.67 days and possessed largest number of ears per plant (1.37 , table 3).Plant height was in max value (212.63 cm) in G1 line. The superiority in these traits perhaps for utilizing their genetical and physiological ability in transformation photosynthesis products for growth and elongation of stem cells besides the importance of the efficient genotype (Al-Fahdawi et al ,2006).Number of rows.ear^{-1} and no.grains.ear^{-1} are important grain yield components in maize and the results showed significant differences among lines in these traits.G12 line gave high mean of number no.rows.ear^{-1}(18.13 cm) while G10 gave highest mean of number of grains.ear^{-1}(775.47 grain).The differences in genetic factors may be affected and shifted their behavior besides the large number of genes controlling these traits (Hamdan and Baktash ,2011).G2 line gave higher value of ear length (17.97) cm,1000 grain weight (259.75 gm) and ash content (1.33%).Ear length contributed positively in increasing grain yield through enhancing either grains number and grains weight especially in case of G2 line. Also carbohydrates should translocate from source to sink (small number of grain) and need to increase their 1000 grains weight (Al-Assafy ,2002 and Al-Zuhairy and Al-Zubaidy ,2012).Maximum grain yield was in G8 line (13607.31 Kg\ha) which significantly differed from G3 ,G5 and G12.High grain yield in G8 results from high range in other grain components: number ears.plant^{-1} ,no.grains.ear^{-1} and 1000 grain weight(table 3).Also Eessa (1984) confirmed that major grain yield components have important role in increasing grain yield. Quality traits showed significant differences among lines, especially in protein and oil content.G5 line gave maximum protein content (12.07%) which perhaps explains by the differences in physiological activities (Egli ,2001). While G4 line was superior in oil content (4.67%).The relations between starch and oil content in grain for each genotype has a large effects on the maize grain oil content (Kadhum and Ramadhan .2013).Studied lines revealed significant differences in other grain quality traits (fiber content ,nitrogen free extract and total digestible nutrients).Lesser ratio of fiber content 13.01% was in G14 than others. Whenever decreased fiber content the feed value of grain would increased. Highest values of nitrogen free extract (70.17%)were recorded for each G6 and G7 lines which mostly correlated positively with improving soluble carbohydrate and grain feed energy. These results are agree with the results stated by Rodrigues et al (2014).Total digestible nutrients has deep effect on grain yield quality as G4 and G14 gave highest the values (89.87 and 89.61% respectively) which may results from improving quality traits especially oil content in G4 line and low fiber content in G14 (table 3) .That which in turn reflected positively of increasing percentage of total digestible nutrients these results are agree with Martin et al (2012).

Table (2):Analysis of variance represents by mean sum of squares of studied traits

d.f.	S.O.V.		
	Rep.	Lines	Error
	2	13	26
Traits			
Days to 50%silking	108.64	15.36 **	3.39
Plant height (cm)	1759.67	547.21**	35.8
No.ears.plant^{-1}	0.13	0.01**	0.00
No.rows.ear^{-1}	17.67	4.00**	0.53
Ear length(cm)	26.16	4.19**	1.07
no.grais.ear-1	91973.90	11207.86**	3094.47
1000grain weight (gm)	10593.70	1325.43**	311.46
Specific weight (hect.)	4114.80	1779.82 ns	785.72
Grain yield (Kg/he)	84922594.8	7185637.73**	2496128.48
Protein content (%)	3.04	0.39**	0.13
Oil content (%)	0.93	0.22**	0.03
Ash content(%)	0.27	0.01**	0.007
Fiber content (%)	19.42	1.51**	0.28
NFE cntent (%)	1.40	0.99**	0.12
TDN content (%)	0.43	0.31**	0.05

NFE: nitrogen free extract , TDN: total digestible nutrients
*:significant at 0.05 level ,**:significant at 0.01 level

