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Estimating genetic diversity of Maize Inbred Lines Using biometrical methods

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ABSTRACT

The field experiment was conducted in the agricultural research stationAbu-Ghraib/Iraq in mid-March 2013 using six inbred lines of maize planted in randomized complete block design RCBD in three replicates. Inbred lines were introduced into the fulldiallelcross-program according to Model-I, Method-1 of (Griffing, 1956).Crosses of (ART-B21 × ART-B26) and (ART-B26 × ART-B21) showed higher heterosis for grain yield and Number of kernels per row. Inbreeds were distributed into four separated groups. Lines ART-B46 and ART-B37occupied one group. Lines ART-B21, ART-B26 and ART-B46 showed high diversity reflected on studied traits of their crosses. The first two components that explained (0.811) of the total variation were determined from principal component analysis and were used for clustering genotypes.PCA results showed a strong correlation between the grain yield and each of ear height and Number of kernels per row.

Introduction

Plant breeder's choice of germplasmsource determines thepotential improvement of traits underselection in the breeding programme. The success ofany breeding method depends

on the availability ofgenetic diversity in the base population(Al-Rawi,et al. , 2013). Utilisationof diverse parents in hybridisation programmes havebeen observed to produce better hybrids. The yield of the cultivars and other quantitative traits is one of the most important traits in determining the validity of cultivars grown in a specific environment(Elsahookie & Al-Rawi, 2011). The quantification of genetic diversity through biometrical procedure made it possible to choose genetically diverse parents for hybrid production.

Determination of genetic variability of parental combinations is an important step for successful breeding and genetic programs. Genetic diversity is one of the useful tools to select appropriate genotypes/lines for hybridization.(Hassan, A.,et al. 2018). Single character evaluation by statistical analysis methods may cause incomplete and sometimes incorrect interpretations. Hence it is very important to analyze morphological, biochemical and/or molecular traits simultaneously.(Aydın, 2007).

Multivariate statistical methods especially cluster analysis is a tool to classify varieties with similar conditions with respect to set of variables has gained increasing interest in recent years(Sandeep, 2018). Hierarchicalcluster analysis highlights the nature of relationshipbetween any type of samples described by any typeof descriptors(Olawuyi, et al , 2013). It could be used as a basis for selectionof parental types that could result to superior hybrids.

Cluster analysis is used to estimate the genetic diversity, determine quantitative characters loci and determine subgroups that are similar within one group and the possibility of classifying genotypes wither they were relative or not(Shrestha, 2016), which helps to classify those genotypes according to the convergence and divergence depending on calculated distances between them in cluster map. Many studies were conducted to classify genotypes into groups in order to use them in breeding programs(A.Subramanian and N. Subbaraman. 2010 , Chukwu, Okporie, Onyishi, & Obi, 2015 , Bibi et al., 2015, Shrestha, 2016,Kandel, Ghimire, Ojha, & Shrestha, 2018).Cluster analysis is frequently used to classify maize (*Zea mays* L.) accessions and can be used by breeders and geneticists to identify subsets of accessions which have potential utility for specific breeding or genetic purposes(Zaman, 2013).Principal component analysis (PCA) is one of multi-variances analysis methods that aims to reduce data into linear

combination and transform it into few dependent variables that shows most variance of that original data and presenting them in perpendicular diagram explaining most results according to the correlation between studied traits .(Sapkota, 2017), (Mounika, Ahamed, & Umar, 2018), (Bibi et al., 2015), (2003) .). KAMARA et al.used PCA to identify traits of maize (Zea mays L.) that accounted for most of the variance in the data. Maryam Ashofteh Beiragi, (2012)used PCA and cluster analysis to group kale populations According to principal component analysis, four principal components (PC) had Eigen values >1 which accounted for 67% of the total variance in the data on early planting date and 73% of the total variance in the data on delayed planting date. Based on cluster analysis, the 18 corn hybrids were separated into four and five major groups with each having two or more subgroups on early and delayed sowing dates respectively.(M. & M., 2006) identified traits that were the main sources of variation of genetic diversity among 106 Slovakian barley accessions Used the method of cluster analysis , the results showed grouped germplasm accessions into two large distinct clusters, the first one of which, with several exceptions, comprising old, and the second one, new genotypes. Principal components 1 and 2 accounted for about 72.8% of variability in germplasm accessions; mainly plant height, lodging, and grain yield accounted for this portion of variability.