Table (3):means of studied traits

lines	Days to 50% silking	Plant height (cm)	No.ears .plant^{-1}	No.rows.ear^{-1}	Ear length (cm)	No.grais.ear^{-1}	1000 grains weight (gm)	Specific weight (hectoliter)	Grain yield (Kg.h^{-1})	Protein content (%)	Oil content (%)	Ash content (%)	Fiber content (%)	NFE cntent (%)	TDN content (%)
G1	58.33	212.63	1.27	17.47	16.37	639.8	202.07	11030.96	768.8	11.94	3.8	1.17	13.13	69.96	88.78
G2	59.33	185.87	1.2	14.87	17.97	546.07	259.75	10999.82	771.87	10.94	4.17	1.33	14.73	68.84	89.01
G3	58.33	176	1.17	15.27	16.47	614.93	210.63	9368.14	807.47	11.49	4.33	1.15	13.78	69.25	89.33
G4	62.67	171.17	1.17	14.53	16.7	525.33	248.54	10500.91	782.13	10.82	4.67	1.16	13.21	70.14	89.87
G5	61.33	196.67	1.1	15.73	17.27	670.8	214.75	9524.23	811.73	12.07	4.2	1.1	13.17	69.47	89.20
G6	63.33	199.57	1.2	15.73	17.83	637.67	205.18	10737.83	803.4	11.28	3.73	1.19	13.62	70.17	88.77
G7	59.33	200.07	1.2	16.8	15.43	644.2	214.88	11745.50	801.73	11.15	4.17	1.27	13.25	70.17	89.21
G8	62	202.23	1.3	16.73	16.33	663.13	227.82	13607.31	799.33	10.98	4.23	1.21	13.95	69.63	89.27
G9	56.67	183.63	1.37	16.87	15.03	627.27	223.94	12966.11	791.2	11.66	4.3	1.17	13.54	69.33	89.27
G10	59.33	189.07	1.23	17.87	17.73	775.47	182.29	11146.70	793.33	11.07	4.1	1.25	14.86	68.71	88.97
G11	58.67	167	1.27	16.73	15.47	591.53	242.81	12615.64	782.13	11.31	3.83	1.02	14.79	69.05	88.87
G12	63	185.43	1.23	18.13	13.73	615.93	212.19	8036.38	713.93	11.32	4.4	1.19	14.18	68.91	89.34
G13	63	190.8	1.37	17.07	16.43	666.93	195.23	12029.52	777.47	11.35	3.97	1.2	14.92	68.57	88.82
G14	57.67	207.8	1.27	17.93	16.9	689.8	220	12713.62	786.67	11.35	4.5	1.16	13.01	69.97	89.61
LSD0.05	3.85	12.52	0.14	1.52	2.16	116.28	36.89	NS	58.59	0.75	0.37	0.18	1.20	0.708	0.47

NFE: nitrogen free extract , TDN: total digestible nutrients

Cluster analysis:

The significant statistical differences among lines in most studied traits may refer to their genetically differences from each other therefor cluster analysis classified lines (through pattern similarity according their differences) by using hierarchical .cluster analysis clustering classified studied lines in to six groups (Fig.1).First group consisting of 5 lines (G1,G2,G4,G6 and G10)(table 4).Second and fifth group are consisting of two lines of each other :G7 and G13 (group 2)and G3and G5(group 5).While the third group consisting of three lines (G9,G11 and G14).G8 and G12 lines differ from other lines and each one has located at separate group. Forth and Sixth group for G8 and G12 respectively, which indicate of large genetic distance from other studied lines. These findings are corresponding with what obtained by Abdelhameed and Baktash (2014) ,Kumar (2016) and Al-Talabany (2016) about existence genetic distance among maize genotypes.

Table (4) : Names and numbersof lines for each group of cluster analysis

Groups	Number of lines	Lines name
I	5	G1,G2,G4,G6,G10
II	2	G7,G13
III	3	G9,G11,G14
IV	1	G8
V	2	G3,G5
VI	1	G12