The objective of this study was tousing cluster analysis to simplify data by collecting genotypes into convergent groups in addition to shorthand big number of variations to less number of factors through which can get the total variation of those variables through the analysis of principle component.

Materials and methods:

Genetic materials and experimental procedures

The experiment was conducted in the agricultural research stationAbu-Ghraib in mid-March 2013 The experimental material comprised of six inbred lines of maize (L1=DAQ, L2= ART-B263 L3=HNG9, L4=UMGW4, L5=ART-B374 and L6=ART-B34).obtained from the Public Authority for Agricultural Research. Inbred lines were introduced into the fulldiallelcross-program according to Model-I, Method-1 of (Griffing, 1956).the single crosses seeds obtained from the previous season were planted manually on (6 /8/2013). Genotypes were planted in randomized complete

block design RCBD in three replicates. All agricultural processes e.g. fertilizer applications, irrigation; soil preparation, and weed control were done according to the science recommendations . Data were collected for Number of days from planting to 50% tasseling (DT) and silking (DS), plant height (PH), ear height (EH), number of rows per ear (NRE), Number of kernels per row (NKR), weight of 100 kernel (HKW) and grain yield per plant (GY)Heterosis was estimated according to the first generation deviation from best parents (BP) as a percentage using the following equation:

$$Heterosis\% = (\bar{F}1 - BP)/BP \times 100$$

Cluster analysis was done using the agglomerative method to simplify data by putting genotypes in closed groups according to the studied traits that showed the same respond. According to the below formula and SPSS analyzing program v.20, data were analyzed.

Distance=$\sqrt{\sum(yi - \bar{y}\imath)^2}$

Principal component analysis (PCA) was estimated using following formula

$C1=a_{1i}\ x_1+a_{2i}\ x_2+ \ldots..+a_{mi}x$

Results and Discussion:

Cluster Analysis

Presented dendrogram in figure (1) demonstrating the distribution of the used parental lines into Two basic groups according to the cluster analysis. The first group included lines L1 and L6, while the second group was divided into three subgroups. Lines L 3 and L5 occupied a separate group, while Lines L2 and L4 were found in one group. If any

genotype located in separated group that's indicating that this group is genetically diverted from other studied genotypes.