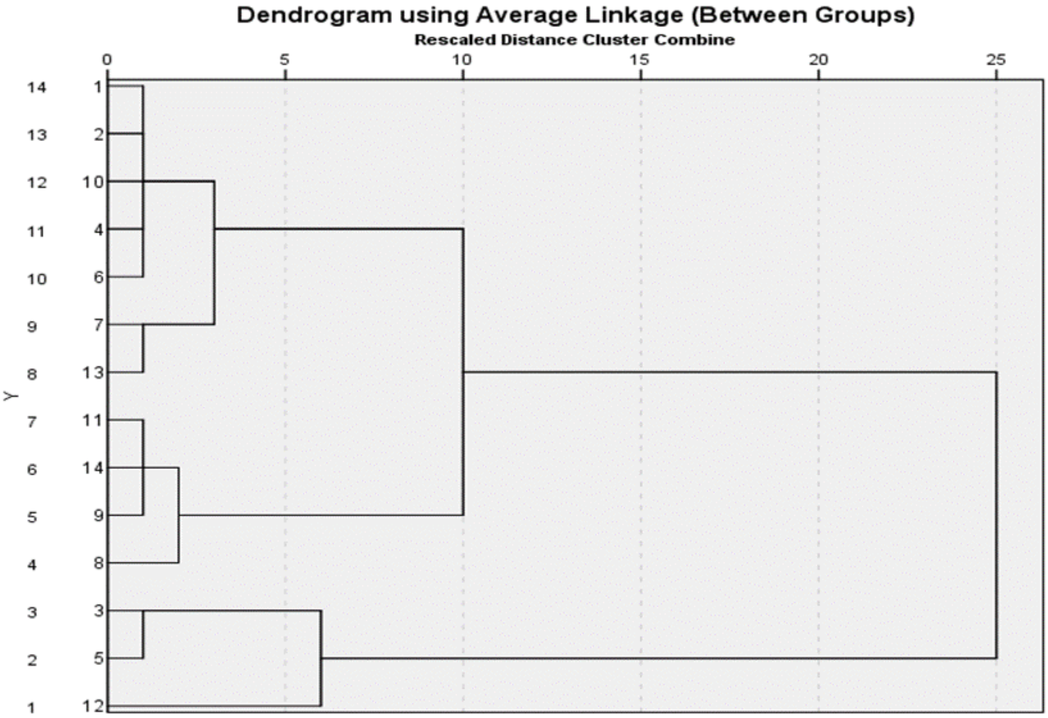

Fig.1 Dendogram showing the genetic relationship among studied lines based on morphological traits, material,Distance and Coefficient.

The stages of formation cluster diagram through merge lines depending on Euclidean distance in fig.1 .and table (5).First stage starts by merging G1 with G2 line to form group (1) which posses lesser Euclidean distance representing as coefficients in table (5).The merging of lines in groups continuous till the maximum coefficient. The latest merge lines possessed largest coefficients. The coefficient ranged from 13823.88 for G1+G2 and 9547033.93 for G1+G3.The high values of coefficient (Euclidean distance)between G1 and G3 refers to high divergence between them and be useful in breeding program and vise versa (Hamadallah ,2011).The average means of studied traits varied among created groups.G8 line of forth group gave high value of plant height (202.23cm), no.ears.plant^{-1}(1.3) ,no.grains.ear^{-1} (663.13) ,grain yield (13607.31 Kg\ha) and NFE (69.63%) which can be regarded in improving these traits in breeding programs. While the sixth group composed of the G12 line which was superior in no.rows.ear^{-1} (18.13), oil content (4.4%) ,fiber content (14.18%) and total digestible nutrients (89.34%) could be useful as a source of improving quality traits. However the third group (G9 ,G11 and G14) possessed the biggest no.ears.plant^{-1} (1.3) , 1000grain weight (228.91 gm) and shortest duration to silking (57.67 day).Therefore, it can be important in improving flowering earliness. Average quality traits means were superior in the fifth group (G3 and G5 lines) in specific grain weight (809.6hectoliter) and protein content (11.79%).Besides second group (G7 and G13) for crude ash content (1.23%) which considered as a source of improving quality traits. Otherwise first group (G1 ,G2 , G4 ,G6 and G10) was superior in ear length (17.32 cm)and low value of fiber content (13.91%) and high mean of total digestible nutrients (89.08%). It can be conclude that the superiority in a large number of agronomic traits may leads to improve maized quality traits (Al-Joboory ,2016).

Table (5): Estimated coefficients among groups of cluster analysis

Stage	Cluster 1	Cluster 2	Coefficients
1	1	2	13823.88
2	11	14	21481.14
3	3	5	27962.57
4	1	10	57093.45
5	4	6	71900.05
6	7	13	82301.17
7	9	11	96572.43
8	1	4	231337.75
9	8	9	734301.91
10	1	7	1093870.84
11	3	12	2004511.87
12	1	8	3668531.47
13	1	3	9547033.93

Table (6): Average studied traits of formed groups

Traits	I	II	III	IV	V	VI
Days to 50%silking	60.6	61.17	57.67	62	59.84	63
Plant height (cm)	191.66	195.43	186.14	202.23	186.33	185.43
No.ears.plant^{-1}	1.21	1.28	1.3	1.3	1.13	1.23
No.rows.ear^{-1}	16.09	16.93	17.18	16.73	15.5	18.13
Ear length(cm)	17.32	15.93	15.8	16.33	16.87	13.73
No.grais.ear^{-1}	624.87	655.57	636.2	663.13	642.87	615.93
1000grains wieght(gm)	219.57	205.05	228.91	227.82	212.69	212.19
Specific weight (hectoliter)	783.91	789.6	786.67	799.33	809.6	713.93
Grain yield (Kg.h^{-1})	10883.24	11887.51	12765.12	13607.31	9446.19	8036.38
Protein content (%)	11.21	11.25	11.44	10.98	11.79	11.32
Oil content (%)	4.09	4.07	4.21	4.23	4.27	4.4
Ash content (%)	1.22	1.23	1.12	1.21	1.13	1.19
Fiber content (%)	13.91	14.09	13.78	13.95	13.47	14.18
NFE content (%)	69.57	69.37	69.45	69.63	69.36	68.91
TDN content (%)	89.08	89.01	89.25	89.27	89.26	89.34

D^2 Analysis:

D^2 statistical analysis aims to calculate genetic distances between and within groups. Lesser distance (28.77) between G1 and G14 lines (table 7) refers to the similarity of these lines and crossing between them should be avoided. While longest distance (42649.61) was between G13 and G5 lines ,which indicates of such lines variation and crossing them may be useful for obtaining hybrid vigor in the studied traits (Kumar et al ,2015). Analysis of variance for the significant dispersion revealed high significant differences of the distances among genotypes (table 8) which indicates the existence of genetic variation. Studied traits lines classified in to three groups according to plot Z1 against Z2 according to their traits.Group1 (7 lines) ,group2 (5lines) and group3 (2 lines) (Fig.2 and table 9).The dissimilarity would be high if the distance between lines was long. Hence maximum distance was between G13 and G5 lines followed by G13 with G4 , G13 with G7 ,G9 with G4 and G13 with G6.The shortest distances were between G6 with G7 lines in the second group followed by G1 with G14 in the first group, which refers to the similarity among genotypes. The distances between and within groups were calculated in table (10).Diagonal values represent the distances of group. Longest distance (141) was in the first group, which consist of longer number of lines than other groups. Shortest distance recorded in the third group (66.66) which mean that first group lines differ from each other. The up diagonal elements refer to distances among groups which were in the highest values between second and third group which clarify importance of diversity. These groups lines may be useful in exploitation hybrid vigor in crossing program towards desired traits.The results were agreed with findings of Alam et al (2013) and Seshu et al (2014).The variation in of studied lines in the genetic distances reflected on their ability and agronomic traits. Table (11) refer to the differences of groups in the studied traits and these differences focused on grain yield in the third group gave the highest grain yield (12497.82 Kg/ha), protein content (11.51%) ,fiber content (14.23%) ,no.grains.ear^{-1} (647.1) and no.ears.plant^{-1}(1.37).While second group distinguishes by higher ear length (16.74cm) ,specific weight (801.29 hectoliter) ,oil content (4.22%), nitrogen free extract (69.84%) and total digestible nutrients (89.27%).First group possessed the shortest duration to silking (59.76 day) and greatest no.rows.ear^{-1} (17.11), 1000 grain weight (220.99gm) and ash content (1.19%).Therefore, can be useful of improving flowering earliness. Also the first group can be used grain yield and it’s components besides the importance the second group lines in in quality traits of breeding programs.The results are along with Singh et al (2005).

Table (7):D^2 of studied lines

Lines	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14
G1	0	16011.23	11818.09	6075.50	19007.89	2748.09	2734.05	1745.57	8339.10	12966.39	8004.27	10478.34	20073.15	28.77
G2		0	2404.79	13867.75	8909.08	15992.99	16534.18	12522.84	18642.13	687.97	3028.33	991.30	15043.05	16429.82
G3			0	5644.03	2561.74	8228.40	8599.58	12331.76	22800.34	3715.70	5789.31	3151.58	24471.01	12120.44
G4				0	5656.73	890.75	976.39	11339.65	25259.57	14501.62	13219.97	12062.03	36825.04	6102.95
G5					0	10235.72	10604.82	22929.69	38769.94	12137.54	15837	11220.38	42649.61	19282.06
G6						0	40.87	7584.34	19578.21	15227.46	12244.71	12540.61	32653.46	2777.84
G7							0	7719.07	19717.92	15686.92	12643.87	12961.68	33128.48	2717.22
G8								0	2866.82	8813.03	3963.53	7009.61	10240.86	1841.76
G9									0	12967.62	6758.88	11683.29	4442.83	8492.51
G10										0	1183.76	204.71	10053.80	13355.76
G11											0	774.06	6738.93	8319.99
G12												0	10336.97	10808.19
G13													0	20474.15
G14														0