Figure 1.Dendrogram of 6 maize inbred lines based on different traits

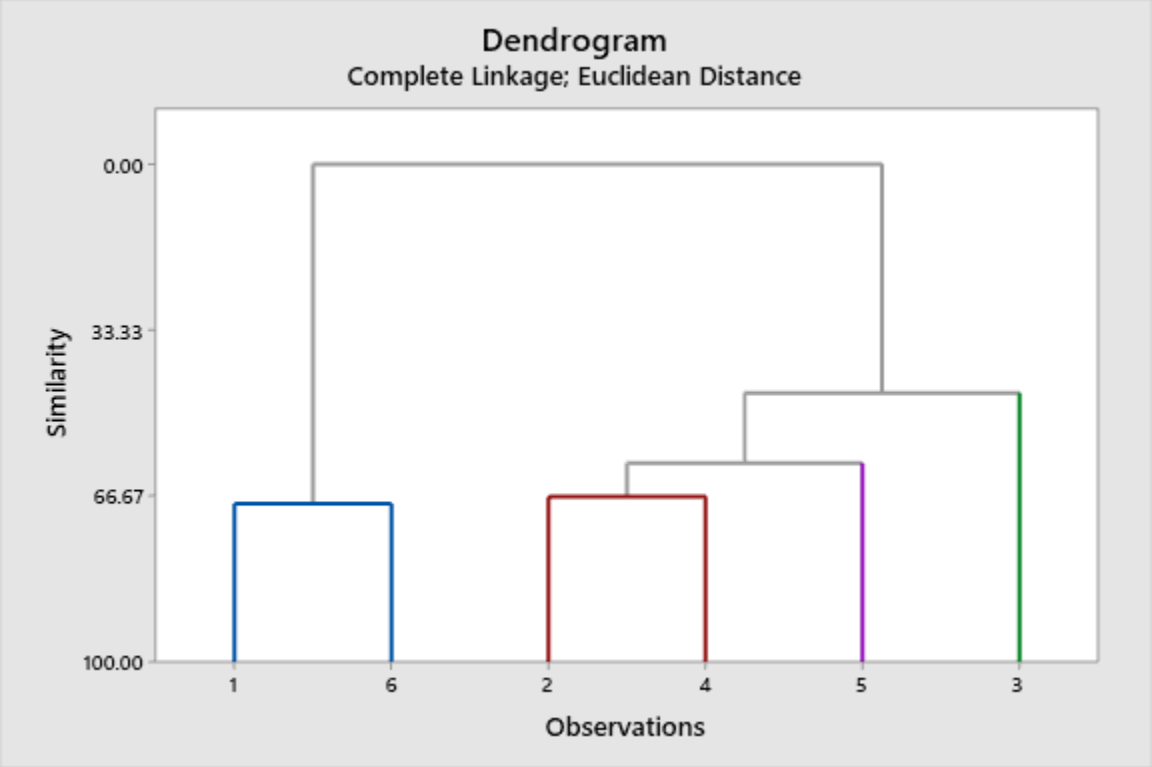

Table (1) results resenting stages of the cluster figure which formed five stages. Starting with the first stage which occupied the lowest distance by merging line (L1) with line (L6) in one group which gave the lowest value of the factor (59.227), while the second stage was merged line (L2) with (L4) and gave factor value of 64.67 and so on for the

rest stages. Low distance means close lines hence such genotypes should be avoided in making hybridization between them.

Table 1: Ability factor value between groups according to cluster analysis

Stage	Cluster 1	Cluster 2	Coefficients
1	1	6	59.227
2	2	4	64.672
3	2	5	81.138
4	2	3	124.406
5	1	2	388.805

Obtained factors values are indicating the nature of the diversity between groups, where the value of the small factor mean that the groups are similar to each other while big value refers to the reduction of homogeneity. Results were in consistency with the hybrid vigour results, where crosses L5×L3 and L3×L5 showed the highest percent of hybrid vigour for the flowering (sillking and tassiling) and as mentioned previously both lines L3 and L5 were occupied separated groups which genetically divert and that clearly presented in the high values of hybrid vigour of both genotypes. The cross L2×L4 had positive value (1.14) of DT which again confirmed by the results of cluster analysis (Figure 1), it can be noticed that the second stage gave less distance between the lines 2 and 4 reached 64.67 for that reason it is not recommended to cross between mentioned lines 4 and 2. Regarding the PH, the cross L2×L3 showed highest positive hybrid vigour which is due to that the two lines were occupied stage four which is coming before the last stage with value reached 124.406 caused an increase in hybrid vigour due to a slight increase in distance. The two crosses L5×L2 and L2×L5 had the lowest positive hybrid vigour which confirmed by cluster analysis, where stage three that containing lines 5 and 2 gave low distance (81.13). From the cluster analysis results, it can be seen that line 3 occupied separate group which reflected on the hybrid vigour for the trait of the main EH which gave when crossed with line 1 high hybrid vigour shown in both crosses L3×L1 and L1×L3. For the NRE, line 3 crossed with line 4 showed highest hybrid vigour for the trait reached 30.07 and 30.00 for both L4×L3 and L3×L4 consequently. Also the two crosses L5×L2 and L2×L5 presented lowest hybrid vigour for the same trait which is due to the low companied distance they have as the third stage which contained line 2 and 5 gave distance value of 81.13. For the yield trait and the NKR; the two crosses L2×L1 and L1×L2 showed highest hybrid vigour which was a result of both lines gave the highest factor value reached 388.805 that occupied stage 5 (the last) and that is due to being

genetically different compared to the other used lines and having desirable gene set that is not exited in other lines. That was reflected on the performance, so the cross between the two lines in two ways gave highest hybrid vigour for the two above mentioned traits. Hybrid vigour for the HKW was shown the highest value in the two hybrids L5×L1 and L1×L5 which might be due to the line 5 was in separate group and line 1 had highest distance with line 2 and this formed stage 5.

From all previously mentioned it can be noticed that the lines 1, 3 and 5 demonstrated clear genetic diversity reflected in hybrid vigour, so it is recommended to use those lines and cross them with other lines to get benefit of the segregations or in improving a certain trait or producing multiple lines. Using such technology can improve selection programs by collecting desired allels especially if biotechnology was not available.

Table 2.Heterosis for the traits expressed as percentage of increase over and decrease under better parent (BP %)

F1	DT	DS	PH	EH	NRE	NKR	HKW	GY
L1×P2	-9.61	-11.57	5.11	25.32	28.75	43.15	22.06	124.82
L2×P1	-9.03	-10.52	4.10	25.76	29.26	40.25	16.59	111.39
L1×P3	-11.83	-13.20	3.49	30.22	16.42	37.46	26.23	102.44
L3×P1	-13.44	-14.22	4.76	28.92	17.42	30.54	26.23	90.32
L1×P4	-2.89	-4.31	7.50	13.81	16.53	22.89	1.61	57.11
L4×P1	-4.63	-6.98	4.49	10.69	18.82	15.73	13.54	62.40
L1×P5	0.532	0.00	7.51	15.79	25.86	21.78	39.45	113.73
L5×P1	-10.76	-12.18	10.13	16.05	23.79	23.08	38.16	110.61
L1×P6	-3.23	-3.56	10.84	9.52	18.75	20.66	21.28	74.99
L6×P1	-7.53	-8.13	8.84	16.06	17.97	23.48	23.03	76.11
L2×P3	-7.92	-10.20	11.70	24.78	29.99	-1.12	24.32	68.45
L3×P2	-9.61	-11.57	9.94	23.51	19.75	1.60	13.16	47.67
L2×P4	1.14	-1.61	7.79	11.91	17.05	27.42	27.27	106.46
L4×P2	-5.20	-7.53	9.55	7.08	16.83	24.88	26.06	99.96
L2×P5	-5.64	-7.89	2.53	-0.85	11.41	19.72	21.71	67.16
L5×P2	-7.92	-10.52	4.29	13.59	8.78	20.79	25.04	72.62
L2×P6	-2.25	-3.16	6.59	9.53	22.38	17.84	16.39	68.64
L6×P2	-2.25	-3.68	5.81	15.88	21.65	14.84	11.43	55.75
L3×P4	-6.94	-8.06	9.16	11.28	30.07	21.54	25.61	113.96
L4×P3	-7.53	-10.21	8.77	13.52	30.00	17.52	22.62	97.64
L3×P5	-13.83	-15.42	10.53	10.47	25.63	14.59	18.26	71.31
L5×P3	-16.49	-17.91	9.36	9.57	25.33	19.43	16.69	75.89
L3×P6	-8.52	-9.96	6.35	13.57	11.57	8.67	11.25	36.03
L6×P3	-11.17	-11.94	6.74	7.71	11.03	11.78	11.59	35.16
L4×P5	-1.73	-3.23	3.91	15.99	19.59	24.29	12.52	76.08
L5×P4	-0.859	-2.15	5.85	18.75	19.59	24.49	12.52	76.38
L4×P6	0.00	-1.61	4.09	9.98	20.12	17.84	9.21	57.75
L6×P4	-0.572	-1.61	6.43	9.53	19.89	19.59	4.74	56.14

L5×P6	-7.36	-3.46	10.13	12.36	19.95	3.62	14.78	50.47
L6×P5	-8.94	-9.86	9.55	16.66	19.72	5.45	16.42	52.63
L.S.D	2.17	2.31	3.56	4.45	0.48	1.32	1.08	6.37

Principal component analysis (PCA):

All studied traits were subjected to PCA to minimise data size and convert it to symmetrical traits using correlation matrix between traits. Table (7) demonstrate Eigen, Proportion and Cumulative values and it can be noticed that the main component pc1 formed 71.1% of the total variation and the second component pc2 has formed around 10% of the total component, so both (first and second) components formed 81.1% which is enough to draw the diagram that shows these values distribution.