Table (8) :Analysis of Covariance of D^2

S.O.V	Df	SS	Ms	Fca.	Ftab.
Variaties	13	3.99E+14	3.07E+13	10856011.1	**
Error	25	70724564.25	2828982.57		
Total	38	3.99E+14			

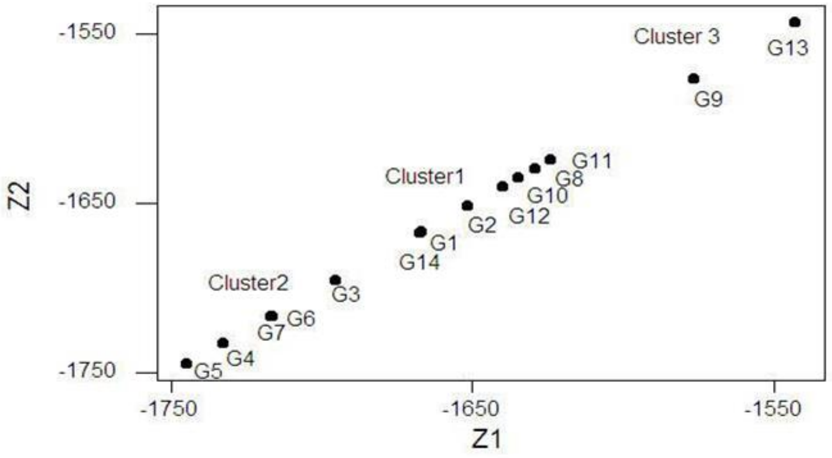

Fig.2 The diagram of plot Z1 against Z2

Table (9):The created groups and their lines

Groups	Lines
cluster 1	G1,G2,G8,G10,G11,G12,G14
cluster 2	G3,G4,G5,G6,G7
cluster 3	G13,G9

Table (10) :Squire root of mean distance among and within created groups

	cluster 1	cluster 2	cluster 3
cluster 1	141	103.91	109.43
cluster 2		106.32	172
cluster 3			66.66

The principle component analysis is characterized by calculating the participation proportion each trait from the total variation. Using of such knowledge to determine at what level the correlation value is important. Accordingly the analysis of principle components of fifteen multivariate traits showed the importance of six PC that each one has eigenvalue more than 1 and explained 88% of total variation among studied lines (table 12).The number of grains.ear^{-1} took part of the greatest variation (0.405) followed by no.rows.ear^{-1} (0.386) from the total variation (0.244) of first principle components. While second principle component explained 0.184 from total morphological variation among studied lines and the most traits that contributed of these differences were nitrogen free extract (0.472), plant height (0.377) and specific weight (0.373).Third principle component illustrated 0.14 of total variations and the major traits affected were no.rows.ear^{-1} (0.394) ,total digestible nutrients (0.328) and oil content (0.319). while other coefficients of principle components were 0.124 , 0.11 and 0.075% from the total variation for the four ,five and six principle components analysis, respectively. The most important affected traits were grain yield and no.ears.plant^{-1} of pc4 (0.411) and protein content for pc5 (0.429) and days to silking (0.329) and plant height (0.309) for the pc6.It can be concluded that the most variation was in the first principle component than other components followed by second principle components. These results are agree with (Nestory and Reuben , 2016).

Table (11) :Means of created groups of D^2 analysis

Traits	Groups		
	1	2	3
Days to 50%silking	59.76	61	59.83
Plant height (cm)	192.86	188.69	187.23
No.ears.plant-1	1.25	1.17	1.37
No.rows.ear-1	17.11	15.61	16.97
Ear length(cm)	16.36	16.74	15.73
No.grais.ear-1	645.96	618.59	647.1
1000grains weight (gm)	220.99	218.80	209.58
Specific weight (hectoliter)	773.72	801.29	784.33
Grain yield (Kg/he)	11450.06	10375.32	12497.82
Protein content (%)	11.27	11.36	11.51
Oil content (%)	4.148	4.22	4.13
Ash content (%)	1.19	1.18	1.19
Fiber content (%)	14.09	13.41	14.23
NFE cntent (%)	69.30	69.84	68.95
TDN content (%)	89.12	89.27	89.04

Table (12) :The ratio of principle component variation

1.1238	1.6492	1.8539	2.1744	2.7540	3.6526	Eigenvalue
0.075	0.110	0.124	0.145	0.184	0.244	Proportion
0.881	0.806	0.696	0.572	0.427	0.244	Cumulative

Table (13) : Coefficients of principle components of studied traits

Traits	PC1	PC2	PC3	PC4	PC5	PC6
Days to 50%silking	-0.078	-0.120	-0.158	-0.350	-0.266	0.329
Plant height (cm)	0.263	0.377	0.039	-0.00	-0.306	0.309
No.ears.plant^{-1}	0.285	-0.212	0.215	0.411	0.007	0.172
No.rows.ear^{-1}	0.386	-0.051	0.394	0.009	-0.157	-0.027
Ear length(cm)	-0.002	0.208	-0.550	0.048	-0.126	-0.176
No.grais.ear^{-1}	0.405	0.166	0.020	-0.034	-0.220	-0.414
1000grains wieght(gm)	−0.392	-0.133	-0.042	0.293	0.160	0.215
Specific weight (hectoliter)	-0.004	0.373	-0.334	0.236	0.155	-0.327
Grain yield (Kg.h^{-1})	0.151	0.052	-0.020	0.676	0.050	0.084
Protein content (%)	0.193	0.262	0.183	-0.278	0.429	-0.040
Oil content (%)	-0.342	0.006	0.319	0.066	-0.301	-0.381
Ash content (%)	0.033	-0.069	-0.204	0.078	-0.604	0.105
Fiber content (%)	0.165	-0.510	-0.256	0.034	0.006	-0.152
NFE content (%)	-0.168	0.472	0.078	0.092	-0.058	0.379
TDN content (%)	-0.379	0.096	0.328	0.097	-0.228	0.280

The score plot of traits in Fig.3 is important in the use of outlier plot to identify any point that is above the reference line.It can significantly affected the results of the analysis. Therefore if identify an outlier in data ,then should examine the observation to understand why it is unusual and correct any measurement or data entry error consider removing data that are associated with special causes and repeating the analysis.(Jackson ,1991).

The results in Fig.3 showed no outliers and all points are below the reference line.First principle component measures long-term stability trait.Number of grains.ear^{-1} and number rows.ear^{-1} are most stable traits and have a large effect in the divergence among lines because of their higher coefficients that excluded on one principle component than others and overwhelmingly illustrated variation than other traits.Most coefficients of plot pc1 against pc2 laid in the upper positive part in the diagram which refer to the important of pc1 and pc2 score of principle component analysis.

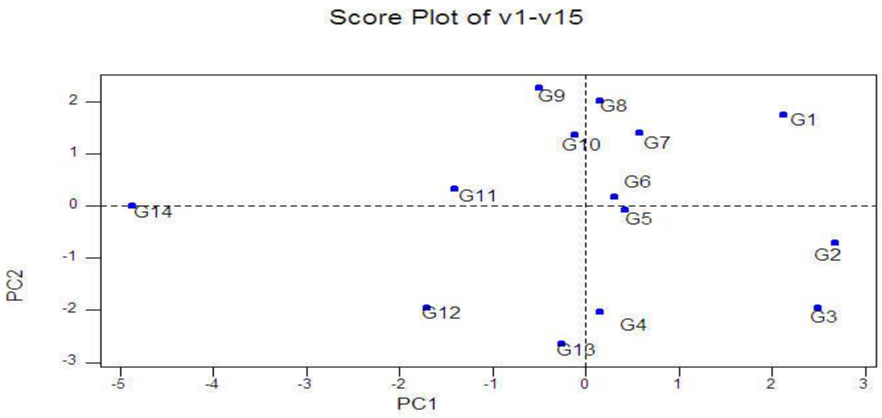

Fig.3:The diagram of principle components for the studied traits of lines.

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