Table 3: Eigenvalue, Proportion and Cumulative variability of different factors based on Principle component analysis of different traits for maize

Eigenvalue	5.6876	0.7984	0.6533	0.5407	0.1780	0.1308	0.0075	0.0039
Proportion	0.711	0.100	0.082	0.068	0.022	0.016	0.001	0.000
Cumulative	0.711	0.811	0.892	0.960	0.982	0.999	1.000	1.000
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
DT	0.361	-0.247	-0.505	0.272	-0.042	0.087	-0.187	0.660
DS	0.372	-0.234	-0.451	0.245	-0.109	0.069	0.156	-0.709
PH	-0.303	-0.716	0.159	-0.060	0.186	0.575	0.036	-0.009
EH	-0.315	0.271	0.010	0.799	0.416	0.117	-0.004	-0.038
NRE	-0.363	-0.317	0.125	0.350	-0.648	-0.358	-0.282	-0.042
NKR	-0.347	0.399	-0.415	-0.186	-0.317	0.532	-0.349	-0.090
WHG	-0.358	-0.195	-0.451	-0.256	0.478	-0.471	-0.318	-0.115
GY	-0.399	0.006	-0.354	-0.037	-0.172	-0.100	0.798	0.193

The main components (pc1 and pc2) were used in drawing the diagram (Figure 2) that shows the correlation between the different traits. Vectors length and their angles cosine used in collecting traits within different groupspresents the correlation between those groups through a matrix of the correlation.

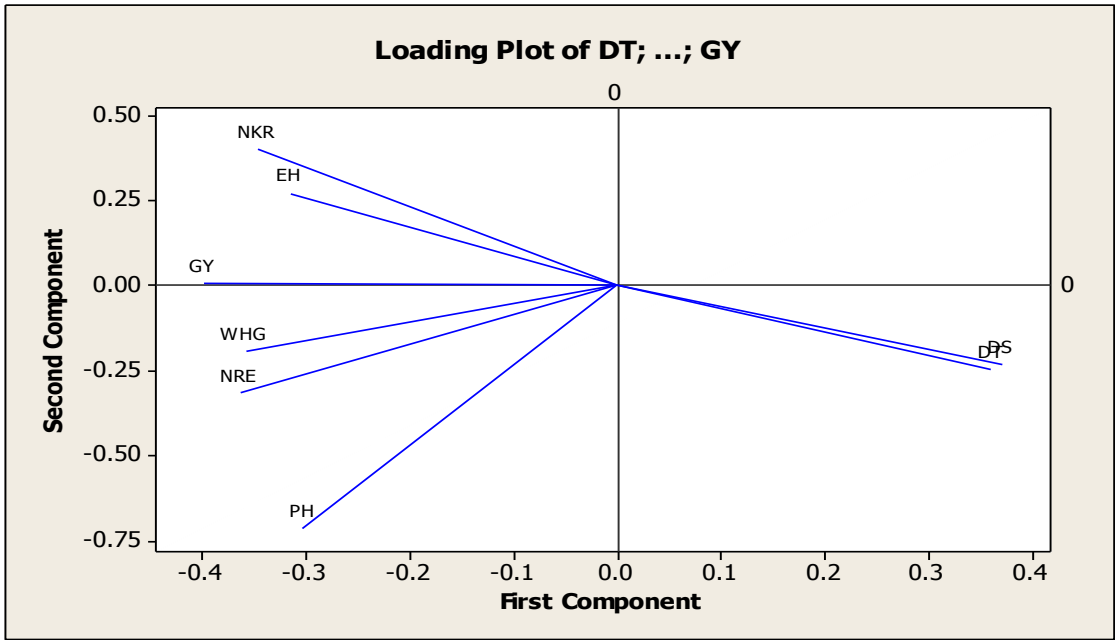

Figure 2: Biplot between PCs 1 and 2 showing contribution of various traits in variability.

These vectors helped to select traits that could be used to improve the plant yield, it is presented from the diagram that there is a strong correlation between plant yield and each of EH and KNR so the selection for grain number trait helps to increase the yield where the yield is considered as a quantitative trait which is complicated in inheriting and controlled by many genes. Pathak (1974) mentioned that the selection for yield components has more impact in increasing yield than selecting for the yield itself, hence it is necessary to select for one or more trait instead of the yield. Also, a negative correlation appeared between the yield and the HKW so the increasing in grain weight could result in reduction in the yield. Another negative correlation between the yield and the NRE and plant height appeared and this means that the selection for those two traits could be useless in improving plant yield.

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